Long-Term Administration of Gonadotropin-Releasing Hormone in Men with Idiopathic Hypogonadotropic Hypogonadism

A Model for Studies of the Hormone's Physiologic Effects

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The effect of long-term administration of gonadotropin-releasing hormone (GnRH) for induction and maintenance of sexual maturation was characterized in 23 men with idiopathic hypogonadotropic hypogonadism. Twenty-two men achieved normal adult male serum testosterone concentrations (575 ± 33 ng/dL; $p < 0.0001$ compared with the baseline mean of 61 ± 6 ng/dL) that were sustained in 21 men for up to 36 months with bolus doses of GnRH varying from 25 to 300 ng/kg body weight administered every 2 hours. Pulsatile luteinizing hormone (LH) secretion occurred in all 23 men, with mean levels of LH (14.7 ± 1.3 mIU/mL) and follicle-stimulating hormone (11.3 ± 1.3 mIU/mL) within or above the normal range for adult men. Mature sperm were observed in the ejaculates of 20 men, with counts ranging from less than 1×10^6 to 96×10^6/mL. Increasing responsiveness of the pituitary-gonadal axis to GnRH was shown in 6 men. Men with idiopathic hypogonadotropic hypogonadism present a useful model to study the onset and maintenance of reproductive function in men.

MEN WITH idiopathic hypogonadotropic hypogonadism have an isolated deficiency of gonadotropin-releasing hormone (GnRH) secretion that prevents them from undergoing puberty. They have otherwise normal endocrine function (1), and their hypogonadism can be reversed with GnRH replacement therapy (2, 3). Initial attempts at hypothalamic hormone replacement in men with idiopathic hypogonadotropic hypogonadism who received this peptide infrequently and in high doses were not successful in achieving and maintaining normal gonadal function (4-8). Subsequently, other investigators reported that a GnRH dose of 25 ng/kg body weight (1 to 2 μg) injected intravenously every 2 hours produced a pattern of gonadotropin secretion similar to that seen in normal puberty (9, 10). We then showed that subcutaneous administration of GnRH in this regimen over a 3-month period to men with idiopathic hypogonadotropic hypogonadism normalized both pituitary and gonadal function and initiated the early changes of sexual maturation (3).

We have now evaluated the administration of low-dose GnRH as an effective long-term therapy for men with idiopathic hypogonadotropic hypogonadism and as a model for the study of the physiologic effects of GnRH. A regimen of subcutaneous GnRH was first established in 23 consecutive men that initiated a pattern of pituitary and gonadal hormone secretion similar to that seen in normal men. Evidence of developmental changes in the pituitary-gonadal axis similar to those occurring during normal puberty (11-14) was then evaluated during the initial months of therapy. Finally, administration of GnRH for periods of greater than 1 year was evaluated to ensure that this therapy remained effective for sufficient time to permit full maturation of the male reproductive axis and to sustain normal pituitary and gonadal function including mature spermatogenesis. We conclude that long-term GnRH administration in men with idiopathic hypogonadotropic hypogonadism produces pituitary-gonadal activity similar to that seen in normal pubertal and adult males and provides a valuable model to examine the roles of GnRH and other modulators of the male reproductive axis during both sexual maturation and adulthood.

Methods

STUDY POPULATIONS

Normal Men: Twenty normal male volunteers between the ages of 18 and 35 were chosen for evaluation of normal pituitary gonadotropin and gonadal steroid secretion on the basis of the following criteria: a normal history and physical examination; normal serum concentrations of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone; testicular volumes of greater than 15 mL by Prader orchidometer; and normal findings on semen analysis ($> 30 \times 10^6$ sperm/mL, > 60% motility, > 2 mL in volume). Normal volunteers were admitted to the Clinical Research Center of the Massachusetts General Hospital, where they had blood sampling for LH and FSH assays at 10-minute intervals for 24 hours. The normal ranges for mean serum LH, FSH, and testosterone concentrations in this population were determined from pooled samples formed from equal aliquots of each of the 145 samples obtained during an admission. The number of LH pulses occurring per 24 hours as well as the mean LH pulse amplitude were determined in each man.

Men with Hypogonadotropic Hypogonadism: Twenty-three consecutive men with idiopathic hypogonadotropic hypogonadism between the ages of 18 and 52 years were selected on the basis of the following criteria: failure to undergo puberty by age 18 (20 patients) or isolated idiopathic loss of gonadotropin secretion after puberty (3 patients); a serum testosterone concentration of less than 110 ng/dL in the presence of low or normal circulating gonadotropin levels; normal levels of thyrotropin (note that 1 patient had concomitant primary hypothyroidism and was receiving L-thyroxine), prolactin, growth hormone, and cortisol on baseline and after stimulation with an intrave-

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nous injection of thyrotropin-releasing hormone (200 μg) and insulin-induced hypoglycemia (glucose < 40 mg/dL); and normal findings on computed tomography of the hypothalamic-pituitary region. In those men receiving androgens or human chorionic gonadotropin before the study, hormonal therapy was discontinued for at least 3 months before their baseline evaluation and initiation of the GnRH replacement. This study was approved by the Subcommittee on Human Studies of the Massachusetts General Hospital, and all men provided written informed consent.

