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IDENTIFICATION AND QUANTITATION OF INSULIN IN THE FETAL RABBIT BRAIN. Ruben Schechter, Lynn Karycki, Arnold Kahn and Sherin Devaskar. St. Louis University and the Pediatric Research Institute, Cardinal Glennon Children's Hospital. Dept. of Pediatrics, St. Louis, MO

It has been known for sometime that adult/neonatal rat and fetal rabbit brain contain an insulin-like substance whose precise identity, however, has remained in doubt. In an effort to clarify this issue, fetal rabbit brains (22-25-27d gestation; term~31d) were collected and either sliced for peroxidase-anti-peroxidase (PAP) staining or extracted with an acid ethanol for SDS-PA gel and ELISA determinations. The monoclonal antibody used for PAP and ELISA analyses recognized insulin but not IGF-I.

Brain slices stained by the PAP technique for insulin showed no reactive cells despite using antibody concentrations as high as 1:20 (pancreatic islets were positive at 1:10,000 dilution). On the other hand, iodinated brain proteins separated by SDS-PAGE and located by autoradiography revealed a protein band that co-migrated with authentic porcine insulin (Mr~6000).In addition, ELISA analysis demonstrated the presence of ~100ng of insulin per mg brain extract or 20g wet brain. This level of insulin was constant throughout the stages of fetal life studied. Why the peptide could not be detected by PAP staining remains unknown but might result from the presence of low amounts of antigen, antigen "masking" or antigen denaturation. We conclude that insulin is present in the fetal brain but in relatively minute amounts. The biologic function of the peptide within the brain remains to be studied.

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GROWTH PATTERNS AND ENDOCRINE STATUS IN CHILDREN WITH OPTIC NERVE HYPOPLASIA. Sanging Shu, Geoffrey P. Redmond, George R. Beauchamp, Robert P. Cruse and Gregory G. Louis (Sponsored by Paul G. Dyment) Cleveland Clinic Foundation, Depts. of Endocrinology, Ophthalmology and Neurology, Cleveland, Ohio USA

Optic nerve hypoplasia (ONH) is known to be associated with hypothalamic-pituitary dysfunction including growth hormone deficiency (hGH-D), panhypopituitarism and diabetes insipidus (DI). Previous series have focused on specific subgroups and probably did not include the full range of patients with ONH.

We have examined growth and endocrine data on 66 subjects (38M, 28F) with ONH aged 1 to 33 yrs (mean 9.5 ± 7.3 yrs). Several distinct patterns of growth and endocrine function were found: 1) Normal height arbitrarily defined as rank ≥ 5% ile was present in 68%. All 7 adults were in this group demonstrating that at least some with ONH achieve normal adult height. 2) Growth failure occurred in 21 subjects (32%). Its onset was 2 years in 10/21, between 2 and 3 years in 4/21, and unknown in 7/21. This age of onset is younger than reported by G. Costin, et al. Growth hormone therapy was able to normalize the growth in 2 subjects. Among this subgroup 6/21 were hypothyroid, 8/21 had definite cortisol deficiency and 3/21 had spontaneous hypoglycemia. hGH-D was documented in 21% of all ONH subjects, significant DI was present in 11% of subjects. Two additional abnormal growth patterns were noted. 3) Early or precocious puberty occured in 4/21 growth deficient children, supporting the observation of C.A. Huseman, et al. 4) Progressive obesity and normal growth with hGH-D similar to craniopharyngioma occurred in 2 subjects.

Conclusions: 1) Children with ONH should be followed carefully for emergence of growth and endocrine abnormalities from infancy. 2) A brief period of normal growth associated with puberty or obesity should not mislead the physician into assuming later growth will be normal.

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USEFULNESS OF THYROTROPIN BINDING INHIBITORY IMMUNOGLOBULIN (TBII) MEASUREMENT IN CONGENITAL HYPOTHYROIDISM. Dorothy I. Shulman, Jack A. Strzelecki, Barry B. Bercu, Allen W. Root. University of South Florida College of Medicine, All Children's Hospital, Dept. of Pediatrics, St. Petersburg, FL 33731.

Seven infants with congenital hypothyroidism (TSH 12-603 uIU/mL) and their mothers were screened for TBII. Two siblings with transient congenital hypothyroidism (T4-6.3, 6.9 ug/dL; TSH-397, 211 uIU/mL) were found to have significant elevation of serum TBII index (90,100;n1<10) at birth equal to that measured in the mother's serum. Technetium scan in the first-born child at 14 days revealed no thyroid gland. Repeat scan at 2 years after withdrawing thyroxine therapy was normal. In light of presumed maternal antibody-mediated hypothyroidism in the first child, hypothyroidism was predicted correctly in the second child and thyroxine therapy initiated on day 1 of life. In contrast, mild elevation of TBII index (12,13) was found in serum of two additional infants but not in maternal serum (TBII <1,7) suggesting that elevated TSH concentrations might be crossreacting in the TBII assay in these two infants. Twelve additional children (11-primary hypothyroidism, 1-thyroid hormone resistance) with elevated TSH concentrations (12-1355 uIU/mL) were tested for TBII. Four were positive (TBII 13-21). TBII correlated significantly with TSH concentrations (r=0.73, p <.01). TSH concentrations <100 uIU/mL were not associated with a positive TBII. TBII may prove useful in screening infants with congenital hypothyroidism caused by maternal TSH-blocking antibodies. However, since high concentrations of TSH may crossreact in this assay maternal serum should be simultaneously assessed.

