146

DISORDERS OF HYPOTHALAMO-PITUITARY FUNCTION AFTER HEAD INJURY IN CHILDREN. A. Ruszczyńska-Wolska, T.E. Romer, M. Ginalska-Malinowska, B. Rymkiewicz-Kluczyńska, Department of Endocrinology, Child Health Center, Warsaw, Poland.

Head trauma can damage the hypothalamus as well as pituitary gland, which can in effect lead to disorders in secretion of tropic hormones or to disfunction of the pituitary itself. The aim of the study was evaluate the frequency and types of hormonal disorders after head injury in children. The study was conducted on 77 patients (0.8-8 yr.) with collection of auxological data and hormonal stimulation tests (the insulin-hypoglicemia, the GRF 1-29, the TRH and GnRH test and serum and urine osmolality) which were carried out from 1 month to 7 yr. after the injury was sustained. Results are presented on tables.

Tab. 1. Type and number of hormonal disorders. * of all children after injury

	Number of cases		% of effected patients
GH peak response	< 5 ng/ml	10	13
	(5-10)ng/ml	23	29,8
	Total	33	42,8
LH FSH↑	5		6,5
ADH↓	2		2,6
GH↓ LH↑ FSH↑	7		9,1
GH↓ Cort.↓	2		2,6
Total	49		63,6*

Tab. 2. GH response to GHRH 1-29 stimulation in 37 patients with GH response (10 ng/ml in the insulin-hypoglicemia test

	Number of patients	Percentage
GH) 10 ng/ml	31	83,8
GH (10 ng/ml	6	16,2

Tab. 3. The number of hormonal disorders in relation to the severity of the sustained head injury.

Type of injury	Total number of cases	Number of disorders	Percentage
Serious injury with prolonged loss of consciousness	47	36	76,6
Injury without loss of conciousness	30	13	43,3

Only 8/49 patients with hormonal disturbances had clinical symptoms - 3 showed height deficiency, 3 precocious puberty and 2 - symptoms of diabetes insipidus. Fourty one patients showed biochemical evidences of hormonal disfunction only. Our data confirmed that the regulation of GH secretion is the most sensitive to injury - mainly on the hypothalamic level, and indicates that routine hormonal diagnostic tests are justified, especially in children who have suffered serious head trauma with prolonged loss of consciousness.

147

ESSENTIAL HYPERNATREMIA AS A RARE CAUSE OF CHILDHOOD OBESITY

C. Livieri , M. Bozzola , R. Lorini , F. Severi
Department of Pediatrics, IRCCS Policlinico S.Matteo, University of Pavia, Pavia, Italy

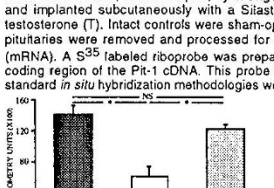
We describe two unrelated patients , a boy and a girl both referred at the age of 4 yrs because of sudden onset obesity, polyphagia, hypodipsia, hypersomnolence, mood alteration with outbrusts of hysterical laughter or cry, episodes of muscular weakness. They were found to be suffering from an hypotalamic syndrome of unknown origin. Both showed no sense of thirst even with chronic hypernatremia and hyperosmolality, severe acrocyanosis , profuse sweating , episodes of enuresis with polyuria and excretion of inappropriately dilute urine. ADH determination, performed when the patients were in good metabolic control, was in the lower normal limits. Other endocrinological investigation showed hyperprolactinemia and low GH response to provocative stimulation in the two patients. EEG revealed non-specific slow wave changes in the boy and multifocal high amplitude spikes and sharp transients in the girl. X rays of skull CT scan and MNR were normal. A defective osmoreceptor function is suspected in both patients.

●148

PIT-1 GENE EXPRESSION IN THE ANTERIOR PITUITARY IS MODULATED BY CHANGES IN CIRCULATING LEVELS OF TESTOSTERONE.

S. González-Parra*1, J.A. Chowen*2, L.M. García-Segura*2 and J. Argente1,
Autonomous University. The Hospital of Niño Jesús. Division of Growth, Endocrinology & Metabolism1. Cajal Institute, C.S.I.C.2. Madrid, Spain.

