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follicle-stimulating hormone
gonadal dysgenesis
gonadotropin-releasing hormone
luteinizing hormone
puberty

Estimation of GnRH Pulse Amplitude during Pubertal Development

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Summary

Fourteen children between 2.5 and 16 years of age were studied to provide a quantitative estimate of the changes in gonadotropin-releasing hormone (GnRH) pulse amplitude in hypophysial portal plasma during puberty. Responses to physiologic doses of synthetic GnRH were measured [induced luteinizing hormone (ΔLH) and induced follicle-stimulating hormone (ΔFSH)] and compared with spontaneous fluctuations in gonadotropins [spontaneous luteinizing hormone (Δ_sLH) and spontaneous follicle-stimulating hormone (Δ_sFSH)]. One to four low-dose (0.0125 or 0.025 μg/kg IV) pulses of GnRH were given every 2 hr between 0800 and 1600 or 2200 and 0400 hr. Maximal peripheral plasma concentrations of GnRH one min after pulses averaged 107 ± 25 pg/ml (S.E.) (0.0125 μg/kg dose) and 218 ± 33 pg/ml (0.025 μg/kg dose). In early pubertal children,the maximal ΔLH was similar to or less than the maximal nocturnal Δ_sLH (maximum, ΔLH 7.0 ± 0.2 *versus* maximum Δ_sLH 7.0 ± 1.3 mIU/ml in boys, 7.0 ± 1.2 *versus* 16.0 ± 3.0 mIU/ml in girls). Luteinizing hormone (LH) responses were low or undetectable in children whose bone ages were less than 10 years. When discernible, LH pulse frequency was similar during daytime and nighttime sampling periods in early pubertal boys. However, two hourly injections of GnRH given during the day did not simulate the initial nocturnal rise in LH. Overall mean ΔFSH and Δ_sFSH were similar in three prepubertal female patients (3.0 ± 0.2 *versus* 2.8 ± 0.2 mIU/ml). ΔFSH was greater than Δ_sFSH in two patients with gonadal dysgenesis (bone ages, 2.5 and 5 years) and in one prepubertal girl. The gonadotropin responses seen in early pubertal children suggest that the amplitude of nocturnal GnRH pulses is equal to or greater than that previously reported in normal men.

Speculation

Direct measurement of hypophysial portal plasma concentrations of GnRH in human beings is impractical. Nevertheless, detailed comparison of spontaneous fluctuations in plasma follicle-stimulating and luteinizing hormones with gonadotropin responses induced by a known concentration of exogenous gonadotropin-releasing hormone (GnRH) should provide reasonable estimates of GnRH pulse amplitude. The current studies suggest that: (1) in early pubertal children, the amplitude of nocturnal GnRH pulses equals or exceeds that of normal men; (2) the initial nocturnal rise in plasma LH characteristically noted in early pubertal boys is the result of a transient increase in the frequency of GnRH secretion; and (3) if GnRH is secreted episodically before puberty, GnRH pulse amplitude is low.

Since the initial synthesis of gonadotropin-releasing hormone (GnRH) (14) in 1971, several groups of investigators have studied the effects of IV administered GnRH. Responsiveness to GnRH has been shown to depend on dosage, route, rate, and frequency of administration, age, sex, sexual maturity, stage of menstrual cycle, and time of day (7–12, 20, 21, 23). These studies have shown that luteinizing hormone (LH), but not follicle-stimulating hormone (FSH) release increases strikingly at puberty in both sexes, whereas FSH release is greater in prepubertal girls than boys.

Information about GnRH secretion into the hypophysial portal system has been obtained via direct measurement in several animal species (1, 3, 6, 18, 22). Carmel *et al.* (3) have measured portal blood GnRH in monkeys and found it to vary between 10 to 800 pg/ml. In humans, such measurements are impractical, and information has been obtained via indirect techniques. We have previously used responses to synthetic GnRH to estimate the hypophysial portal plasma concentration of GnRH in men and to gain information about its secretory pattern. On the basis of these studies, we concluded that the portal plasma concentration of GnRH varied between <30 and 300 pg/ml (8), an estimate similar to those obtained by direct measurement in animals. A similar study in prepubertal children would be difficult to interpret because of the shallow dose response curve for LH in these children (7, 9). In this study, we have utilized the striking FSH responses of prepubertal and hypogonadal children, as well as the marked day/night difference in LH secretion characteristic of early puberty to estimate GnRH pulse amplitude.

