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 structural 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.

GROWTH HORMONE (GH) DEFICIENCY IN ADULTHOOD: SHORT AND LONG TERM EFFECTS OF GH SUBSTITUTION. J.O.L. Jørgensen, N.E. Skakkebaek 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 abnormalities most of which become normalized after GH substitution.

Neurosteroids

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, P450_{sc}. Rat neonatal forebrain cultures can mimic adrenal steroidogenesis by converting ³H mevalonolactone, a precursor of Chol, to ³H Chol, ³H Preg, and ³H 20-OH Preg. To determine whether P450_{sc} mediates this conversion, we analyzed RNA from rat brains, primary cultures of glial cells, and C6 glioma cells for P450_{sc} mRNA by RNase protection assays, but found none. However P450_{c11b} (11 β hydroxylase), but not P450_{c11a} (aldosterone synthase) nor P450_{c17} (17 α hydroxylase) was detected. Only by RT/PCR analysis could we detect P450_{sc} mRNA, but not P450_{c17} nor P450_{c11a} mRNAs. P450_{sc} mRNA is most abundant in the cortex, but is also found in the amygdala, hippocampus and midbrain of both male and female rats. P450_{c11b} 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 P450_{sc} but not P450_{c11b} mRNA. Western blotting and immunocytochemistry show that P450_{sc} protein is almost as abundant in our cultures as in Y-1 cells, while P450_{sc} 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.

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 3 α -hydroxy A-ring reduced metabolites of progesterone and deoxycorticosterone, allopregnanolone and allotetrahydroDOC respectively. We have shown that these 3 α -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 amide 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 well a discussion of their pharmacological, physiological, and clinical significance.

Maria Dorota Majewska

Medications Development Division, National Institute on Drug Abuse
Rockville, MD, USA

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. Tetrahydropregesterone (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 contractility. 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

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

Rik Derynck, Ellen Filvaroff, Adrian Erlebacher, and Stephen Gitelman
Departments of Growth and Development, and Anatomy, and
Department of Pediatrics (S.G.), University of California at San Francisco,
San Francisco CA 94143.

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*.

In one set of studies, we have evaluated the role of endogenous TGF- β responsiveness in the maintenance of the differentiated phenotype of myoblasts and osteoblasts. We therefore abrogated the responsiveness to TGF- β in established osteoblasts and myoblasts that can differentiate into myotubes in culture. Our results indicate an important role of continuous TGF- β responsiveness in these well-differentiated cell types. In other studies we focussed on the biological activities of vgr-1/BMP-6, a TGF- β family member which is specifically expressed in hypertrophic cartilage, but for which no functional information is as yet available. Transfected CHO cells overexpressing murine vgr-1/BMP-6 were derived and recombinant vgr-1/BMP-6 was purified to serve as a source of the factor thus allowing us to evaluate its role in osteogenesis and chondrogenesis in culture. In addition, transfected CHO cells overexpressing vgr-1/BMP-6 were inoculated into mice and the effect of vgr-1/BMP-6 on the phenotype of the resulting tumors was evaluated by histological analysis. Studies of this type should allow us to define the natural role of the different TGF- β family members in the mesenchymal differentiation pathways.

41

EFFECTS OF 17 β ESTRADIOL ON HUMAN CARTILAGE CELL PROLIFERATION AND DIFFERENTIATION. O. Blanchard, L. Tsagris and M.T. Corvol, INSERM U30, Hôpital Enfants Malades, Paris, 75015, France.

