● 122

QUANTITATION OF CORTISOL (F) PRODUCTION AND ELIMINATION RATES IN PREMATURE AND TERM NEONATES: EVIDENCE FOR PULSATILE SECRETION. D. L. Metzger, N. M. Wright, A. D. Rogol, J. D. Veldhuis, J. R. Kerrigan. Departments of Pediatrics, Pharmacology and Internal Medicine, University of Virginia, Charlottesville, Virginia 22908, USA.

Pulsatile secretion of cortisol has not been documented in the newborn infant. Using repeated blood sampling and deconvolution analysis, we investigated F secretory and elimination dynamics in a clinically stable group of 5 premature [gestational age (GA) 24-34 wk] and 5 term neonates. Blood samples were obtained through umbilical arterial cannulae at 15-min intervals for a 6-h period. All plasma F determinations were >55 nmol/L (2.0 μg/dL), and pulsatile F secretion was observed in all infants. On average, 4 ± 1 discrete F secretory episodes were detected during the study. No significant differences were noted between the 2 groups of subjects with regard to 6-h mean plasma F, plasma corticosteroid-binding globulin, F secretory burst frequency, mass of F secreted per burst, F production rate (FPR) or plasma F half-life. However, the premature infants had a significantly longer F secretory burst half-duration ($p = 0.007$) and a lower maximal F secretory rate ($p = 0.018$) than the term infants. The 2 most premature infants had significantly greater mean plasma F and FPR than the other 3 premature and all the term infants.

Extrapolating to 24 h and correcting for distribution volume of F and for body surface area, we estimate FPR to be approximately 17-24 nmol/m²/24 h (6.6-8.8 mg/m²/24 h) for neonates of ≥34-wk GA. These values are consistent with estimates of FPR in older children and adults determined using either deconvolution analysis or stable isotope-dilution methods.

* 123

MOLECULAR-PHENOTYPIC CORRELATIONS IN THE ANDROGEN INSENSITIVITY SYNDROME. CA QUIGLEY, A DEBELLIS, MK EL-AWADY, EM WILSON, FS FRENCH. Laboratories for Reproductive Biology, University of North Carolina at Chapel Hill, NC 27599.

The androgen insensitivity syndrome (AIS) is a classic example of a hormone resistance disorder. In the presence of a 46,XY karyotype and normal or increased androgen levels, affected individuals have a female or ambiguous external genital phenotype, resulting from abnormalities of the intracellular androgen receptor (AR), required to mediate the action of androgens. Using polymerase chain reaction, denaturing gradient gel electrophoresis and dideoxy sequencing, we have identified single nucleotide mutations in the AR gene causing amino acid (AA) substitutions in the AR in 16 AIS subjects (9 complete [CAIS], 7 partial [PAIS]). To determine whether a correlation exists between the specific molecular defect and its clinical expression, we have analyzed these mutations and 70 reported by others, with respect to the location, nature and functional effects of the AA change. The majority (89%) of AA alterations occurred in the steroid-binding domain (SBD), 70% in the central region, encoded by exons E, F and G of the AR gene. Mutations in exons D and E predominantly cause CAIS (CAIS:PAIS = 4.4:1). In contrast, mutations in exons F, G and H, encoding the C-terminal region of the SBD, cause CAIS or PAIS with fairly equal frequency (CAIS:PAIS = 1.2:1). This suggests that the region encoded by exons D and E, plays a particularly critical role in AR function. Highly charged residues Arg (basic) and Asp (acidic) are the most frequently altered (57% of CAIS; 44% of PAIS). The phenotype resulting from the AA change appears to correlate with the severity of the change: conversion of Arg to a markedly different AA, such as Cys, produces the CAIS phenotype, while a conservative change, Arg to His (also a basic AA), is associated with evidence of androgen action in vivo and retention of transactivation capacity by the AR, when analyzed *in vitro*. A number of sites in the AR gene appear to be mutational "hot spots" (Arg774, Arg855, Val866): although CAIS or PAIS may occur with different mutations at the same site, no single mutation has yet been found that produces CAIS in one kindred and PAIS in another. Analysis of recreated mutant receptors indicates that retention of androgen-dependent transactivation activity *in vitro* correlates in general with retention of AR function *in vivo*. These data may enable prediction of likely response to androgen in subjects with mutations causing PAIS.

● 124

GERM CELL LOCALIZATION OF TESTICULAR GROWTH HORMONE RELEASING HORMONE (GHRH). P Breyer, C. Srivastava, M. Peredo, J. Rothrock, M. Collard*, O.H. Pescovitz, Department of Pediatrics, Indiana University, Indianapolis, IN 46202 and *Utah State U., Logan, Utah 84322.

Hypothalamic releasing hormones have been found outside of the CNS, however, their extrahypothalamic function remains largely unknown. We have identified a testicular GHRH-like peptide and mRNA in human and rat testis. Human testis, like hypothalamus and placenta, contains an abundant 750bp GHRH mRNA. Rat testis has been shown to produce a larger mRNA of 1750bp. This study was designed to localize GHRH production in testis. Rat testes were fractionated following collagenase and trypsin digestion. Germ cells were further separated by sedimentation, using a staput device. Total RNA was extracted from each cell population by the guanidinium thiocyanate method. Northern analysis, using a ^{32}P-labeled riboprobe complementary to a GHRH cDNA (gift of R. Evans), revealed an abundant 1750bp transcript in spermatocytes, round spermatids and, to a lesser extent, in Sertoli cells. No transcript was detected in RNA from elongating spermatids, Leydig cells, or epididymis. Further localization by *in situ* hybridization using this probe, revealed prominent labeling of early spermatogenic cells. Because human germ cell cancers are the result of dysregulated growth of undifferentiated spermatogenic cells, we probed the Tera-2 and NT_2D_1 human germ cell cancer lines with the GHRH riboprobe. Like hypothalamus and human testis, a 750bp transcript was clearly detectable in both cell lines. To determine if GHRH might play a role as an autocrine mitogenic factor in these cancers, we evaluated thymidine incorporation into Tera-2 cells following treatment with GHRH (100nM). There was a 52% increase in thymidine incorporation suggesting that GHRH can induce DNA synthesis in these cells. We conclude that GHRH is produced in normal and cancerous germ cells and speculate that it may function as a local testicular growth factor.

