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PRETREATMENT IGF-I CORRELATES WITH GROWTH RESPONSE TO GH IN HYPOPITUITARISM: THE IMPORTANCE OF ACID SEPARATION.

Previous investigations using plasma IGF-I in children with growth hormone deficiency (GHD) as a predictor of the response to growth hormone treatment have given variable and usually negative results. When the growth response to the first year of growth hormone treatment (0.1 mg/kg TIW) was compared to pretreatment plasma IGF-I values measured after acid chromatography (AC) in a group of 72 children with classic GHD, a correlation of -0.67 ($p < 0.001$) was obtained. In a subgroup of 33 of these children, we were able to compare IGF-I RIA after AC to direct plasma assay using a commercial kit to measure IGF-I. With both RIA's, the IGF-I values in these GHD patients were generally below the normal range. The correlations obtained in the 33 patients with GHD between the growth rate in the first year of GH treatment and these two IGF-I RIA's were -0.07 (NS) for the direct RIA, and -0.65 ($p < 0.001$) for AC. These results stress the importance of AC in the measurement of IGF-I, and show that properly performed, IGF-I is the strongest single predictor of response to GH treatment in GHD children.

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SERUM IGF BINDING PROTEINS ELECTROPHORETIC PROFILES IN CHILDREN WITH COELIAC DISEASE

Subjects with impaired nutritional status are known to have high hGH levels and very low IGF I levels. With a view to studying the regulation of the different molecular forms of IGF binding proteins (BPs), Western blotting was used to analyse the sera of 19 4-to 15-year-old children with coeliac disease before and/or during treatment, and to compare their BP profiles with those of hypopituitary (n=7) and obese (n=7) children of the same age. The untreated coeliac disease patients (n=14) were all stunted in height ($\bar{m}$ = -3.6 SD) and weight ($\bar{m}$ = -2.1 SD) and had high hGH ($\bar{m}$ = 28 ng/ml) and low IGF (0.30 U/ml) levels. In 12, the electrophoretic profiles closely resembled those of the hypopituitary patients with significantly decreased 42- and 39K BPs (which are the subunits of the 150 K [IGF-BP] complex) and a strongly enhanced 34 K BP. In the treated subjects (n=7), the clear resumption of growth (+ 8.2cm in 8 months) was accompanied by a slight rise in IGF levels and a gradual change in the BP profiles which became normal in only two out of the three patients on gluten-free diet over one year. In the obese cases, negligible GH levels accompanied normal IGF levels, normal 39- and 42K, and diminished 34K BPs. These findings indicate 1) that the amount of 39- and 42K BP in the serum is more closely linked to IGF than to GH levels and 2) that their slow rise in patients on a gluten-free diet may favour growth resumption by increasing the bioavailability of IGFs.

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SOMATOMEDIN BINDING PROTEIN (SmBP) IN NORMALS AND IN GROWTH HORMONE DEFICIENCY (GHD)

The major SmBP in serum is a 150 kD glycoprotein. Its acid--stable subunit was isolated from human plasma Cohn fraction IV by a 3 steps procedure. With the purified protein a RIA was developed which recognizes the complete 150 kD complex in nativ serum. In normal controls there was a considerable increase from 25.7 nmol/l (median, range 14.7 - 44.7) in newborns to 49.4 nmol/l (28.8 - 84.7) after 8 weeks of age. Thereafter SmBP increased continuously to 110.5 nmol/l (77.9 - 156.9) at puberty and declined to 98.9 nmol/l (73.4 - 132.9) in adults. Correlation with IGF-I and IGF-II (by RIA) was non-linear. The sum of both IGF-I and IGF-II correlated linearly with SmBP ($r = 0.89$) with a regression line intersecting the abscissae at 19.7 nmol/l (about 148 ng/ml) of total IGF-I and -II. In 70 patients with GHD (max. GH <10 ng/ml in 2 standard tests) 68 had subnormal levels of SmBP (mean SDS for age: -5.52). SmBP increased significantly on treatment with hGH. Conclusions: 1. The 150 kD SmBP is the major BP form in postnatal life. 2. Total serum Sm levels (IGF-I + IGF-II) are primarily determined by SmBP concentration. 3. SmBP is strongly GH dependent. 4. SmBP is a more specific parameter for the diagnosis of GHD compared to IGF-I or -II particularly below age 5.

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POTENTIAL AUTOCRINE ROLE OF IGF-I IN THE MAINTENANCE OF DIFFERENTIATED ADRENAL FUNCTIONS.

