105

CORTISOL PRODUCTION RATES DO NOT INCREASE DURING CRITICAL ILLNESS IN PRETERM INFANTS

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Background: Controversy exists whether early adrenal insufficiency is present in preterm infants (PIs) or not. Urinary cortisol production rates (CPRs) provides an integrated index of adrenal function. The aim of this prospective study was to compare CPRs between well and ill PIs< 30 weeks gestational age.

Methods: PIs were classified according to clinical criteria and the score for neonatal acute physiology (SNAP). All major neonatal glucocorticoid metabolites (GMs) (n=14) were profiled by gas chromatography-mass spectrometry in 24-h urinary samples. Excretion rates of GMs were summed to calculate CPRs (μg/kg/d/mg creatinine). We developed an own non invasive urine collection procedure using specially manufactured cellulose nappies and extraction by hydraulic press.

Results: CPRs were determined in 17 well (27.9 ± 1.8 wk; 1078 ± 321g) and 44 ill (27.3 ± 1.6; 984 ± 257) PIs. Medians of SNAP and CPRs of ill (well) PIs were given in the table, p<0.05[1].

AGE	1st day	2nd day	3rd day	5th day	2nd wk	3rd wk	4th wk
SNAP	22(15)[1]	20(9)[1]	19(8)[1]	12(7)[1]	6.5(5)[1]	6 (3)[1]	5(5)[1]
CPRs	35 (40)	42(38)	48(53)	47(41)	72 (48)	73(37)[1]	54(26)

During the observational period SNAP and CPRs behaved inversely in ill PIs. CPRs were not different in ill vs well PIs during critical illness.

Conclusion: Concerning severity of illness, CPRs in ill PIs might reflect inadequate stress reaction. This could reveal persistence of intrauterine mechanisms of cortisol regulation. Supported by DFG grant (HE 3557/1–1) to M.H. and S.A.W.

106

ASSOCIATION OF TWO TUMOR NECROSIS FACTOR POLYMORPHISMS WITH THE INCIDENCE OF SEVERE INTRAVENTRICULAR HEMORRHAGE IN PRETERM INFANTS

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Background: A systemic perinatal inflammatory response is known to be a risk factor of severe intraventricular hemorrhage (IVH) in preterm infants. Molecular markers predicting susceptibility to cerebral morbidity in premature infants are missing. Genetic polymorphisms in the tumor necrosis factor (TNF) -gene are known to modify TNF expression.

Aims: To study the incidence of biallelic polymorphisms of the TNF alpha promoter region and NcoI polymorphism of the TNF beta gene in premature infants with severe IVH.

Methods: Study subjects: A double blinded retrospective cohort study was carried out on stored Guthrie blood spot cards. 27 premature infants <32 weeks of gestational age with sonographic finding of severe IVH at day 7 postnatal age (grade III IVH or IVH with apparent periventricular hemorrhagic infarction) and 102 healthy newborn infants (>32+0 weeks of gestation, no signs of severe IVH on ultrasound examination at 7 days postnatal age) were included in the study. TNF allele distribution of the study population was also compared to cohorts of healthy adult volunteers. **Laboratory investigation:** To detect microsatellite nucleotide polymorphism in the TNF gene, polymorphism of the TNF alpha promoter-308 region and NcoI polymorphism of TNF beta gene was assessed using polymerase chain reaction (PCR) followed by melting curve analysis or NcoI digestion.

Results: The overall allele frequency and genotype distribution of the -308 TNF-alpha polymorphism were comparable with values found in controls and no difference was found with regards to gender. The overall incidence of the TNF beta 2 allele was higher in the IVH group compared to the control group (81.5% vs.63.2%; p=0.01). Allele distribution of a polymorphic site within the TNF beta locus in the male patient group significantly differed from the distribution in the control group (TNF beta 1/2 frequency: 13.3% / 86.7% vs. 36.8% / 63.2%; p=0.01). Male patients showed a significantly higher prevalence of the homozygous genotype for the TNF beta 2 allele (80% vs. 44%; p=0.04).

