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M.C. Postel-Vinay, J.P. Clot*, C. Benelli*, B. De Gallé*, INSERM U.30, Hôpital des Enfants-Malades, Paris, France.

PYRUVATE DEHYDROGENASE ACTIVITY IS STIMULATED BY GROWTH HORMONE : A NEW TOOL TO MEASURE RESPONSIVENESS OF HUMAN MONONUCLEAR CELLS TO THE HORMONE.

No rapid effects of growth hormone (GH) in isolated human cells have been measured until now. Human mononuclear cells were used to examine the effects of human GH (hGH) on the activity of the pyruvate dehydrogenase (PDH) complex. Cells ($8x10^6$/ml) were incubated at 37°C for various times in the presence of 10^{-7}M hGH or porcine insulin. PDH activity was determined in sonicated cells by measuring $^{14}CO_2$ liberation from 1-^{14}C-pyruvate. Increase in basal PDH activity was linear as a function of time (up to 20 min), of pyruvate concentration (up to 0.25 mM) and of proteins (up to $4x10^6$ cells). Ten subjects, aged 5 to 40 years, were studied. Basal PDH activity represented 24 % of the total PDH activity (20.54 ± 1.19 pmol/min/10^6 cells). Treatment of mononuclear cells by hGH led to an increase in basal PDH activity which was maximal (53 ± 7% above control value) at 15 min. Later on, activation progressively decreased and was no longer detectable at 30 min. Total PDH activity (measured in the presence of 0.5 mM $CaCl_2$ and 20 mM $MgCl_2$) was unaffected by hGH treatment. Incubation of cells with insulin also increased basal PDH activity (65 ± 9% above control value) at 15 min, without changing total PDH activity. In conclusion, hGH as well as insulin,is able to stimulate PDH activity of human mononuclear cells. This hormonal effect allows the rapid evaluation of the cellular responsiveness to GH in various pathophysiologic situations. No effect of GH on PDH activity was observed in cells prepared from two Laron dwarfs.

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Schnabel D., Th. Danne, H. Bröslcke, D. Hartmann*, A. Grüters, D. l'Allemand, H. Helge, B. Weber
University Children's Hospital Berlin, W. Germany
Institut Pasteur de Lyon, France

MEASUREMENT OF 15N-RETENTION AND TYPE I AND TYPE III COLLAGEN SERUM LEVELS PREDICTS RESPONSE OF TREATMENT WITH HUMAN GROWTH HORMONE (hGH)

The prognostic value of 15N-(stable isotope nitrogen)-retention (mass-spectrometry) and serum levels of fragments of type I collagen (bone and interstitial fibrils) and type III procollagen (interstitial fibrils) (both by RIA) was studied in 20 hGH deficient children (median age: 10 ys). Patients showing good responses to hGH-therapy (increases above 1 SDS of height for chronological age after 1 year of treatment) had higher mean collagen levels during treatment (Type I: 214 ± 14 vs. 200 ± 14 ng/ml; Type III: 43.3 ± 13.7 vs. 39.5 ± 8.0 ng/ml) than those with smaller responses. The median increase after 3 days of hGH (2IU/m²/die) was higher in those with better responses (Type I: 22 vs. 10 ng/ ml; Type III: 13.5 vs. 7.2 ng/ml). Cumulative renal 15N-excretion decreased from pretreatment median values of 0.91 to 0.54 mg/15N/kg. However, 15N-retention (in %) was identical for good and poor hGH responders (44 %). Short-term administration (3 x 2IU/m²) in patients with Turner's Syndrome (n=5) showed no median change of type III collagen (1.7 ng/ml) and 15N retention (9.2%). Therefore, longterm hGH-treatment with such doses in Turner patients are not likely to induce optimal growth responses. Furthermore, these results suggest studies of both 15N-retention test, as well as type I and type III collagen serum levels to predict the response before initiation of longterm hGH-treatment.

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J.J. van der Werff ten Bosch and A. Bot*.
Department of Endocrinology, Growth and Reproduction, Faculty of Medicine, Erasmus University, Rotterdam, Netherlands.

GROWTH HORMONE HASTENS BONE MATURATION DURING 'PUBERTY' IN HYPOPITUITARY BOYS.

Skeletal maturation rate (SMR) during spontaneous or induced (pseudo-) puberty was studied in boys with hypopituitary dwarfism, 30 treated with hGH, 26 without. Bone age (BA, Greulich & Pyle) was estimated at the time when pubertal signs were first noted or when androgen substitution was begun. When final height was reached pubertal growth was considered to have ended and BA assumed to be maximal (19 y). SMR (mean 0.91, SEM 0.058) was negatively correlated with initial BA (mean 11.2, SEM 0.37) in boys without hGH; in only 4/26 SMR exceeded 1 y/y. For boys with hGH there was no correlation between SMR (mean 1.54, SEM 0.070) and initial BA (mean 11.5, SEM 0.26); in 26/30 SMR exceeded 1 y/y.
Conclusions: androgen-induced skeletal maturation occurs faster in presence of hGH than in its absence; this explains earlier finding of similar final heights of hypopituitary boys with and without hGH-treatment. Hence hGH-treatment must aim at greatest possible height before puberty.

