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STRUCTURE AND FUNCTIONAL ANALYSIS OF THE HUMAN GROWTH HORMONE RECEPTOR. William I. Wood, Department of Molecular Biology, Genentech, Inc. South San Francisco, CA 94080

The human growth hormone (GH) receptor contains an extracellular, hormone-binding domain of about 245 amino acids, a single transmembrane domain, and a cytoplasmic region of 350 residues. Defects in the gene encoding the growth hormone receptor (including deletions, truncations, splicing variants, and single amino acid changes) have been identified in individuals with Laron-type dwarfism demonstrating that the receptor is required for normal growth. The extracellular domain of the receptor functions as a soluble GH binding protein; it is produced either by proteolysis of the membrane-bound receptor or by translation of an alternatively spliced mRNA. Detailed structurally analysis of the ligand-binding interaction based the co-crystallization of human GH and its binding protein shows that one molecule of GH binds to and dimerizes two receptor molecules. Functional expression of the receptor in FDC-P1 cells (a mouse premyeloid cell line) results in cells that require GH stimulation for survival. Functional analysis in this response system with GH antagonists indicates that dimerization of receptor is the first step in the GH signaling pathway. The expression of truncated forms of the GH receptor shows that at least 84 % of the cytoplasmic domain is not required for GH receptor mediated signaling in FDC-P1 cells.

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GROWTH HORMONE (GH) DEFICIENCY IN ADULTHOOD: SHORT AND LONG TERM EFFECTS OF GH SUBSTITUTION. J.O.L.Jørgensen, N.E. Skakkebæk and J.S. Christiansen, Medical Department M (Endocrinology and Diabetes), Aarhus Kommunehospital, Aarhus and Department of Growth and Reproduction, Rigshospitalet, Copenhagen, Denmark

GH secretion continues in adulthood and it is well recognized that GH exerts distinct actions also in adults. The advent of biosynthetic GH has prompted renewed interest in the adult GH-deficient (GHD) patient. Our experience with GH replacement in these patients can be summarized as follows:
Short-term effects: On a 24-h basis GH normalizes resting energy expenditure (EE). This is associated with a lowering of protein EE from a supranormal level. Lipid oxidation is increased from a subnormal state. GH also acts to decrease both oxidative glucose disposal and overall insulin sensitivity. Finally, insulin induced hypoglycemia is offset by GH by an increase in peripheral lipid consumption. The overall effects of GH seems to be glucose and protein sparing at the expense of lipids. Long-term effects: GH therapy normalizes serum IGF-I levels and peripheral thyroid hormone metabolism. Highly significant increments in muscle mass and reductions in fat mass are consistent findings together with increased exercise capacity and muscle strength. GH also restores the decreased extra cellular volume, which may cause transient perturbations in terms of acral swelling and discomfort. Data from other studies indicate improvements in psychological well-being and quality of life. These findings are preserved during more than 3 yrs of treatment. Conclusions: Adult GHD is associated with distinct abnormalites most of which become normalized after GH substitution.

Neurosteroids

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NEUROSTEROID BIOSYNTHESIS: GENES FOR ADRENAL STEROIDOGENIC ENZYMES ARE EXPRESSED REGIONALLY IN THE RAT BRAIN. S. H. Mellon and C. F. Deschepper, Dept. of Ob, Gyn & Repro Sci and The Metabolic Research Unit, Univ. of Calif, San Francisco, CA 94143 USA and Clinical Research Institute of Montreal, Montreal, Quebec, Canada H2W1R7

Neurosteroids are steroids that are made in and act on neural tissue in an autocrine/paracrine fashion. Steroidogenesis in classical endocrine tissues (e.g., adrenal, gonads, placenta) is initiated by conversion of cholesterol (Chol) to pregnenolone (Preg) by the mitochondrial cholesterol side chain cleavage enzyme, P450scc. Rat neonatal forebrain cultures can mimic adrenal steroidogenesis by converting ^{3}H mevalonolactone, a precursor of Chol, to ^{3}H Chol, ^{3}H Preg, and ^{3}H 20-OH Preg. To determine whether P450scc mediates this conversion, we analyzed RNA from rat brains, primary cultures of glial cells, and C6 glioma cells for P450scc mRNA by RNase protection assays, but found none. However P450c11ß (11ß hydroxylase), but not P450c11AS (aldosterone synthase) nor P450c17 (17α hydroxylase) was detected. Only by RT/PCR analysis could we detect P450scc mRNA, but not P450c17 nor P450c11AS mRNAs. P450scc mRNA is most abundant in the cortex, but is also found in the amygdala, hippocampus and midbrain of both male and female rats. P450c11ß mRNA, also, is found mainly in the cortex of male and female rats, but is more abundant in the female than male hippocampus. Purification of our mixed primary glial cultures shows that Type-1 astrocytes synthesize P450scc but not P450c11ß mRNA. Western blotting and immunocytochemistry show that P450scc protein is almost as abundant in our cultures as in Y-1 cells, while P450scc mRNA is orders of magnitude less abundant suggesting that the protein is very stable in the brain. Thus the synthesis of classic steroids (glucocorticoids and progestins) and neurosteroids (allopregnanolone) can be regulated *in situ* in the brain, and do not require extra-neural substrates.

