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VARIABILITY OF THE 5' FLANKING REGION OF IGF II mRNA ACCORDING TO TISSUE AND STAGE OF DEVELOPMENT

A human cDNA library was tested using IGF I cDNA (which exhibits many homologies with IGF II cDNA) and oligonucleotides synthesized according to the human liver IGF II cDNA sequence. The library (2.10^6 initial recombinants) was constructed from placental material using a method described previously (Le Bouc et al. FEBS Lett. 196:108-112, 1986). 250 000 recombinants were screened with the synthetic IGF II probe. Five clones were selected which hybridized also with human IGF I cDNA. The IGF II cDNA coding sequence was identical to those previously described for liver cDNA. The non-coding 5' end was quite different, starting from the splicing zone between the first and the second exon, several nucleotides upstream from the ATG initiation codon. However, these sequence was identical to one of those found in foetal rat liver IGF II cDNA. Moreover one of the five clones contained yet a third sequence not previously described. These results suggest that from one tissue to another (or according to the stage of development), the expression of the IGF II gene varies with the use of different exons, but yields the same protein. The differences seen in the non-coding regions raise questions concerning 1) the regulatory rôle of these non-coding regions during protein biosynthesis and 2) the mechanism of selection of an exon, i.e. of a specific promoter or of specific splicing during the processing of the RNA primary transcript.

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EXPRESSION OF THE INSULIN LIKE GROWTH FACTOR GENES IN HUMAN FETAL AND ADULT TISSUES.

We have investigated the occurrence of Insulin-like Growth Factor I (IGF-I) and IGF-II specific messenger RNAs in human fetal tissues (liver,kidney,lung,heart,adrenal,brain,muscle,jejunum,thymus,spleen,pancreas) and adult liver and kidney. In human fetal tissues and in the adult kidney IGF-I mRNA is barely detectable as a 7.6 kb band. In adult human liver a 7.6 kb mRNA species can be readily detected, along with species of around 1.1 kb and a broader zone of hybridization between 2.5 and 5 kb. In rat and mouse liver a similar pattern is observed. The pattern of hybridization with IGF-II cDNA contrasts sharply with these results: by far the highest abundance of IGF-II specific mRNA is found in fetal tissues, notably the liver (species of 6.0, 4.8, 4.1 and 2.1 kb). Tissue specificity is not very prominent. In human adult liver only a 5.3 kb IGF-II mRNA species can be detected, while the pattern in adult human kidney resembles that of fetal tissues. Combination of these data with the analysis of the human IGF-II gene has revealed that the different IGF-II mRNAs arise from the development and tissue-specific activation of at least three different promoters in the IGF-II gene. Considering the relative smallness of the preproIGF molecules encoded (less than 200 amino acid residues), the length of the mRNA transcripts is remarkable. The significance of this phenomenon, e.g. with respect to mRNA stability and translatability, requires further investigation.

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SERUM IGF II LEVELS IN CHILDREN AND ADOLESCENTS OF CONSTITUTIONALLY VARIANT STATURE.

A specific assay for IGF II using binding proteins extracted from cerebrospinal fluid (JCEM 1986) was used to investigate a population of children and adolescents of constitutionally variant stature with normal GH secretion following provocative stimuli. The results were compared with those of a control population of the same age. In all three groups of subjects (control, short and tall subjects) IGF II levels increased from infancy until puberty as it was the case for IGF I, but with a weak slope and without further significant increase of IGF II during puberty ; control values were 691 ± 165 ng/ml before 5 years, 963 ± 247 ng/ml in 5-11 years (P_1) and 1009 ± 182 ng/ml in pubertal subjects. The tall subjects had higher levels than controls ($p < .02$). Among the short subjects, only the youngest had lower levels than controls of the same age ($p < .05$). For the three groups of subjects taken together there was a positive correlation between IGF II levels and 1) height age ($r = .50$, $p < .001$) and 2) bone age ($r = .44$, $p < .001$). A close correlation was seen between IGF I and IGF II levels ($p < .001$). This is not the case in the situations where excess or deficiency of GH secretion is demonstrated.