Pit-1, or GHF-1, is a transcription factor specific to the anterior pituitary and is involved in the expression of the GH, PRL and TSH-ß-subunit genes. The expression of these genes is modulated by changes in the steroid environment. Therefore, we asked the question as to whether this effect could be mediated, at least in part, by changes in Pit-1 expression. Adult male rats were castrated and implanted subcutaneously with a Silastic capsule that was either empty or that contained testosterone (T). Intact controls were sham-operated. Four days later, animals were sacrificed and pituitaries were removed and processed for *in situ* hybridization to detect Pit-1 messenger RNA (mRNA). A S^{35} labeled riboprobe was prepared by *in vitro* transcription of a 915 bp insert of the coding region of the Pit-1 cDNA. This probe was applied to 12 µm sections of the pituitaries and standard *in situ* hybridization methodologies were followed. Specific labeling was found only in the anterior pituitary, with the posterior and intermediate pituitaries serving as negative controls. Densitometric analysis of the tissue sections (8 per animal) was performed by using an automated image analysis system. Castrated animals had significantly lower levels of Pit-1 mRNA when compared to intact animals. Replacement with physiological levels of testosterone inhibited this decline (ANOVA: p<0.005). There was no significant difference between intact controls and castrated T-replaced animals.



PIT-1 mRNA in the anterior pituitary

Conclusion: Changes in circulating levels of sex steroids modulate the expression of Pit-1. Hence, the effects of sex steroids on the synthesis of certain anterior pituitary hormones may be mediated, at least in part, through the modulation of this specific transcription factor.

149

ACTIVATION OF NICOTINIC CHOLINERGIC RECEPTORS MODULATES THE SOMATOSTATINERGIC SYSTEM IN THE RAT HYPOTHALAMUS

V. Barrios*1, S. González-Parra*1 and E. Arilla*2 (Introduced by J. Argente), Autonomous University. The Hospital of Niño Jesús. Division of Growth, Endocrinology & Metabolism1. Department of Biochemistry & Molecular Biology. University of Alcalá de Henares, Madrid2, Spain.

Acetylcholine potentiates the excitatory effect of somatostatin (SS) on brain neurons and nicotine, a cholinergic drug, modifies catecholamine turnover in the hypothalamus. The hypothalamus shows a high concentration of nicotinic cholinergic receptors and a dense innervation of SS-positive nerve terminals. In light of these findings, we studied the effect of intravenous (i.v.) nicotine injection (0.3 mg/kg) on SS peptide levels and receptor binding in the hypothalamus of male Sprague-Dawley rats. A second experimental group was pretreated with mecamylamine (5 mg/kg), a centrally acting antagonist of nicotinic cholinergic receptors, in order to evaluate whether the effects of nicotine on the studied system involved the activation of these receptors. Control rats received an i.v. saline injection. The rats were killed 4 min after i.v. administration, and the hypothalamus was dissected to isolate SS and its membrane receptors.

Results: Nicotine produced an increase in somatostatin-like immunoreactivity (SLI) and in SS receptors. When the rats were pretreated with mecamylamine, the effects of nicotine were inhibited. Mecamylamine alone did not influence either parameter.

	SLI (ng/mg protein)	SS receptors	
Groups		Bmax (fmol/mg protein)	Kd (nM)
Saline	19.65 ± 2.14	152 ± 34	0.97 ± 0.13
Nicotine	160.76 ± 16.86 *	347 ± 26 *	0.95 ± 0.20
Mecamylamine plus nicotine	17.55 ± 2.06	163 ± 14	0.94 ± 0.17
Mecamylamine plus saline	18.98 ± 1.54	173 ± 10	1.04 ± 0.07

* $p < 0.01$ *vs* control

Conclusions: 1. These results suggest that the rat hypothalamic somatostatinergic system is regulated by nicotine-like acetylcholine receptors. 2. The somatostatinergic system may be involved in some of the neuroendocrine effects of nicotine.

●150

NEONATAL TESTOSTERONE MODULATES THE NUMBER AND RESPONSIVITY OF GROWTH HORMONE-RELEASING HORMONE (GHRH) NEURONS.

J.A. Chowen*1, S. González-Parra*2, L.M. García-Segura*1, and J. Argente2,
Cajal Institute, C.S.I.C.1. Autonomous University. The Hospital of Niño Jesús2. Madrid, Spain.