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NEUROACTIVE STEROIDS. Steven M. Paul, M.D., Section on Molecular Pharmacology, Clinical Neuroscience Branch, National Institute of Mental Health, 9000 Rockville Pike, Bethesda, MD 20892, USA

It is now well accepted that certain steroids rapidly alter the excitability of neurons via non-genomic mechanisms, resulting in behavioral effects within minutes following parenteral administration. Almost 50 years ago Selye first identified the sedative/anesthetic properties of pregnane and androstane steroids including the 3α-hydroxy A-ring reduced metabolites of progesterone and deoxycorticosterone, allopregnanolone and allotetrahydroDOC respectively. We have shown that these 3α-hydroxy A-ring reduced metabolites are potent ligands of central and peripheral $GABA_A$ receptors. Using biochemical and electrophysiological techniques we have shown that these steroids selectively augment the inhibitory properties of GABA by binding to sites that are distinct from those for barbiturates and benzodiazepines. We have termed these $GABA_A$ receptor-active steroids -- "neuroactive steroids." The latter include any natural or synthetic steroid that rapidly alters the excitability of neurons. Some neuroactive steroids are also neurosteroids in that they are synthesized de novo within the CNS. Several neuroactive steroids have recently been shown to have excitatory properties, and some like the synthetic amidine steroid R5135 and pregnenolone sulfate antagonize GABA receptors. Pregnenolone sulfate has also recently been shown to augment the actions of glutamate at the NMDA receptor. Thus, steroids can rapidly modulate both inhibitory and excitatory neurotransmitters via direct interaction with their receptors. Examples of both inhibitory and excitatory neuroactive steroids will be presented as will a discussion of their pharmacological, physiological, and clinical significance.

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$GABA_A$ RECEPTORS AND ACTIONS OF NEUROSTEROIDS

GABA acting via $GABA_A$ receptors exerts inhibitory actions on neurons. Certain endogenous steroids (neurosteroids) are modulators of $GABA_A$ receptors. Tetrahydroprogesterone (THP) and tetrahydrodeoxycorticosterone (THDOC) behave as allosteric agonists of the $GABA_A$ receptors, while pregnenolone sulfate (PS) and dehydroepiandrosterone sulfate (DHEAS) act as antagonists. The neurosteroids alter ligand binding, chloride transport and electrophysiological responses to GABA, acting at unique steroid recognition sites at the $GABA_A$ receptors. Certain behavioral effects of the neurosteroids are consistent with their GABA-agonistic or -antagonistic properties. For example, THP and THDOC manifest hypnotic and anxiolytic actions, while PS reduces barbiturate-induced sleep time. Physiologically changing levels of GABAergic steroids, during development, stress, phases of ovarian cycle or pregnancy may alter $GABA_A$ receptor function, thus influencing neuronal excitability and CNS arousal. For example, pregnancy and puerperium are associated with alterations of $GABA_A$ receptor function that seem attributable to steroid actions. GABA-ergic steroids also regulate uterine contractibility. This phenomenon may play a role in quieting the uterus during pregnancy and in its activation during parturition. (Review: Majewska, Prog.Neurobiol.38:379, 1992.)

Bone and Mineral Metabolism

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THE ROLE OF TGF-β FAMILY MEMBERS IN MESENCHYMAL DIFFERENTIATION.

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The TGF-β superfamily is a large family of structurally related dimeric growth and differentiation factors. Depending on the nature of the factor and the cell type, these factors induce changes in cell proliferation, synthesis of extracellular matrix components, cell-matrix interactions and expression of differentiation-specific markers. In addition to these activities, the expression of the TGF-β superfamily members at sites of cellular differentiation during development implicates these proteins as important mediators of cell differentiation and tissue development. We have concentrated on the role of distinct members of the TGF-β superfamily in the differentiation of mesenchymal cells into muscle, bone and cartilage cells. Our general approach to define the function of these factors in mesenchymal differentiation is to alter the expression levels of either the ligands or the corresponding receptors and to evaluate the consequences for cellular differentiation both in vitro and in vivo.