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SPATIO-TEMPORAL PATTERNS OF HORMONE AND HORMONE TRANSCRIPTION FACTOR GENE EXPRESSION DURING THE DEVELOPMENT AND MATURE FUNCTION OF THE NEUROENDOCRINE SYSTEM. Larry W. Swanson, Dept. of Biological Sciences, Hedco Neuroscience Bldg., mc 2520, Univ. of Southern Calif., Los Angeles, CA 90089-2520, USA

The core of the neuroendocrine system consists of the hypothalamus and pituitary, and the mechanisms that interrelate these two organs. We have used this system as a model for studying the spatio-temporal patterns of gene expression that may be involved in establishing a functional system during the development of the rat embryo, as well as in the modulation of gene expression during different functional states of the adult organism. In the embryo, we have examined the spatio-temporal pattern of expression for the various genes that are involved in the synthesis of the classical anterior pituitary hormones, and it was found that there is an interesting compartmental pattern of expression which is also characterized by distinct temporal patterns of expression. In addition, we have correlated these patterns with the pattern of expression of two putative transcription factors that are thought to be involved in regulating the expression of growth hormone, thyrotropin releasing hormone, and prolactin. In the adult rat, it is now becoming clear that individual hypothalamic neurosecretory neurons can not only synthesize multiple neuropeptides, but that the genes regulating the synthesis of these neuropeptides may be differentially regulated by different physiological and behavioral factors that fall under the broad category of stress.

Abnormalities of Steroidogenesis and Metabolism

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MOLECULAR BASIS OF CONGENITAL ADRENAL HYPERPLASIA DUE TO 3β-HYDROXYSTEROID DEHYDROGENASE DEFICIENCY J. Simard[1], Y. Morel[2], E. Rhéaume[1], R. Sanchez[1], F. Mebarki[2], N. Laflamme[1], M.I. New[3] & F. Labrie[1].1 MRC Group in Molecular Endocrinology, CHUL Research Center & Laval Univ., Québec, G1V 4G2, Canada. 2 INSERM U329 & Dept. of Pediatrics, Univ. de Lyon, France, 3 Dept. of Pediatrics, Div. of Pediatric Endocrinology, New York Hospital-Cornell Medical Center, New York,USA.

Classical 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase (3β-HSD) deficiency is an autosomal recessive form of congenital adrenal hyperplasia (CAH). In contrast to CAH due to 21-hydroxylase and 11β-hydroxylase deficiencies, which impair steroid formation in the adrenal cortex exclusively, classical 3β-HSD deficiency affects steroid biosynthesis in the gonads as well as in the adrenals. Classical 3β-HSD deficiency is thus characterized by varying degrees of salt-losing in newborns of both sexes, associated with pseudohermaphroditism in males, while females exhibit normal sexual differentiation or mild virilization. To elucidate the molecular basis of classical 3β-HSD deficiency exhibiting various levels of severity of symptomatology, we determined the nucleotide sequence of each of the two highly homologous 3β-HSD genes in 13 classic 3β-HSD deficient patients from 10 unrelated families previously described by us and/or by Drs M.G. Forest, U. Heinrich, T. Moshang, S. Pang, A.P. Van Seters, S.C. Wallis and M. Zachmann. The 12 point mutations characterized were all detected in the type II 3β-HSD gene, which is the gene predominantly expressed in the adrenals and gonads. No mutation was detected in the type I 3β-HSD gene mainly expressed in the placenta and peripheral tissues, thus providing the basis for the well recognized intact peripheral intracrine steroidogenesis in these patients. Our findings also provide a molecular explanation for the enzymatic heterogeneity ranging from the severe salt-losing form to clinically inapparent salt-wasting form of classical 3β-HSD deficiency.

