

93 INCREASE IN SPECIFIC BINDING (SB) OF ERYTHROCYTE (RBC) RECEPTORS FOR INSULIN-LIKE GROWTH FACTOR 2 (IGF-2) WITH GROWTH HORMONE TREATMENT OF HYPO-PITUITARY CHILDREN. D. Colm Costigan, Constantin Polychronakos, Barry I. Posner, Harvey J. Guyda. Mc Gill University, Montreal Children's Hospital, Dept. of Paediatrics, Montreal.

Specific receptor binding for IGF's is measurable in younger (lighter) RBC's. An aliquot of such cells of similar age (fraction A), can be reproducibly obtained by dextran gradient centrifugation from 5-10 ml of blood. (JCEM 1983 57: 436). We now report IGF-2 SB to these RBC's from normal children and those with growth hormone deficiency (GH def.). Inter- and intra-assay variability of the procedure is 11%. The per cent of total RBC's in fraction A did not differ in the 2 groups of children. Normals (Group 1), were 5 male volunteers (14.7 ± .6 yr, Mean and SEM) and 10 children (3 female) with constitutional short stature (11.4 ± 1.6 yrs) who had normal 6 hr GH profiles and plasma IGF-1 values. 11 GH def. children (Group 2), aged 12.7 ± 1.4 yr, (6 girls), had samples taken after 2 mos without hGH therapy (2a), and again during 10 mos of hGH (2U,3/wk) (2b). RESULTS: Group 1 had IGF-2 SB of 10.2 ± 0.6% (per 3x10⁹ cells). IGF-2 SB was less in Group 2a at 6.6 ± 0.8% (P<0.002). With hGH therapy (2b), IGF-2 SB rose to 10.4 ± 1.0% (P<0.02), a value not different from that in Group 1. Corresponding plasma IGF-2 values showed a positive correlation with IGF-2 SB (R=0.58, P<0.01). 3 patients have had a 2nd 2 month interruption of hGH. They had a similar response; off hGH, 6 ± 2.7%: on hGH, 10.6 ± .96%: Thus GH deficiency is associated with a reversible decrease in IGF-2 SB on the RBC. These data suggest that plasma IGF-2 may upregulate its own receptor.

94 HUMAN PLACENTAL LACTOGEN PROMOTES CELL PROLIFERATION, DNA SYNTHESIS, AND SOMATOMEDIN RELEASE BY ISOLATED HUMAN FETAL CONNECTIVE TISSUES.

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Since placental lactogens have been implicated in the control of fetal metabolism, and in the release of somatomedins (SM) by fetal tissues, we examined the actions of human placental lactogen (HPL) on the growth of isolated human fetal myoblasts, fibroblasts and cartilage explants, and on their release of immunosayable SM-C. Myoblasts were derived from skeletal muscle and fibroblasts from the skin of abortuses delivered between 12 to 17 weeks after prostaglandin induction. Costal cartilage explants were obtained from one hemithorax. After myoblasts and fibroblasts were plated at low density (0.3 x 10³ cells per well) HPL (50-1000 ng ml⁻¹) caused a dose-dependent increase in cell number over a 7 day incubation, becoming statistically significant between 50 and 250 ng/ml with individual experiments. After sub-confluent myoblasts and fibroblasts were growth-restricted by exposure to medium containing 1% fetal calf serum, HPL caused a significant increase in [³H] thymidine uptake during a subsequent exposure at similar concentrations. Human growth hormone (hGH) failed to increase thymidine incorporation by myoblasts or fibroblasts, while neither HPL or hGH affected isotope uptake by cartilage explants. Culture medium conditioned for 44 h by exposure to myoblasts or fibroblasts was assayed for SM-C by RIA after extraction with acid-ethanol. The SM-C present in conditioned medium was significantly elevated after exposure of cells to HPL (250 ng ml⁻¹) but not hGH. The results show that HPL is mitogenic for cultured human fetal myoblasts and fibroblasts and that this may be due partly to an increase in SM-C release by the cells.

95 NEONATAL EVOLUTION OF IGFs AND SERUM THYMIDINE ACTIVITY. Rose-Marie Schimpff, Mauro Bozzola and Jurgen Zapf. INSERM U188, Paris, France; Medizinische Klinik, Zurich, Switzerland.

Serum growth-promoting activity measured as 3H-THY incorporation into lymphocytes (TA) was measured simultaneously with RIA of IGFs I and II in the same samples of cord blood and of capillary blood collected 30 min. and 24 hrs after vaginal delivery in newborns (16 F, 17 M) with birth weight and length in the normal range. Values found in cord blood were lower than in normal adult serum: TA: 0.87 ± 0.06 U/ml, IGF I: 45 ± 3 ng/ml, and IGF II: 228 ± 22 ng/ml. The values observed in the capillary blood at 30 min., more elevated than those in the cord blood, agree with a production of growth factors by the newborn. The 24 hrs evolution was discrepant: decreasing for IGF I (16 ± 1.8 ng/ml), unchanged for IGF II (260 ± 28 ng/ml) and increasing above normal adult level for TA (1.84 ± 0.22 U/ml). Each of these factors could play a role in the fetal growth, as suggested by the positive correlation (p<0.05) observed between their levels and the birth weight. Non-IGF factors involved in TA play probably a major role in the neonatal period since they are higher in newborns than in adults.

