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THE EFFECTS OF ZINC ON LACTOGEN STIMULATION OF Nb2 CELLS USING AN ELUTED STAIN ASSAY (ESTA).

Ionic zinc (Zn) has been reported to enhance binding of human growth hormone (hGH), but not prolactin (PRL), to the human PRL receptor at a concentration of ~50uM (1). We have investigated the effect of zinc on hGH bioactivity using a lactogenic bioassay.The potencies of selected doses of both hGH and PRL in the presence of increasing concentrations of $ZnCl_2$ were investigated with an Eluted Stain Assay (ESTA) which used Nb2 cells. This colorimetric bioassay is based upon the reduction of a yellow tetrazolium salt, MTT, to its purple formazan by lactogen-activated Nb2 cells. Zinc enhanced the bioactivity of hGH but not PRL. Progressive enhancement of a low dose (0.5mU/L; 9pM) of hGH (IRP 80/505) was observed over the Zn concentration range of 6-100uM; higher Zn concentrations were inhibitory. Potentiation of bioactivity was observed only with low doses of hGH; with >2.5mU hGH/L, 50uM Zn inhibited the response. The bioactivity of PRL was consistently inhibited by 50uM Zn (PRL range 0.2-50mU/L; 0.4-105pM), inhibition being greater with higher PRL doses. **We conclude that** in this precise and sensitive bioassay, zinc has a differential effect on hGH and PRL bioactivities which concords with the radioligand studies but potentiation of bioactivity was only observed with low hGH doses. It was optimal at ~50uM Zn but was far less than might have been anticipated from the binding studies, when the affinity increased by 8000-fold. This discrepancy was not explained by significant endogenous Zn in the bioassay medium (only 2uM by Atomic Absorption Spectroscopy). Our findings therefore do not support the earlier suggestion (1) that Zn is crucial for the lactogenic bioactivity of hGH.

1) Cunningham B.C, Bass S, Fuh G, Wells JA 1990 Science 250:1709-1712.

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SHOULD GROWTH HORMONE DOSES BE INCREASED DURING PUBERTY?

Physiological growth hormone (GH) secretion increases during puberty and it has been suggested that similar changes should be made to the therapeutic schedules of GH-insufficient children receiving GH therapy. We have tested this hypothesis in 50 GH-insufficient children aged between 9.7 and 14.5 years who were randomised to continue with biosynthetic human GH at a dose of 15 U/m^2/week (Group A) or to an increased dose of 30 U/m^2/week (Group B), at breast stage 2 development in girls and at a testicular volume of 8 mls in boys. All children had received GH treatment prior to randomisation for at least 1 year. There was no difference between the groups prior to randomisation in terms of age, height and height velocity. Puberty was entered at a similar mean age: 11.8 years (SEM 0.3) in A and 12.0 (SEM 0.2) in B. Despite a 100% increase in GH dose similar growth accelerations of 7.2 (SEM 0.5) (A) and 7.1 (SEM 0.4) cms/year (B) were observed. The net height gain 2 years into the study was similar at 12.7 and 13.8 cms respectively. Both doses of GH led to a significant acceleration in the time taken to progress between genitalia stage 2/breast stage 2 and genitalia stage 4/breast stage 4 compared to Tanner standards (average acceleration 0.3 years). These data suggest that increasing the dose of GH in puberty to mimic the physiological situation does not lead to a significant improvement in growth rate and statural gain. Both doses appear to accelerate the rate at which the individual progresses through puberty which might have important implications for the total height gain expected.

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BODY COMPOSITION MEASUREMENT IN GROWTH DISORDERS BY DUAL ENERGY X-RAY ABSORPTIOMETRY. J.W. Gregory, A. Aynsley-Green and P.J. Smith, Department of Child Health, Medical School, University of Newcastle upon Tyne, NE2 4HH, U.K.