125

A MUTATION IN THE CYP11B2 GENE IN A PATIENT WITH CORTICOSTERONE METHYLOXIDASE II (CMO II) DEFICIENCY. L. E. Cohen, J. A. Majzoub, and L. Muglia, Division of Endocrinology, Children's Hospital, Boston, MA 02115, USA

CMO II deficiency is a defect in the final step in aldosterone biosynthesis. The CYP11B2 gene encodes a cytochrome P450 enzyme which catalyzes this 18-oxidation reaction, as well as the preceding 11-hydroxylation and 18-hydroxylation steps in the zona glomerulosa of the adrenal gland. A newborn female, born to consanguineous parents, presented with severe salt-wasting. Laboratory evaluation revealed an increased level of 18-OH-Corticosterone consistent with the diagnosis of CMO II deficiency. Segments of the patient's CYP11B2 gene, as well as that of her parents and a normal control, were amplified by PCR using specific oligonucleotide primers in areas that differed from the 93% homologous CYP11B1 gene. The PCR products were analyzed by Southern blot and revealed a large mutation of the patient's CYP11B2 gene 5' of exon 6. Downstream of exon 6, there are no other gross mutations. This mutation differs from those previously described in the literature. Further evaluation of this defect may reveal valuable insight into the mechanism of aldosterone biosynthesis.

● 126

ACTIVATING MUTATION IN THE STIMULATORY G PROTEIN GENE IN AN INFANT WITH ADRENOCORTICONODULAR DYSPLASIA

B. Boston, M. Bliziotes*, S. Mandel, and S. LaFranchi Department of Pediatrics, Oregon Health Sciences University and *Portland VAMC, Portland, OR.

Infantile Cushing's Syndrome is a rare condition caused by excess cortisol production. We describe an activating mutation in the gene coding for the stimulatory G protein ($G_s$alpha) in DNA from adrenal tissue in an infant with ACTH independent Cushing's Syndrome. Activating mutations in exons 8 and 9 of the $G_s$alpha gene have been associated with growth hormone secreting pituitary tumors and McCune-Albright syndrome. Specifically, point mutations coding for an arginine to cysteine or an arginine to histidine substitution at site 201 in exon 8 have been described.

The patient in this study was Cushingoid in appearance with poor linear growth by 3 months of age. Evaluation revealed elevated serum cortisol despite low and high dose dexamethasone suppression and undetectable baseline ACTH levels (<3 pg/ml). He underwent bilateral adrenalectomy with subsequent steroid replacement. Adrenal histology revealed bilateral adrenocortical dysplasia with nodular elements.

Genomic DNA was extracted from adrenal tissue and amplified by PCR with primers for $G_s$alpha exon 8. Using allele specific oligonucleotide hybridization, the amplified DNA was probed for the presence of point mutations which correspond to the known mutations in exon 8. An oligonucleotide probe corresponding to the cysteine substitution at site 201 hybridized with amplified DNA from the patient's adrenal tissue. A "wild type" probe coding for arginine bound to both leukocyte DNA from a normal human control and patient DNA while the probe for the arginine to histidine mutation bound to neither patient nor control DNA.

We conclude that the arginine to cysteine activating mutation in exon 8 of $G_s$alpha is present in the adrenal tissue of this infant with Cushing's Syndrome. This mutation may be involved in the pathogenesis of adrenocorticonodular dysplasia in some patient's with infantile Cushing's Syndrome.

127

SALIVARY 17α-HYDROXYPROGESTERONE (17α-OHP) AS AN INDEX OF ADEQUACY OF TREATMENT IN CHILDREN WITH CONGENITAL ADRENAL HYPERPLASIA. O.Fofanova,M.D., E.Kuznetsova,M.D. Pediatric Department, All Russian Research Centre for Endocrinology, Russian Academy of Medical Sciences, Dm.Uljanova str, 11, Moscow, 117036, Russia.

The study was carried out to evaluate the usefulness of circadian rhythms of 17α-OHP concentration in the saliva as a criterion of the efficacy of therapy in children with congenital adrenal hyperplasia (CAH). The circadian patterns of saliva 17α-OHP in prepubertal children with CAH due to 21-Hydroxylase deficiency were studied before and after 6 months of prednisolon treatment. Saliva samples were taken every 4 hours over 24-hour period. The pretreatment measurement revealed a pronounced diurnal rhythm of saliva 17α-OHP, with the persistently elevated levels throughout the day and night, reaching a maximum of 6,89±0,61 ng/ml at 8.00 and a mimimum of 1,31± 0,53 ng/ml at 20.00 ($p<0,0001$). All patients on therapy showed a marked supression of 17α-OHP levels in saliva throughout the all time points and the maintenance of the diurnal rhythm of the steroid with the maximal levels at 8.00(1,43±0,37 ng/ml) and minimal at 20-24.00(0,43±0,17 ng/ml, $p<0,001$). In conclusion, the use of salivary 17α-OHP assays, and especially it's circadian patterns in monitoring of therapy in children with CAH is valuable and perspective.