

470 STEROID 21-HYDROXYLASE GENE ANALYSIS IN CONGENITAL ADRENAL HYPERPLASIA. John A. Phillips III*, Karla J. Matteson†, Pamela J. Orlando*, and Walter L. Miller. *Vanderbilt Univ., Nashville, TN and Univ. of California, San Francisco.

Congenital adrenal hyperplasia (CAH) is usually due to an autosomal recessive defect in 21-hydroxylation of steroid hormones. The human gene for 21-hydroxylation, P450c21B, is closely linked to its inactive homologue, P450c21A. We have cloned a human P450c21 cDNA and used it to determine the restriction patterns of 51 members of 10 different families, each with 2 or more CAH cases. Following digestion of genomic DNA with EcoRI or TagI, patterns reported by others to indicate P450c21B gene deletions (absent 12- or 3.7-kb fragments) were seen for 6/20 CAH and 6/20 normal P450c21B alleles. Following digestion with BglII, EcoRI, KpnI, TagI, or XbaI normal P450c21B gene patterns were seen in all CAH samples with at least 2 enzymes. Linkage analysis indicates the P450c21 alleles co-segregate with CAH (no recombinants seen in 15 informative meioses). We conclude 1) P450c21B gene "deletions" detected by others using single enzyme digestions may, in some cases, represent gene conversions, polymorphisms or unequal crossover products rather than simple deletions and 2) while prenatal diagnosis of CAH is feasible by linkage analysis in 8/10 families studied to avoid errors the patterns of all family members should be studied before inferring the CAH status of the fetus from restriction patterns.

471 LACK OF METABOLIC EFFECT OF GROWTH HORMONE (GH) 36 HOURS AFTER INJECTION Martin Press, Sonia Caprio, Morey Haymond, William Tamborlane. Depts of Peds, Yale Univ, New Haven CT & Mayo Clinic, Rochester MI.

Since GH is only given three times a week we studied whether metabolic effects were still apparent 36h after a shot. Six GH deficient boys aged 13-17 received a stepwise euglycemic insulin clamp (8, then 40 $\mu\text{M}/\text{m}^2/\text{min}$ for 90 mins each) combined with ^2H -glucose infusion to independently measure hepatic and peripheral insulin sensitivity. Children were studied before and after 7-19 weeks of GH treatment (0.05 mg/kg IM qod) which increased growth rate from 4.6±0.9 to 8.9±1.4 cm/yr ($p<0.02$). At the time of the second study, 36h after the last shot, GH levels had returned to baseline (2.0±0.5 vs 1.7±0.3 ng/ml) while somatomedin C was still markedly elevated (1.29±0.25 vs 0.29±0.06 U/ml, $p<0.01$). There was no difference in fasting plasma glucose (79.7±3.4 vs 75.9±1.8 mg/dl) or insulin (7.4±2.0 vs 6.0±1.4 $\mu\text{U}/\text{l}$), although basal glucose turnover was slightly higher (2.48±0.28 vs 2.11±0.27 mg/kg.min, $p<0.05$). However, hepatic glucose production suppressed to the same extent during the low dose clamp (71 vs 85%), and insulin stimulated glucose uptake during the high dose clamp actually tended to be higher after GH treatment (9.4±1.9 vs 7.9±0.9 mg/kg.min). In contrast, in three children studied 12h after a GH shot, insulin sensitivity was decreased. Thus, 36h after GH, no adverse effects on carbohydrate metabolism were detectable, and some children were actually more insulin sensitive, possibly as a result of insulin-like effects of somatomedins.

472 HYPOTHALAMIC HYPOSOMATOTROPINISM IN CHILDREN WITH SHORT STATURE. M. Pugliese, F. Lifshitz, P. Fort, C. Cervantes, Dept. of Peds., Cornell Univ. Med. Coll. and Dept. of Pediatrics, North Shore University Hospital, Manhasset, NY.

