Evaluation of the Gonadotropic Responsiveness of the Pituitary to Acute and Prolonged Administration of LH/FSH-Releasing Hormone (LHRH) in Untreated Patients With Congenital Adrenal Hyperplasia

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The pituitary gonadotropic responsiveness to acute and prolonged administration of LH/FSH-releasing hormone (LHRH) were assessed in 6 patients with untreated congenital virilizing adrenal hyperplasia (partial 21-hydroxylase deficiency). The oldest subjects had normal response in comparison to females at the midfollicular phase, to the acute infusion of 25 ug LHRH regarding both gonadotropins whereas LH secretory area was decreased during the prolonged (100 ug LHRH in 8 hours) infusion with normal FSH secretion. The two youngest subjects, with higher steroid levels in our series, were either unresponsive on both ways of testing or presented pre-pubertal response.

UNTREATED female patients with congenital virilizing adrenal hyperplasia (CAH) provide an interesting model to study the influence of prolonged exposure to high levels of sex hormones on the pituitary responsiveness to LHRH. Previous work published regarding gonadotropin regulation in subjects with CAH have dealt with patients on good or poor glucocorticoid control.[1,2,3] Therefore, we studied their response to LHRH with a technique presented by the authours in a previous publication.[4]

MATERIALS AND METHODS

Six untreated patients with CAH (partial form of 21-hydroxylase type), all female, chronological age varying from 12–28 yr, were studied. Two patients, MJPS and JRN, with the mildest form of the disease, had menarche at the ages of 18 and 15 respectively, with very irregular and scanty menses afterwards. One patient, CNA, first seen at the age of 6, was treated, however poorly, up to the age 12, when glucocorticoid was stopped; 3 yr afterwards the patient was referred to the Unit.

The experiments had been approved by the chairman of the Department of the Medical School and the Hospital das Clinicas at which these experiments were conducted. Informed consent was obtained from the patients or their parents, for testing and ovarian biopsy. Clinical and laboratory data are presented in Table 1.

The following steroids were measured in plasma: testosterone, androstenedione, dehydroepiandrosterone, progesterone, 17-hydroxyprogesterone, estradiol and estrone by specific radioimmunoassay, after separation on 5 × 1 cm Sephadex LH-20 columns.

A single IV bolus injection and a prolonged infusion of synthetic luteinizing hormone-releasing hormone (LHRH) was made, with an interval of 1 wk between them, the sequence being randomized in the subjects. For the performance of the single IV bolus injection — acute tests, the patients were given 25 ug of LHRH as a single rapid intravenous injections after an overnight fast and blood samples were collected at −5, 0, 5, 10, 15, 20, 25, 30, 40, 50, and 60 min after the injection, for the determination of serum LH and FSH. In the prolonged test, LHRH was administered by constant intravenous infusion, after an overnight fast, through an indwelling catheter in one arm. Blood was obtained for hormone measurements from an indwelling needle, in the other arm. LHRH was infused at a rate of 0.21 ug/min, for 8 hours in 1000 ml saline solution. Blood was sampled before and at 60, 120, 180, 240, 360, and 480 min when the infusion was stopped. In the acute post-prolonged infusion test, a 25 ug bolus of LHRH was administered following the prolonged LHRH infusion. The blood samples were obtained at the same intervals as in the acute test: 5, 10, 15, 20, 30, 40, 50, and 60 min following LHRH injection, except that no blood was drawn at 25 min. LH and FSH were measured in every serum sample by radioimmunoassay[5,6] against the second IRP-H.M.G. as the standard but expressed in terms of LER-907 preparation which has a content of 35 IU FSH and 219 IU LH per mg. All values were reported as ng/ml. The pattern of pituitary response was compared to that obtained in similar tests performed in the mid-follicular phase of the menstrual cycle in 8 regularly cycling women aged 19–36 yr, previously published.[4] The standard unpaired Student test was utilized in the statistical analysis of the gonadotropin data.

The change in plasma LH and FSH following LHRH infusion were analyzed by mean levels prior to LHRH administration (basal), peak level attained (peak), maximum increment over basal levels (Δ) and relationship between basal and maximum increment as the percent gonadotropin response above basal, i.e., the relative maximum response. The calculation of the relative maximum gonadotropin response (Δ/Basal × 100 = Δ%) permitted evaluation of the gonadotropin response to the LHRH independent of factors influencing the basal level. The areas under the response curves in the 60 min (acute test), 480 min (prolonged test) and 480–540 min (acute post-prolonged test) of gonadotropin concentrations above basal were used as an index for comparing relative quantitative changes of gonadotropin secretion. The units used to express this function of gonadotropin secretion were ng/ml. min^{-1}.

