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EFFECTS OF CONTINUOUS GHRH INFUSION ON GH SECRETION IN GH-DEFICIENT PATIENTS WITH HYPOTHALAMIC-PITUITARY ABNORMALITIES

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To evaluate residual pituitary GH, GH responses to continuous 180-min infusion of increasing doses of GHRH 1-29 followed by iv bolus injection of GHRH were studied in 28 hypopituitary patients, 21 of whom had pituitary abnormalities on MRI. Pituitary hypoplasia, stalk agenesis and ectopic posterior pituitary lobe in 7 patients with isolated GH deficiency (IGHD) and 6 with multiple pituitary hormone deficiencies (group I); isolated pituitary hypoplasia in 8 IGHD (group II); normal pituitary gland morphology in 7 IGHD (group III). Pituitary volume was not significantly different in the groups I and II.

The study consisted of 1) 0.9% saline infusion for 30'(50ml/h) from 0830-0900h; 2)GHRH 200 ng/kg body weight/h from 0900-1000h, GHRH 400 ng/kg/h from 1000-1100h and GHRH 600 ng/kg/h from 1100-1200h; 3)iv bolus dose of GHRH (2ug/kg) at 1200h. Blood samples for GH and IGF-1 levels were obtained every 15 min until 1200h and then after 5,10,15,20 and 30' following GHRH bolus.

A slight increase of GH was observed in group I while GHRH infusion significantly increased GH secretion in the groups II and III. Mean GH pulse amplitude as well as mean GH height were significantly lower in group I (1.08±0.35, 3.12±0.94 ng/ml) than in group II (4.74±0.53, 14.89±1.50 ng/ml, p=0.0007) and III (6.78±2.42, 22.93±5.41 ng/ml p=0.0092, p=0.004). A similar trend was also observed for the mean integrated total GH areas over 0-line in the group I (30.24±9.55 ng/ml),II (202.79±33.87 ng/ml, p=0.0003) and III (193.32±27.93 ng/ml, p=0.0004).

Spontaneous GH peaks occur during saline infusion in the group III suggesting the presence of two releasable pituitary GH pools. The GH secretion pattern was quite different in groups II (late GH reponse) and III. GH response to the iv bolus dose of GHRH was indicative of pituitary desensitization. Basal IGF-1 was in the normal levels in group III and no variations were detected during GHRH infusion.

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GROWTH HORMONE INSUFFICIENCY IN TURNER SYNDROME: IS BODY WEIGHT THE KEY FACTOR? S. Cianfarani*, F. Vaccaro*, A.M. Pasquino**, S.A. Marchione**, F. Passeri**, G.L. Spadoni*, S. Bernardini*, A. Spagnoli*, B. Boscherini*. Departments of Paediatrics, * "Tor Vergata" University and ** "La Sapienza" University, Rome, I-00173, Italy.

The age related decline in spontaneous growth hormone (GH) secretion has been suggested to cause growth failure in girls with Turner syndrome (TS). We studied 23 girls (mean age: 11.1 yrs with 95% confidence intervals (CI) 9.9 to 12.3) diagnosed to have Turner syndrome by karyotype analysis. 15 prepubertal age-matched subjects (mean age: 11.6 yrs with CI 10.4 to 12.8) with growth retardation due to familial short stature and/or constitutional growth delay were chosen as controls. Spontaneous 12-hour nocturnal GH secretion was assessed by RIA at 30 minutes intervals. Plasma IGF-1 levels were determined by RIA after acid-ethanol extraction. In TS, the percentage of ideal body weight was significantly higher than controls (mean: 127.5 with CI 116 to 139 in TS, and 100.3 with CI 96 to 104.5 in controls; $P = 0.0005$), and correlated with bone age ($r = 0.62$, $P < 0.005$). Spontaneous GH secretion was significantly lower in TS than controls (mean: 3.2 ng/ml with CI 2.5 to 3.9 in TS, and 5.4 ng/ml with CI 4.8 to 6.0 in controls; $P < 0.0001$). No significant difference was found in IGF-1 levels. In controls, GH concentrations correlated with bone age ($r = 0.56$, $P < 0.05$), whereas in TS no correlation was found. Interestingly, in TS GH levels negatively correlated with percentage of ideal body weight ($r = -0.43$, $P < 0.05$). Our results, confirming that obesity is a common finding in girls with TS, at least in the age range of our patients, suggest that overweight might be the key factor in determining the subnormal spontaneous GH secretion. On the basis of our previous observations showing a close inverse relationship between body weight and serum IGFBP-1 levels in TS, it might be hypothesized that obesity, probably by increasing insulin secretion, would reduce IGFBP-1 levels eventually leading to an enhancement of IGF negative feed-back effect on GH secretion.

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NOCTURNAL BIOACTIVE FSH PULSES ARE EVIDENT IN PRE (PERI) PUBERTAL BOYS.

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Secretion of both LH and FSH is pulsatile and maximal during the night in early peripubertal boys. To evaluate the pulsatile pattern of bioactive FSH (B-FSH), we assessed serum concentrations of B-FSH in four Tanner Stage I boys with constitutional growth delay (mean age 15y 3m, mean bone age 12y 6m) and compared their data to previously published data from 6 Tanner Stages II-III pubertal boys (Hassing et al, JCEM, 1990, 70:1082). Each of the four boys had a 12 h overnight blood sampling (every 20 min) followed by administration of 1µg/kg nafarelin sc. Serum I-LH and I-FSH were measured by RIA and B-FSH was measured by the rat Sertoli cell aromatase induction assay using hLH-I3 and hFSH-I3 standards and expressed as ng/ml. The mean serum I-LH, I-FSH and B-FSH were 0.63±0.06, 0.40±0.15 and 1.8±0.2 ng/mL ($p<0.05$, B vs I FSH). B-FSH pulse frequency was similar to I-LH pulse frequency (0.43±0.1 and 0.43±0.05 pulses/boy/h) and to previously published data in mid pubertal boys. ΔI-LH release was 3.3±0.7, ΔI-FSH was 2.4±0.6 and ΔB-FSH was 4.2±1.4 ng/mL. We conclude that during the peripubertal period in boys, serum B-FSH is secreted in a pulsatile manner and the concentrations exceed those of serum I-LH or I-FSH..