STUDY PROTOCOL

Baseline Studies in Hypogonadotropic Men: A detailed history and physical examination were obtained from each man to evaluate any evidence of spontaneous sexual maturation. Each patient was then admitted to the Clinical Research Center, where he had 16 to 24 hours of blood sampling for LH and FSH assays at 10- or 20-minute intervals to assess endogenous gonadotropin secretory patterns. Mean baseline serum concentrations of LH, FSH, testosterone, and estradiol were determined from pools containing equal aliquots of each sample drawn during an admission.

Gonadotropin-Releasing Hormone Dose Regimen: We administered GnRH subcutaneously to hypogonadotropic men via a portable infusion pump (Ferring Laboratories Inc., Ridgewood, New Jersey) at 2-hour intervals to simulate the LH pulse frequency observed in normal men (2, 15-18). An initial dose of 25 ng/kg per bolus was chosen on the basis of previous studies that showed intravenous administration of this dose produced LH pulses similar to those resulting from endogenous GnRH secretion in normal men (9, 19). However, when 25 ng/kg was administered subcutaneously to men with idiopathic hypogonadotropic hypogonadism, it was not uniformly sufficient to initiate normal gonadal steroidogenesis (3). Therefore, GnRH doses were increased progressively in each man, allowing at least a 2-month period at each dose (25, 50, 100, 150, 200, and 300 ng/kg) for evaluation of responses, until serum testosterone concentrations were maintained consistently within the midrange observed in the normal adult men. Five patients also received doses of 75 ng/kg.

Responses to GnRH administration were monitored during biweekly admissions to the Clinical Research Center. During each admission, interval histories were obtained, physical examinations were done, and blood samples were drawn for LH and FSH determinations at 20-minute intervals for 8 hours during administration of four doses of GnRH. In addition, serum LH, FSH, testosterone, and estradiol concentrations were determined from pools formed from equal aliquots of each of the 25 samples obtained during an admission. Mean values of serum LH, FSH, and testosterone concentrations from the first and last admissions during administration of the GnRH dosage that normalized serum testosterone levels were used to reflect hormonal levels achieved during therapy. Serum samples were screened for anti-GnRH antibodies at 6-month intervals.

Spermatogenesis: Samples for semen analysis were requested monthly once an ejaculate was present. Serum LH and FSH concentrations at the time of the maximum sperm count during therapy were compared among men with sperm counts of less than 1×10^6/mL, those with counts of 1×10^6 to 10×10^6/mL, and those with counts greater than 20×10^6/mL.

Radioimmunoassays: Serum gonadotropin and sex steroid concentrations were determined by previously described radioimmunoassays (20, 21). The cross reactivity of the alpha subunit of LH in the LH radioimmunoassay was 4%.

COMPARISONS OF TREATED AND NORMAL MEN

Mean serum LH, FSH, and testosterone concentrations of the 20 men with hypogonadotropic hypogonadism who were documented to have mature spermatogenesis during therapy were compared with the mean levels of these hormones in the 20 normal men. Mean serum LH, FSH, and testosterone concentrations in the 20 normal men were also compared with those of the men with idiopathic hypogonadotropic hypogonadism subdivided according to their seminal fluid analysis as outlined above.

EVALUATION OF CHANGES IN PITUITARY AND GONADAL RESPONSIVENESS ACROSS SEXUAL MATURATION

Patients continued to have biweekly monitoring of gonadotropin and sex steroid responses while receiving the GnRH dose that initiated normal gonadal steroidogenesis. Changes in pituitary and gonadal responses and in GnRH dose requirements were evaluated by studying seven consecutive men who had at least 12 months of continuous GnRH administration and who had had no evidence of sexual maturation before initiation of therapy (no history of spontaneous puberty and baseline testicular volumes of 3 mL or less). After 6 to 12 months of sexual maturation, during which time normal steroidogenesis was maintained, the GnRH dose in each man was progressively decreased to levels that had been previously unable to elicit normal steroidogenesis before sexual maturation. Thus, LH, FSH, and testosterone concentrations were determined in serum pools of two biweekly admissions in each of three stages of study. In each case, at least 2 weeks were allowed to pass after dose adjustments, for equilibration of the pituitary-gonadal axis, before each period of evaluation. Stage 1 was defined as the final two biweekly admissions on a GnRH dose that was unable to produce normal serum testosterone concentrations before sexual maturation; stage 2 was the second and final biweekly admissions on the optimized GnRH dose (that is, the GnRH dose that produced normal serum testosterone concentrations); stage 3 was the second and third biweekly admissions after reinstatement of the GnRH dose that had previously been unable to produce normal serum testosterone concentrations during stage 1.