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ATYPICAL 11β-HYDROXYLASE DEFICIENCY: ABSENCE OF RENIN SUPPRESSION AND HYPERTENSION DESPITE HIGH DOC. Phyllis W. Speiser, Robin L. Nemery, Diane Chow, Kumiko O. Martin, Maria I. New, The New York Hospital-Cornell Medical Center, Department of Pediatrics, New York, NY 10021.

Congenital adrenal hyperplasia due to 11β-hydroxylase (11β-OHase) deficiency usually produces hypertension and virilization. The hormonal profile is typified by elevated serum and urinary DOC and compound S, and suppressed renin (PRA) and aldosterone (aldo). We report 2 cases in which extreme elevation of serum and urinary precursors of 11β-OHase produced neither hypertension nor suppression of PRA and aldo. These findings correlated with low number of mineralocorticoid binding sites per cell (MRBS) in peripheral blood leukocytes. DOC suppressed following dexamethasone administration, and additional DOC infusion caused transient hypertension and expected renin suppression. We postulate that down-regulation of MRBS is an adaptive response to high DOC, inhibiting mineralocorticoid effects. Normal basal aldo levels indicate intact glomerulosa 11β-hydroxylation, suggesting a defect in tissue-specific expression of the enzyme, rather than a structural gene defect.

		(ng/dl)				
BASAL	BP	: DOC	S	ALDO	: PRA	MRBS
Pt 1 (7yM)	100/60	: 2045	567	22	: 15	90
Pt 2 (12yF)	92/54	: 120	3150	29	: 11	
Mean Normal	100/65	: 20	80	9	: <8	200

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ACQUISITION OF ALDOSTERONE BIOSYNTHETIC CAPACITY IN CONGENITAL ADRENAL HYPERPLASIA WITH HOMOZYGOUS DELETION OF THE GENE ENCODING P450/c21. Phyllis W. Speiser, Perrin C. White, Maria I. New, The New York Hospital-Cornell Medical Center, Dept. of Pediatrics, New York, N.Y. 10021.

Salt-wasting congenital adrenal hyperplasia due to 21-hydroxylase (21-OHase) deficiency has been associated with deletion of the active structural gene for the adrenal enzyme. We report a patient with apparent homozygous deletion of the active adrenal 21-OHase gene (OH21B), i.e., complete absence of 3.7 kb Taq I fragment on genomic blot hybridization using 21-OHase cDNA as a probe (PNAS,81:7505,1984), in whom aldosterone biosynthesis was absent in infancy and childhood, but was present during adolescence. Three older sibs had died of salt-wasting crisis in infancy. The neonatal period in the proband was complicated by shock, hyponatremia and hyperkalemia, treated successfully with parenteral fluids, hydrocortisone, and mineralocorticoid supplements. While receiving salt-retaining hormones at age 12 years, the patient was unable to mount an adequate response to a low sodium diet: renin was elevated (23-29 ng/ml/hr) but pH_1 aldosterone was only 1 mcg/m^2/day, and serum aldo was undetectable. At age 19 years after months of discontinued mineralocorticoid therapy , low salt testing was repeated: on day 3 sodium balance was achieved when renin was 115, and pH_1 aldo was now 8.6, with serum aldo 10.9 ng/dl. This case suggests that a structural 21-OHase gene defect is necessary, but not sufficient to produce the salt-wasting phenotype. The mechanism by which 21-hydroxylase activity for aldosterone biosynthesis is acquired in this patient with a homozygous deletion of the OH21B gene remains to be elucidated.

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INSULIN-LIKE GROWTH FACTOR-II (IGF-II) RECEPTORS IN RAT NEUROBLASTOMA CELLS. Marlene A. Sturm, Hung Pham, and Ron G. Rosenfeld. Stanford University School of Medicine, Department of Pediatrics, Stanford, CA.

The presence of high concentrations of IGF-II in cerebrospinal fluid and brain, as well as the prominence of receptors in cortical tissues, make the CNS an attractive site for the study of IGF-II binding and action. B-104 is a transformed rat neuroblastoma cell line whose neuronal qualities include neurotransmitter synthesis and the ability to project axodendritic processes. We observed steady-state binding of 125-I-IGF-II to B-104 membranes between 1.5 and 4 hrs at optimal conditions of 25°C, pH 7-8. Specific binding averaged 12.2±4.0% per 100 ug/ml membrane protein, compared with 125-I-IGF-I binding of 10.1±2.9%. 125-I-IGF-II binding was minimally inhibited (<20%) by insulin concentrations as high as 100 ug/ml. In the presence of unlabeled IGF-II (0.5-5ng/ml), 125-I-IGF-II binding was increased by as much as 50% over baseline; at higher concentrations, 125-I-IGF-II binding was inhibited, with 50% displacement at 50 ng/ml. We observed a similar increase in 125-I-IGF-II binding in the presence of unlabeled IGF-I at concentrations ranging from 1-400 ng/ml. When 125-I-IGF-II was cross-linked to membranes, solubilized, and immunoprecipitated with a specific antibody for the rat IGF-II receptor, a classical type II receptor (Mr~240K) was visualized upon electrophoresis. These results demonstrate the presence in B-104 cells of a specific high affinity type II receptor, as well as a membrane-bound binding protein, whose existence in conditioned medium was confirmed by activated charcoal assay. B-104 should serve as an appropriate and intriguing in vitro model for future study of IGF receptors and action in the CNS.