Exposure to sex steroids during the fetal and neonatal periods plays an important role in the organization of the hypothalamus. Since the rate of growth of the pubertal and adult animal is also affected by neonatal sex steroid exposure, we asked the question as to whether this phenomenon is due to an effect of these steroids on those hypothalamic neurons involved in stimulating GH, *i.e.*, GHRH neurons. To address this question, the following experimental groups were studied:

Group name	IM	MAC	MCOAO	MCOAT	MCTAO	MCTAT	FNOAO	FNOAT	FNTAO	FNTAT
Treatment on day 0	Sham castrated	Sham castrated	Castrated - Oil	Castrated - Oil	Castrated - TInj	Castrated - TInj	Oil	Oil	TInj	TInj
Treatment on day 60	Sham castrated	Castrated SImp	SImp	TImp	SImp	TImp	SImp	TImp	SImp	TImp

Where M=male; F=female; Oil=injection with 100 µg oil; TInj=injection with 250 µg T in 100 µg oil; SImp=empty Silastic capsule; TImp=Silastic capsule containing T. Growth was charted throughout development and was significantly affected by these treatments. Animals were sacrificed (day 75) and the brains removed and processed for *in situ* hybridization for GHRH mRNA. The total number of GHRH cells and the relative level of GHRH mRNA (analyzed by an automated image analysis system) were assessed in anatomically matched slides. Neonatal T had a significant effect on the number of detectable GHRH neurons in the hypothalamus (ANOVA: $p<0.0001$). Animals exposed to neonatal T had significantly more GHRH neurons than those that were not. Adult T-treatment did not affect

Group	IM	MAC	MCOAO	MCOAT	MCTAO	MCTAT	FNOAO	FNOAT	FNTAO	FNTAT
Number of GHRH neurons	602 ± 47	530 ± 55	315 ± 11	345 ± 12	417 ± 21	623 ± 88	297 ± 25	331 ± 51	404 ± 27	442 ± 48
Grains/cell	102 ± 7	75 ± 2	67 ± 5	86 ± 3	72 ± 6	105 ± 8	58 ± 1	75 ± 4	73 ± 2	110 ± 12

the number of detectable GHRH neurons, but significantly influenced levels of GHRH mRNA (ANOVA: $p<0.0001$). Furthermore, adult T-treatement had a significantly greater effect in those animals that had received neonatal T when compared to those animals that did not receive neonatal T (2-way ANOVA: $p<0.05$). These results suggest that one way in which exposure to sex steroids during the neonatal period affects the growth axis is by increasing the number of hypothalamic GHRH neurons, as well as to modulate the ability of these neurons to respond to changes in circulating levels of testosterone.

151

MOLECULAR ANALYSIS OF THE PROOPIOMELANOCORTIN (POMC) GENE IN 3 CASES OF CONGENITAL ISOLATED ACTH DEFICIENCY.

J.-C. Carel, I. Tardivel, X. Bertagna, P.F. Bougnères and J.-L. Chaussain, INSERM U342 and Pediatric Endocrinology, Hôpital Saint Vincent de Paul, Paris, FRANCE.

We studied the POMC gene in 3 cases (1 boy, 2 girls) of isolated ACTH deficiency with manifestations of hypocortisolism before 6 months of age, undetectable ACTH after stimulation with LPH and/or CRF, normal secretion of the other pituitary hormones and normal appearance of the pituitary on C.T. scan or M.R.I.. One patient was born to consanguineous parents and one girl had an affected brother who died in the neonatal period. DNA from the 3 patients digested with EcoRI, BglII and PstI revealed a normal pattern after hybridization with POMC-genomic probes encompassing exons 1 and 3. After digestion with SacI and hybridization with an exon 1 probe, a 10/15 kb polymorphism was detected and compatible with linkage of the disease to the POMC gene in the two families studied. PCR amplification of exons 1, 2 and 3 using primers in the flanking intronic sequences gave products of the expected size in the 3 patients. Direct sequencing of exon 2 which contains the transcription initation site and 15% of the coding sequence revealed no difference with controls and with the published sequence. Sequencing of exon 1 and 3 is under progress. We conclude that these 3 cases of congenital ACTH deficiency are not due to deletions in the POMC gene or point mutations in exon 2.