

83

P. Tassoni*, F. Zappulla*, M. Cau*, C. Colli*, M.T. Tosi*, P. Tonini*, A. Balsano*, E. Frejaville*, S. Donati*, E. Cacciari.
Department of Pediatrics, S. Orsola - Malpighi Hospital, University of Bologna, Italy.
VARIABILITY OF ARGININE L-DOPA AND SLEEP TEST PERFORMED TWICE IN THE SAME PATIENTS.

We performed arginine and sleep tests (blood samples from latter collected every 30' for 12 hours) twice within two weeks in 21 short patients with mean C.A. 11.15 years (range 7.5 - 15.91 years); 14 males, 7 females; 12 prepubertal, 9 pubertal. Mean height SDS was -2.28 ± 0.65 ($r = -3.85, -1.37$); predicted height was 166 cm. for males, 152 cm. for females. We calculated % difference between same parameters of double tests (a1, a2) according to formula $|(a1-a2)/(a1+a2)/2| \times 100$. Results were following: % differences:

arginine peak (1)	arginine area (2)	sleep value (3)	sleep peak (4)	sleep area (5)	sleep peaks >5 (6)	sleep peaks >5 (7)
89.18	86.96	38.88	42.93	34.51	43.41	102.11
± 54.28	± 56.62	± 29.78	± 17.2	± 22.3	± 60.26	± 51.15

Despite great variability between all tests, variations between physiological and pharmacological tests are highly significant (1,2 versus 3,4,5,6). In 22 short patients with mean C.A. 11.44 ± 2.72 ($r = 4.16-15.5$ years), 13 M, 9 F, 11 impubertal and 11 pubertal, mean height SDS -2.34 ± 0.92 ($r = -5.34, -1.17$), predicted height 168 cm (males), 151 cm (females), arginine and/or L-Dopa tests were performed twice. Differences in double tests overall:

arginine peak (n.43)	arginine area (n.43)	L-Dopa peak (n.20)	L-Dopa area (n.20)
76.24 \pm 55.6	75.6 \pm 53.6	80.8 \pm 58.1	77.4 \pm 57.1

In conclusion sleep test showed less variability than pharmacological tests.

84

C. Åström*, SA Pedersen*, W. Trojaborg*, NE. Skakkebaek
Department of Clinical Neurophysiology, Rigshospitalet
Copenhagen University Hospital, Denmark, and the Department of Paediatrics, Hvidovre Hospital, Denmark.

DEEP SLEEP AND GROWTH HORMONE SECRETION IN ISOLATED GH-DEFICIENT PATIENTS.

Studies of normals have shown that the nocturnal secretion of growth hormone (GH) is coupled to deep sleep (stage 4), but administration of human growth hormone releasing factor has failed to modify sleep parameters in normals. The aim of the present study was to examine whether GH-deficient patients have normal sleep. 8 pituitary dwarfs with isolated growth hormone deficiency (peak value in clonidin stimulation test: less than 3 ng/ml), age 18-28 years old were examined. The sleep was recorded 2 consecutive nights with EEG, EOG and EMG according to Rechtschaffen and Kales' criteria. The sleep records were manually scored by an independent scorer as well as automatically scored.

There was a reduction of stage 4 sleep (mean: 35 min) compared to 13 age and sex matched controls (mean: 57 min, $p < 0.01$). Total deep sleep (stage 3+4) was also reduced ($p = 0.02$; Mann Whitney U test). There was a trend towards REM sleep time reduction, but the difference was not significant. Total sleep time (TST) was significantly increased (mean TST: 559 min, range 429-770 min, $p < 0.01$). The increased TST was related to an increase in stage 2 NREM sleep. The abnormally low deep sleep is at minimum a biological marker in these patients. It is possible that the poor GH-secretion in GH-deficient patients is related to a reduction of deep sleep, making the sleep-process triggering the hypothalamo-pituitary GH secretion.