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11ß-HYDROXYLASE AND HYPERTENSION. P.C. White, Cornell University Medical College, New York, NY 10021, USA

In humans, the *CYP11B1* and *CYP11B2* genes on chromosome 8q22 encode steroid 11ß-hydroxylase isozymes that are 93% identical in amino acid sequence. *CYP11B1* is expressed at high levels in the adrenal cortex, is up-regulated by ACTH and encodes an enzyme with 11ß-hydroxylase activity. *CYP11B2* is expressed at low levels in the normal adrenal cortex but at higher levels in aldosterone secreting tumors, is up-regulated by angiotensin II and encodes an enzyme with 11ß- and 18-hydroxylase as well 18-oxidase activities. Thus, *CYP11B1* is required for cortisol synthesis whereas *CYP11B2* is required for aldosterone synthesis. **Steroid 11ß-hydroxylase deficiency** (failure to convert 11-deoxycortisol to cortisol) causes a hypertensive form of congenital adrenal hyperplasia. This autosomal recessive form of genetic hypertension presumably results from accumulation of deoxycorticosterone and related metabolites with mineralocorticoid activity. We have now characterized a total of nine mutations in CYP11B1 causing this disorder. Eight are point (three nonsense and five missense) mutations and one is a single base pair deletion causing a frameshift (other investigators have reported one additional frameshift mutation). We have used an *in vitro* transfection assay to show that all five missense mutations causing 11ß-hydroxylase deficiency abolish enzymatic activity. In principle, deletions of *CYP11B1* could be generated by unequal crossing over between *CYP11B1* and the adjacent *CYP11B2* gene but no such deletions were found among the deficiency alleles in this study. Seven of the ten known mutations are clustered in exons 6 to 8, a non-random distribution within the gene ($P<0.04$). This may reflect the location of functionally important amino acid residues within the enzyme or an increased tendency to develop mutations within this region of the gene. **Glucocorticoid-suppressible hyperaldosteronism** is an autosomal dominant form of hypertension in which aldosterone synthesis is ACTH regulated and there are high levels of 18-hydroxy- and 18-oxocortisol, the latter a 17α-hydroxylated analog of aldosterone. In all 16 families examined thus far by us and by others, an unequal crossover has occurred generating a chromosome with a third *CYP11B* gene. This third gene is a chimera with a 5' end (including regulatory sequences) corresponding to *CYP11B1* and a 3' end corresponding to *CYP11B2*. The breakpoint of the crossover is always somewhere between introns 2 and 4. *In vitro* expression of the corresponding chimeric cDNAs demonstrate that the chimeric enzyme retains the ability to synthesize aldosterone only if the last five or more (out of nine) exons correspond to *CYP11B2*. Thus, glucocorticoid-suppressible hyperaldosteronism is caused by abnormal expression and regulation of an enzyme with the ability to synthesize aldosterone from deoxycorticosterone. The observed distribution of breakpoints apparently reflects functional constraints on enzymatic activity. It is not yet known if abnormal regulation of *CYP11B2* is responsible for any cases of essential hypertension.

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STEROID 5α-REDUCTASE TYPE 2 DEFICIENCY. David W. Russell, Department of Molecular Genetics, UT Southwestern Medical Center, Dallas, TX 75235, USA

The enzyme 5α-reductase converts testosterone to dihydrotestosterone and is required for male phenotypic sexual differentiation. There are two 5α-reductase genes in man that encode isozymes (designated types 1 and 2) with distinct biochemical and pharmacological properties and tissue distributions. Mutations in the gene encoding the type 2 isozyme cause male pseudohermaphroditism. The characterization of 27 mutations at the molecular and biochemical levels has revealed amino acids in the protein that participate in substrate and cofactor (NADPH) binding, as well as determinants of protein stability. An unexplained feature of this disease has been the occurrence of partial virilization at puberty in affected males. Determination of the developmental expression patterns of the two 5α-reductase isozymes by immunoblotting suggest that the observed virilization may be caused by expression of the type 1 isozyme in the liver and skin. This result suggests that dihydrotestosterone may act in a true endocrine fashion in addition to the autocrine and paracrine mechanisms typically ascribed to this androgen.

