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INDUCTION OF TESTICULAR GROWTH AND SPERMATOGENESIS BY PULSATILE IV LRH.

In hypogonadotropic hypogonadism (HH) gonadotropin treatment often fails to induce testicular growth. When the gonadotrophs are capable to secrete LH and FSH, pulsatile LRH should be able to induce testicular development.
17 male pts, aged 14.2-26.0 yrs, were treated with pulsatile LRH: 5 pts with Kallmann's S., 3 postcraniopharyngectomy, 8 with idiop. HH and 1 with MPHD. LRH was administered iv with a presumed physiologic pulse interval (pi) of 90 min. Depending on the individual response the dose increased stepwise from 2 to 20 µg per pulse. In 14 pts treatment was preceded by 2 periods of 4 wks of LRH with pi of 180 min. and 30 or 45 min.resp. LRH was replaced by hCG twice/wk.
During pi of 90 min.all 17 pts showed an increase of LH, FSH and Testosterone into the normal adult range. At a pi of 180 min.the LH/FSH ratio was lower; in 3 pts only FSH increased. LRH with a pi of 30 or 45 min.did not result in desensitization; LH was higher than at a pi of 90 min. All pts showed a clear testicular growth. At the end of LRH treatment 11 pts had spermatozoa in the ejaculate; 1 pt only showed sperm production during hCG and 2 pts were still azoospermic after 1.7 and 2.3 yrs on hCG.
Conclusion: Pulsatile LRH iv is a feasible way to induce testic. growth as well as spermatogenesis. HCG is able to maintain or even improve this development.

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IMPAIRED RESPONSE OF GHRH MEASURED IN PLASMA AFTER L-DOPA STIMULATION IN PATIENTS WITH IDIOPATHIC DELAYED PUBERTY: NORMALISATION WITH START OF PUBERTY.

In order to investigate the regulation of GH secretion in patients with idiopathic delayed puberty (IDP) either prepubertal (stage P1) or early pubertal (P2) GHRH levels in plasma were measured after stimulation with L-Dopa in a group of 16 patients with IDP. The results were compared to those obtained in 12 patients with constitutional short stature (CSS) at the same stage of puberty who underwent L-Dopa test for insufficient height. Plasma GHRH levels were measured after extraction by RIA. After L-Dopa intake the peak of GH was mean ± SEM 8.6 ± 1.4 ng/ml in IDP and 12.0 ± 0.8 ng/ml in CSS (NS). The peak of GHRH after L-Dopa was 41 ± 10 pg/ml in IDP and 96 ± 25 pg/ml in CSS ($p<0.02$). Basal plasma GHRH levels were measured in five patients with IDP before and after 3 x 1500 IU of hCG. Testosterone levels rose to 4.8 ± 0.9 ng/ml, no changes in plasma GHRH levels were observed (25 ± 13 pg/ml). Oxandrolone was given in 6 patients with IDP. Six months after when puberty was clearly started with an increase in growth velocity (6.3 ± 0.7 cm/6 months) peak GHRH levels during L-Dopa stimulation test increased significantly ($p<0.02$) from 48 ± 12 pg/ml to 142 ± 33 pg/ml, GH peaks being respectively 8.4 ± 3.2 pg/ml before, 11.4 ± 1.9 after. These results suggest an hypothalamic dysfunction in patient with IDP, reversible with the start of puberty. They indicate a relationship between the wellknown partial and transitory GH deficiency found in some adolescents having a pubertal delay and their secretion of GHRH.

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MECHANISM OF GROWTH HORMONE (GH) SECRETION IN PHARMACOLOGICAL STIMULATION TESTS

Availability of radioimmunoassay for growth hormone releasing hormone (GHRH) in plasma has made it possible to measure circulating GHRH concentrations during stimulation of growth hormone (GH) secretion. It has been previously shown that administration of L-dopa increases plasma levels of GHRH, followed by an elevation of plasma GH concentration. Ornithine or clonidine do not seem to affect plasma levels of GHRH. To elucidate the role of GHRH in the stimulation of GH secretion during pharmacological stimulation tests we measured plasma immunoreactive GHRH and GH levels in 8 healthy adult men during insuline induced hypoglycemia (IH) and after oral administration of clonidine of L-dopa. Plasma samples for the determination of GHRH levels were purified with octa-decyl-silica cartridges and assayed by a radioimmunoassay specific for the midportion of human GHRH. Mean GH response (SEM) after administration of L-dopa was 8.67 ug/l (2.9), 2.22ug/l (1.0) in clonidine test and 24.33 ug/l (4.4) during insuline induced hypoglycemia. Mean GHRH responses respectively were 9.64 ng/l (2.6), 6.65 ng/l (2.3) and 4.25 ng/l (3.4).
The plasma GHRH peaks preceded or coincided with GH peaks in all subjects after L-dopa administration. In the clonidine test and IH no correlation was found between the GHRH and GH peaks. These results confirm that the effect of L-dopa on GH secretion is mediated by GHRH, whereas other factors than GHRH seem to play a major role in GH stimulation by IH and clonidine test.

