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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(7) platelets maximum specific binding of J-125-IGF-1 was 1.6% with an unspec. binding of 0.3%. 1/2 max.binding was shown at 20 ng/ml IGF-1,the affinity constant was 0.67x10 (9)xM(-1). 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 $^{125}$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 $^{125}$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 ($^{3}$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 51-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. m | 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 m | 21.3 | 23.1 | 21.2 | 22.8 | 25.6 | 36.2 | 15 | 7 |
| IGF-II | New Pts. m | 63.5 | 64.8 | 58.5 | 61.8 | 71.4 | 53.5 | 21 | 15 |
| nmol/l | f | 74.8 | 68.7 | 59.9 | 61.0 | 77.5 | 50.5 | 10 | 7 |
| | Transfer m | 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. m | 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 m | 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. m | 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 m | 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 differenciates 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 amonium 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