RESULTS

Mean plasma steroid levels, withdrawn immediately before the two tests, are indicated in Table 2. The two patients with the mildest form of the disease (MJPS and JRN) presented lower plasma testosterone and androstenedione and in JRN the lowest 17-hydroxyprogesterone levels as well as the highest urinary 17-OHCS of all subjects studied. The possibility that JRN had an 11-B hydroxylase deficiency rather than a 21 was discarded by the normal blood pressure

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Table 1. Clinical and Laboratory Data on Six Patients With Virilizing CAH (Partial 21 Hydroxylase Deficiency)

Patient	Age (yr)	Ht (cm)	Wt (Kg)	Bone Age	Virilism Age of Onset	Degree*	Urine 17OHCS§ umole/m²/24 hr	Urine 17KS‖ umole/24 hr	Ovaries¶ Size	Ovaries¶ Cysts
MJPS	28	151	51.6	Adult	16	†	10.7	281.9	N	0
JRN	20	153	53.0	Adult	14	•	16.6	93.4	•	•
MJSM	26	148	50.0	Adult	8	†	11.0	241.8	N	•
JDM	18	143	44.9	Adult	7	†	5.2	151.9	N	0
ACA	12	143	47.5	Adult	4	‡	5.5	428.7	<	0
CNA**	15	148	56.4	Adult	2	†	6.2	92.8	‡	•

All patients presented heterosexual precocious pseudo-puberty with pubic and axillary hair and primary amenorrhea except 2 subjects (MJPS and JRN).

* – clitoral enlargement.

† – penile clitoris + fusion of labia majora (urogenital sinus).

‡ – penile urethra + total fusion of labia majora.

§Method of Silber and Porter.[5] Normal values for children above 5 yr and adults: 8.3–17.2 umole/m²/24 hr (unpublished data).

‖Method of Drekter et al.[6] Normal values for adults females: 20.0–58.8 umole/24 hr.

¶Evaluated by pneumogynecography and/or ovarian biopsy. N, normal; + to + + + enlargement; < decreased; 0, cysts absent; + cysts present.

**On irregular glucocorticoid replacement from age 6–12 yr.

values and high plasma renin activity (6 ng/ml/hr—normal mean ± SEM: 0.5 ± 0.2) and aldosterone levels (17 ng/dl—normal values 8.3 ± 1.0) with an 120 mEq Na intake/day. On the other hand, the two youngest patients (ACA and CNA) with the more intense androgenic features in our series, had the highest levels of plasma testosterone and estradiol. Regarding the serum LH responsiveness to the gonadotropin-releasing factor the patients were divided in 2 groups: One consisting of the youngest subjects, ACA and CNA, 12 and 15-yr-old respectively, with no response and another with the remaining 4 subjects, older ones, who presented a significant response (LH-responsive patients).

Acute LH-RH Tests

The results are indicated in Fig. 1 and Table 3. The comparison of the LH response between the normal females and the LH-responsive CAH patients demonstrated that the statistically significant differences were few being observed only in the basal values which were significantly lower and the relative maximum response significantly higher in the latter and no difference in the total secretory area being observed when both groups of subjects were compared. Regarding FSH responses no differences were present, in all parameters studied, between the 2 groups.

The 2 LH-unresponsive CAH patients also presented lower basal LH values than that found in the normal females at the midfollicular phase and did not release such gonadotropin on LHRH stimulation. Similarly, the basal FSH values were greatly below the normal range and the gonadotropin levels fluctuated during the infusion, without a definite pattern of response.

Prolonged Infusion of LHRH

The results shown in Figs. 2 and 3 and Table 3 indicate that the gonadotropin response patterns to prolonged LHRH infusion in 7 normal females at the mid-follicular phase and the LH-responsive CAH patients were similar. Both gonadotropins reached mean peak levels within 4 hr, followed by a decline up to the time when the 25 ug bolus of LHRH was given IV, except for mean FSH values which because of the variability in response only decreased in the last sample. As after the IV bolus injection of LHRH, very few statistically significant changes were noticed in these subjects in relation to the controls after prolonged LHRH infusion. In effect, only basal LH

Table 2. Mean Plasma Steroid Concentrations in Six Patients With Untreated CAH

Patient	Testosterone ng/ml	Androstenedione ng/ml	Dehydroepandrosterone ng/ml	Progesterone ng/ml	17-OH-Progesterone ng/ml	Estradiol pg/ml	Estrone pg/ml
MJPS	3.03	8.3	40.7	9.7	90	65	276
JRN	2.98	8.4	11.8	4.8	18	51	250
MJSM	4.56	26.6	13.2	2.7	98	46	223
JDM	5.62	20.0	12.0	5.0	114	67	260
ACA	7.89	52.0	37.6	5.7	115	95	339
CNA	7.13	9.1	6.7	6.0	236	109	158
Normal Range*	0.20–0.88	0.20–2.10	2.6–7.6	0.2–0.7	<1	62–120	50–75

*For females at mid-follicular phase.