Transmembrane Signalling Diseases

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THE MCCUNE ALBRIGHT SYNDROME: A GENETICALLY DETERMINED SIGNAL TRANSDUCTION DISORDER. A. M. Spiegel, A. Shenker, and L. S. Weinstein, NIDDK/NIH, Bethesda, MD 20892, USA

The McCune Albright syndrome (MAS) is a sporadic disorder with pleiotropic manifestations including autonomous endocrine hyperfunction, polyostotic fibrous dysplasia, and *cafe-au-lait* skin pigmentation. Since the endocrine abnormalities in MAS (e.g. gonadotropin-independent precocious puberty, growth hormone hypersecretion, hyperthyroidism, hypercortisolism) are all consistent with constitutive activation of the cAMP 2nd messenger system, we screened tissues from patients with MAS for mutations in the G protein α subunit (Gs-α) that regulates cAMP formation. We found missense mutations that lead to constitutive activation of Gs-α (either Arg 201-> His or Arg 201-> Cys) in affected endocrine tissues from all subjects with MAS studied. The mutations were found in a mosaic distribution consistent with a somatic mutation occurring early in embryogenesis. Gs-α mutations were also found in dysplastic bone samples, and in various nonendocrine tissues such as liver and heart. Mutations in the latter may be responsible for previously

underappreciated manifestations of MAS such as hepatobiliary disease and early death. Identification of constitutively activating mutations of Gs-α as the cause of most if not all of the manifestations of MAS has important implications for our understanding of cAMP regulation of cell function, and ultimately, for treatment of MAS.

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GROWTH HORMONE INSENSITIVITY SYNDROME. U. Francke, Howard Hughes Medical Institute and Departments of Genetics and Pediatrics, Stanford University, Stanford, CA 94305, USA

Growth hormone insensitivity syndrome (GHIS or Laron syndrome) is an autosomal recessive disorder caused by mutations in the gene for the growth hormone receptor (GHR). This gene, located on chromosome region 5p13.1-p12 (1) encodes a 620 amino acid protein with a centrally located hydrophobic transmembrane domain. Proteolytic cleavage of the extracellular domain that includes the growth hormone binding site generates the circulating GHBP. One GH molecule binds to two GHBP molecules resulting in dimerization of the receptors, possibly an essential step for signal transduction.

In order to detect GHR mutations in patients with GHIS, a screening procedure has been devised (2,3). All nine exons and surrounding splice junctions of the GHR gene are amplified by PCR with primers designed from the published sequence. Each PCR product is analysed for altered melting behavior by denaturing gradient gel electrophoresis (DGGE). A GC-clamp is added to one primer to ensure complete analysis of the entire exon and splice site regions. PCR products that are positive by DGGE screening are sequenced either directly or after subcloning. In our laboratory, Dr. Mary Anne Berg has identified two nonsense mutations (R43X and R217X), two splice junction mutations (189-1 G-to-T and 71+1 G-to-A) and two frameshift mutations leading to translational stop codons (46 del TT and 230 del TA) all of which involve either exon 4 or exon 7 (3). The most unusual mutation - that she has detected in over 50 affected individuals from Ecuador - involves a single nucleotide substitution in codon 180 that does not change the amino acid encoded (E180E). The A-to-G transversion, however, creates a new 5' splice site which is exclusively used and leads to an in-frame deletion of 24 nucleotides from exon 6 in the patients' mRNA (2). Since the GH binding and receptor dimerization sites would remain intact but no response to GH has been reported in these patients, the mutant receptor protein is postulated to undergo abnormal folding and intracellular degradation. The E180splice mutation has not been detected in any patients outside of Ecuador.

In general, the mutations are unique to particular families or geographic areas. Only R43X has recurred on different haplotype backgrounds. All mutations we have identified so far are predicted to result in complete absence of a functional receptor protein. Thus, it is not surprising that affected individuals who are homozygotes for particular mutations or compound heterozygotes for two different mutations are clinically indistinguishable. The variable GHBP levels, by indirect immunochemistry, that have been reported among individuals homozygous for the same mutation, remain unexplained. Curiously, all reported mutations involve the extracellular domain. The intracellular domain encoded by one small and one very large exon may present a less favorable target for mutations, or such mutations could cause a different phenotype.