MATERIALS AND METHODS

All studies were done in the Clinical Research Center after obtaining written informed parental consent. These studies were approved by the Human Investigation Committee of the University of Michigan. Clinical information is summarized in Table 1. Gonadal dysgenesis was diagnosed by physical examination, increased basal FSH, karyotype, and surgical exploration in patient 2. Four patients (patients 3 to 6) had bone ages of <10 years, complete absence of secondary sexual development, and prepubertal responses to standard GnRH testing (2.5 μg/kg IV) (12). Five patients (patients 4 to 6, 13 and 14) were growth hormone (GH) deficient based on failure to respond to two provocative tests of GH reserve and subsequent response to GH therapy. These patients had no other hormone deficiencies. Six patients (patients 7 to 12) had early signs of pubertal development and pubertal responses to a standard GnRH test, but the onset of sexual development had been delayed.

All patients were studied over 3-day protocols. Patients were admitted to the Clinical Research Center on the afternoon or evening of the day before testing to allow acclimatization to the unit. On the next day, a heparin lock was placed in a forearm vein and left in place during the entire study. Blood was obtained every 20 min for 6 to 24 hr depending on the patient's size and blood volume. Sampling was divided into equal diurnal and nocturnal periods. Two hourly IV injections of normal saline (3.0 ml) were used as a control for the subsequent pulsatile GnRH injections. LH and FSH were measured on all specimens. The third day, low-dose pulses of GnRH (0.0125 or 0.025 μg/kg IV) were given every 2 hr. Patients with gonadal dysgenesis received one or two 0.0125 μg/kg pulses in addition to the 0.025 μg/kg pulse because of increased sensitivity to GnRH previously noted in this syndrome (7, 9, 21). The remaining patients all received 0.025 μg/kg pulses.

Table 1. *Clinical characteristics, responses to standard GnRH tests (2.5 μg/kg IV) and diagnoses of study patients*

Group	Patient/sex	CA/HA/BA[1]	Mean basal LH/FSH (mIU/ml)	Maximal increment LH/FSH (mIU/ml)	Diagnosis
Gonadal dysgenesis	1/F	5.5/3/5	3.5/21.5	21.4/71.7	45X/46XXqi
	2/F	2.5/2.5/2.5	2.5/21.5	27.4/61.7	46XY
Prepubertal	3/M	10/7/8	1.2/1.4	8.1/3.2	C.S.S.[2]
	4/F	11.5/8.7	1.9/3.9	8.5/15.6	G.H. Def.
	5/F	9.5/4/3.5	2.6/1.8	2.8/16.7	G.H. Def.
	6/F	7.5/4.5/7	1.0/2.9	8.0/21.9	G.H. Def.
Early pubertal	7/M	14.5/10/12	3.7/4.2	37.2/2.7	D.A.
	8/M	14.5/13/12	4.4/2.2	37.5/3.6	D.A.
	9/M	15/11/11.5	5.5/3.2	19.6/5.3	D.A.
	10/M	16/15.5/14	5.9/4.0	14.5/0.9	D.A.
	11/M	14/11/13	7.5/6.5	33.8/4.4	D.A.
	12/F	14/10.5/11	12.2/17.7	36.5/12.3	D.A.
	13/F	14.5/8/14	7.9/16.9	13.2/9.0	G.H. Def.
	14/F	12.5/5.5/11	9.2/3.9	45/8.6	G.H. Def.

[1] CA/HA/BA, chronologic/height/bone ages in years.

[2] C.S.S., constitutional short stature; G.H. Def., isolated growth hormone deficiency; D.A., delayed adolescence.

