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ASSESSMENT OF GROWTH HORMONE PITUITARY RESERVE AND MAGNETIC RESONANCE IMAGING FINDINGS IN SHORT CHILDREN. A.Dammacco, S. Pesce, A. Acquafredda, A. Lorusso*, C.F. Andreola*, A. Carella* and F. Dammacco, Div. Ped. Endocrinology, Osp. "Giovanni XXIII"; CCR* and Neuroradiology*, University of Bari, Bari, Italy.

Growth hormone (GH) pituitary reserve and magnetic resonance imaging (MRI) findings were studied in 22 short prepubertal slow growing children (aged 6-11 yrs; males 14). We assessed GH responses to a combined administration of arginine (0.5g/kg) plus GH-releasing hormone (GHRH, 1ug/kg) (Arg+GHRH test) and mean spontaneous nocturnal GH secretion (MGHC, normal >3 ng/ml). Subjects were divided into 3 groups, comparable in ages, heights and growth rates: group I, 5 GH deficient children, with reduced GH pituitary reserve (Arg+GHRH GH peaks <20 ng/ml; mean±SE values=8.4±0.8 ng/ml) and low MGHC (2±0.2 ng/ml); group II, 9 GH deficient patients, with normal GH pituitary reserve ((Arg+GHRH GH peaks >20 ng/ml; 58.3±7.9 ng/ml) and low MGHC (1.9±0.3 ng/ml); group III, 8 slow growing children, with normal GH-pituitary reserve (Arg+GHRH peak: 51.1±13.2 ng/ml) and normal MGHC (5.4±0.8 ng/ml). MRI examination was performed to measure height, width, length and volumes of the pituitary gland. Results. All children showed a normal pituitary anatomy without stalk interruption; mean gland measurements were not significantly different between the 3 groups (height: 2.7±0.5, 3.5±0.3, 3.1±0.3 mm in groups I, II, III, respectively; volumes: 111.6 ± 32, 124.5 ± 15 and 126.3 ± 20 mm³, respectively). These data show that in subjects with normal pituitary anatomy and intact stalk the GH pituitary reserve is not correlated with magnetic resonance imaging findings.

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TARGETED MUTATION OF THE MOUSE CRH GENE IN EMBRYONIC STEM (ES) CELLS. L. J. Muglia¹, N. G. Copeland², N. A. Jenkins², and J. A. Majzoub¹, ¹Division of Endocrinology, Children's Hospital, Boston, MA 02115, ²NCI-Frederick Cancer Research and Development Center, Frederick, MD 21702, USA

Corticotropin releasing hormone (CRH) is known to be a major regulator of the HPA axis, but its role in relation to other modulators of ACTH secretion, i.e. vasopressin and catecholamines, is unclear. CRH has also been found in the cerebral cortex and immune system, where its functions are poorly understood. To address these questions, and also to analyze the role of CRH in development of the HPA axis we are constructing a mammalian model of CRH deficiency. We have cloned and sequenced the mouse CRH gene, and mapped its location in the mouse genome to the proximal region of chromosome 3 by interspecific mouse backcrosses. No known mutations near this region have a phenotype suggestive of CRH deficiency. We have constructed a vector which replaces the entire coding region of the CRH gene with the neo^r gene and has the herpes thymidine kinase gene flanking CRH sequences. This vector was linearized and electroporated into 6x10⁷ ES cells, which subsequently underwent selection with G418 and gancyclovir. 250 doubly resistant clones were obtained, and 2 targeted mutations with disrupted CRH genes were obtained from 87 screened. These targeted clones will be injected into blastocysts to obtain chimeric animals, which will then be bred to obtain animals hetero- and homozygous for CRH deficiency.

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L. Iughetti, S. Bernasconi, F. Facchinetti*, A. R. Genazzani*. Depts. Ped. and Ob-Gyn, Universities of Parma and Modena* - Italy. BASAL AND STIMULATED ALPHA-MSH PLASMA LEVELS THROUGHOUT PUBERTY.

α-Melanocyte-stimulating-hormone (α-MSH) is a peptide derived from a two steps cleavage of Pro-opiomelanocortin (POMC) and produced in adults by a residual zone of the original intermediate lobe. It has recently been shown that α-MSH is not only detectable in healthy adult subjects, but also that plasma levels change during menstrual cycle, concomitantly with sex steroids modifications. In addition it has been demonstrated that the peptide secretion is controlled by a dopaminergic inhibitory tone. In order to evaluate whether plasma α-MSH levels vary with advancing pubertal development and the dopaminergic tone influence the peptide release in children, 12 prepubertal and 15 pubertal subjects of both sexes underwent a domperidone stimulation (i.v. bolus dose of 0.3 mg/kg, max 10 mg). α-MSH and ACTH 1-13 plasma levels were determined before and after domperidone administration. Both peptides were measured by RIA after plasma extraction on Seppak Cartridges and reverse phase HPLC fractionation. In prepubertal children basal α-MSH (2.8±0.8 fmol/ml) and ACTH 1-13 (6.0±1.6 fmol/ml) levels were lower, though not significantly, than those in pubertal subjects (4.8±0.9 and 8.2±1.4 respectively). After domperidone both α-MSH (8.1±2.5) and ACTH 1-13 (20.7±5.2) plasma levels significantly (p < 0.05) increased in prepubertal subjects, whereas no changes were detected in pubertal ones. The results of the present study demonstrate that: 1) plasma α-MSH levels are detectable throughout childhood; 2) a functional activity of pituitary neurointermediate lobe is more evident in prepubertal than in pubertal subjects, suggesting a modified dopaminergic tone and/or anatomical changes during puberty.