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STRUCTURAL VARIANTS OF SOMATOMEDINS IN HUMAN PLASMA

Six somatomedin-like peptides were purified from human plasma and Cohn fraction IV by a six-step procedure including ethanol precipitation, reversed-phase extraction, gel filtration, chromatofocusing and reversed-phase HPLC. The apparent isoelectric points (pI) of the major components were 8.2 (Sm III) and 6.3 (Sm V) as expected for IGF-I/SmC and IGF-II resp. The pI-values of the minor components were 9.2 (Sm I), 8.7 (Sm II), 6.7 (Sm IV), and 6.15 (Sm VI). They were composed of single peptide chains with apparent MW (SDS-PAGE) of 6,800 (Sm I, II, IV) and 6,400 (Sm III, V, VI). All peptides were equipotent in a bioassay (SO incorporation into porcine cartilage) and in a competitive protein binding assay. According to their potencies in various RRA and RIA systems Sm I, II, and III were classified as IGF-I/SmC-like and Sm IV, V, and VI as IGF-II-like. These findings were confirmed by amino acid sequencing of the first 20 N-terminal residues so far. Minor differences, however, were observed. The data provide further evidence for the existence of structural variants of IGF-I/SmC and IGF-II in the human.

97 HUMAN FETAL AND ADULT CHONDROCYTES: CLONAL GROWTH PROMOTION BY INSULIN LIKE GROWTH FACTORS I AND II, INSULIN AND GROWTH HORMONE.

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A direct assay for cellular growth was used. It measures the capacity of isolated chondrocytes to proliferate in a clonal manner starting from a single cell suspension in a semisolid system (0.8% methylcellulose, BM Whissler Medium, 5% heat inactivated serum, 1000 inserted chondrocytes / ml). This assay was used to measure the clonal proliferation of human fetal (20th week of pregnancy, n=10) and adult articular chondrocytes (20 yrs, n= 10) in response to insulin like growth factors I and II (IGF I/II), biosynthetic human insulin (BHI) and human growth hormone (hGh). Both BHI (100 ng/ml) and hGh (100 ng/ml) did not stimulate fetal and adult chondrocytes to proliferate in a clonal manner. IGF I (25 ng/ml) stimulated clonal growth of fetal chondrocytes (60 ± 12 colonies/1000 inserted cells; m ± 1 SD), however IGF II (25 ng/ml) was significantly more effective (116 ± 12 colonies/1000 inserted cells; p < 0.05). In contrast IGF I was more effective in adult chondrocytes (40 ± 5 colonies/1000 inserted cells) than IGF II (24 ± 2 colonies/1000 inserted cells; p < 0.05).

These results support the concept that IGF I is the predominant adult and IGF II is the predominant fetal somatomedin, and insulin and hGh are no direct stimuli for chondrocyte growth at least in vitro.

98 GLYCOGENIC EFFECTS OF SmC, MSA AND EGF IN FETAL RAT HEPATOCYTES. Michael Freemark, A. Joseph D'Ercole and Stuart Handwerker, Duke Univ. Med. Center and Univ of North Carolina, Depts of Pediatrics, Durham and Chapel Hill, North Carolina, USA.

The role of peptide growth factors in the regulation of fetal metabolism is poorly understood. Since somatomedin-C (SmC) and multiplication-stimulating activity (MSA) have insulin-like actions in postnatal tissues, we have compared the effects of these growth factors on glycogen metabolism in cultured fetal rat hepatocytes with those of insulin and epidermal growth factor (EGF). SmC (25-375 ng/ml) stimulated dose-dependent increases in ¹⁴C-glucose incorporation into glycogen (14-73%) and total cellular glycogen content (11-34%) during 4 hours of incubation. MSA and insulin also stimulated glycogenesis but with potencies only 4.5 and 6.7% that of SmC, respectively. The concentrations of SmC, MSA and insulin which caused half-maximal stimulation of ¹⁴C-glucose incorporation were 6.7 nM, 150 nM and 100 nM, respectively. The dose-response curves of SmC, MSA and insulin were parallel and their maximal effects were not additive, suggesting a common mechanism of action. In contrast, EGF (2.5-150 ng/ml) stimulated glycogen synthesis but its dose-response curve was not parallel to that of SmC and its maximal effect was only 40% that of SmC or insulin. In addition, EGF and insulin had additive effects at maximal concentrations. These findings suggest that SmC and EGF have direct anabolic effects on fetal carbohydrate metabolism. The glycogenic actions of insulin and MSA in fetal liver may be mediated through binding to a receptor which also binds SmC. Grants HD07447 and HD06301.