STATISTICAL TESTS

All results are expressed as the mean ± SE. The presence of gonadotropin pulses was determined by the criteria of Santen and Bardin (16), which require a 20% increase from nadir to peak serum concentrations of gonadotropins. To decrease further the risk of false-positive detection of pulses, we also required that each pulse have an amplitude of at least 2 mIU/mL and contain at least two time points if sampling was done at 10-minute intervals. Pulse amplitude was calculated as the difference between nadir and peak gonadotropin values. Two-tailed *t*-testing was used to determine significant differences in serum LH, FSH, and testosterone concentrations between normal men and men with hypogonadotropic hypogonadism. Paired one-tailed *t*-testing was used to analyze differences in serum LH, FSH, and testosterone concentrations and testicular volumes in hypogonadotropic men between different stages of GnRH therapy. Two-tailed *t*-testing was used to determine differences in LH, FSH, and testosterone concentrations and testicular volumes between hypogonadotropic men with low sperm counts and those with normal sperm counts. In instances when a variable was not normally distributed, differences were analyzed with the Mann-Whitney test.

Results

NORMAL MEN

Pooled serum samples from 24-hour sampling periods showed a range of luteinizing hormone (LH) levels from 3.6 to 18.9 mIU/mL (mean, 9.0 ± 0.9), of follicle-stimulating hormone (FSH) levels from 2.1 to 19.8 mIU/mL (mean, 7.0 ± 1.1), and of testosterone levels from 329 to 969 ng/dL (mean, 622 ± 40). The mean amplitude of LH pulses seen in normal men ranged from 4.5 ± 0.8 to 16.3 ± 2.5 mIU/mL (mean, 10.5 ± 0.7), and LH pulses occurred at frequencies of 6 to 19/24 hours (mean, 10.9 ± 0.7). Pulses of FSH were rarely seen.

MEN WITH IDIOPATHIC HYPOGONADOTROPIC HYPOGONADISM

Baseline Characteristics: Table 1 shows the baseline characteristics of the hypogonadotropic men. Three men

Table 1. Baseline Characteristics and Sperm Counts in 23 Men with Idiopathic Hypogonadotropic Hypogonadism*

Patient	Age	Initial Serum Testosterone Level	Initial Testis Volume	Maximal Testis Volume	History of Puberty	Anosmia	GnRH Dose Required	Duration of Normal Testosterone Level	Maximal Sperm Count
	yrs	*ng/dL*	*mL*	*mL*			*ng/kg*	*wks*	$n \times 10^6$/*mL*
1	18	108	12	23	−	−	25	79	96
2	24	44	2	23	−	−	100	81	78
3	24	62	14	23	+	+	100	47	73
4	27	63	20	25	+	−	25	58	50
5	23	81	11	23	−	−	25	22	22
6	27	107	15	23	+	−	25	20	4
7	23	79	2	15	−	−	200	16	3
8	29	40	2	15	−	+	150	92	3
9	25	32	3	12	−	−	50	65	3
10	28	67	2	10	−	+	25	56	2
11	25	28	1	12	−	+	100	49	1
12	27	31	3	11	−	−	100	135	< 1†
13	30	35	2	11	−	−	200	113	< 1†
14	41	41	2	11	−	−	150	111	< 1
15	25	31	2	11	−	−	150	101	< 1
16	25	43	3	15	−	+	150	27	< 1
17	25	49	2	12	−	+	150	142	< 1
18	22	49	2	9	−	−	300	98	< 1†
19	18	90	2	20	−	−	50	40	< 1
20	18	26	2	14	−	+	150	16	< 1
21	18	72	2	5	−	−	25	. . .	ND‡
22	52	96	8	12	−	−	200	. . .	ND
23	18	23	3	9	−	+	§	. . .	ND

* History of puberty refers to whether a history of partial or complete progression through puberty was present (+) or absent (−). Gonadotropin-releasing hormone (GnRH) dose required indicates the dose of GnRH that normalized serum testosterone concentrations. Duration of normal testosterone indicates the number of weeks that the serum testosterone level had been normal at the time of the maximal sperm count. Maximal sperm count indicates the maximal count attained during GnRH therapy.

† Patient had a history of unilateral cryptorchidism.

‡ ND = not determined.

§ Anti-GnRH antibody developed before the GnRH dose was optimized.

gave a history of partial or complete progression through puberty with subsequent idiopathic loss of sexual function. All others gave a history of no spontaneous initiation of any sexual maturation. Testicular volumes ranged from 2 to 20 mL with 17 men having testicular volumes of less than 3 mL. Sixteen men had no LH pulses detected by frequent blood sampling for 24 hours. Seven men had LH pulses of decreased amplitude during sleep or wakeful periods, or both, as previously described (2). The mean baseline serum concentration of LH was 2.1 ± 0.5 mIU/mL ($p < 0.005$ compared with normal); FSH, 3.2 ± 0.4 mIU/mL ($p < 0.02$ compared with normal); and testosterone, 59 ± 6 ng/dL ($p < 0.001$ compared with normal). Baseline serum estradiol concentrations were less than 20 pg/mL.