The two early pubertal GH-deficient girls (patients 13 and 14) received pulses at 2200 hr to evaluate responsiveness shortly before the expected nocturnal increase in LH secretion. All other patients received pulses between 0800 and 1600 hr. GnRH (Warner-Lambert/Parke, Davis and Co.) was diluted with normal saline to 0.0125 or 0.025 μg/kg/ml. Injections were given rapidly IV (<5 sec), and the line was flushed with 3.0 ml normal saline. LH and FSH were measured every 20 to 30 min before and after pulse injections. Plasma GnRH was measured before and 1, 2, 8, and 20 min after the first and last pulse injections. Blood for GnRH measurement was obtained from an indwelling catheter in the opposite arm.

Plasma GnRH was measured in duplicate 200 μl specimens by a previously described radioimmunoassay (4, 8). Mean sensitivity of the assay was 1 pg/tube or 5 pg/ml plasma. Duplicate 100 or 200 μl samples were used for LH and FSH determinations, respectively, and all samples from a patient were measured in the same assay (15–17). Gonadotropin concentrations are reported as mIU/ml of the second International Reference Preparation—Human Menopausal Gonadotropin (IRP-HMG). One mIU of the first IRP-HMG (WHO 69/104) is equivalent to 3.9 mIU of the second IRP-HMG in the LH assay and to 1.8 mIU of the second IRP-HMG in the FSH assay. One mg of LER-907 is equivalent to 232 IU (LH) and 46 IU (FSH).

Calculations for all assays were performed by the computer program described by Duddleson *et al.* (5). Intraassay coefficients of variation were 21.5% (LH) and 19% (FSH) for values below 5 mIU/ml, 12.5% (LH), and 11.4% (FSH) for values between 5 and 10 mIU/ml, 10.9% (LH), and 5.8% (FSH) for values between 10 and 20 mIU/ml, and 8.1% (LH) and 7.2% (FSH) for values between 20 and 30 mIU/ml. A significant spontaneous LH or FSH secretory increment was defined as a rise from nadir to peak which was at least twice the corresponding intraassay coefficient of variation. Induced increments were considered significant only if they occurred within 20 min of a GnRH pulse, reached a maximum within 40 min for LH and 60 min for FSH, and exceeded twice the intraassay coefficient of variation. Students paired or when appropriate unpaired *t* tests were used to determine significance levels ($P < 0.05$ or greater) after log transformation of data. Response areas were computed by triangulation.

RESULTS

PLASMA GnRH

The mean plasma concentrations of GnRH after low-dose pulses are shown in Figure 1. Basal plasma GnRH values were at or below assay sensitivity (5 pg/ml). Peak plasma GnRH was achieved rapidly and occurred at one min in all but one patient (patient 13, whose peak value was at +2 min). A rapid decline was seen in plasma GnRH with values approaching basal within 20 min.

