Pharmacodynamics of Aqueous Leuprolide Acetate Stimulation Testing in Girls: Correlation between Clinical Diagnosis and Time of Peak Luteinizing Hormone Level

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We assessed the pharmacodynamics of a 3-hour leuprolide stimulation test in 11 girls with precocious puberty to determine an optimal single sampling time. Luteinizing hormone level following leuprolide stimulation was near maximum by 30 minutes in girls with central precocious puberty, whereas it continued to rise slowly in girls with nonprogressive puberty. *(J Pediatr 2012;161:757-9)*

Central precocious puberty (CPP) is defined as premature activation of the hypothalamic-pituitary-gonadal axis resulting in early sexual maturation. The gonadotropin-releasing hormone (GnRH) agonist stimulation test using leuprolide acetate (LA [Lupron]; Abbott Laboratories, Chicago, Illinois) plays an important role in the evaluation of children with suspected CPP. A pubertal response of luteinizing hormone (LH) to LA stimulation testing is considered diagnostic for CPP, whereas a preponderant follicle-stimulating hormone (FSH) response supports a diagnosis of the premature thelarche form of nonprogressive puberty (NPP-PT).[1] The first LA stimulation protocols measured LH and FSH out to 24 hours,[2-4] but later studies emphasized the early LH rise.[5-8] In this study, we examined the early pharmacodynamics of LH and FSH concentrations after LA stimulation in girls with various presentations of puberty to provide practical clinical guidance.

Methods

Females with a history of breast development before age 8 years were recruited for this study. Subjects were categorized into 4 subgroups based on clinical characteristics, specifically the type and temporal sequence of secondary sexual characteristics: CPP (breast development preceding development of pubic hair), CPP-Rx (previously diagnosed CPP, receiving depot leuprolide therapy with suboptimal suppression), NPP-PT (nonprogressive isolated breast tissue at repeated observations with onset before age 5 years), and NPP-variant (a presumed nonprogressive pubertal variant demonstrating androgen effects [pubic or axillary hair] before feminization, with congenital adrenal hyperplasia excluded).

Subjects underwent LA stimulation testing in the General Clinical Research Center. An intravenous catheter was placed in the antecubital vein, and blood was withdrawn at 0, 15, 30, 45, 60, 90, 120, 150, and 180 minutes after LA injection (20 $\mu g/kg^2$). LH and FSH levels were measured by immunochemiluminometric assay (Esoterix, Calabasas Hills, California), with a sensitivity threshold of 0.02 IU/L and interassay variability of 10.7% for LH and 9% for FSH.[7] Estradiol was obtained at 0 minutes and performed by radioimmunoassay (Esoterix), with a sensitivity of 5 pg/mL. This study was approved by the Stanford University Administrative Panel on Human Subjects in Medical Research.

Results

Subjects had a mean age of 6.9 ± 2.0 years at diagnosis of precocious puberty and 7.7 ± 1.7 years at study testing (Table I; available at www.jpeds.com). The pretest diagnosis was corroborated by a peak LH level >8 IU/L in the subjects with CPP and <8 IU/L in those with NPP. LH approached maximal levels earlier in girls with CPP versus girls with NPP, reaching 93% of maximum at 30 minutes. In contrast, LH levels were lower in girls with NPP and rose more slowly, reaching only 63% and 55% of maximum in NPP-PT and variant, respectively, at 30 minutes (Figure 1 and Table II). The 30-minute and peak LH levels were tightly correlated (R^2 = 0.987). The percentage of peak LH reached by 30 minutes was correlated with peak LH level itself (R^2 = 0.318); that is, the higher the peak, the earlier the LH rise.

Girls in the CPP and CPP-Rx groups achieved 61% of maximal FSH at 30 minutes, in contrast with 43% by those in the NPP-PT and variant groups (Figure 2; available at www.jpeds.com). Compared with LH values, the 30-minute and peak FSH levels were not as tightly correlated (R^2 = 0.420), and likewise the percentage of maximal FSH

CPP	Central precocious puberty
FSH	Follicle-stimulating hormone
GnRH	Gonadotropin-releasing hormone
LA	Leuprolide acetate
LH	Luteinizing hormone
NPP	Nonprogressive puberty
NPP-PT	Nonprogressive puberty, premature thelarche form

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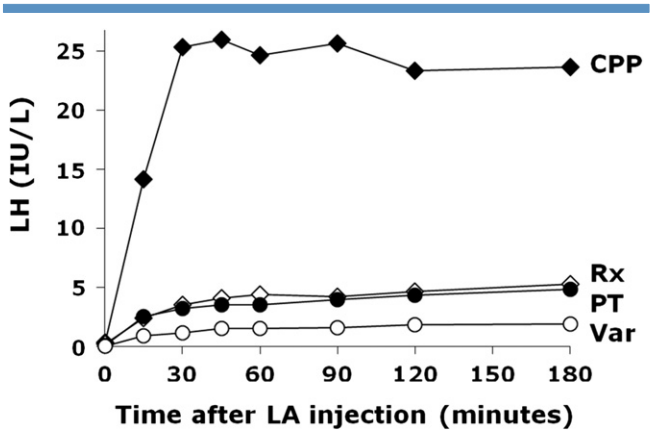