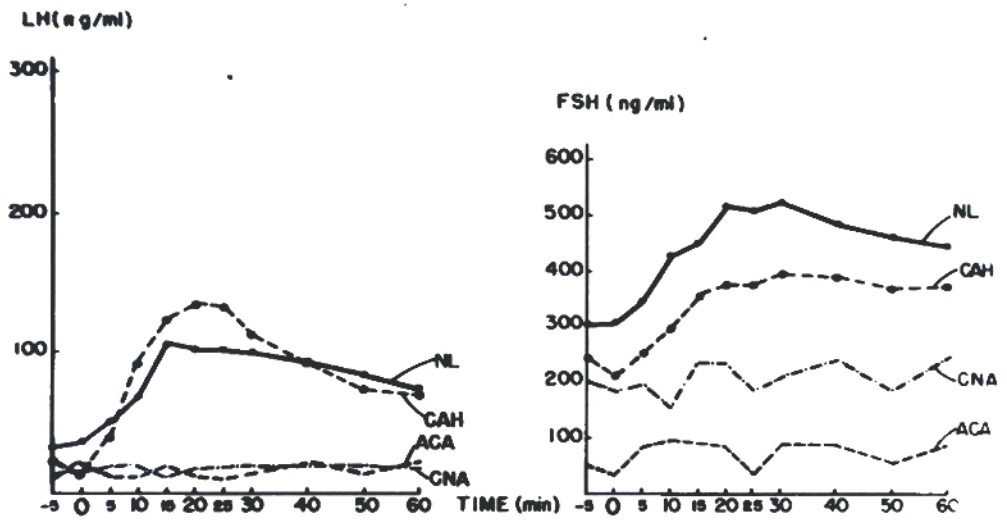

Fig. 1. Mean serum LH and FSH levels after the acute administration of 25 ug LHRH in 8 normal (NL) adult females at the mid-follicular phase and 4 responsive CAH patients. The results in the 2 non-responsive patients with untreated CAH are also shown (ACA and CNA).

levels and secretory areas were significantly lower in CAH subjects than in normals but not the other LH parameters. Regarding the FSH response, only the relative maximum response was significantly greater in the CAH patients in relation to the normal females but not the total secretory area.

The non-responsive patients, as for the acute LHRH test, presented a very small degree of responsiveness to the prolonged LHRH infusion (ACA's LH secretory area: 4,560 ng/ml × min $^{-1}$; CNA's area: 14,850 ng/ml × min $^{-1}$ whereas normal females at the mid-follicular phase had a mean value of 252,557

Table 3. LH and FSH Responses in Normal Females and in 4 LH-responsive Patients With CAH (mean ± SEM)

Administration	No. of Subjects	Basal (ng/ml)	Peak (ng/ml)	Δ (ng/ml)	Δ %	Area (ng/ml × min $^{-1}$)
LH Acute (25 ug)	8 Normal	35	120	85	245	3,279
LHRH		±5	±19	±15	±32	±608
	4 CAH	18*	202	183	971*	7,043
		±1	±44	±44	±221	±1,941
Prolonged infusion	7 Normal	40	797	756	1,882	252,557
(100 ug LHRH)		±4	±83	±80	±180	±31,546
	4 CAH	22*	559	537	3,112	163,575*
		±5	±104	±103	±1,137	±25,688
Acute (25 ug)	7 Normal	576	764	187	44	5,028
After prolonged infusion		±78	±61	±29	±15	±1,495
	4 CAH	416	869	453	104	10,156
		±37	±154	±126	±29	±4,606
FSH Acute (25 ug)	8 Normal	310	562	252	85	9,666
LHRH		±22	±61	±61	±20	±2,798
	4 CAH	280	542	262	94	11,910
		±26	±80	±86	±22	±3,427
Prolonged infusion	7 Normal	314	1,139	825	274	253,708
(100 ug LHRH)		±37	±136	±121	±47	±45,005
	4 CAH	244	1,928	1,684	688*	458,485
		±3	±440	±440	±177	±119,451
Acute (25 ug)	7 Normal	718	1,324	605	85	19,857
After prolonged infusion		±71	±208	±130	±17	±3,942
	4 CAH	1,533*	2,349	816	48	26,659
		±228	±463	±237	±11	±8,023

*$p < 0.05$.

