

5 N.Lahlou[†], J.L.Chaussain, A.M.Piot[†], M.Roger, J.E.Toublanc, A.V.Schally* Fondation de Recherche en Hormonologie and Hôpital Saint-Vincent de-Paul, Paris, France, V.A. Medical Center, New Orleans, La, USA. DIFFERENTIAL EFFECT OF D-TRP-6-LH-RH IN MICROCAPSULES (LRH-M) UPON LH AND LH_α RELEASE DURING LONG TERM TREATMENT OF PRECOCIOUS PUBERTY (PP).

LH_α secretory episodes are simultaneous with LH pulses. However short treatments with LH-RH agonists increase LH_α release while decreasing immunoreactive (IR) LH levels. The effect of intramuscular LRH-M (60 µg/kg every 28 days for 1 year) was investigated in 17 girls with PP. LH_α was measured by a specific RIA, IR-LH by a RIA in which the crossreactivity of LH_α relatively to LER 907 was 100%. LH_α levels (\bar{x} ±sem, ng/ml) were 0.49±0.16 before LRH-M and significantly increased at 3 weeks, 3 and 6 months of treatment, respectively 4.2±0.42, 2.4±0.34, 1.8±0.21, whereas IR-LH was unchanged. LH_α returned to basal levels at 12 months, whilst IR-LH was significantly lowered. LH_α peaks after LH-RH test were before LRH-M and after 3, 6 and 12 months respectively 10.8±1.4, 7.8±1.1, 5.3±0.3 and 4.8±1.0. The ratios of LH_α peaks to IR-LH (ng LER 907) peaks were respectively (%) 4.3±0.6, 20.3±2.1, 16.3±1.8 and 14.2±3.1 (differences from basal p<0.01). This study brings evidence that - LH_α accounts for a part of IR-LH in subjects treated with LH-RH agonists - LH_α secretion is strongly stimulated for several months whilst plasma IR-LH decreases - LH_α response to LH-RH is maintained throughout the study demonstrating a differential effect of LRH-M on the regulatory mechanisms of a subunit and LH secretion.

6 J.P.Bourguignon, A.Gerard[†], G.Debougnoux[†], P.Franchimont[†], Pediatric Clinic and Radioimmunoassay Laboratory, University of Liège, CHU, Liège, Belgium. A GnRH SUPERAGONIST, BUSERELIN, REDUCES GnRH RELEASE FROM THE RAT HYPOTHALAMUS IN VITRO.

Single retrochiasmatic hypothalami of male rats aged 15, 30 and 50 days were studied in incubation chambers where culture medium (0.5 ml DMEM) was renewed every 7.5 min and collected for the RIA of GnRH. Using the anti-GnRH antibody RR5 provided by A. Root, no cross-reactivity was observed in the presence of buserelin concentrations < 100 nM. When the 50-day hypothalamus were incubated in the presence of 50 µM veratridine, a depolarizing agent, the mean release of GnRH (\pm SD) was 17.8 ± 4.4 pg/7.5 min. From the first time buserelin (10 nM) was added, GnRH release fell to 7.5 ± 2.5 pg/7.5 min. A similar inhibition was observed at 15 and 30 days of age, persisted as long as buserelin was present and was reversible when the agonist was no more added. The spontaneous release of GnRH was significantly reduced by several buserelin concentrations between 0.01 and 10 nM. In contrast, GnRH release did not change in the presence of other analogs (Leu⁸-GnH, picolylamide¹⁰-GnRH) or when buserelin had been previously heated at 90° for 90 min. Finally, the occurrence of spontaneous intermittent pulses of GnRH release, which was observed in control hypothalamus of 30- and 50- day-old rats, was reduced to less than 1 pulse/3 h in the presence of buserelin 1 or 10 nM. An hypothalamic action of this GnRH agonist is suggested by these data and should be considered in the evaluation of the therapeutic and possible side effects of buserelin.

7 J.R. Ducharme, G. Renier*, J. Gaulin*, W. Gibb*, R. Collu* Research Unit on Reproductive and Developmental Biology, Pediatric Research Center, Hôpital Ste-Justine, Montreal, Canada. STUDIES ON THE INFLUENCE OF GROWTH HORMONE AND GROWTH FACTORS ON TESTICULAR STEROIDOGENESIS

Growth hormone (GH) treatment can increase testicular LH receptor concentration and testicular responsiveness to LH in hypophysectomized rats. To verify whether GH and growth factors (somatomedin C or IGF₁ and IGF₂) exert a direct control on Leydig cell function, their effect on cAMP and testosterone (T) production by highly purified immature porcine Leydig cells (L) in primary culture was studied. Porcine GH (pGH) (10⁻⁷-10⁻⁹ M) induced a significant increase in cAMP accumulation and T production and significantly enhanced the action of hCG (60 mIU/ml) on T release. Human GH (hGH) had a similar effect at 10⁻⁷ M but not at 10⁻⁹ M indicating some species specificity. This effect was partially blocked by a specific anti-hGH antibody. hGH also increased IGF₁ production by L while IGF₁ also enhanced basal and hCG stimulated T production by L. Finally, receptors to IGF₁ and IGF₂ have been detected and characterized in L. These data indicate that 1) GH has a stimulatory effect on steroidogenesis of L in culture. 2) Their effect is exerted directly on the Leydig cell and is not mediated by another cell-type, and 3) hCG and GH seem to enhance T production by separate mechanisms and to interact at different receptorial sites. The mechanisms by which this action is exerted and the possibility that growth factors may be involved in this action remain to be determined.