Initiation of Normal Pituitary and Gonadal Function:

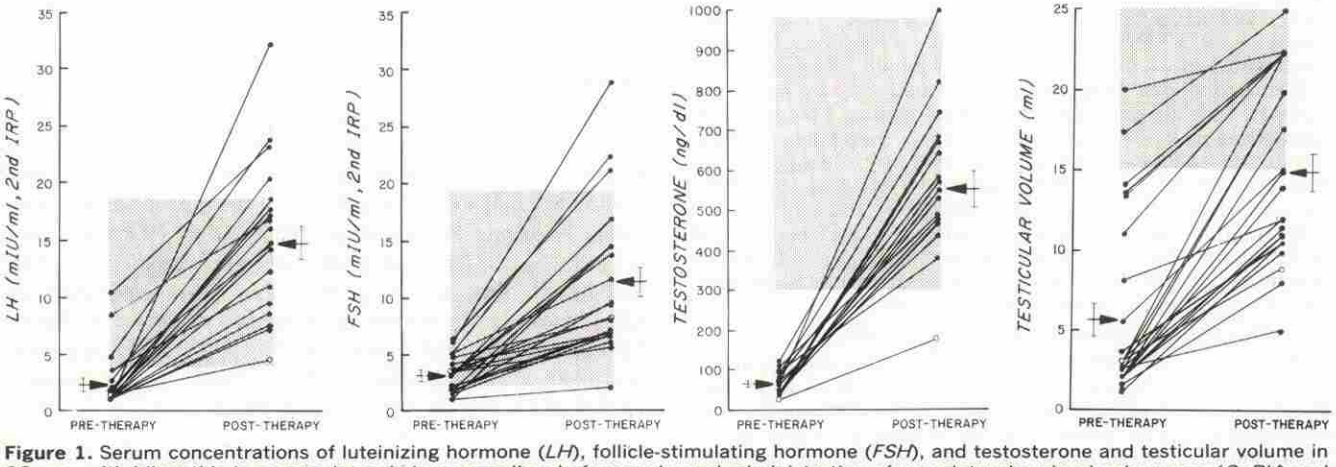

Figure 1. Serum concentrations of luteinizing hormone (*LH*), follicle-stimulating hormone (*FSH*), and testosterone and testicular volume in 23 men with idiopathic hypogonadotropic hypogonadism before prolonged administration of gonadotropin-releasing hormone (*GnRH*) and while receiving optimized doses of GnRH. The shaded areas indicate the range of values determined from our normal men. Mean (± SE) values of the 23 men before and during optimal GnRH administration are indicated by the arrows to the sides of each graph and the differences were statistically significant ($p < 0.0001$). The open circles refer to the man who developed an anti-GnRH antibody before achieving normal serum testosterone levels. 2nd IRP = second international reference preparation.

Table 2. Serum Luteinizing Hormone, Follicle-Stimulating Hormone, and Testosterone Levels in Men with Idiopathic Hypogonadotropic Hypogonadism Who Achieved Mature Spermatogenesis on Therapy with Gonadotropin-Releasing Hormone*

	Follicle-Stimulating Hormone	Luteinizing Hormone	Testosterone
	mIU/mL	*mIU/mL*	*ng/dL*
Patients ($n = 20$)			
Sperm count $< 1 \times 10^6$/mL ($n = 9$)	21.0 ± 4.8†‡§	21.7 ± 3.2§‖	583 ± 67
Sperm count 1 to 10×10^6/mL ($n = 6$)	8.5 ± 1.5	14.2 ± 2.6 ¶	454 ± 100
Sperm count $> 20 \times 10^6$/mL ($n = 5$)	5.5 ± 1.1	11.7 ± 1.8	526 ± 112
Normal men ($n = 20$)	7.0 ± 1.1	9.0 ± 0.9	622 ± 40

* All values are at the time of the maximum sperm count. Data are given as means ± SE.
† $p < 0.05$ compared with hypogonadotropic men with sperm counts of 1×10^6 to 10×10^6/mL.
‡ $p < 0.02$ compared with hypogonadotropic men with sperm counts $> 20 \times 10^6$/mL.
§ $p < 0.01$ compared with normal men.
‖ $p < 0.05$ compared with hypogonadotropic men with sperm counts $> 20 \times 10^6$/mL.
¶ $p < 0.03$ compared with normal men.