Surgical biopsies from 27 girls and 18 boys aged between 2 and 8 years were used to prepare high density primary cultures of chondrocytes in serum-free culture medium. The children were considered normal in term of skeletal growth. Cell multiplication and collagen type II biosynthesis and secretion by the cultured cells were studied in the absence or in the presence of 10^{-10} M 17 β estradiol or testosterone. Chondrocyte multiplication, performed in the presence of 10ng/ml of bFGF, was never modified by the addition of sex steroid hormones. By contrast, when estradiol or testosterone were added to the cells at confluency, a significant increased of type II collagen peptide was recovered in the treated cells as compared with non treated ones. Cycloheximide (10^{-7} M) did not completely inhibit the increased amount of type II collagen peptide recovered in hormone treated cells. At the mRNA level, as evaluated by northern blot analysis, the steroids induced only a two fold increase of type II collagen transcripts. The stimulating effect of 17 β estradiol was always higher than the one of testosterone. A possible effect of testosterone through its metabolic conversion into estrogens was suggested, since the cultured chondrocytes appeared to contain aromatase activities. No significant increase of chondrocyte collagen content was observed when the steroid hormones were added during the division phase of the culture. Interleukine 1 (IL1) which is known to stimulate the collagenase secretion by chondrocytes, was added at the concentration of 50ng/ml, to confluent chondrocytes cultured from two prepubertal girls. A significant decrease of chondrocyte collagen type II was found and was not observed in the presence of 17 β estradiol added at 10^{-9} M during three days. These data suggest that sex steroid hormones are acting on the differentiation more than the proliferation of chondrocytes. Sex steroid hormones may maintain cartilage cell phenotype by modulating both the synthesis and the degradation of chondrocyte specific proteins.

42

BONE MASS GROWTH DURING NORMAL PUBERTAL DEVELOPMENT. G.E. Theintz, B. Buchs, R. Rizzoli, D. Slosman, H. Clavien, P.C. Sizonenko and J.Ph. Bonjour. Divisions of Biology of Growth & Reproduction, Clinical Pathophysiology and Nuclear Medicine, University Hospital, Geneva, Switzerland.

Bone mineral density (BMD, gr/cm²) and content (BMC, gr) were determined at the levels of the lumbar vertebrae (L2-L4), femoral neck (FN) and midfemoral shaft (FS) using DEX absorptiometry at 1-yr interval in 198 healthy adolescents (98 females, 100 males) aged 9-19 yrs. BMD/ BMC values were related to age, height, weight, pubertal stage and to environmental variables such as calcium intake and the level of physical activity. In females, the gain in BMD/BMC was sustained from 11 to 14 yrs but fell dramatically after 16 yrs and/ or 2 yrs after menarche. In males, the BMD/ BMC gain was elevated from 13 to 17 yrs and then declined markedly, remaining significant between 17 and 20 yrs for L2-L4 BMD/BMC and FS BMD only. Large interindividual variations were observed between height increments and bone mass gain with a relationship evolving according to a loop pattern. Multiple regression analysis showed that weight was the strongest predictor of bone mass at each site. Nutrition and exercise improved the R² of the overall model by only 6% at L2-L4 and 4.1% at FN. Their effect on FS was negligible (+0.7%). During the normal pubertal development of average subjects, the effects of environmental factors appear to be outweighed by the large gain of bone mass due to puberty itself. These effects should therefore be easier to demonstrate in prepubertal children: this point appears of relevance for future intervention programmes aiming at maximizing bone mass gain during growth.

Final Height Symposium

43

FINAL HEIGHT ATTAINMENT IN NORMAL CHILDREN WITH SHORT STATURE TREATED WITH GROWTH HORMONE. International Task Force (ITF) Report, H. Guyda, Division of Endocrinology and Metabolism, Montreal Children's Hospital, Montreal, Canada.