8 C.-J. Partsch*, P. Heidemann and W.G. Sippell Endocrine Unit, Departments of Paediatrics, Universities of Kiel and Göttingen, Germany. DIAGNOSTIC ADMINISTRATION OF PULSATILE GnRH IN MALE ADOLESCENTS WITH ANTERIOR PITUITARY INSUFFICIENCIES In an attempt to select the appropriate long-term substitution therapy for ultimately attaining fertility, the inducibility of pituitary gonadotropin (Gn) production by short-term pulsatile administration of GnRH was studied in 9 male adolescents with anterior pituitary deficiencies (APD) including severe hypogonadotropic hypogonadism (HH). Clinical data (ranges): CA 14.3-23.3 yrs, BA 8.5-18 yrs, testis vol. 1.0-10 ml. ACTH, TSH and GH deficiency was present in 8, 6 and 9 patients (pts), respectively. Study protocol: (1) Spontaneous nocturnal Gn profile sampled every 20 min, (2) 1st GnRH bolus test, (3) pulsatile GnRH (5 µg every 90 min iv) for 36 h, sampled 30 min after each pulse, (4) 2nd GnRH bolus test. Testosterone (T) determination before (2) and after (3). LH and FSH levels during (1) were prepubertally low in all pts, ranging from 0.9-3.2 and 0.9-3.0 mIU/ml, respectively. No spontaneous pulses were detected. During (3), mean LH and FSH levels were significantly higher (p<0.00025) in 7 and 9 pts, respectively. Spearman rank correlation of LH and FSH during (3) revealed highly significant increases of LH (p<0.01) in 4 and of FSH (p<0.002) in 5 pts. In all but one pt, T did not increase after (3). It is therefore likely that the HH in 5 out of 9 pts tested is predominantly of hypothalamic, in 4 of pituitary origin. We suggest to use this or a similar protocol applying short-term pulsatile GnRH stimulation in order to select those APD patients whose HH is due to a hypothalamic lesion and thus can be treated by pulsatile GnRH rather than hCG/hMG for achieving fertility.

9 M.G.Forest, Y.Morel* and A.Asensio*. INSERM-U.34, Hôpital Debrousse, 69322 Lyon Cedex 05, France. BIOACTIVE LUTEOTROPIN (LH) IN URINE: NYCTHEMERAL VARIATION IN BIOLOGICAL POTENCY IN MID-PUBERTY.

A previous work (Arch.Fr.Pediatr.1985,42,579) has shown the usefulness of immunoassayable (I) urinary LH levels for assessing gonadotropic function in children and adolescents. To further investigate the physiological significance of this parameter we have assessed urinary LH biopotency (B) and compared the values to that of I/LH. The bioassay for LH was performed by mice interstitial cell testosterone production (MICT) assay. Samples were tested in quadruplicate at 2 different dilutions. Sensitivity was 30-40 µIU per tube; intra- and inter-assay CV were 5.5% and 7.5% (mid-range). The dose-response slope of 2 standards (a highly purified hCG preparation and the MCR 68/40 LH standard used in RIA) and 3 urines were parallel. The bioassay and RIA were performed on fractionated 12 h/12 h (night/day) urine collections (n=50; 8-17 yr). Bioactive LH was detected in all samples and significant correlations (r=0.6 to 0.7) were observed between B and I/LH in all pubertal groups. However, the B/I ratio varied during pubertal maturation: close to 1 in prepuberty, increasing to 2-3 in early and mid-puberty. More striking was the varying B/I ratio between day and night at the period when the sleep associated surges of LH take place. This was reflected by a night/day ratio significantly higher (p < 0.001) for B/LH (4.07±0.99) than I/LH (2.54±1.3) in stage P2-P3. In conclusion. 1) Urinary I/LH is bioactive, 2) the pubertal rise in B/I ratio reported in plasma is also observed in urine, 3) a pubertal night/day variation in B/I ratio appears superimposed.

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THE RELATIONSHIP BETWEEN HEIGHT VELOCITY AND 24 HOUR GROWTH HORMONE SECRETION

We have performed 24 h growth hormone (GH) profiles on 52 children of varying stature, sampling intermittently at 15-20 minute intervals. GH pulse amplitudes and areas under GH pulses were compared with height velocity.

The relationship between height velocity SDS (y) and any one of the parameters of GH secretion (\bar{x}) was described by the asymptotic regression $y = a - b(e^{-cx})$, where a, b and c are constants. This was confirmed by regressing height velocity SDS against the log of the GH secretion parameters yielding a straight line (r = 0.73 p<0.001).

These data would predict that the growth of third centile children growing at a normal velocity could be augmented by increasing circulating GH concentration. We have tested this hypothesis by administering synthetic GH (Somatomorm:Kabi), 2 units s.c. nocte 6 nights out of 7, to 17 short children. Pretreatment height velocity SDS of -0.49 (SD 0.39) rose to ±2.86 (0.89) over 6 months of therapy.

We conclude that height velocity is controlled by GH pulse amplitude. We can find no discontinuity between GH deficiency and normality indicating that GH secretion in childhood is a continuum.