Hypsomatotropinism can be due to a pituitary defect of growth hormone (GH) secretion, a hypothalamic defect of GH-releasing hormone (GHRH) secretion or to a Neurosecretory Defect (NSGHD). Twenty-nine patients, aged 5-17 years, with height below the 5th percentile, delayed bone age and no organic disease were studied. GH status was evaluated by [1] a combined hormonal stimulation test (CHST) of sequentially administered insulin, GnRH, TRH, and L-Dopa, [2] 8-hour overnight GH test (OGH) and [3] IV (1-44)hp GHRH-NH₂ infusion. Fourteen patients with growth rate (GR) of 3.7±1.1 cm/yr had a normal GH response on CHST (12.9 ± 4.5 ng/ml) and GHRH (24.6±12.6) but subnormal OGH (2.6±0.7) compatible with NSGHD. Five patients with GR of 2.8±0.6 cm/yr had a subnormal GH response on CHST (2.6±1.4) and OGH (1.6±0.3) but a normal GH response to GHRH (11.3±5.7) compatible with GHRH deficiency. Two patients with a GR of 3.0 cm/yr had subnormal responses on CHST (2.3), OGH (1.3) and GHRH (1.6) compatible with pituitary GH deficiency. Eight patients with GR of 4.9±1.3 cm/yr had normal responses on all 3 tests. GH deficiency is mainly hypothalamic in origin. The most common form is the NSGHD with a clinical spectrum from a virtually normal growth rate down to severe growth stunting. GHRH deficiency is a less common, but is more common than a pituitary GH deficiency. This study demonstrates that combined physiologic and pharmacologic tests of GH reserve can determine the origin of the disorder.

473

QUANTITATION OF URINARY SOMATOMEDIN C (SmC/IGF1)

Teresa Quattrin, Christine Albini and Margaret H. MacGillivray SUNYAB, Buffalo Children's Hospital, Dept. of Pediatrics, Buffalo Plasma SmC/IGF1 concentrations do not correlate well with growth velocity of healthy infants and children, nor with linear growth rates of GH deficient patients during GH therapy. The aim of this study was to obtain quantitative information about the excretion of SmC/IGF1 in timed urine collections obtained from children with normal and abnormal growth. 84 children (ages 2-17 years) collected 12 hour overnight urine specimens. Group I included healthy controls. Group II comprised children with height >2 S.D., growth < 5 cms/year, and peak GH > 8 ng/ml to stimuli. Group III consisted of GH deficient children. 50 ml aliquots of urine were dialyzed and concentrated 50 fold by lyophilization using a modification of the method of Hansen. SmC/IGF1 was measured by a radioimmunoassay (Nichols Institute). The quantity of SmC/IGF1 excreted was standardized for weight (mU/kg).

Results: Urine SmC/IGF1 mU/kg (mean ± se)

	Group I	n	Group II	n	Group III	n
All	27.5 ± 2.2	31	8.6 ± 1.6	29	8.4 ± 1.2	24
Prepubertal	27.3 ± 2.8	17	7.3 ± 1.9	13	6.0 ± 1.7	11
Pubertal	27.8 ± 3.7	14	9.6 ± 2.5	16	10.5 ± 1.4	13

Healthy children (Group I) excrete significantly greater amounts of SmC/IGF1 than children with pathologic growth (groups II and III, $p < .01$). Pubertal and prepubertal children had similar quantities of SmC/IGF1.

Urine SmC/IGF1 in Hypopituitary Patients Pre and Post GH Injection

Baseline	n	0-12 h Post Injection	n	13-24 h Post Injection	n
10.9 ± 1.9	13	24.2 ± 5.6	13	24.9 ± 10.5	6

Conclusion: Pathologically short children who are GH deficient or GH sufficient by standard provocative tests, excrete less urine SmC/IGF1 than control subjects. Measurements of urine SmC/IGF1 may increase our understanding about the interplay between this growth factor and GH, sex steroids and nutrition.