85

Z. Hochberg, D. Lewinson*, Z. Harel*, P. Shenzer*, M. Silbermann*.
Departments of Pharmacology and Morphological Sciences*, Rappaport Family Inst. for Research in the Medical Sciences Technion-Israel Institute of Technology, Haifa, Israel.

THE IMPORTANCE OF GROWTH HORMONE (GH) IN THE EPIPHYSEAL CARTILAGE AND BONE CHANGES OF HYPOTHYROIDISM (HT).

To evaluate the role of GH in the growth retardation of HT, 7-weeks-old female rats were rendered HT by methimazol for 7 weeks (Gr1). This resulted in serum $T_4 < 1 \mu\text{g/dl}$ (ctr $2.4 \pm 0.4 \mu\text{g/dl}$), growth arrest and 96% depletion of pituitary contents of GH (from 78 ± 3 to $3 \pm 3 \mu\text{g/pit}$). Supplementation with L-thyroxine (T_4) for the last 2 weeks (Gr2) restored pituitary GH to $23 \pm 4 \mu\text{g/pit}$ and accelerated rats' growth from -3 g/wk to $+9 \text{ g/wks}$. Treatment of HT rats with hGH for the last 2 weeks (wks 12-14 of life) (Gr3) resulted in intermediate growth of $+3 \text{ g/wks}$.

Combined treatment with T_4 and hGH (Gr4) increased growth further to 12g/wk. The proximal tibiae were dissected and the epiphysis, metaphysis and growth plate were evaluated qualitatively and measured morphometrically. In the control group (ctr) the metaphyseal trabecular bone was $54 \pm 2\%$ of the total bone. In Grs 1-4 it was 18 ± 2 , 36 ± 3 , 24 ± 2 and $34 \pm 2\%$ respectively. The epiphyseal trabecular bone in ctr and Grs 1-4 was 43 ± 3 , 30 ± 2 , 46 ± 2 , 41 ± 2 and $36 \pm 2\%$ resp. The width of the compact tibial midshaft was 597 ± 21 , 373 ± 14 , 432 ± 19 , 330 ± 16 and 453 ± 4 microns resp. The width of the epiphyseal growth plate was 189 ± 6 , 140 ± 3 , 262 ± 14 , 126 ± 5 and 300 ± 9 microns resp. While the HT plate was noted for more resting cells, less and smaller proliferating cell and arrest of cartilage remodelling into metaphyseal bone hGH replacement resulted in only minor improvement and T_4 replacement enhanced proliferation and remodelling. Combined treatment resulted in further increase of proliferation. It is concluded that GH-deficiency is responsible for only a small part of the epiphyseal cartilage and bone changes of HT, and that GH requires thyroid hormones for its full expression.

86

M.C. Postel-Vinay, J.-F. Hocquette*, A. Amar-Costesec*, B. de Hemptinne* and J.B. Otte*. INSERM U.30, Hôpital des Enfants-Malades, Paris, France and ICP, UCL, Brussels, Belgium.
GROWTH HORMONE RECEPTOR OF HUMAN LIVER.

Human livers, obtained from donors at time of transplant, were homogenized in 250 mM sucrose and fractionated by differential and isopycnic centrifugation. Specific binding of ^{125}I -human growth hormone (hGH) was measured in total particulate fractions prepared from 12 livers. Values range from 0 to 50.5% of total radioactivity/mg protein. Binding affinity constant is $2.0 \pm 0.3 \times 10^6 \text{ M}^{-1}$ and binding capacity varies from 14 to 53 fmol/mg protein. No relationship is observed between the level of hGH binding and age or sex of the donors. Binding sites are specific for hGH. Dissociation of the hormone-receptor complex is extremely slow and does not exceed 25% of the bound hormone after 24 h. Cross-linking of ^{125}I -hGH to plasma membrane receptors yields two major bands of 110 and 55 Kd, after subtraction of hGH MW. After differential centrifugation of the liver homogenate, GH binding sites are recovered in approximately equal proportion in the nuclear and the microsomal fractions. In gradient subfractions from untreated microsomes, the GH binding activity displays a distribution pattern very similar to that of marker enzymes for endoplasmic reticulum. However, when digitonin-treated microsomes are centrifuged to equilibrium, a noticeable proportion of the binding activity undergoes a density shift similar to that of plasma membrane constituents. Solubilization of a total particulate fraction with Triton X-100, followed by chromatography on an hGH-affinity column, leads to partial purification of the receptor.