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K. Damm Jørgensen*, S. Jensen* (Introd. by N.E. Skakkebæk).
Departments of Pharmacological and Clinical Research, Nordisk Gentofte A/S.

EFFECT OF GROWTH HORMONE TREATMENT ON ULTIMATE WEIGHT AND LENGTH OF TURNER MICE.

Turner mice (xo) and xx mice with the same genetic background were treated for a 6-month period with daily subcutaneous doses of 0.5 IU Norditropin^R or placebo, starting at the age of 5 weeks to 5 months. The Turner mice which were treated with Norditropin^R reached a significantly higher ultimate body weight (about 38g) and ultimate length (nose to tip of the tail - about 21.5cm) compared to the placebo-treated Turner mice (about 28g and 19cm). The body weight remained constant after cessation of treatment. There was no correlation between age at the start of treatment and ultimate weight or length.
Upon sacrifice it was found that the femoral and tibia bones from the Norditropin^R-treated Turner mice were significantly longer compared to placebo mice. The ultimate weight and length of the Norditropin^R-treated xx mice, as well as the length of their bones, were not significantly different from the comparable parameters in placebo-treated xx mice. Both xo and xx mice reached their ultimate weight significantly faster when they were treated with Norditropin^R compared to placebo (means: 22.1 and 19.7 weeks respectively after start of dosing).

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R. Sandhu*, P.J. Pringle*, P. Hindmarsh*, R.G. Stanhope, J. Holly*, R. Biddlecombe*, L.H. Rees* & C.G.D. Brook.
St. Bartholomew's Hospital and The Middlesex Hospital, London.

BIOACTIVE GH CONCENTRATIONS ARE LOWER THAN IMMUNOREACTIVE GH IN DELAYED PUBERTY.

We have compared levels of serum growth hormone (GH) in children with growth disorders using the Nb2 node lymphoma (Nb2BA) bioassay and immunoradiometric (IRMA) assay. Seven children (2 tall, 2 short normal, 3 GH insufficient) showed identical secretory patterns by time series analysis but Nb2BA GH was nearly twice as high as IRMA GH. There was a high correlation between IRMA and Nb2BA but daytime samples showed a better correlation than those obtained at night. It is suggested that some residual stimulation of Nb2BA by prolactin occurred in the serum despite the addition of a 1:1000 dilution of prolactin monoclonal antibody.

Four boys with delayed puberty were also studied. Although the GH profiles were identical, GH concentrations were lower in Nb2BA than IRMA. All 4 boys grew on low dose oxandrolone (1.25-2.5mg/day) and analysis of GH profiles by the two methods showed an increase in the ratio of Nb2BA:IRMA GH to 1.5:1. These data suggest that differences between bioactivity and immunoreactivity may be relevant to the measurement of GH concentrations in patients with delayed puberty.

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L.Audí,M.L.Granada*,A.Sanmartí*,A.Lucas*,I.Salinas*, J.Reverter*,J.M.Cuatrecases*,A.Carrascosa,M.Foz* .
Hormonal and Endocrine Units,Hospital Germans Trias i Pujol,Universitat Autònoma,Barcelona , Spain .

INTEGRATED CONCENTRATIONS OF GH IN CHILDREN WITH CONSTITUTIONAL GROWTH DELAY.RESULTS FROM SEVEN DIFFERENT RIAs

Integrated plasma concentrations of GH were studied in 14 children with constitutional growth delay using 7 different RIAs.
GH levels in 24h pool plasma samples measured in the same samples by the different assays varied widely(up x3 the lowest value).The assay which uses monoclonal Abs (HYBRITECH)gave the lowest values; those using polyclonal Abs gave in general higher values(x2 the monoclonal assay)but they also varied widely among themselves(x2 between lowest and highest),hierarchy being : SERONO $<$DPC $\leq$SORIN $<$ BEHRING $<$BIOMERIEUX $\leq$CIS .
The BIOMERIEUX(B) and SORIN(S) assays were applied to all 30' samples in addition to the pool 24h,day and night samples.The GH levels in 24h pool samples from the 14 children were($\bar{M}\pm SD$):4.6 ± 2.7 ng/ml according to the B assay(11.4 to 1.2) and 2.9 ± 1.2 according to the S assay(5.7to0.8).The average ratio between the values given by the two assays(B/S)was 1.51 ± 0.32(2.0 to 1.0).A highly significant correlation was observed between the two assays(r=0.97)but the divergence was maximal at the highest levels of GH and a positive correlation between the B/S ratio and the GH concentrations has been documented($r=0.72, p<0.01$).There is no correlation between B/S ratio and the maximum GH concentration attained(B/S=1.27 ± 0.18).The last is similar in GH response to Propanolol+Exercise.
In conclusion,the results of the GH integrated concentrations are strongly influenced by the RIA used,probably because they measure different forms of the GH molecule .