474 BIOSYNTHETIC METHIONYL-HUMAN GROWTH HORMONE (BMHGH) TREATMENT AFFECTS IMMUNE FUNCTIONS (IF) IN GROWTH HORMONE-DEFICIENT CHILDREN. Robert Rapaport, Bruce Petersen, Kathryn A. Skuza, Melinda Heim, Steven Goldstein (Spon. by F. Behrle). UMD-New Jersey Medical School, Children's Hospital of New Jersey, Department of Pediatrics, Newark, NJ; Eli Lilly & Co., Indianapolis, IN

We have previously reported transient depression of % B cells, Phytohemagglutinin (PHA) responsiveness and T Helper/Suppressor cell (T H/S) ratios in GH-deficient children during treatment (Tx) with pituitary derived human growth hormone (PHGH) (J Pediatr 109:434, 1986). In vitro, incubation of peripheral blood lymphocytes (PBL) with GH resulted in decreased % B cells. The PBL of children undergoing GH Tx had increased baseline and GH stimulated proliferation.

In the current study, we evaluated IF during Tx with BMHGH (kindly provided by Eli Lilly & Co.) in 7 children, ages 4-15, 2 female. Four pts. had been part of the previously cited report. Cell surface markers (SM) and interleukin-2 receptors (IL-2r) were measured by flow cytometry. All pts. had normal baseline IF. Data obtained during 6 months of Tx were analyzed.

% B cells decreased significantly at 1 month of Tx (mean change vs baseline -3.9 ± 3.2, $p<0.05$ by two-tailed paired T test) and remained below baseline in most pts. at 3 and 6 months, confirming our in vitro and in vivo findings with PHGH. % T total cells increased (mean change +3.5 ± 3.4, $p<0.05$) at 1 month. There were no other statistically significant changes in SM. Mitogen responses to PHA and concanavalin-A (Con-A) increased in 5/7 and 6/7 pts. at 1 day-1 month of Tx, in agreement with our in vitro data. At 3 months of Tx PHA and Con-A responses decreased in 2/7 and 5/7 children respectively. IL-2r expression was diminished at 1 day-1 month in 6/7 pts. Most of these changes were transient and none was clinically evident.

We provide first evidence that BMHGH treatment does affect IF in children. The effects of BMHGH on IF appear to be similar to those of PHGH. These data further support our concept of the presence of a GH-immune network that needs to be considered when treating children with human growth hormone.

475 VASOPRESSIN mRNA IN THE SUPRAOPTIC AND SUPRACHIASMATIC NUCLEI: APPEARANCE AND CIRCADIAN REGULATION DURING DEVELOPMENT. Steven M. Reppert & George R. Uhl, Children's and Neurology Services and Howard Hughes Medical Institute, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114

We have studied and quantified the developmental appearance and regulation of hypothalamic vasopressin (preproressophysin) mRNA using *in situ* hybridization. Timed pregnant rats were housed under controlled environmental lighting conditions. At various ages, fetuses or pups were decapitated, and the brains immersion-fixed. Coronal (10 μm) sections through the central supraoptic nuclei (SON) and suprachiasmatic nuclei (SCN) were subjected to *in situ* hybridization using a 40-base ³⁵S-labeled oligonucleotide probe directed against vasopressin mRNA. Vasopressin mRNA levels in the SON were reliably detected on day 16 of gestation (day 0 = sperm positive), while mRNA levels in the SCN were detectable on day 21. These developmental patterns correlate well with the immunohistochemical appearance of preproressophysin translation products previously reported in these nuclei. A prominent day-night rhythm of vasopressin mRNA levels was first evident in the SCN on day 21 of gestation; no such rhythm was present in the SON at any developmental stage examined. Thus, vasopressin mRNA levels in the SCN are already under specific circadian control when the nuclei are morphologically immature and virtually devoid of neuropil. The circadian regulation of vasopressin mRNA levels in SCN during fetal life represents one of the earliest and clearest examples of regulated gene expression in the mammalian brain. Supported by PHS grant HD14427 and March of Dimes Grant #1-945.