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COPULSATILITY OF GROWTH HORMONE AND PROLACTIN SECRETION IN GROWTH HORMONE DISORDERS.

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A dynamic neurosecretory relationship may exist between somatotropin (hGH) and prolactin. Regulation of both hormones occurs at the CNS, hypothalamic and pituitary level. Simultaneous serial measurements of both hormones were analyzed for determination of their temporal relation and the possibility to differentiate hypothalamic from pituitary defects in growth hormone disorders. Secretory patterns were analyzed in 45 children with hGH inadequacy on the basis of suboptimal response to three standard provocative agents. Integrated measurements were obtained at 20 minute intervals, from 2000-0800, during sleep. Prolactin was measured with a solid-phase ^{125}I RIA. hGH measurements utilized the Tandem-R hGH (Hybritech) method. Analysis of co-secretory dynamic relationships by pulse amplitude and integrated area under the curve (AUC) revealed characteristic but not consistent patterns for hypothalamic or pituitary dysfunction. The pattern of diminished hGH and facilitated prolactin suggests hypothalamic dysfunction, whereas diminution of both peptides suggests a pituitary abnormality. A significant correlation ($r=0.36$, $p=0.014$) was noted for the number of hGH and prolactin peaks. No significant relationship to gender or maximum hGH value was seen. Using the Cluster pulse detection program, the mean interpulse interval for hGH was 159 min. and for prolactin 105 min. Analysis of pulse interaction using the AnCoPuls program revealed significant copulsatility for prolactin pulses trailing hGH pulses by 80 min. ($p<0.004$). This suggests a relationship of the neurosecretory mechanisms for the two hormones in these children at either the intra-pituitary or more likely supra-hypothalamic level.

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LHRH SECRETION OF IMMORTALIZED HYPOTHALAMIC NEURONS IS STIMULATED BY N-ACETYLASPARTYLGLUTAMATE.

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The acidic dipeptide N-Acetylaspartylglutamate (NAAG) is thought to be an endogenous ligand of the excitatory amino acid receptor system. Because excitatory amino acids stimulate release of LHRH in vivo, we tested the hypothesis that NAAG might stimulate LHRH release from immortalized LHRH neurons in culture. GN-10 cells, grown to semiconfluence in 24-well plates, were incubated with NAAG, ß-NAAG, or glutamate. LHRH secretion was evaluated by enzyme immunoassay. Both NAAG and glutamate elicited LHRH secretion in a dose-dependent manner. 10^{-9} M NAAG increased LHRH secretion significantly compared to controls (72 ± 45 [SD] vs 13 ± 17 pg/mL, $p<0.005$) , whereas a 100-fold higher concentration of glutamate was required to achieve significant stimulation (46 ± 21 vs 8 ± 11 pg/mL, $p<0.005$). ß-NAAG was inactive at all concentrations (10^{-13} to 10^{-4} M). To examine whether the stimulation of LHRH release observed with NAAG could be due to enzymatic cleavage of NAAG into NAA and glutamate, GN-10 cells were incubated for up to 2 hours with NAAG radiolabelled with ^3H-Glu, and ^3H-NAAG and ^3H-glu separated by HPLC. No ^3H-glu was detected. We conclude that NAAG is not degraded by GN-10 cells, and that NAAG is a potent stimulus for LHRH release at concentrations at which glutamate is inactive.

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KETOCONAZOLE TREATMENT OF TWO ADOLESCENTS WITH CUSHING'S DISEASE.

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We evaluated the effect of ketoconazole (KT) treatment in two adolescents with pituitary dependent Cushing's disease. Case 1:a girl, aged 12.9 years, was given orally KT (200 mg/day), which induced a significant decrease of plasma cortisol levels for 3-4 months (mean±SD: 29.1±8.3 at start vs 6.2±1.2 µg/dl at 3 months, $p<0.01$; blood samples were taken at 4-h intervals, 8 a.m.-12 p.m.) but not after 6 months(24.2 ± 4.0 µg/dl), despite an increase of KT dose up to 600 mg/day. Mean plasma ACTH levels were 36.2±4.7, 23.2±8.5 and 30.2±14.2 pg/ml, at start, 3 and 6 months, respectively. Similarly, in Case 2, a boy aged 14.8 years, KT (400 mg/day) reduced mean plasma cortisol levels for 3 months (25.8±2.3 vs 18.8±2.7 µg/dl;$p<0.005$) but not after 6 months (30.7±5.3 µg/dl), again despite an increase of KT dose up to 1000 mg/day. Mean ACTH levels remained unchanged for 3 months (45.1±5.3 at start vs 40.6±12.8 pg/ml at 3 months), but increased after 6 months (73.5±3.8 pg/ml;$p<0.005$ vs initial values). Both patients showed a good clinical improvement with reduction of body weight and normalization of blood pressure for six months; KT was well tolerated. Coincident with the reoccurence of clinical symptoms, magnetic resonance features of pituitary microadenoma became evident at six months and both patients underwent transsphenoidal surgery. Our experience shows that Ketoconazole may have a short reducing effect on plasma cortisol in children with pituitary dependent Cushing's disease while awaiting therapeutic surgery.