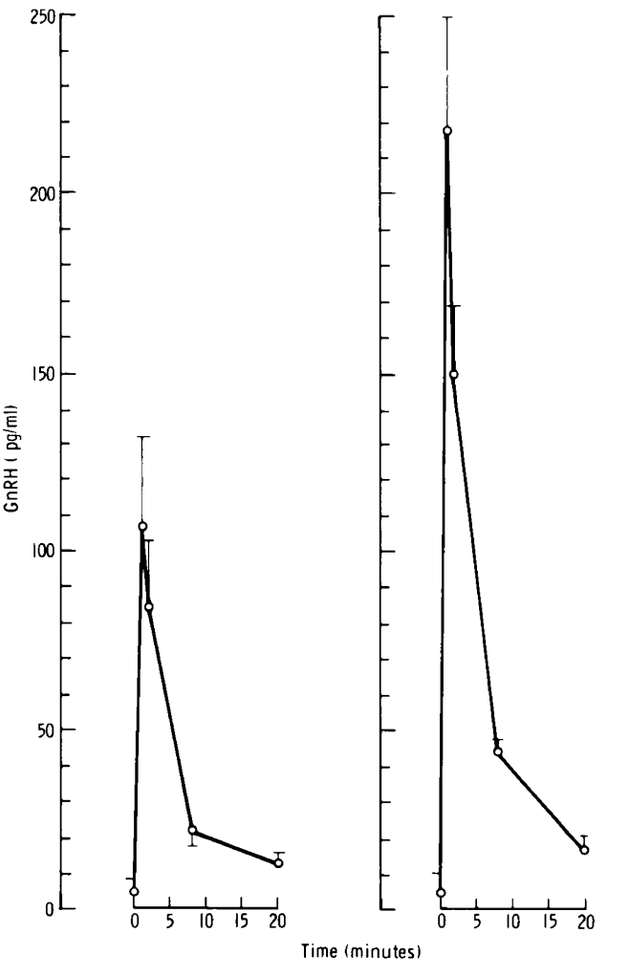

Fig. 1. Mean plasma GnRH concentrations after low dose, 0.0125 μg/kg (*left*) or 0.025 μg/kg (*right*) IV pulse injections of synthetic GnRH. *Vertical lines*, S.E.

CHILDREN WITH GONADAL DYSGENESIS

Inconsistent day/night differences in plasma FSH and LH were noted in children with gonadal dysgenesis, but both had increased basal FSH values for age. The high FSH concentrations at night in patient 1 decreased throughout the sampling period and were lower the next morning whereas the opposite pattern was present in patient 2. After GnRH pulse injections, significant FSH increments occurred which exceeded spontaneous increments (7.5 ± 1.1 mIU/ml *versus* undetectable) (Fig. 2). LH increments of lesser magnitude were also seen after pulses. Response areas for both FSH and LH were greater after the 0.025 μg/kg pulses than after the 0.0125 μg/kg pulses: 137 *versus* 78 mIU·min/ml for LH and 765 *versus* 350 mIU·min/ml for FSH.

PREPUBERTAL CHILDREN

The four prepubertal children (Fig. 3) showed no significant day/night difference in mean LH (3.2 ± 0.6 *versus* 3.4 ± 0.8 mIU/ml) or FSH (3.3 ± 0.1 *versus* 3.4 ± 0.8 mIU/ml). Patients 4 and 5 had FSH responses to the GnRH pulses, but only the FSH response of patient 5 exceeded the variability noted in FSH during the control period. The maximal peripheral plasma concentration of GnRH was 277 pg/ml in patient 5. Neither patient 4 nor 5 had LH increments distinguishable from those seen during the control period. Patients 3 and 6 had no detectable responses to low-dose pulses, although FSH increased slightly after pulses in patient 6 from 2.2 ± 0.1 to 2.8 ± 0.1 mIU/ml.

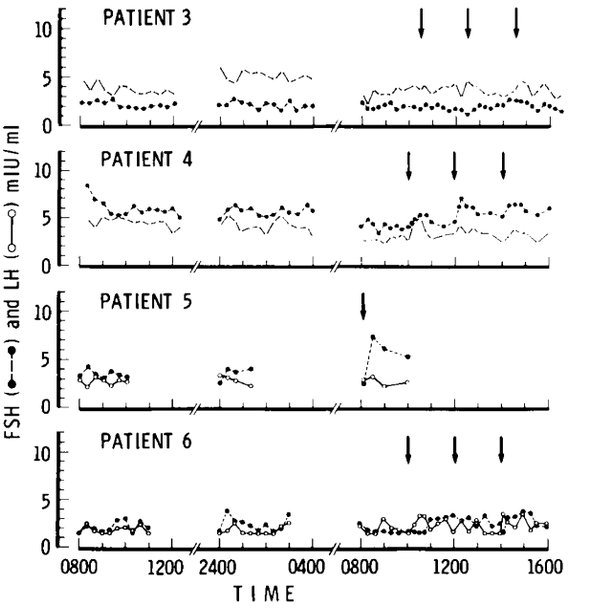

Fig. 3. Plasma FSH (- - - -) and LH (——) patterns in four prepubertal patients. *Arrows*, 0.025 μg/kg IV doses of GnRH.