The dual energy x-ray absorptiometry (DEXA) technique to measure bone density may also provide estimates of body composition, abnormalities of which are a well recognized consequence of growth hormone deficiency. To assess the precision of the method in clinical practice, we have compared estimates of total body water by DEXA (Hologic QDR 1000/W) with those obtained from skinfold thickness and bioelectrical impedance measurements in 10 patients aged (range) 14.0 to 17.9yrs, receiving growth hormone therapy. Total body water (kg) derived from estimates of lean tissue (assuming 73% hydration) by DEXA was compared with those values obtained by skinfold and impedance measurements as follows:

		skinfolds	impedance
r		0.99	0.92
bias		0.45	0.60
limits of agreement		-0.91 to 1.81	-3.39 to 4.59
95% CI for limits:	lower	-1.76 to -0.07	-6.06 to -0.73
	upper	0.97 to 2.65	1.93 to 7.25

We conclude, that given the small numbers of patients studied thus far, estimates of body composition by DEXA have a high degree of precision when compared to those obtained from the skinfold thickness method though less so when compared with bioelectrical impedance. Measurement of body composition by DEXA may therefore be of value in the assessment of the effects of growth hormone therapy on body composition.

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A ROLE FOR IGF-1 AND ITS BINDING PROTEINS IN NEURONAL RESCUE FOLLOWING ASPHYXIAL INJURY. P. Gluckman, J. Guan, E. Beilharz, E. Sirimanne, O. Miller and C. Williams, Research Centre for Developmental Medicine and Biology, University of Auckland, Private Bag 92019, Auckland, New Zealand.

Unilateral asphyxial neuronal injury was induced in rats by carotid ligation followed by inhalational asphyxia. Infarction and neuronal loss occurs in the ligated hemisphere. In situ hybridisation showed enhanced expression of IGF-1 by glia and of its binding proteins BP-2 and BP-3 within 24-72 hours of injury although the distribution of expression showed differences. In contrast IGF-2 and BP-5 were induced much later not appearing for 7-10 days post injury. BP-4 was suppressed on the side of injury. IGF-1 was administered to adult rats via the lateral ventricle. Treatment 2 hours after injury led to a dose dependent reduction in infarction from 87% to 26% ($p<0.05$) over the dose range 5 to 50μg/rat. Functional testing showed protected somatosensory function in the treated rats compared to sham controls. The effect of IGF-1 was not mimicked by des 1-3 IGF-1 suggesting an important role for the induced BP-2 or BP-3. Insulin had a weaker effect than IGF-1 and no protective effect of IGF-2 was observed: compatible with an action at the type 1 IGF receptor. Treatment prior to injury had no effect suggesting that IGF-1 acts via interfering with apoptosis. These observations suggest endogenous IGF-1 production is enhanced following neuronal injury as a protective response and suggests that exogenous IGF-1 might be a potential neuronal rescue therapy.

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HUMAN COLON CARCINOMA CELLS (CACO2) EXPRESS IGF RECEPTORS AND SYNTHESIZE IGF-II: POTENTIAL AUTOCRINE OR PARACRINE ACTION OF IGF-II.