Doses ranging from 25 to 300 ng/kg per bolus were required to produce normal pituitary and gonadal function (Table 1). All 23 men achieved levels of LH (14.7 ± 1.3 mIU/mL; $p < 0.0001$ compared with baseline) and FSH (11.3 ± 1.3 mIU/mL; $p < 0.0001$ compared with baseline) that were within or above the normal adult range (Figure 1). The pattern of LH secretion in response to subcutaneous GnRH administration was pulsatile in all men, as assessed during biweekly 8-hour admissions (Figure 2). Twenty-two men achieved normal circulating levels of testosterone (575 ± 33 ng/dL; $p < 0.0001$ compared with baseline; Figure 1). The single remaining man developed an anti-GnRH antibody before his GnRH dose was optimized, and he was removed from the study. Testicular volumes increased from a baseline value of 5.4 ± 1.2 to 15.0 ± 1.2 mL ($p < 0.0001$) during therapy (Figure 1). No statistically significant correlation was found between the dose of GnRH required to initiate normal steroidogenesis and the baseline testicular size, circulating gonadotropin or testosterone levels before treatment, or the presence of LH pulses. However, of the 6 men with initial testicular volumes of greater than 5 mL, 4 produced normal circulating testosterone levels with the minimal GnRH dose employed (25 ng/kg per bolus). Of the 17 men with testicular volumes of less than 5 mL, only 2 responded to a dose of 25 ng/kg per bolus.

Spermatogenesis: Mature sperm appeared in the ejaculate of 20 of our men, with counts ranging from less than 1×10^6 to 96×10^6/mL (Table 1). The remaining 3 men did not provide specimens for semen analysis. Five men achieved sperm counts of greater than 20×10^6/mL, and 4 of them had an initial testicular volume of greater than 10 mL. Only 1 of the remaining 18 men had an initial testicular volume of greater than 10 mL (Table 1). In addition, of the 3 men who gave a history of partial or complete spontaneous pubertal development, 2 achieved normal sperm counts (Table 1). Finally, neither of the men with a history of unilateral cryptorchidism achieved sperm counts above 1×10^6/mL (Table 1).

There was a significant positive correlation between sperm count and both the initial testicular volume ($r = 0.55$; $p < 0.02$) and the maximum testicular volume ($r = 0.64$, $p < 0.005$) in the men who provided semen specimens and did not have a history of cryptorchidism. There was no correlation ($r = 0.07$) between the maximum sperm count and the number of weeks that the man had a normal testosterone level. In addition, those men with sperm counts of less than 1×10^6/mL had significantly higher serum FSH levels than men with counts of 1×10^6 to 10×10^6/mL ($p < 0.05$) or men with counts of greater than 20×10^6/mL ($p < 0.02$; Table 2). Serum LH concentrations in the men with sperm counts of less than 1×10^6/mL were also significantly higher than LH levels in men with counts greater than 20×10^6/mL ($p < 0.05$) but were not different from those of men with sperm counts between 1×10^6 and 10×10^6/mL. There was no significant difference in the serum testosterone concentrations of these three groups (Table 2).

COMPARISON OF TREATED AND NORMAL MEN

Mean serum LH and FSH concentrations in the 20 hypogonadotropic men who were documented to have mature spermatogenesis during therapy were 17.0 ± 1.0 and 13.4 ± 2.7 mIU/mL, respectively, and were significantly higher than LH and FSH levels of normal men ($p < 0.001$ for LH and $p < 0.05$ for FSH). However, those patients with sperm counts of greater than

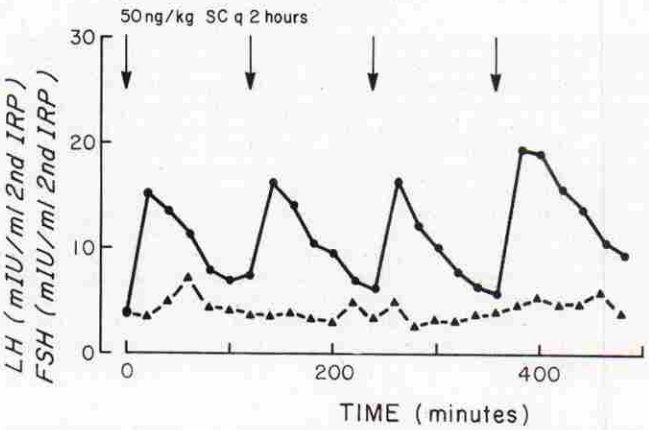

Figure 2. Serum luteinizing hormone (*LH*; *circles*) and follicle-stimulating hormone (*FSH*; *triangles*) concentrations determined at 20-minute intervals for 8 hours in a representative patient receiving long-term subcutaneous therapy with gonadotropin-releasing hormone (*GnRH*) at 2-hour intervals. The arrows indicate subcutaneous 1-minute bolus doses of GnRH. The patient's testosterone level was 884 mg/dL, his estradiol concentration was less than 20 pg/mL, and his testes were 10 mL (right) and 12 mL (left) in size. 2nd IRP = second international reference preparation.