87

G. Massa*, M. Maes, JP. Thissen*, JM. Ketelslegers*
University of Louvain, School of Medicine, Brussels, Belgium.

SEXUAL DIMORPHISM OF HEPATIC GROWTH HORMONE (GH) RECEPTORS IN RATS IS NOT DUE TO DIFFERENCES IN OCCUPANCY BY ENDOGENOUS GH.

To ascertain that the sexual dimorphism of liver GH receptors in rats is not due to differences in their occupancy by endogenous GH, specific ^{125}I -bovine GH binding was determined on homogenates from male and female animals, before and after MgCl_2 treatment to remove endogenous GH. In both sexes total and free GH binding increased with age (table). In 3 day-old rats free receptors were low and increased slightly after MgCl_2 . Until 3 weeks of age, no sex difference was present. In adult rats, however, female animals had more GH receptors than males, whether endogenous GH was removed or not. In conclusion: 1) in neonatal rats, the low GH binding is not due to occupancy by GH; 2) in adult rats, the sexual dimorphism of GH binding is not due to differences in occupancy by GH.

Specific hGH bound (% of total radioactivity/mg protein; mean \pm SE)

age	sex	N	+ MgCl_2 (total)		- MgCl_2 (free)	
			mean	SE	mean	SE
3 days	F	8	2.92	0.34	N.D.	1.90
	M	8	2.59	0.38	N.D.	1.50
3 weeks	F	8	5.12	0.54	4.07	0.39
	M	8	5.92	0.52	5.31	0.62
2 months	F	8	18.83	1.92	11.22	0.91
	M	8	9.20	0.89*	5.79	0.56*

N.D.: not detectable
* : $P < 0.01$ vs F

88

Z. Zadik, H. Landau, Z. Hochberg, Y. Limoni, A. Kowarski, E. Lieberman.
Kaplan Rehovot: Hadassah Jerusalem; Rambam Haifa, Univ. Maryland USA; Soroka, Beer Sheva. Israel.
FREQUENCY OF NEUROSECRETORY DYSFUNCTION (NSD) IN REHOVOT, ISRAEL.

111 children with heights below 2.5 SD for age (Panner) were identified by screening of 7500 children, 6-14 years of age, in the Rehovot region. The total population of this age group is 40,000 in the area studied. The 111 children of short stature (ss) included: 8 skeletal disease, 2 Down Syndrome, 4 Thalassemia, 4 Turner, 3 celiac, 4 classical growth hormone deficiency (CGHD), 4 intrauterine growth retardation, 4 systemic disease, and 78 without obvious underlying disease. In 35 of the 78 subjects the 24 h integrated concentration of growth hormone (ICGH) was in the hypopituitary range ($< 3.2 \text{ ng/ml}$) and were thus diagnosed as having Neurosecretory Dysfunction (NSD). A one-year growth response to somatotropin (recombinant GH, BioTropin, Bio-Technology General, Israel) of NSD patients, 19 of whom remained prepubertal throughout the study, was compared to CGHD patients. The response of both groups was similar, as summarized below:

Patient	Height velocity (cm/yr)			
	CA	CA/BA	Pre	1 year
Group (N)				
NSD (19)	11.4 \pm 2.2	1.4 \pm 0.3	3.4 \pm 1.0	7.5 \pm 1.3
CGHD (19)	10.9 \pm 3.6	1.6 \pm 0.3	2.8 \pm 1.2	9.5 \pm 2.3

The frequency of NSD patients (45%) among ss without underlying pathology in the Rehovot region was consistent with that found in NSD diagnosed patients referred from other Israeli centers (35/78 and 99/221, respectively). The overall frequency of NSD in the population screened was 4/1000 children.