EARLY PUBERTAL BOYS

Figure 4 shows the control day gonadotropin secretory patterns and responses to low dose GnRH pulses in five early pubertal boys and for comparison an adult male. Patient 11 did not receive pulses because of technical problems. Mean control day LH and LH responses are shown in Table 2. All patients had a significant day/night difference in mean LH, but not FSH (data not shown). The youngest patient studied (patient 11) showed the greatest day/night difference, and the oldest patient (patient 10) showed the least day/night difference in mean LH. Three of the four patients studied continuously for 24 hr during the control day (patients 8, 9, and 11) showed a progressive rise in LH over 2½ to 3 hr beginning around 2200 hr. This pattern was not seen for FSH. Mean spontaneous LH increment (Δ_sLH) and mean maximal Δ_sLH were greatest at night. LH pulse frequency was similar during the diurnal and nocturnal sampling periods (130 and 142 min, respectively).

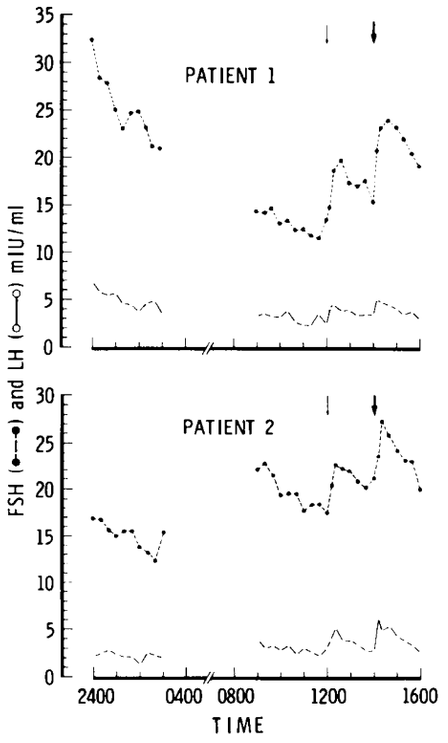

Fig. 2. Plasma FSH (- - - -) and LH (——) patterns in two patients with gonadal dysgenesis. *Arrows*, 0.0125 μg/kg (↓) or 0.025 μg/kg (↓) IV doses of GnRH.

Low-dose pulses of GnRH (0.025 μg/kg) elicited significant LH increments after 11/12 pulses (patient 9 did not respond to the third pulse). The mean maximal induced LH increment (7.0 ± 0.2 mIU/ml) was almost identical to the mean maximal Δ_sLH (7.0 ± 1.3 mIU/ml) seen at night.

FSH increments during the day and night control periods were low or undetectable and did not differ significantly. FSH responses to GnRH pulses were similarly low and did not differ significantly from spontaneous increments.

EARLY PUBERTAL GIRLS

Figure 5 shows the gonadotropin secretory patterns and responses to GnRH pulses for three early pubertal girls. Mean control day LH and LH responses are listed in Table 2. In contrast to the findings in early pubertal boys, day/night differences in plasma LH and FSH were inconsistent. This may reflect the more advanced pubertal development of female patients in this study. When discernible, LH pulse frequency was similar during the day and night (135 and 108 min, respectively). As in boys, the mean spontaneous LH increment was greatest at night, but the shorter sampling periods did not allow delineation of the transition phase between day and night secretory patterns.

Responses to 0.025 μg/kg pulses of synthetic GnRH were studied during the day in one patient (patient 12) and at 2200 hr in the other two (patients 13 and 14). Spontaneous LH increments occurred before the first two pulses in patient 12 and precluded evaluation of gonadotropin responses. Pulse responses were less than the spontaneous nocturnal increments in all patients. The mean maximal LH response to pulses was significantly less than the mean maximal spontaneous nocturnal LH increment. Spontaneous FSH increments, although of smaller magnitude, were similar during day and night, and did not differ from the mean induced FSH increment.