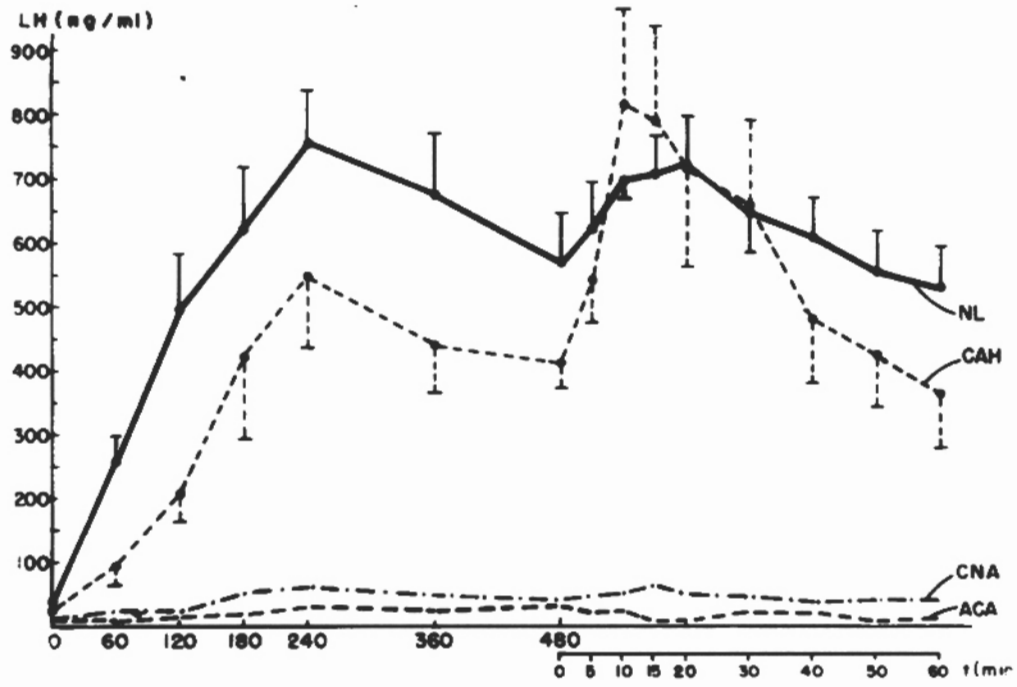

Fig. 2. Serum LH levels (mean ± SEM) during continuous infusion of LHRH (0.21 ug/min) followed by a 25 ug pulse in 7 normal (NL) females (at the mid-follicular phase of the menstrual cycle) and 4 responsive patients with CAH. The results in the 2 non-responsive patients with untreated CAH are also shown (ACA and CNA).

ng/ml × min^{-1}). As for FSH, patient ACA, with very low basal values, did not show an increase during the prolonged gonadotropin-stimulating infusion. CNA also with low basal FSH values had a normal relative maximum increment but a decreased secretory area (177,720 ng/ml × min^{-1}) relative to the normals.

Acute LH-RH test following prolonged LH-RH infusion:

As shown in Figs. 2 and 3 and Table 3, all parameters and LH and FSH secretion were similar in the normal females at the mid-follicular phase and in the LH-responsive CAH patients, except for the significantly higher basal FSH values, in the latter (at the end of the prolonged infusion).

The LH-unresponsive CAH subjects, as expected, did not respond to the acute infusion.

There was a negative and significant correlation between the basal plasma estradiol levels and the LH secretory area in the prolonged LHRH infusion ($r = -0.92$; $p < 0.01$), expressed by the equation: LH area = $352298 - 3325.8 \times E_2$.

On the other hand, there was no correlation between any of the other plasma steroids measured and LH secretory area.

DISCUSSION

In congenital virilizing adrenal hyperplasia, the increased adrenal sex hormones concentrations should be associated with suppression of gonadotropins secretion from the expected pituitary-sex hormones relationship. However, in the LHRH-responsive patients, the ovaries were of normal adult size or enlarged (in one of the patients) as noticed by pneumogynecography and/or at the time of surgical biopsy the ovary, presumably because of the presence of adequate concentrations of FSH. This observation suggests that prolonged secretion of increased amounts of adrenal steroids including androgens and estrogens, occuring in patients who are untreated long enough, may produce maturation of the central nervous system, perhaps clinically evident as advanced bone age. Consequently, it would occur the release of gonadotropins[9] and initiation of the puberal LH "program".[10]

On the other hand, the effect of the increased circulating adrenal sex steroids is paradoxical: In