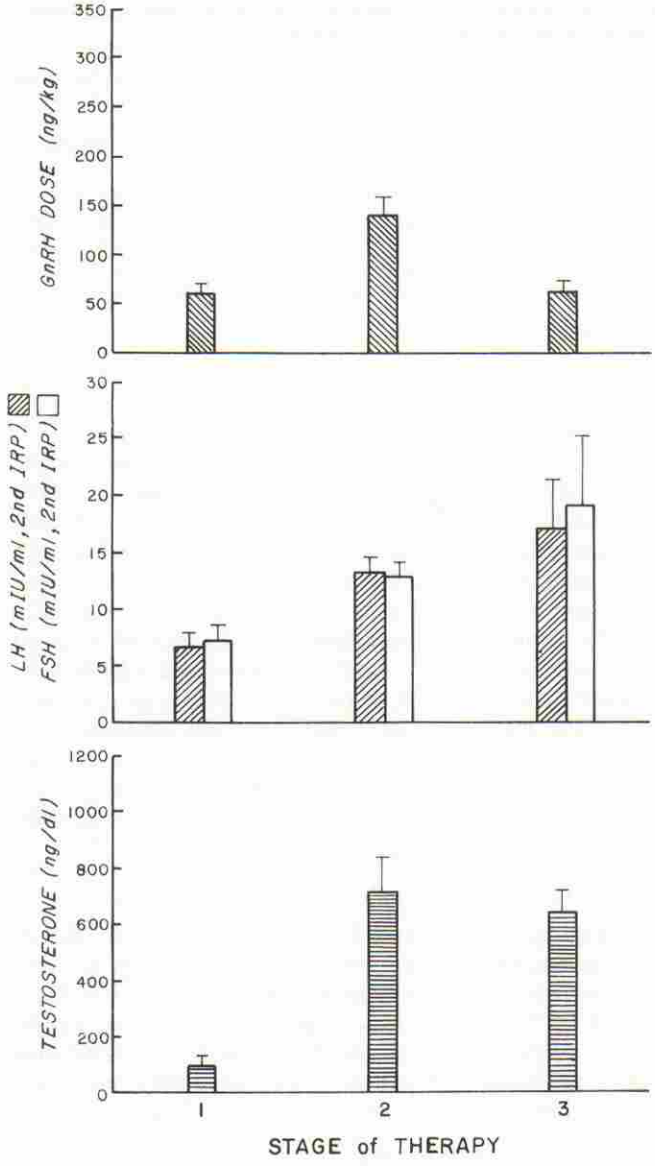

Figure 3. Doses of gonadotropin-releasing hormone (*GnRH*) and mean (± SE) luteinizing hormone (*LH*), follicle-stimulating hormone (*FSH*), and testosterone concentrations determined on serum pools from 6 men with idiopathic hypogonadotropic hypogonadism who had increased pituitary-gonadal responsiveness during three stages of GnRH therapy: stage 1 was during administration of a GnRH dose incapable of normalizing serum testosterone concentrations before sexual maturation; stage 2 was during the first and last biweekly admissions on the optimized GnRH dose; stage 3 was during reinstatement of the GnRH dose that had been unable to produce normal steroidogenesis before sexual maturation. The rise in LH from stage 1 to stage 3 was not statistically significant ($p < 0.07$), whereas the rises in testosterone and FSH from stage 1 to stage 3 were statistically significant ($p < 0.005$ for testosterone and $p < 0.05$ for FSH). 2nd IRP = second international reference preparation.

20×10^6/mL had no significant differences in serum LH and FSH concentrations when compared with normal men (Table 2). Detailed GnRH dose adjustments were not attempted in the other groups of hypogonadotropic men (Table 2) to determine if LH and FSH levels could be matched to those of normal men. The mean LH pulse amplitude in the 20 patients who had mature sperm in their ejaculates was 15.3 ± 1.7 mIU/mL (range, 7.4 to 29.1) as determined when maximum sperm counts were observed. This pulse amplitude was greater than the mean LH pulse amplitude of 10.5 ± 0.7 mIU/mL (range, 4.5 to 16.3) observed in the normal men ($p < 0.03$), though there was considerable overlap. As with the normal men, FSH pulses were rarely seen. There were no significant differences between serum testosterone concentrations of any of the three groups of hypogonadotropic men when compared with the testosterone levels of normal men (Table 2).

CHANGES IN PITUITARY AND GONADAL RESPONSES TO GONADOTROPIN-RELEASING HORMONE DURING SEXUAL MATURATION

The mean serum testosterone concentration in the 22 hypogonadotropic men during the first month of administration of their optimized GnRH dose was 589 ± 64 ng/dL. With continued administration of the same dose, testosterone levels rose further, to a mean maximum of 945 ± 211 ng/dL ($p < 0.01$) with peak values occurring between the second and fifth months of treatment with this optimized dosage. In 5 men the serum testosterone concentrations increased to above 1000 ng/dL before GnRH doses were decreased. Serum testosterone concentrations remained stable after this period of time for the duration of therapy (up to 36 months).

