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INSULIN-LIKE GROWTH FACTOR-1 RECEPTOR INTERNALISATION INTO HUMAN PLATELETS

Specific IGF-1 binding has been demonstrated on isolated human platelets. Maximal specific binding activity of J-125-recomb.-IGF-1 was measured after 8 h incubation at 10°C in 0.1 M Hepes-buffer pH 7.4 (cont. electrolytes). For 4x10<sup>7</sup> platelets maximum specific binding of J-125-IGF-1 was 1.6% with an unsp. binding of 0.3%. 1/2 max. binding was shown at 20 ng/ml IGF-1, the affinity constant was 0.67x10<sup>9</sup> M<sup>-1</sup>. IGF-1 internalisation experiments were performed by preincubation with J-125-IGF-1 and dissociation of labeled hormone after cell placement into IGF-1 free medium or displacement by inactive IGF-1. Increasingly incomplete dissociation of J125-IGF-1 was demonstrated for preincubation times > 1h. These results are confirmed by diminished displacement of cell-bound J-125-IGF-1 by unlabeled hormone after a preincubation period of > 1h. Preliminary results indicate that lysed platelets contain remarkable amounts of IGF-1. - It has to be considered that platelets serve as a reservoir from which IGF-1, following a local injury, is released after aggregation, and together with PDGF might stimulate fibroblast growth in wound healing.

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IGF-I BINDING TO RED BLOOD CELLS OF SHORT AND TALL BOYS. COMPARISON WITH GH-BINDING PROTEIN LEVELS.

Binding of <sup>125</sup>I-IGF-I to red blood cells (RBC) was determined in 12 prepubertal boys aged 7-9 yrs, 6 with short stature (≤ 2 SD height) and 6 with tall stature (≥ 2 SD height). Concomitantly blood hGH, IGF-I and GH-binding protein (BP) was determined. The results (mean ± SD) are:

	Short Stature	Tall Stature	P
Plasma hGH (ng/ml)	3.6± 5.2	1.4± 1.4	N.S.
Serum IGF-I (nM/l)	10.6± 6.6	26.0± 10.1	<0.01
IGF-I receptor (No/cell)	3.9± 0.6	4.1± 0.5	N.S.
IGF-I receptor Kd (nM)	0.3± 0.09	0.4± 0.15	N.S.
GH-BP (%)	55.4± 13.2	102.4± 14.1	<0.001

In the present pilot study it was found that <sup>125</sup>I-IGF-I receptor binding does not correlate with extreme short or tall heights as does serum IGF-I and GH-BP. The ability of IGF-I to stimulate uptake of (<sup>3</sup>H) amino-iso-butyric acid (AIB) by fibroblasts of the same patients is being performed.

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PLASMA INSULIN-LIKE GROWTH FACTOR (IGF)-I AND II LEVELS AND BIOASSAYABLE SOMATOMEDIN ACTIVITY IN CHILDREN WITH CEREBRAL GIGANTISM (SOTOS SYNDROME).

Cerebral gigantism (Sotos syndrome) is characterized by a large birth size, excessive statural growth, advanced bone age, mental retardation and dysmorphic features. In this study 30 plasma samples of 14 children with Sotos syndrome were assayed for IGF-I and II and bioassayable somatomedin activity (SM-act). Immunoreactive IGF-I was determined in unextracted plasma and compared to the Nichols Institute references. Immunoreactive IGF-II was measured with a nonequilibrium RIA, using the tyrosylated eight amino acid C-peptide region of IGF-II (CP-II) and an antiserum against this fragment (kindly donated by Dr. R. Hintz). SM-act was determined with the porcine cartilage bioassay. All but 3 values of IGF-I were within the ± 2 SD range for age. When the data were expressed as a Z-score for age, mean IGF-I decreased from -0.1 (range -1.0 to +1.0, n=6) at 0-3 years to -1.1 (range -2.0 to 0.3, n=7) at 3-5 years. IGF-II levels were generally within the reference range. SM-act was usually low between 1 and 5 years of age (mean -2.2 SD, range -5.0 to 3.6, n=11) and in the lower normal range thereafter. These IGF-I and SM-act results are in contrast to the data in constitutional tall stature. The relatively low somatomedin levels between 1 and 5 years of age concur with the deceleration of growth which is usually seen after the first year of life in children with Sotos syndrome.