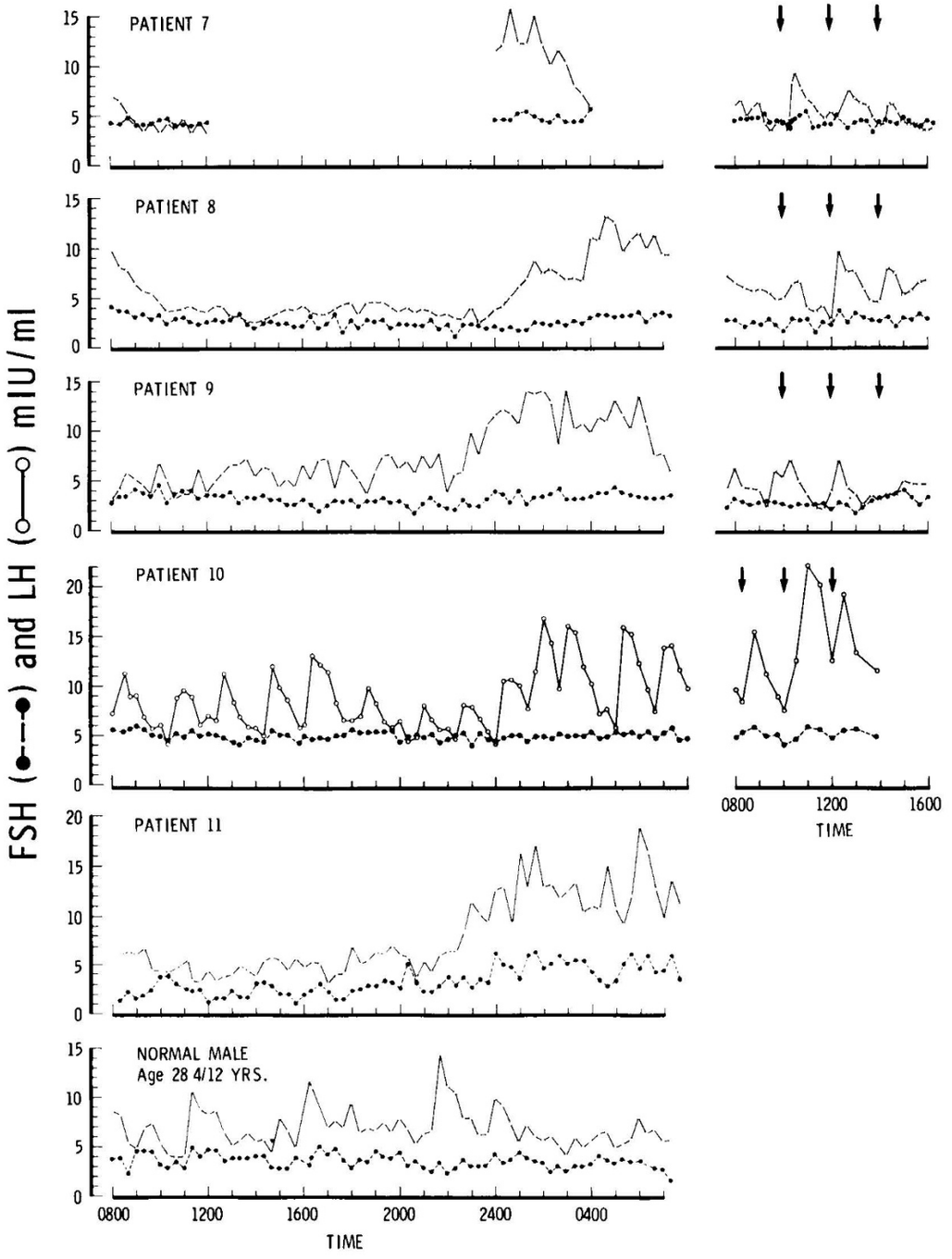

Fig. 4. Plasma FSH (- - - -) and LH (——) patterns in five early pubertal boys and a normal adult male. *Arrows*, 0.025 μg/kg IV doses of GnRH.

DISCUSSION

Measurement of GnRH in peripheral plasma has been impractical because the marked dilution that occurs between the hypophysial portal system and the peripheral circulation results in concentrations well below the sensitivity of current assays. Direct measurement of portal blood GnRH has been accomplished in several animal species (1, 3, 6, 18, 22). Carmel *et al.* (3), for example, found portal blood GnRH to vary between <10 to 800 pg/ml in ovariectomized monkeys, and Sarkar *et al.* (22) found levels between <6 to 950 pg/ml during the spontaneous preovulatory LH surge in rats.