Seven of the men without evidence of puberty before therapy, who received courses of GnRH of sufficient duration to develop and maintain normal serum testosterone concentrations for at least 6 months, were evaluated in detail for changes in pituitary and gonadal responses during GnRH-induced sexual maturation. In six of the seven, a dose of GnRH that was unable to produce normal serum testosterone concentrations before sexual maturation was able to maintain normal serum testosterone concentrations when reinstituted after sexual maturation (Figure 3, lower panel; 148 ± 44 ng/mL in stage 1 compared with 634 ± 80 ng/mL in stage 3; $p < 0.005$). Mean pooled FSH levels also showed a rise when the lower GnRH dose was reinstituted after sexual maturation as compared with FSH levels before sexual maturation (Figure 3, middle panel; 7.2 ± 1.2 mIU/mL in stage 1 compared with 19.3 ± 6.0 mIU/mL in stage 3; $p < 0.05$). Although mean pooled LH levels rose after sexual maturation as compared with levels before sexual maturation, the difference did not achieve statistical significance (Figure 3, middle panel; 6.8 ± 2.3 mIU/mL in stage 1 compared with 17.0 ± 4.5 mIU/mL in stage 3; $p < 0.07$). Serum estradiol concentrations were below the assay detection limit (20 pg/mL) in all samples used for statistical evaluation of stage 1 of therapy, in 4 of the 14 samples for stage 2, and in 3 of the 14 samples for stage 3. Therefore, although an increase in serum estradiol levels was evident, it could not be statistically evaluated.

DURATION OF EFFECTIVE THERAPY IN HYPOGONADOTROPIC MEN

Optimized doses of GnRH were administered for 5 to

36 months to the 22 men who achieved normal adult gonadal steroidogenesis. Normal circulating testosterone (> 400 ng/dL) and estradiol (< 20 to 32 pg/mL) levels were maintained throughout this time in 21 of the 22 men. The remaining man developed a circulating anti-GnRH antibody between the fourth and fifth months of therapy. This man and the single man who developed an anti-GnRH antibody before achieving a normal testosterone level were the only men who developed anti-GnRH antibodies. Once normal mid-range adult serum testosterone concentrations were achieved, they were maintained without further increases in the GnRH dose for the remainder of therapy.

Discussion

In the present study, we report the effectiveness of long-term, low-dose, pulsatile administration of GnRH as a therapy for men with idiopathic hypogonadotropic hypogonadism and as a model for the study of the physiologic effects of GnRH in humans. We have previously reported successful induction of puberty in 6 hypogonadotropic men achieved by administering GnRH subcutaneously over a 3-month period at a dose of 25 ng/kg per bolus every 2 hours (3). However, in 2 men in this initial report, normal serum testosterone levels were not achieved with this dose. In view of the variable subcutaneous absorption of hormones (19, 22-24) and the varied clinical and biochemical presentations of idiopathic hypogonadotropic hypogonadism (25-31), we suspected that GnRH dose requirements might differ among patients. In addition, the recent experience of Morris and colleagues (32), in which 12 of 17 hypogonadal men treated with pulsatile GnRH failed to achieve normal serum testosterone concentrations, led us to evaluate more thoroughly the efficacy of low-dose, pulsatile GnRH administration in our patients.

Normal pituitary gonadotropin and gonadal steroid secretion with accompanying sexual maturation was successfully initiated in 22 of our 23 patients who received 25- to 300-ng/kg bolus doses of GnRH injected subcutaneously at 2-hour intervals by an automatic infusion pump. This group included the 2 men who had not responded to 25-ng/kg boluses with normal testosterone levels in our earlier study (3). The only early treatment failure was due to the appearance of an anti-GnRH antibody. Although heterogeneity of clinical parameters has been recognized in the men with idiopathic hypogonadotropic hypogonadism (25-31), neither the presence of LH pulses during the baseline 24-hour blood sampling period nor the initial testicular size correlated significantly with the dose of GnRH required to normalize serum testosterone levels in our patients. Once serum gonadotropin and sex steroid concentrations achieved normal levels, they remained so throughout the duration of therapy (up to 36 months) in all but 1 man. Loss of LH response in this second treatment failure was also due to the development of an anti-GnRH antibody, as has been reported in 2 other men during chronic GnRH therapy in other studies (33, 34). The discrepancies between our results and those reported by Morris and colleagues (32) may relate to differences in the patient populations, regimens of therapy, or patient compliance.

Mature spermatogenesis was achieved in all men who provided a specimen for semen analysis. Normal sperm counts were most likely to develop in men who either had an initial testicular volume of greater than 10 mL or who had a history of some spontaneous pubertal development. There was a positive correlation between both the initial and maximal testicular volumes and the maximal sperm count. These results are similar to those in the recent report by Finkel and coworkers (35), who noted that treatment with human chorionic gonadotropin was more likely to produce normal sperm counts in men with onset of hypogonadotropic hypogonadism after puberty than in men with onset before puberty.