Cultured bovine adrenal cells (BA) contained IGF type I receptors which have been characterized by cross-linking (125 KD) and affinity experiments (KD = $1.4 \pm 0.3 \times 10^{-9}$ M). IGF-I had small mitogenic effects but potentiated the effect of fibroblast growth factor (FGF). Moreover, IGF-I enhanced in a time- and dose-dependent manner ($ED_{50} \simeq 10^{-9}$ M) the number of angiotensin-II (A-II) receptors (2-3 fold), the cAMP response to ACTH (3 fold) and the steroidogenic response to both hormones (4-6 fold). Moreover, BA cells cultured in serum-free medium secreted, under basal conditions, an IGF-I-like material (5 ± 1.5 ng/10^6cells/48 h, n = 7), the secretion of which was stimulated 8, 4.5 and 3 fold by ACTH, A-II and FGF respectively but not by growth hormone. The effect of FGF and those of ACTH or A-II were additive. Further characterization of the IGF-I-like material was achieved by affinity chromatography (using monoclonal anti-IGF-I antibodies) of conditioned medium from cells which were incubated for 48 h with labeled amino-acids.

The present results demonstrated that 1) BA cells contained IGF type I receptors and this peptide is required for the maintenance of BA differentiated function; 2) BA secreted an IGF-I like peptide, the secretion of which is regulated by the specific trophic hormone of these cells. Thus, IGF-I might play an autocrine role in the maintenance of differentiated adrenal functions and in their regulation by the specific trophic hormones.

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PRODUCTION OF LABELLED IGF I BY ISLETS CELLS IN CULTURE : BIOCHEMICAL CHARACTERIZATION.

It has been shown previously that neonatal and fetal rat islets in culture secrete IGF I as measured by specific radioimmunoassay. We hereby report the partial purification and biochemical characterization of IGF I produced and secreted by fetal rat islets in culture. Fetal islets were incubated in RPMI 1640 with 1% fetal calf serum with or without (3H) leucine and (35S) methionine. The conditioned media were acidified to pH 2.7 and chromatographed on Biogel P 100 in acid to separate IGF I from its binding proteins. The quantity of immunoreactive IGF I present in the media increased with time and plateaud after 48 hours in culture reaching a value of 1 ng IGF I for 300 islets. Part of the total radioactivity (3%) comigrated on Biogel P100 with immunoreactive IGF I. This fraction was further purified by HPLC on a microbondapak C18 column and eluted with a linear gradient of acetonitrile. Material which comigrated with pure IGF I (39% acetonitrile) was then purified by chromatofocalisation on PBE 96 column. Part of the radioactivity (30%) eluted at the same basic pH (8.55) than IGF I produced by other fetal rat cells in culture and was immunoprecipitable by anti-IGFI Ab. In conclusion: labelled material was produced and secreted in vitro by islet cells with identical Mol Weight, hydrophobicity and isoelectric point than native IGF I.

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SERUM BONE GLA-PROTEIN (BGP) AT THE AGE OF TWO MONTHS IS MUCH HIGHER IN BREAST-FED INFANTS THAN IN FORMULA-FED INFANTS.

BGP, a protein produced by osteoblasts which has been shown to be a marker of bone growth, was determined by radioimmunoassay in 27 exclusively breast-fed and 6 formula-fed infants at the age of 2 months (mean 62.6 days, SD 2.4) as part of a prospective study of nutrition and growth in normal infants. Knee-heel length was measured with electronic calipers at the age of 1 and 2 months. In breast-fed infants the mean concentration was 274 ng/ml (SD 95) which is ten fold higher than values in 6-10 year old children previously published by us. In formula-fed infants the mean concentration was significantly lower (77 ng/ml, SD 46, $p<0.001$). There was no significant difference ($p>0.05$) in knee-heel length and knee-heel length velocity between the breast-fed and the formula-fed infants. The mean knee-heel length was 173 mm (SD 8 mm) and 168 mm (SD 11 mm), respectively. The growth velocity was 0.37 mm/day (SD 0.12) and 0.38 mm/day (SD 0.09), respectively. In conclusion BGP is much higher in breast-fed infants, and this cannot be explained by differences in length or length velocity. Thus BGP should be used with caution as a marker of linear growth in infants. Histomorphometrical parameters of bone formation rates are related to BGP levels in adults. It remains to be seen if bone structure in infants is related to mode of feeding.