We previously estimated hypophysial portal plasma concentrations of GnRH in normal men by comparing spontaneous and induced gonadotropin secretory patterns with peripheral plasma concentrations of GnRH achieved after low-dose pulse injections or during constant infusions of synthetic GnRH (8, 11). In close agreement with direct measurements in lower species, we reported that GnRH probably varied between <30 and 300 pg/ml in hypophysial portal plasma. In this study, we have extended this approach to estimate GnRH pulse amplitude during human puberty.

In prepubertal children, gonadrotropin secretion is low and relatively constant. Whether gonadotropins are secreted episodically before puberty is unsettled. Penny *et al.* (19) were able to detect small but significant LH pulses in three prepubertal children, and Kulin *et al.* (13) found that gonadotropin excretion in urine was greatest at night in prepubertal children. At the onset of

Table 2. *Mean (± S.E.) plasma LH concentrations and spontaneous (Δ_s) and induced (Δ) LH increments in early pubertal patients (mIU/ml)*

	Mean concentration		Mean Δ_s			Maximal Δ_s		
	Day	Night	Day	Night	Mean Δ	Day	Night	Maximal Δ
Boys	5.4 ± 0.7[1] (4.2–8.0)[6]	10.0 ± 0.7[7] (7.5–12.0)	3.5 ± 0.4 (1.5–7.2)	5.0 ± 0.1[2] (1.6–10.0)	4.7 ± 0.9[3] (1.8–14.6)	4.2 ± 1.1 (1.6–7.2)	7.0 ± 1.3[2] (4.4–10.1)	7.0 ± 0.2[4,5] (4.4–14.6)
Girls	8.0 ± 0.9 (6.8–9.8)	13.6 ± 2.1 (9.5–16.3)	5.6 ± 2.4 (2.0–9.3)	14.8 ± 3.8 (7.3–22.3)	7.0 ± 1.2 (5.3–9.4)	6.8 ± 2.4 (4.4–9.3)	16.0 ± 3.0 (12.0–22.3)	[7]

[1] Mean ± S.E.
[2] Significantly greater than daytime values.
[3] Significantly greater than daytime Δ_s.
[4] Not significantly different than maximal nocturnal Δ_s.
[5] Significantly greater than daytime maximal Δ_s.
[6] Numbers in parentheses, range.
[7] Same as mean Δ.

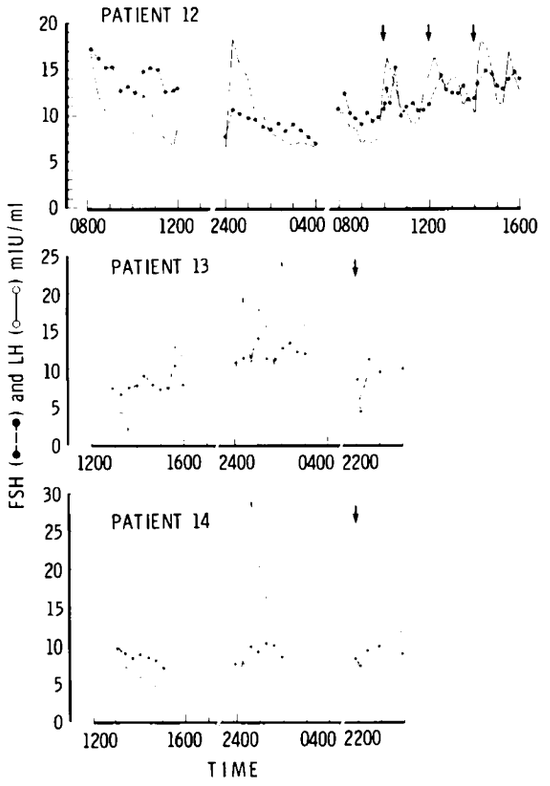

Fig. 5. Plasma FSH (- - - -) and LH (——) patterns in three early pubertal girls. *Arrows*, 0.025 μg/kg IV doses of GnRH.