The mandate of the ITF was defined in order to determine the outcome in terms of final height attainment in short normal children of normal birth weight who have been treated continuously with GH for at least three years. After considerable

discussion, the ITF developed a questionnaire for this purpose, which was provided to all investigators who had indicated by preliminary survey that they had data that could be made available for analysis. Several significant issues were recognized that would make data collection and analysis difficult. These included the limited number of children who have attained final height, the influence of puberty onset occurring during GH therapy, the enormously variable protocols that have been employed with both pituitary-extracted and biosynthetic growth hormone, the absence of a suitable control group that would span the genetic heterogeneity of the study populations, and the interruption of therapy in some children as a result of the CJD crisis in 1985. Data analysis will include initial bone age (BA) and mid-parental height to assess genetic potential, one-year pre-treatment growth velocity (GV) to determine height SDS at onset, height attained at two years after initiation of continuous therapy, and final height attained after at least a total of three years of GH therapy (BA fused and/or GV < 2 cm/yr.). Historical controls will be developed from untreated normal short stature children who have reached adult height by analysis of databases in several countries, including Germany, Sweden, and Denmark. A comparative analysis will also be made of those children with the diagnosis of GHD that have been treated with GH and attained final height, but who retest as 'normal' following discontinuation of GH therapy. It is anticipated that this task force will be able to provide at least a preliminary final height outcome statement in regard to short children who have received growth hormone for a significant period of their childhood. It will also recommend that a similar analysis be conducted in the future in regard to psychological and educational outcome variables in these children.

44

HEIGHT DEVELOPMENT IN TURNER SYNDROME (TS): RESULTS OF AN INTERNATIONAL SURVEY CONDUCTED BY ESPE/LWPES. M.B. Ranke for the ESPE/LWPES, Section for Paed. Endocr., Univ. Children's Hospital, D-7400 Tuebingen, Germany.

Recent attempts to improve height in TS through growth hormone therapy have stimulated worldwide interest in the spontaneous growth and final height of these patients. Published data show that women with TS reach a mean height of approx. 20 cm. below the normal female population, even if the karyotypes differed. TS-specific age-related growth curves, however, show distinct variability, particularly during childhood. This is probably due to the bias introduced by the circumstances leading to the diagnosis, e.g. pre-natal diagnosis through karyotyping, early diagnosis through dysmorphology and/or organ abnormalities, diagnosis during childhood following persistent short stature, delayed or absence of puberty, or infertility in adult life. Thus, data on adult height, drawn from patients whose diagnosis was made very late, cannot govern the height prognostics used for patients who are recognized much earlier. Height prognosis in TS, therefore, needs to be further individualized by taking parental height, karyotype, size at birth, degree of dysmorphology, bone age and ethnically-representative growth curves into consideration. Current data supports the view that GH therapy can improve both height in childhood and in adult life in TS.

45

A PREDICTION MODEL FOR THE FIRST YEAR GROWTH RESPONSE TO GROWTH HORMONE IN TURNER SYNDROME (TS) BASED ON KIGS (Kabi Pharmacia International Growth Study) DATA. M.B. Ranke*, O. Guilbaud, A. Price, A. Wallström for the KIGS International Board. *Section for Paed. Endocrinol., Univ. Children's Hospital, D-7400 Tuebingen, Germany.

In order to optimize treatment with GH, a large-scale, complex analysis on the response to GH in terms of auxological data, as well as treatment modalities is essential.¹ We analyzed 175 TS patients <11 yrs. The median values were: CA=7.9 yrs; HT SDS=-2.5 SD; target HT=0.0 SD; Weight for Height index (WHI %)=97; GH dose (IU/kg/wk)=0.8; No. of GH inj/wk=6. Out of 10 potential predictors, only the following were validated: No. of inj/wk, GH dose, CA and WHI. The corresponding regression equation is: HV (cm/yr)=9.02 + [0.36 x No. inj/wk] + [2.01 x GH dose (IU/kg/wk)] - [0.25 x CA (yrs)] - [0.036 x WHI (%)] ; r²=0.37; error SD=1.26 cm/yr). We concluded firstly that the mode of treatment rather than the auxology determines growth in TS, early treatment being possibly advantageous. Secondly, in contrast to GHD, WHI is negatively correlated to the growth response, thus pointing to a different body composition in TS. Thirdly, we found the growth response to GH in TS was less predictable with the variables used. Lastly, other predictors eg. degree of dysmorphology, may improve the prediction model.