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RESPONSE OF PLASMA CONCENTRATIONS OF GROWTH HORMONE (GH)RELEASING HORMONE, GH, INSULIN, AND SOMATOSTATIN (SRIF) TO A MIXED MEAL IN CHILDREN.

GH-releasing hormone (GHRH) is detectable in the peripheral plasma but its possible functions and sources remain to be elucidated. We therefore measured the response of GHRH, GH, insulin and SRIF to a mixed meal (800 kcal; 50% carbohydrates,35% protein, 15% fat) in 7 short normal children (8.4 -12.6 years) undergoing 24h spontaneous GH secretory pattern investigation. Blood samples were drawn at 0; 30; 60; 90; 120; 150 and 180 minutes. All hormones were measured using specific RIAs. In response to the mixed meal there was a significant increase of plasma GHRH with peak values occurring between 60 and 150 min (10.2 ± 1.2 pg/ml vs 25.6 ± 4.5 pg/ml;$p < 0.01$). Plasma GH values increased within 30 to 150 minutes suggesting spontaneous GH bursts. Plasma insulin levels increased between 60 and 90 minutes (9.4 ± 1.2 µU/ml vs 49.5 ± 4.4 µU/ml; $p < 0.001$). A significant rise of plasma SRIF was found which showed a biphasic pattern. There was no correlation between increments of plasma GHRH and the other hormones. In contrast,in 2 obese children we could find no plasma GHRH increase. Our results support the concept that circulating GHRH might act as a peripheral hormone and posssibly originates from the gastrointestinal tract.

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THE IMMUNE RESPONSE TO GRF (1-44) NH_2 AND GRF (1-29) NH_2 AS A PROBE OF CONFORMATION IN VIVO

Treatment with peptide hormones leads to the development of specific antibodies (Abs). Structural homology does not prevent induction of high affinity Abs. GRF 1-44 NH_2 and GRF 1-29 NH_2 are linear peptides. Formation of amphilic secondary structures on cell surfaces can be important for biological action and immune response. Guinea pigs were immunized against 1-44 NH_2 and 1-29 NH_2. The concentrations, the affinities and the sizes of IgG-Abs-GRF complexes were determined by ultracentrifugation. The native hormone is a fair immunogen which induced specific Ab concentrations of 593 ± 41mg/l after 4 weeks, comparable to insulin and hGH in the same system. 85% of these Abs bind to epitopes of the amino-acid residues 30-44 with high affinity ($3.8\pm0.9\ 10^9$ l/M), only 15% bind to epitopes of sequence 1-29 with lower affinity ($6\pm0.3\ 10^8$ l/M). Abs to 1-29 NH_2 had lower concentrations (36.4 ± 8mg/l) and lower affinities towards the native hormone ($4 \pm 0.2\ 10^8$ l/M) and the 1-29 NH_2-fragment ($3\pm0.2\ 10^8$ l/M). These data support the concept of amphilic secondary structures. 1-44 NH_2 is a divalent antigen which forms 7S and 10S antigen-Ab complexes, 1-29 NH_2 forms largely monomeric complexes. One out of 9 children treated with 1-29 NH_2 s.c. for 3 ms developed Abs with low titer and low affinity, 2 after 6 ms with low titer and affinity. Samples drawn 6 ms after discontinuation were negativi.

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MEASURMENT OF ATRIAL NATRIURETIC PEPTIDE (ANP), cGMP, ALDOSTERONE AND VASOPRESSIN LEVELS IN INFANTS.

In our first studies we found elevated plasma levels of ANP and its second messenger cGMP in newborns and in children with cardiac diseases. Despite these high levels there is no effect on the water- and electrolyte excretion. The lacking response to ANP in these patients may be explained by an enhanced release of other volume regulating hormones. To prove this hypothesis we measured plasma and urinary levels of ANP and cGMP as well as urinary excretion of aldosterone (Aldo) and vasopressin (AVP) in 22 healthy infants and 26 infants with cardiac diseases. Urine was collected for 8 hours from 10 p.m. to 6 a.m. In children with cardiac diseases we found significantly higher plasma levels of ANP ($\bar{x}$ = 225 ± 65 pg/ml) and cGMP ($\bar{x}$ = 8,5 ± 4,5 pmol/ml) and urinary levels of cGMP ($\bar{x}$ = 30 ± 5 nmol/8h/kg), Aldo ($\bar{x}$ = 800 ± 20 pg/8h/kg) and AVP (1100 ± 250 pg/8h/kg) than in control infants ($p < 0.001$). There was no correlation between ANP or cGMP levels and water- and sodiumexcretion.
Our results support the assumption that the natriuretic effect of chronically elevated ANP levels is markedly reduced by the simultaneous stimulation of water- and sodium retaining hormones such as Aldo or AVP.