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L. Ghizzoni, S. Bernasconi, L. Iughetti, C. Volta, F. Facchinetti*, A. R. Genazzani*, Department of Ped. and Ob-Gyn, University of Parma and Modena*, ITALY. β-ENDOPHIN SECRETION IS NOT INFLUENCED BY HYPERGLYCEMIA OR HYPERINSULINEMIA IN OBESE CHILDREN.

An increased opiodergic tone has been demonstrated in obese subjects and ascribed to the metabolic derangements of obesity. Based on the different beta-endorphin (β-EP) responses to oral (OGTT) and intravenous (IVGTT) glucose tolerance test, it has been suggested that the increased β-EP levels detected in obese adult patients are not only originated from the pituitary but also from peripheral sites as the gastrointestinal tract. To assess whether β-EP levels are affected by the hyperglycemia and/or hyperinsulinemia obtained by the enteral or parenteral administration of glucose in children, 24 obese children (14 F and 10 M, aged 6-17 yr) underwent either an OGTT (1.75 g/kg of glucose) or IVGTT (0.5 g/kg of glucose). Blood samples were obtained before and 0,30,60,90 and 120 min after the po glucose administration and -15, -1, before and 1,3,5,7,10,15,30,60, min after the iv glucose dose for measurements of blood insulin, β-EP and glucose levels. Insulin plasma levels significantly increased following both tests. In contrast, β-EP blood concentration did not vary after either the po or iv administration of glucose. No significant correlation between insulin and β-EP plasma levels was found. The results of the present study suggest that β-EP secretion of obese children is not influenced by metabolic factors such as hyperglycemia and/or hyperinsulinemia as that of obese adult subjects.

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STIMULATED GH SECRETION AFTER GHRH PRETREATMENT IN OBESE CHILDREN: EVIDENCE FOR SOMATOSTATIN INCREASE?

The impairment of GH secretion present in obesity could be due to an increase of the somatostatinergic tone or to a reduced pituitary production rate. To further study this problem, we evaluated 27 prepubertal obese children (18 M, 9 F; mean age 10.9±2.5 yrs) in which we studied the GH response to GHRH (n=6), insulin hypoglycemia (n=6), clonidine (n=7) and arginine (n=8) after GHRH pretreatment (1 ug/kg 120 min before the tests) and we compared the results obtained with those of 26 short normal children matched for age and pubertal status. The main results can be summarized as follows: 1) no difference was present in baseline Igf1 levels in the two groups. 2) GHRH pretreatment and all the following stimuli are able to elicit a significant GH response in obese as in controls, with the exception of arginine which in the latter group did not induce a significant GH increase. 3) No difference was found among the GH responses to the second stimuli in obese children, while in controls peak values after arginine were lower than after GHRH and clonidine. 4) The comparison between normal and obese children showed similar baseline values but higher GH levels in controls after all stimuli, but arginine after which no difference was present between the 2 groups. We conclude that the pattern of GH neuroendocrine control is similar in normal and obese children; the different behaviour after arginine, which is supposed to act through somatostatin inhibition, might be due to a chronic increase in somatostatinergic tone responsible for the lower stimulated GH levels in obesity.

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THE RATE OF CORTISOL DECREASE DURING CLONIDINE TEST INFLUENCES THE AMOUNT OF SUBSEQUENT GH RESPONSE.

INTRODUCTION. Glucocorticoids enhance or suppress the Clonidine (CL) induced GH release depending to the time of administration and CL has been claimed to decrease Cortisol (F) levels in normal adults. Our purposes were 1) to establish if results in adults are relevant in a more physiological setting in children and 2) to detect circumstance in which a low GH responsivity to CL could be explained by a peculiar pattern of HAA activation. SUBJECTS AND METHODS. 17 prepubertal subjects without GH deficit (7 females, 10 males; age 6.916 to 12.883 yrs; H-SDS: -3.065 to -0.527; BMI-SDS: -1.542 to 2.505) whose height or height growth velocity falls below the 3rd centile for age were randomly assigned to one of the following study groups: A (-15 min. CHR, 0 min CL, n:5) B (0 min CL, CHR 60 min., n:6) or C (0 min CL, 120 min CHR, n:6) (dosage in every group: CL 150 ug/m² po, CHR 4ug/kg iv). Blood samples were gained 0,15,30,45,60,90,120 min after CL and 0,5,15,30,45,60,90,120 min after CHR. F and GH were measured in every sample by RIA. RESULTS. No statistical differences in F and GH peak height between groups were detected. The F decrease rate (basal-nadir F, FD) during CL test was significantly and negatively related to GH peak (GHP) or GH Delta (GHD) (DF vs. pGH, p=0.0407, r=-0.5004; DF vs. GHD, p=0.0279, r=-0.5479). DISCUSSION. Adopted CL dosis had no effect on maximal CHR-dependent F release. Otherwise our results suggest that GH responses are sensitive to the F decrease rate during CL test. This mechanism could be advocated in the explanation of discordant effects of CL treatment on growth.