(1) Barton, D.E., Foellmer, B.E., Wood, W.I., Francke, U.: Chromosome mapping of the growth hormone receptor gene in man and mouse. *Cytogenet. Cell Genet.* **50**:137-141 (1989).

(2) Berg, M.A., Guevara-Aguirre, J.G., Rosenbloom, A.L., Rosenfeld, R.G., Francke, U.: Mutation creating a new donor splice site in the growth hormone receptor genes of 37 Ecuadorean patients with Laron syndrome. *Human Mutation* 1:24-34 (1992).

(3) Berg, M.A., Argente, J., Chernausek, S., Gracia, R., Guevara-Aguirre, J., Hopp, M., Pérez-Jurado, L., Rosenbloom, A., Toledo, S.P.A., Francke, U.: Diverse growth hormone receptor gene mutations in Laron syndrome. *Amer. J. Hum. Genet.*, revision submitted (1993).

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MOLECULAR GENETICS OF EARLY-ONSET NON-INSULIN-DEPENDENT (TYPE 2) DIABETES MELLITUS. G.I. Bell, Howard Hughes Medical Institute, University of Chicago, Chicago, IL 60637, USA

NIDDM is one of the most common metabolic diseases affecting ~5% of the world population. However despite much research, the genetic and nongenetic factors that contribute to its development remain largely unknown. Careful clinical studies of subjects with NIDDM whose onset occurred during childhood or adolescence have identified a familial form of diabetes termed maturity-onset diabetes of the young or MODY. Its early age of onset, autosomal dominant mode of inheritance and availability of multigenerational pedigrees make MODY an attractive model for identifying diabetes-susceptibility genes using genetic approaches. Such studies have shown tight linkage between DNA markers on chromosome 20 and MODY in a large Michigan family of German origin as well as with DNA polymorphisms in the glucokinase gene on chromosome 7 in French and British families. Although the diabetes-susceptibility gene on chromosome 20 has not been identified, mutations in the glucokinase gene have been shown to be the cause of MODY in ~60% of French subjects with this form of NIDDM. The demonstration that mutations in the glucokinase gene can cause diabetes suggests that NIDDM may be, at least in part, a disorder of glucose metabolism. This implies that genes encoding other glycolytic and gluconeogenic enzymes, especially those that control rate-limiting steps in these pathways, are candidates for contributing to the development of this genetically heterogeneous disorder. Genetic studies of early-onset NIDDM have provided a better understanding of its causes and have pointed to cellular pathways that are important for the maintenance of glucose homeostasis and whose perturbation may contribute to the development of the more common late-onset forms of NIDDM.

Fetal and Placental Endocrinology

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THE ROLE OF THE INSULIN-LIKE GROWTH FACTORS IN NORMAL AND ABNORMAL FETAL GROWTH. P.D. Gluckman, Research Centre for Developmental Medicine and Biology, University of Auckland, Auckland, New Zealand.

Fetal growth (FG) in late gestation (LG) is primarily determined by uteroplacental transfer of nutrients. FG is normally constrained by the capacity of this transfer. In LG, fetal IGF-1 is acutely regulated by glucose, but not by amino acid availability, acting via enhanced insulin release; this provides the mechanism of fetal overgrowth induced by hyperinsulinism. Fetal IGF-1 levels correlate with fetal nutritional status and thus FG. Direct evidence for the role of IGF-1 in FG is provided by embryo transfer experiments in mice selected for high or low IGF-1 levels. The infusion of IGF-1 (60μg/kg/hr) to LG fetal sheep increased IGF-1 4 fold without a measurable change in fetal or placental glucose uptake. However placental lactate production fell ($p < 0.03$). The amino-nitrogen concentration fell ($p < 0.01$) in both mother and fetus as did fetal urea production ($p < 0.05$). These observations suggest that IGF-1 inhibits fetal protein catabolism, promotes fetal anabolism, enhances placental amino acid transfer and alters placental metabolism favourably. It is suggested that glucose availability affects fetal IGF-1 production via altered insulin secretion and this in turn has anabolic and anticatabolic consequences. IGF binding proteins are also acutely modulated by nutrient availability. Fetal IGF-2 in late gestation is under lesser nutritional regulation compatible with a more constitutive role in the regulation of FG. Maternal IGF-1 administration throughout pregnancy in rodents abolishes the physiological constraints on FG without affecting placental growth. Maternal IGF-1 administration reduces fetal urea production in sheep. It is suggested that maternal IGF-1, under nutritional and hormonal control,determines nutrient availability and transfer across the placenta. Thus optimal FG depends on coordinate increases in both fetal and maternal IGF-1.