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EVALUATION OF INSULIN-LIKE GROWTH FACTOR (IGF) II AS A DIAGNOSTIC TOOL IN GROWTH HORMONE DEFICIENCY (GHD)

IGF-I/SmC has proved to be useful for the diagnosis of GHD. Due to low normal levels in early infancy, however, its diagnostic value is limited at this age. We, therefore, investigated, whether serum IGF-II could improve the diagnostic sensitivity in GHD. For its determination a RIA was developed with an antiserum raised against the synthetic fragment IGF-II(33--40) and IGF-II was used as tracer. Cross-reactivity with IGF-I was 0.05% and the sensitivity was 0.02 ng. In contrast to IGF-I normal IGF-II levels were almost constant during development between age 1 and 17 years (5-percentile: 395 ng/ml; 50-percentile: 545 ng/ml; 95-percentile: 753 ng/ml). In 57 patients with GHD diagnosed by GH stimulation tests basal levels of IGF-I and -II were measured. Subnormal values for IGF-II were found in 42 (74%) cases, 5 of which had normal IGF-I; out of 15 (26%) patients with normal values for IGF-II 8 had also normal IGF-I levels. Below 5 years of age (n=8) all patients had subnormal values for IGF-II, 4 of which, however, had normal IGF-I levels. It is concluded that IGF-II is a useful tool for diagnosis of GHD at any age and is superior to IGF in early infancy.

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EVALUATION OF ERYTHROCYTE IgF1 RECEPTORS IN GH-DEFICIENT CHILDREN BEFORE AND AFTER THE START OF GH-THERAPY.

The efficacy of GH-therapy(GHT)in isolated GH-deficient children (GHD)could depend on interactions between GH-SM axis and their receptors(R). The aim of our study is to evaluate the effect of short term (ST) GHT on erythrocyte IgF1 receptors (ER). ER were assayed in 8 never GH treated GHD, 3-12 y. old, before (B), 1 week(1w), 2 and 3 months(m) after the start of GHT(synthetic GH,Saizen,Serono:0.5 U/Kg/week in 3 divided doses s.c). Controls(C) were 6 age-matched normal children. ER were assayed in duplicate modifying Gambir's technique (Diabetes 27: 701,1978). Standard IgF1 was provided by Nüesch and Scheibli(Ciba-Geigy) and tracer IgF1 by Amersham. We evaluated specific binding (SB) and, by Scatchard analysis, the number of receptorial sites per cell (RS). Mann-Whitney test was applied. SB and RS were lower in GHD than in C before and after 1w($p < 0.01$), 2m ($p<0.025$ and<0.01 respectively) and 3m($p<0.01$ and $=0.05$ respectively) of GHT. SB increased after 3m of GHT(B vs 3m:$p < 0.01$, 2m vs 3m:$p < 0.025$). RS increased after 3m of GHT(B vs 3m:$p=0.05$). A regulation of IgF1 R number by SM levels has been hypothesized "in vivo" in monocytes of newborns (Rosenfeld JCEM 48:456,1979) and in GHD because of a IgF1 R decrease after a ST GHT (Rosenfeld JCEM 52:759,1981). Our data do not confirm this effect of ST GHT, showing an increase of SB and RS after a 3 m GHT. Further studies are needed to confirm our preliminary data, to clarify their physiological significance and to elucidate if variations found in not primary target cells for SM could reflect changes in critical target cells.

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11β-HYDROXYLASE DEFICIENCY CONGENITAL ADRENAL HYPERPLASIA (11β-CAH): UPDATE OF PRENATAL DIAGNOSIS

Hormonal measurements in maternal urine and amniotic fluid (AF) throughout pregnancy and/or delivery correctly predicted the postnatal diagnosis of 11β-CAH in 7 fetuses at risk. In the 4 affected ones, maternal urinary excretion of tetrahydro-11-deoxycortisol (THS) was increased by the first trimester (280-2200 µg/day)- before amniocentesis was feaseable, increased to peak levels (518-3400 µg/day) by the third one, but suppressed with dexamethasone. Urinary THS was low after delivery in these mothers, in normal pregnancies and in parents of affected individuals. However, the 3 heterozygous pregnant mothers who carried healthy fetuses excreted 15-260 µg/day of THS. AF concentrations of THS, 11-deoxycortisol (11-DF) and Δ4-androstenedione were markedly elevated in prgenancies with affected fetuses, but normal in healthy ones. Although the AF levels of tetrahydrocortisol (THF) and tetrahydrocortisone (THE) were always normal, the AF THS/THF + THE ratio (which remained constant during gestation in 150 normal women 0.63 ± 0.34) was significantly elevated in all pregnancies with affected fetuses (2.8-4.4) and normal in non-affected ones. Maternal concentrations of serum 11-DF and Δ4-androstenedione, determined sequentially throughout pregnancy, were variable and did not contribute to prenatal diagnosis of the disorder. Affected babies were born pigmented, 2 were big, and the female baby was severely virilized. Two males respectively developed severe neonatal hypertension and seizures. Although serum 11-DF and Δ4-androstenedione were already elevated during the first week of life, urinary excretion of THS was low in the neonatal period. Prenatal diagnosis of 11β-CAH based on hormonal parameters is reliable when sequential maternal urine with combined AF determinations are performed. Because of the clinical severity of this disorder elective abortion might be the final goal, even though prenatal treatment may be attempted.