Figure 1. Mean serum LH concentration at designated times after aqueous LA injection (20 $\mu g/kg^2$) in subjects with CPP (*solid diamond*), CPP-Rx (*open diamond*), NPP-PT (*solid circle*), and NPP-variant (*open circle*). SDs are omitted because of the small numbers in each group. *Rx*, receiving therapy; *PT*, premature thelarche form; *Var*, variant.

reached by 30 minutes was not as strongly correlated with maximal FSH (R^2 = 0.199). The mean rise in FSH after 30 minutes was 7.9 IU/L and was greatest in the NPP-PT group.

Due to the greater LH levels, mean LH/FSH ratio was higher in the CPP group compared with the NPP groups (>1 vs <0.5). In all groups, the LH/FSH ratio at 30 minutes exaggerated the peak LH/peak FSH ratio, because of the slower FSH rise (Table II); for example, in the CPP group, the 30-minute mean LH/FSH ratio was 2.70 versus the mean peak ratio of 1.53. The peak LH/FSH ratio correlated with the percentage of maximal LH achieved by the 30-minute time point (R^2 = 0.431).

Discussion

Currently, LA stimulation is the definitive test for confirming CPP. Despite substantial evidence indicating that a single stimulated gonadotropin sample is informative,[8-14] many centers continue to obtain multiple samples during the LA stimulation test, leading to longer test duration, higher costs, and greater inconvenience to patients. Confusion regarding gonadotropin sampling may be attributed to early LA test protocols which used 24-hour sampling with first sampling point at 3 hours.[2] Unlike GnRH stimulation, in which LH levels peak within 20-40 minutes followed by a decline,[14,15] studies using LA and other GnRH analogues have demonstrated a comparable early rise followed by sustained LH elevation. Recent LA stimulation studies in subjects with normal puberty and CPP have confirmed the robust and reliable rise in LH in the first hour, with an LH peak occurring any time between 1 hour[2,16] and 4 hours.[6,17] Accordingly, investigators have created brief LA stimulation tests, collecting 1 or 2 samples at various times from 0.5 hour to 3 hours, for both diagnostic and monitoring purposes.[10-12,18]

The present study demonstrates that the time of LH peak following LA stimulation varies according to diagnosis, as has been implied previously.[4,6] Girls with CPP exhibit an immediate rise in LH owing to preexisting gonadotropin stores, followed by persistent LH production and secretion for several hours. Subjects with poorly suppressed CPP during therapy demonstrate an intermediate pattern, reaching 80% of peak LH by 30 minutes. In comparison, girls with NPP exhibit a blunted early LH surge, followed by a limited but measurable capacity to sustain new LH synthesis in response to LA. Nonetheless, girls with presumed NPP should be monitored for possible later conversion to LH-predominant CPP, as we observed in one of our subjects.

FSH continued to rise for the entire 3-hour time course in all clinical subgroups, but more so in the NPP-PT group. Because LH rises relatively more quickly than FSH, particularly in CPP, sampling at 30 minutes exaggerated the LH/FSH ratio in all groups. Although robust FSH response and low peak LH/FSH support a diagnosis of NPP-PT,[1] FSH provides limited additional information beyond LH in the diagnosis of CPP. In our opinion, FSH rises sufficiently in early sampling to yield the essential clinical information, but sample time will affect the cutoffs used to distinguish NPP from CPP.

Our pharmacodynamic data after LA stimulation confirm that LH rises more quickly than FSH, and that maximum LH is effectively reached by 30 minutes in girls with CPP, but not in others. This is consistent with rapid secretion of readily releasable gonadotropin stores in true puberty. Thus, a single gonadotropin sample obtained at 30 minutes (or later) can provide a reliable measure of pituitary secretory status and is sufficient for diagnosing CPP. Samples obtained at 1-3 hours exhibit higher FSH levels and marginally higher LH levels in those with NPP, therefore expected gonadotropin levels and LH/FSH ratios

Table II. Percentage of peak LH and FSH achieved at 30, 60, and 120 minutes by group

	LH				FSH				LH/FSH ratio			
Group (n)	Peak, IU/L	30 min, %	60 min, %	120 min, %	Peak, IU/L	30 min, %	60 min, %	120 min, %	Peak	30 min, %	60 min, %	120 min, %
CPP (3)	27.3	93	90	92	18.0	60	80	86	1.53	166	120	93
CPP-Rx (3)	5.5	81	83	81	10.2	62	73	81	0.55	134	119	101
NPP-PT (2)	4.9	63	72	76	24.5	42	56	89	0.22	147	129	117
NPP-variant (3)	1.9	55	73	79	10.8	43	55	92	0.20	142	142	119