The IGFs have been implicated in the development of the intestinal tract. We have studied the human colon carcinoma cell line CaCo2 to gain more insight into the function of the IGFs in the gut.^{125}I-IGF-I and -II bound specifically to CaCo2 cells as measured in competitive binding experiments. The existence of IGF-I receptors and of IGF-II/M6P receptors was further demonstrated by affinity crosslinking studies using DSS as the crosslinking agent. Western blotting of CaCo2 cell extracts using an anti IGF-II/M6P receptor antiserum provided additional evidence for the expression of the IGF-II/M6P receptor. Northern blotting experiments showed specific IGF-I receptor and IGF-II/M6P receptor gene expression in CaCo2 cells: cells were homogenized in 4M guanidiniumthiocyanate and RNA extracted in 5.7 M CsCl. RNA was denatured in glyoxal/DMSO and electrophoresed on 0.8 % agarose. RNA was transferred to a nylon membrane by capillary transfer, fixed and the blots hybridized with cDNA probes. A 614 bp Pst I fragment of the IGF-I receptor cDNA and a 663 bp fragment of the IGF-II/M6P receptor cDNA were labeled with ^{32}P-dCTP using random prime labeling: Autoradiographs of Northern blots showed a 11 kb band with the IGF-I receptor probe. Hybridization with the IGF-II/M6P receptor probe yielded a 9 kb RNA species. In a subset of experiments a Pst I 700 bp fragment of the IGF-I cDNA and a 833 bp Pst I fragment of the IGF-II cDNA, were used for hybridization: no hybridization was detected with the IGF-I probe. However, using the ^{32}P-IGF-II probe bands at 6.4, 5.3 and 5.0 kb were labeled. In addition, CaCo2 cells were cultured until confluency, changed serum-free and cell-conditioned medium was harvested . IGF-II immunoreactivity was measured using an IGF-binding protein blocked radioimmunoassay. CaCo2 cell-conditioned medium contained 1-2 ng/ml IGF-II immunoreactivity. In conclusion, (1) CaCo2 cells express both IGF-I and IGF-II/M6P receptors. (2) CaCo2 cells express IGF-II mRNA and secrete IGF-II immunoreactivity. We hypothesize that in human colon carcinoma cells IGF-II acts as an autocrine growth factor (supported by DFG and DAAD, Bonn, Germany).

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DOES PRETREATMENT WITH GHRH CHANGE THE GROWTH RESPONSE TO GH THERAPY?

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Thirty-six prepubertal children with GHD, 21 boys and 15 girls aged 4.25 to 13.00 years, bone age (BA) from 2.0 to 9.5 years with growth deficiency from -1.36 to -5.62 SD to chronological age, were treated with daily subcutaneous injections of GHRH 1-29 or 1-44 at a dose of 10 µg/kg/day for 6 months. After a six month interval, treatment with biosynthetic growth hormone (Maxomat, Sanofi) at a dose of 0.6 U/kg/week in subcutaneous injections 6-7 times per week was instituted. The growth velocity at various stages of observation and treatment was determined. The results were compared with a control group made up of 14 prepubertal GHD children who had not been treated with GHRH, 11 boys and 3 girls aged 3.75 to 13.83 years, BA 1.5 to 11.0, treated for the first time with biosynthetic growth hormone (Maxomat, Sanofi) according to the same protocol and dose as above.

The growth velocity (GV) during successive periods of observation was found to be:

	Group I - GHRH-treated SDS/BA	Group II - Control SDS/BA	
Before treatment GHRH	(-2.73) ± 1.35 a/ (-1.34) ± 1.64	(-3.14) ± 1.62 b/ - -	a/ t = 3.48, p < 0.01
Treatment interval GH treatment	(-1.76) ± 1.37 a/ b/ (+1.24) ± 2.08 c/	- - (+4.35) ± 3.40 c/	b/ t = 2.93, p < 0.01
			c/ t = 3.90, p < 0.001

GV before any treatment was not statistically different between both groups (t=0.87, p - ns).
BA (t=1.565, p - ns) between both groups before any treatment did not differ statistically. Before GH treatment, BA in patients treated with GHRH was statistically higher than in the control group (t=2.21, p < 0.05), and remained more advanced during GH treatment with the difference between both groups significant (t=2.03, p < 0.05). Our data indicate that in spite of the fact that before any treatment, the studied groups did not differ in terms of BA and GV, before institution of GH therapy, a statistically significant difference was found between BA and GV in both groups. It is difficult to conclude on the basis of our material if the GV in group I during GH treatment was dependent mainly on pretreatment with GHRH or were more advanced BA and/or higher GV before initiation of GH therapy also decisive factors.
In the group treated with GHRH, no correlation was found between the GV in response to GH and the GV observed during GHRH treatment (r = -0.17, p - ns).
The growth response to GHRH does not seem to be a prognostic of response to GH therapy.