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SOMATOMEDINS POTENTIATE INTERLEUKIN 2 INDUCED STIMULATION OF NATURAL KILLER CELL ACTIVITY

Natural killer (NK) activity of peripheral blood lymphocytes (PBL) is reduced in GHD. We, therefore, investigated whether or not IGF-I and IGF-II have any influence on NK activity in the presence or absence of interleukin 2 (IL-2). PBL were obtained by fractionation of blood cells on a Ficoll gradient. After incubation in RPMI 1640 medium with or without IL-2 (100 U/ml), IGF-I and/or IGF-II (25 ng/ml) for 15 h cytotoxicity was measured by a 4-h <sup>51</sup>Cr-release assay utilizing the cell line K562. IGF-I and IGF-II alone or in combination had no effect on NK activity. The stimulatory effect of IL-2 (100%) was not significantly enhanced by further addition of IGF-I (123 ± 48%) or IGF-II (118 ± 36%), if inter-individual means (N=11) were regarded, although in some individuals a significant potentiation (p<0.001) was observed (maximal values with IGF-I: 232%, IGF-II: 194%). In contrast, addition of both IGF-I and -II potentiated the IL-2 effect significantly (p<0.001) even if the inter-individual mean was regarded (146 ± 25%). In 7 out of 11 individuals the combined effect of IGF-I and -II was clearly more than additive. Dose-response curves of IGF-I and -II in the presence of IL-2 exhibited a significant increase of NK activity between 0.1 and 10 ng/ml. It is concluded that (1) somatomedins are modulators of NK cell function and (2) that IGF-I and -II may act synergistically in particular systems.

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SERUM LEVELS OF IGF-I AND IGF-II IN CHILDREN TREATED WITH RECOMBINANT HUMAN GROWTH HORMONE (r-hGH) WITHOUT METHIONINE.

Results of IGF-I and IGF-II measured in 70 GH deficient children (51m, 19f) treated with r-hGH for up to 18 months and grouped according to sex and BA at start of therapy, are shown in the table

Months of therapy	0	3	6	9	12	18	n	IGF↓
BA Group 1: boys<11.5 yrs., girls<10 yrs.								
IGF-I New Pts. n	15.1	22.1	21.6	23.0	29.6	41.0	21	10
nmol/l f	14.6	18.4	21.2	20.2	29.4	33.2	10	7
Transfer n	21.3	23.1	21.2	22.8	25.6	36.2	15	7
IGF-II New Pts. n	63.5	64.8	58.5	61.8	71.4	53.5	21	15
nmol/l f	74.8	68.7	59.0	61.0	77.5	50.5	10	7
Transfer n	81.2	84.8	87.0	65.5	74.8	58.7	15	13
BA Group 2: boys>11.5 yrs., girls>10 yrs.								
IGF-I New Pts. n	13.7	25.2	25.0	29.1	30.0	81.6	7	4
nmol/l f	18.7	27.1	18.9	20.1	26.1	26.2	6	5
Transfer n	27.1	30.9	32.9	35.4	38.0	59.8	8	4
f	34.8	30.4	23.7	31.6	39.8	39.8	3	2
IGF-II New Pts. n	71.1	71.7	76.8	70.5	69.7	74.8	7	6
nmol/l f	73.8	69.6	38.9	64.9	65.2	92.4	6	4
Transfer n	76.6	70.1	78.4	56.2	100.5	53.8	8	7
f	106	125	103	112	94.7	--	3	2

During treatment we observed a significant transient decrease of IGF-I in 25/51m and 14/19f and of IGF-II in 41/51m and 14/19f. After 18 months of therapy, IGF's were normalized in all groups.

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BIOACTIVITY OF THE IGF-1 BINDING SUB-UNIT OF THE LARGE MOLECULAR WEIGHT CIRCULATING COMPLEX IN HUMAN PLASMA.

The biological activity of the partially purified insulin-like growth factor 1 (IGF-1) binding protein (BP) of the large MW circulating complex (145 K) in human plasma was evaluated using our IGF-1 Leydig (LC) cell bioassay model that specifically differentiates under IGF-1 action (Bernier J. Cell. Physiol. 129:257, 1986). This BP inhibits both the 125-IG-1 binding to the specific LC IGF type 1 receptors and the dramatic increase in testosterone secretion (in response to LH) specifically induced by IGF-1. BP also inhibits the IGF-1 dependent increment in LH-HCG receptor number. These BP inhibitions of IGF-1 actions are specific and dose dependent. These data help to further understand the nature of the circulating IGF-1 large MW complex and function of its IGF-1 BP.

Partial purification of the IGF-1 BP subunit included ammonium sulfate precipitation, DEAE sephadex A50, S-300 sephacryl (pH=3.5) and IGF-1 affinity chromatographies then C4 reverse phase HPLC. On SDS-PAGE, DEAE peak 2 (containing BP) cross-linked to 125-IGF-1 lead to two specific bands (39 K and 24 K). This material gel filtrated (S-300, pH=7.4) lead to a 54 K complex. When cross-linking included 125-IGF-1, DEAE peak 2 and 3. SDS-PAGE lead to a major 94 K, a minor 39 K and a constant 24 K band. S-200 gel filtration then recovered a large IGF-1 MW complex, but of 125 K