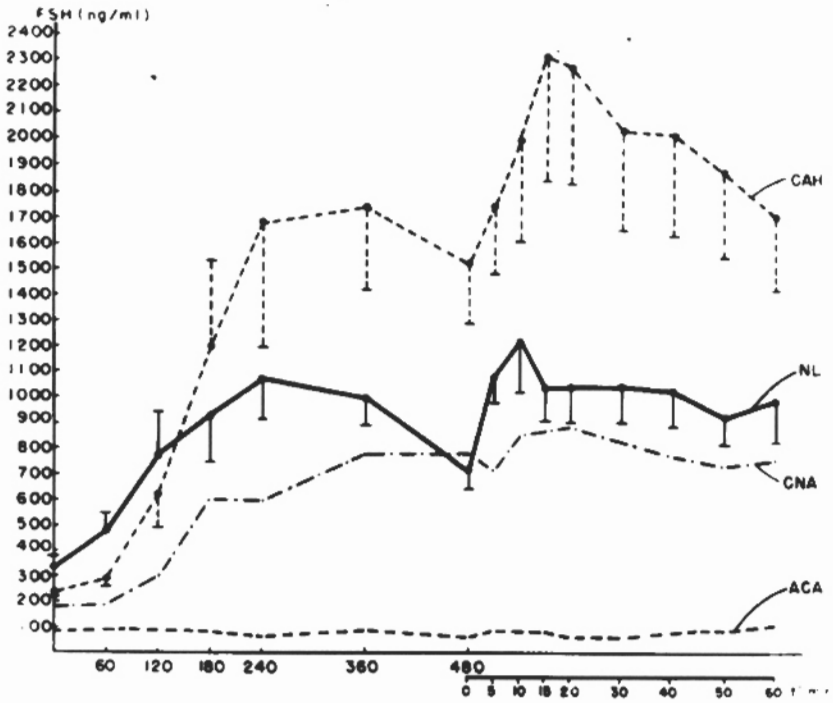

Fig. 3. Serum FSH levels (mean ± SEM) during 8 hr continuons in fusion of LHRH (0.21 ug/min) followed by a 25 ug pulse in 7 normal (NL) adult females (at the mid-follicular phase of the menstrual cycle) and 4 responsive CAH patients. Shown are also the results of the 2 non-responsive patients with untreated CAH (ACA and CNA).

addition to their probable maturing effect on hypothalamic-pituitary gonadotropins, in the LH-responsive older subjects, there was a suppressive action on the cyclic release of gonadotropins by their action on the gonadotropes and/or the hypothalamus since our subjects had primary amenorrhea or very irregular and scanty menses in the patients with the mildest form of the disease. As basal FSH was not decreased in these patients, endogenous hypothalamic LHRH production could be considered as being normal. On acute LHRH infusion, the release of preformed and readily releasable gonadotropins was relatively or completely normal in these subjects as previously also observed by Richards et al.,[3] suggesting that adrenal sex steroids in increased amounts, act essentially at the level of the hypothalamus, as previously suggested.[11]

On the other hand, the prolonged infusion of the gonadotropin-releasing factor, demonstrated that the newly synthetized LH was slightly decreased in relation to the normal females (at the midfollicular phase presenting the same estradiol levels as our patient)[4] but showing the same degree of FSH release. However, the normal acute release of both gonadotropins, in the post-prolonged LHRH infusion, in our LH-responsive CAH patients, is an indication that the mentioned decreased in LH secretion is not dependent on reduced synthesis but on a blockade of its release, being overcome by the acute loading dose of the synthetic hypothalamic stimulator.

Regarding our two youngest patients, with the greatest severity of their androgenic manifestations, LH-unresponsive to the acute and chronic LHRH infusion, from what it was mentioned, their hypothalamus should have maturated, as probably indicated by the advanced bone age. However, their very low basal FSH could suggest decreased endogenous LHRH production and or suppressive effect on the pituitary compatible with the finding of pre-puberal ovaries in the patient never treated (ACA). Accordingly, no releasable gonadotropins were observed with the acute and prolonged LHRH infusion in patient ACA. The other subject, CNA, with the higher basal FSH levels, presented a certain degree of responsiveness to prolonged LHRH, regarding FSH but not LH, a clearly prepubertal pattern, which could have some relation to the previous period of cortisol treatment.

The only difference between these subjects and the remaining ones was the greater severity of the androgenic manifestation, with higher plasma testosterone levels and associated elevated plasma estradiol. However, the high plasma sex steroids in both groups of patients were of such magnitude that it is improbable that their differences could explain why the older patients have a normal response to LHRH while the younger ones did not release gonadotropins or have a pre-puberal response.

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