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Gs-UNIT ACTIVITY IN PSEUDOHYPOPARATHYROIDISM SYNDROME : RESULTS OF A PEDIATRIC MULTICENTER STUDY.

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Pseudohypoparathyroidism (PsHP) is a rare disorder that might be caused by a defect in the stimulatory G-proteine (Gs), which transduce the PTH receptor signal to adenylate cyclase. We investigate 35 patients (29 children and 6 adults) with PsHP syndrome. Clinical features, Ellsworth-Howard test, serum level of intact PTH (PTHi) and Gs biological activity are studied. Gs activity is measured with a technic adapted from that of LEVINE we optimized to applicate in children (Assay variation : 10 %). 24 patients (68 %) have a PsHP type Ia with a decrease of Gs-unit activity, but we don't find any relation between Albright's osteodystrophy (AHO) and Gs-unit defect : 4 children are AHO - and 20 are AHO +, and 10 of them presented an associate hypothyroidism. 2 patients (6 %) have no response to infusion of exogenous PTH with an increased of Gs-unit activity and could be PsHP type Ib. Only one patient is PsHP type II with normal Gs-unit, serum PTHi level are increased in all patients (mean = 218 pg/ml, range 65 - 571) without difference between PsHP groups, or relation with Gs-unit activity. Other patients would be classified in pseudo-PsHP with decrease Gs - biological activity. In other hand, we studied 10 families, in 9 Gs-unit activity defect is found at least in 1 member (7 times mother, 1 both mother and father, 1 father). In conclusion, Gs-unit activity appears to be of interest in the investigation of PsHP syndrome.

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Gs-UNIT ACTIVITY IN VITAMIN D DEFICIENCY RICKETS.

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35 infants, aged 13.4 ± 11.8 months with vitamin D deficiency rickets were included in the study. Calcemia, phosphorus, serum alkaline phosphatases (ALP) level and isoenzymes, osteocalcin, 25 and 1.25 dihydrovitamin D metabolites, biological activity of Gs protein were measured before and after treatment. Gs protein or stimulatory G protein transduce the signal form PTH receptor to adenylate cyclase. Quantitative analysis of Gs-unit biological activity was determined by a technic, we adapted and optimized from that of Levine to applicate in infants (Assay variation = 10 %). The procedure was based on the measure of cAMP generated in vitro. Normal range is 85-110 % of a Gs biological activity of control patients. We observed, as soon as 1 week after treatment a expected signifiant change in all parameter but ALP. Particularly, Gs-unit activity is constantly decreased but one (73 ± 15 %) and increased to normal range after treatment (98 ± 15 %) ($p < 0.002$). Low Gs-units activities observed were similar to those found in our experience about pseudohypoparathyroidism type Ia. Our results are consisting with a PTH renal resistance in infants with vitamin D deficiency rickets, which could be due to low biological activity of Gs protein, in relation with vitamin D metabolite or hyperparathyroidism.

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TRANSIENT FORAMEN OVALE INCOMPETENCE IN NORMAL NEWBORNS.

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In contrast with the generally accepted mechanism of functional closure of the foramen ovale at birth, echocardiography studies in sick newborns revealed the presence of transient interatrial left to right shunts (IALRS) in several patients without structural heart disease. The aim of this study was to assess incidence and natural history of IALRS in healthy newborns. 20 healthy term newborns underwent an echocardiographic and Doppler study daily during the first week after birth. Left atrial and aortic root dimensions were measured, as well as duration and direction of ductal and interatrial shunting. In 11 of these infants a predominant IALRS was detected on the first day of extrauterine life. In one infant IALRS persisted for as long as 6 weeks. Median persistance of IALRS in the remaining 10 infants was 3 days. Ductal left to right shunts were present in 3 infants with and in 2 without IALRS. There was no significant difference between infants with or without IARLS in respect to ductal patency, birthweight or sex. There was a statistically significant relation between lower gestational age and presence and duration of IALRS. We conclude that transient IALRS is common in normal newborns during the first week of extrauterine life. We suggest that this transient foramen ovale incompetence results from a relatively short foramen ovale flap in combination with the relative large extracellular volume as is present in the first days of life.

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CHANGES IN THE CEREBRAL CONCENTRATION OF OXIDIZED CYTOCHROME aa_3 FOLLOWING ISCHAEMIA IN FETAL SHEEP, AS MEASURED BY NEAR INFRARED SPECTROSCOPY.