Still, it is unclear why many men did not achieve normal sperm counts. Mean serum concentrations of gonadotropins were within or slightly above the normal range, and LH was released in a pulsatile fashion with a similar frequency to that observed in normal men and with a slightly greater amplitude. It is conceivable that GnRH-deficient men lack the normal neonatal activation of the pituitary-gonadal axis and that this early testicular stimulation by gonadotropins is important for the future development of quantitatively normal spermatogenesis. Men with idiopathic hypogonadotropic hypogonadism might also lack another substance(s) that is important for spermatogenesis and not stimulated by long-term GnRH administration. Finally, it is possible that a longer duration of therapy would result in higher sperm counts; the average duration of GnRH administration (excluding those men with a history of cryptorchidism) in our series was 83 weeks. This lack of full spermatogenic development in some of our patients during prolonged GnRH therapy is similar to the findings of Finkel and coworkers (35), in which five of seven men with a prepubertal onset of hypogonadotropic hypogonadism without cryptorchidism had sperm counts either below normal or in the lower end of the normal range despite 14 months of treatment with pharmacologic doses of gonadotropin. Continued follow-up of men receiving long-term GnRH therapy should help to clarify this issue.

In those men with sperm counts of greater than 20×10^6/mL, serum gonadotropin concentrations were similar to the levels determined in our normal men. Although serum LH and FSH levels in patients with sperm counts of less than 1×10^6/mL were greater than those observed in our normal men, we did not attempt to adjust the GnRH dose to see if gonadotropin levels in these hypogonadotropic men could be matched to those observed in our normal men. It is possible that normal serum testosterone levels would have been maintained with gonadotropin concentrations similar to those of normal men if lower doses of GnRH had been administered to this group. In all groups of hypogonadotropic men, serum testosterone concentrations were not significantly different from normal values. Thus, in our population of well-characterized hypogonadotropic men given individually adjusted GnRH doses, long-term replacement with a physiologically based regimen of GnRH was an effec-

tive method to achieve and maintain normal adult levels of circulating gonadotropins and sex steroids as well as to initiate mature spermatogenesis.

The fact that most men with some evidence of prior sexual maturation responded to the lowest GnRH dose administered suggested that the responsiveness of the reproductive axis to GnRH increases with puberty. The continued rises in serum testosterone concentrations in men receiving constant doses of GnRH further indicated the possibility of increased pituitary or gonadal responsiveness, or both, during sexual maturation. To evaluate this possibility, we evaluated the data from the initial 6 to 12 months of therapy with the optimized GnRH dose for evidence of maturation of pituitary-gonadal responsiveness to GnRH. Increased pituitary-gonadal responsiveness was shown by greater serum testosterone concentrations in response to the same dose of GnRH administered after sexual maturation as compared with before sexual maturation. This change might have been due to enhanced pituitary responsiveness to GnRH as suggested by the increased levels of gonadotropin secretion that occurred after sexual maturation despite a constant dose of GnRH. Previous studies have also supported this notion by showing that the pituitary gonadotropin response to 100-μg challenges of GnRH increases through puberty in normal children (13). The greater pituitary responsiveness after puberty could not be explained by differences in serum sex steroid levels, because both serum testosterone and estradiol concentrations were greater after sexual maturation and thus would have been expected to result in enhanced negative feedback effects on the pituitary.

Enhanced pituitary-gonadal activity during sexual maturation may also reflect an element of increased testicular responsiveness to gonadotropins. Other investigators have reported increasing testicular responsiveness to LH in seasonal-breeding rams as they become reproductively active each year (36) and to human chorionic gonadotropin in humans throughout puberty (12). The increased testicular steroid secretion in our patients may have been caused by increased gonadal responsiveness, as suggested by Winter and coworkers (12), or by an increase in the bioactivity of the LH secreted throughout puberty, as has been suggested by other groups (37, 38), or by combination of these effects. Because these changes occur in the pituitary-gonadal axis of men with idiopathic hypogonadotropic hypogonadism during induction of puberty with GnRH, this model presents a unique opportunity to study these various components of the maturation of the reproductive system in the human in a controlled experimental circumstance.

In summary, our results indicate that long-term pulsatile GnRH administration in men with idiopathic hypogonadotropic hypogonadism is both an effective therapy for the disorder and a valuable model for the study of the neuroendocrine control of reproductive processes in humans. Administration of a regimen of GnRH designed to mimic normal endogenous GnRH secretion normalized circulating sex steroid levels in all of our patients who had their GnRH dosage properly adjusted and did not develop anti-GnRH antibodies. As therapy progressed, the dose could be decreased in many men with no decrease in serum testosterone concentrations. The initial period of therapy provides an ideal model for studying the maturation of the pituitary-gonadal axis during puberty, because the time of onset of sexual maturation is predictable and GnRH stimulation of the pituitary may be controlled and monitored. After a 6- to 12-month period during which pituitary and gonadal responses and spermatogenesis mature, the hypogonadotropic man provides a valuable model in which to study normal reproductive function in the adult male.

ACKNOWLEDGMENTS: The authors thank the staff of the General Clinical Research Center; Carolyn Albers, Jayne Dorfman, MaryAnn Connors, and Raechel Katzin for their dedicated care of these patients; Drs. Jurgen Sandow and Michael Conn for GnRH antibody determinations; and the physicians who referred these patients.

Grant support: in part by grants HD15788 and RR-1066 from the National Institutes of Health; and by the Vincent Memorial Fund.

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