puberty, pulsatile secretion of LH is easily detectable during sleep, but not while children are awake. Presumably, the nocturnal increase in LH secretion results from increased secretion of GnRH and a secondary increase in responsiveness to GnRH (2). LH responsiveness to synthetic GnRH increases considerably at the onset of puberty in both sexes: FSH responsiveness however, remains relatively constant in boys and decreases strikingly in girls (7). Thus, prepubertal children, in contrast to adults, have relatively or absolutely greater FSH responses. The shallow LH dose response curve to synthetic GnRH did not allow estimation of GnRH pulse amplitude in prepubertal children. However, the increased FSH responsiveness of young hypogonadal children, as well as the marked day/night difference in gonadotropin secretion in early pubertal children, did allow estimation of GnRH pulse amplitude during childhood.

Based on our earlier data (8, 11), the dosage and method of administration of GnRH used in this study were chosen to yield brief pulses of GnRH with peripheral plasma concentrations of approximately 100 and 200 pg/ml (0.0125 and 0.025 μg/kg doses, respectively). The close agreement between predicted and observed GnRH concentrations in this study as well as between the results of the 0.025 μg/kg dose in children and our previous data in adult males is not surprising, since children and adults metabolize GnRH similarly (11). The blood sampling intervals after GnRH injections were the same in this and in our earlier study in men (11). Thus, comparison between children and men would appear valid. Our estimate of GnRH amplitude might be somewhat low, however, because the first samples were not drawn until one min after injection. Furthermore, our estimate is based on the assumption that the pituitary circulation patterns for endogenous and exogenous GnRH are similar. Indeed, endogenous GnRH may not come in contact with all the gonadotrophs stimulated by exogenously administered GnRH, resulting in a somewhat low estimate. Reliability of this indirect assessment of GnRH pulse amplitude is also dependent on relatively unchanging sensitivity of the gonadotrophs to GnRH. Schwarzstein *et al.* (23) suggest that this may not be entirely correct, but could show suppression of responsiveness at noon, a time when our early pubertal males showed good responses to pulses. We feel that these limitations do not preclude the usefulness of this technique, but only underscore the fact that it is an approximation.

Gonadotropin responses to "physiologic" pulses of GnRH were not informative in most of the prepubertal children. The clear FSH response of patient 5 and especially the striking FSH responses of the two patients with gonadal dysgenesis do allow us to infer that GnRH pulse amplitude is low in prepubertal children, probably less than 100 pg/ml. The reason for the high nocturnal FSH during the control night in patient 1 is unclear, but may reflect a brief period of increased endogenous GnRH secretion. Additional control sampling would have been necessary to document the pattern of the nocturnal FSH rise. However, this was impossible because of the patient's small size.

LH responses to "physiologic" pulses of GnRH were most informative in the early pubertal children. Indeed, the LH increments induced by the 0.025 μg/kg dose of GnRH mimicked closely the spontaneous nocturnal increments in LH seen in boys. In girls, however, the induced increments were less than the spontaneous nocturnal pulses of LH. Inasmuch as the 0.025 μg/kg dose induced LH increments in men similar in magnitude to spontaneous LH increments (8, 11), the current findings suggest that endogenous GnRH pulse amplitude is as great or greater in early pubertal children than in men. The limited number of observations and the slightly more advanced sexual maturation of the girls in this study preclude meaningful comparisons between early pubertal boys and girls.

The nocturnal rise in LH in early pubertal boys was characterized by a slow, steady increase over 2 to 3 hr in several patients.

This is illustrated best by the LH pattern of patient 8 (Fig. 4). Two hourly pulses of GnRH given during the day did not mimick this pattern, however. This suggests that nocturnal secretion of LH in early pubertal boys is initiated by an increase in GnRH pulse frequency. Additional studies are needed to confirm or refute this impression. Finally, precise evaluation of GnRH secretion in human beings will depend on development of more sensitive techniques.

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