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Six chronically instrumented, late gestation (119-136 days) fetal sheep were subjected to 30 minutes of total cerebral ischaemia *in utero*. Changes in the concentration of oxidised cytochrome aa_3 ($[CytO_2]$), oxyhaemoglobin ($[HbO_2]$) and deoxyhaemoglobin ([Hb]) were measured using near infrared spectroscopy (NIRS) through optodes surgically secured to the fetal skull. Cortical impedance (CI) was recorded simultaneously as a measure of cortical cytotoxic oedema. Measurements commenced prior to the insult and continued subsequently for 96 hours. Brains were examined histopathologically at the end of the study. Data were analysed by one way repeated measures ANOVA and linear regression and significant results ($p \leq 0.05$) are presented (mean±SEM). An initial fall in $[CytO_2]$ occurred during the insult ($-0.6 \pm 0.3 \mu mol.l^{-1}$) and reperfusion post insult ($-1.4 \pm 0.4 \mu mol.l^{-1}$). A further fall in $[CytO_2]$ commenced 23±3 hours after the insult and continued progressively to a maximum of -4.4 ± 1.1 $\mu mol.l^{-1}$ at 60±10 hours. Changes in $[CytO_2]$ were not temporally related to alterations in $[HbO_2]$, [Hb] or CI. The late fall in $[CytO_2]$ was positively related to % increase in CI following the insult and % neuronal loss.

CONCLUSION: Following transient ischaemia, a maximum late fall in $[CytO_2]$, as measured by NIRS, correlated with the severity of cerebral damage.

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RELATION BETWEEN CEREBRAL HAEMODYNAMICS, CORTICAL IMPEDANCE AND NEURONAL LOSS FOLLOWING BRAIN ISCHAEMIA IN FETAL SHEEP.

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The relation between alterations in cerebral haemodynamics and severity of cerebral injury following ischaemia was investigated. Six chronically instrumented, late gestation (119-136 days) fetal sheep were subjected to 30 minutes of total cerebral ischaemia *in utero*. Changes in the cerebral total haemoglobin concentration ([tHb]) (proportional to cerebral blood volume) were measured using near infrared spectroscopy (NIRS) through optodes fixed to the fetal skull. Cortical impedance (CI) was recorded simultaneously to measure cortical cytotoxic oedema. Measurements commenced prior to the insult and continued subsequently for 96 hours. Brains were examined histopathologically at the end of the study. Data were analysed by one way repeated measures ANOVA and linear regression; significant results ($p \leq 0.05$) are presented as mean±SEM. During ischaemia CI rose (140±10%) and [tHb] fell ($-28 \pm 2 \mu mol.L^{-1}$). After ischaemia [tHb] transiently increased ($24 \pm 1.4 \mu mol.L^{-1}$) for 155±25 minutes. A second hyperaemia ($34 \pm 2.5 \mu mol.L^{-1}$) beginning 14±2 hours post insult preceded a rise in CI by 3.8±1.3 hours. Injury severity, as shown by % increase in CI following the insult and % neuronal loss, was positively related to the duration of the early hyperaemia and negatively related to the delay in onset of the second hyperaemia.

CONCLUSION: Following transient ischaemia, more severe injury was associated with a prolonged early cerebral hyperaemia and earlier onset of a second hyperaemia.

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CARBOHYDRATE DEFICIENT GLYCOPROTEIN SYNDROME (CDGS) - A STORAGE DISEASE OF THE ENDOPLASMIC RETICULUM

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Carbohydrate-deficient Glycoprotein Syndrome (CDGS) is an autosomal recessive congenital disorder that affects many organ systems. It is characterized by specific physical stigmata as well as hypotonia, retardation, hepatopathy, and pericardial effusion. The basic cellular defect is supposed to be a cotranslational asparagine-N-linked oligosaccharide transfer deficiency in the endoplasmic reticulum (ER) (Yamashita et al., 1993, J. Biol. Chem. 268 : 5783-5789).

The cotranslational oligosaccharide transfer is crucial for correct folding of many newly synthesized glycoproteins. If it is inhibited by tunicamycin treatment or mutation of the glycosylation site these proteins experience severe problems in their folding process leading to irreversible aggregation and retention in the ER.

By electron microscopy we found that in CDGS fibroblasts the ER is dilated indicating a possible retention phenomenon. For a more detailed analysis several well characterized viral glycoproteins were expressed in these cells (influenza hemagglutinin, SFV spike glycoproteins). Their glycosylation and folding was investigated by immunoprecipitation with specific antibodies and subsequent analysis by non-reducing SDS-PAGE. We found that cotranslational glycosylation and folding of these model glycoproteins were undisturbed. Endoglycosidase treatments of the samples revealed that the transport to the Golgi was delayed.

Our findings demonstrate that in CDGS the cell biological defect is located at the level of the endoplasmic reticulum. However, they contradict the current hypothesis of a cotranslational glycosylation defect.