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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