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PLACENTAL GROWTH HORMONE AND RELATED PROTEINS. N.E. Cooke, B.K. Jones, A. Misra-Press, M. Urbanek, J.E. Russell, S.A. Liebhaber, Departments of Medicine and Genetics, University of Pennsylvania, Philadelphia, PA 19104, USA.

The human growth hormone (hGH) gene cluster contains the pituitary hGH-N gene as well as 4 placentally expressed genes: hCS-A, hCS-B, hCS-L, and hGH-V (placental GH). The 4 placental genes are coordinately induced during fetal development. There is a developmentally regulated switch in the relative expression of hCS-A and hCS-B. The hGH-V gene produces a maternally circulating hormone that reaches maximal levels during the 2nd and 3rd trimesters, as well as a larger protein, hGH-V2, that remains associated with the cell. hGH-V maintains the full spectrum of GH-like bioactivity but is 7-fold more purely somatogenic than hGH-N. The hCS-L gene has lost its normal exon 2 splice donor and undergoes extensive alternative splicing. Expression of the growth hormone receptor (GHR) has been detected in all 4 layers of the placenta, suggesting a means of signal transduction for the placental GH isoforms. The placental villi selectively express an alternatively spliced form of the receptor lacking exon 3, hGHRd3, while decidua and chorion predominantly express the full-length receptor. Both forms of hGH receptor possess indistinguishable ligand binding activities when expressed in Xenopus oocytes. To study the developmental and tissue-specific regulation of this gene cluster, 150 kb of DNA including and flanking the cluster have been analyzed for DNase I hypersensitive sites (HSS). A set of HSS were identified in syncytiotrophoblastic nuclei and in the nuclei of a GH-secreting pituitary adenoma about 40 kb upstream and >20 kb downstream of the cluster. These sites were absent from a number of cell types that do not express the GH-related genes. Constructs containing the hGH-N gene plus and minus the HSS were used to generate transgenic mice. When linked to the 5'-HSS the hGH-N gene was specifically expressed in the pituitary with serum levels of 2-10 ng/ml hGH-N. The 5'-HSS reformed in the transgene in nuclei from pituitaries of transgenic mice. The possibility that this region, remote from the structural genes, is critical to their activation in transgenic mice suggests a novel mechanism for the control of the members of this cluster.

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PLACENTAL EPIDERMAL GROWTH FACTOR RECEPTORS: FROM PHYSIOLOGY TO PATHOLOGY. D. Evain-Brion, E. Alsat, S. Roulier, C.Bonneton. C.Fondacci Labo. de Physiopathologie du Développement, ENS, 75005 Paris

Alteration of placental development directly interferes with fetal growth. Epidermal growth factor (EGF) plays a major role in placental implantation, growth and differentiation. EGF acts on its placental target cells, ie the trophoblasts, via a specific receptor which belongs to the tyrosine kinase receptor family. Placental abundant EGF receptors (EGFR) localize in the brush border at the fetomaternal interface. EGFR expression is increased *in vivo* and *in vitro* with the differentiation of the syncytiotrophoblast which is the functional endocrine tissue of the placenta. In trophoblast cells in