Conclusion: The study indicates a predominance of the TNF beta 2 allele and genotype of a proinflammatory cytokine like TNF in male gender to be linked to a major neuropathological outcome parameter in premature infants.

107

VASCULAR ENDOTHELIAL GROWTH FACTOR AND TRANSFORMING GROWTH FACTOR BETA-1 IN THE CSF OF NEONATES WITH POSTHEMORRHAGIC AND NON-HEMORRHAGIC HYDROCEPHALUS

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Background: The expression of specific growth factors like vascular endothelial growth factor (VEGF) and transforming growth factor (TGF) beta-1 is of importance during brain development and in the pathogenesis of neurodegenerative disorders. Aims: To study VEGF and TGF beta-1 in the cerebrospinal fluid (CSF) of neonates with posthemorrhagic and non-hemorrhagic hydrocephalus. To determine the interference of inflammatory cytokine interaction with the expression of VEGF and TGFbeta-1, IL-6 and IL-10 CSF concentrations were measured.

Methods: 18 neonates (median birth weight 676g, median gestational age 25+0 weeks) with posthemorrhagic hydrocephalus (PHHC) undergoing serial reservoir puncture, and 9 neonates (median birth weight 2750g, median gestational age 34+3 weeks) with congenital non-hemorrhagic hydrocephalus (CHC) undergoing first shunt surgery, were included in the study. CSF samples of 11 neonates (median birth weight 2230g, median gestational age 33+4 weeks) with lumbar puncture for the exclusion of meningitis served as controls. VEGF, TGFbeta-1, IL-6 and IL-10 concentrations in the CSF were measured by ELISA technique.

Results: VEGF concentrations in the CSF of patients with PHHC were significantly higher (median 377pg/ml, range 101-1301pg/ml) when compared to patients with CHC (median 66pg/ml, range 3–1991; p<0.001) and controls (median 2pg/ml, range 0–12pg/ml, p<0.0001). TGFbeta-1 CSF concentrations did not differ from control infants in all groups. Median IL-6 and IL-10 concentrations in the CSF were found to be low in all patient groups.

Conclusion: Increased release of VEGF in the CSF of neonates with PHHC and CHC may serve as an indicator of brain injury from progressive ventricular dilatation. TGFbeta-1 CSF concentrations are not elevated in the phase of acute fibro proliferative reactions in patients with PHHC.

108

EXPRESSION OF EPITHELIAL SODIUM CHANNEL (ENAC) IN THE AIRWAYS OF HEALTHY TERM INFANTS DURING THE FIRST POSTNATAL DAYS

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Background: ENaC is considered critical for the movement of liquid across respiratory epithelium. Functional impairment of liquid transport may participate in the pathogenesis of respiratory insufficiency. In preterm infants with respiratory distress syndrome ENaC expression is lower than in healthy term infants. We wanted to study how expression of ENaC changes over the first postnatal days.

Methods: We studied 35 healthy term infants (GA 39.5±1.3 wks). Samples of respiratory epithelium were obtained by scraping both nostrils at 1–4, 23–27 and 45–48 hours after birth. The samples were subjected to RT-PCR using real-time PCR for analysis of expression of alpha-, beta- and gamma-ENaC and cytokeratin 18 (CK18) which was used as an epithelial marker. Reactions were performed in singleplex format.

Results: In healthy term infants expression of alpha-ENaC was 8.2±4.0, 7.7±6.6 and 7.5±5.9 amol/fmol CK18 at 1–4, 23–27 and 45–48 hours after birth, respectively. Expression of beta-ENaC decreased significantly during the first 27 postnatal hours from 12.0±8.1 to 4.1±4.2 amol/fmol CK18 (p=0.0001). At 45–48 hours after birth beta-ENaC was 2.7±3.6 amol/fmol CK18. Expression of gamma-ENaC was 19.2±34.9, 8.7±23.6 and 27.5±101.6 at 1–4, 23–27 and 45–48 hours after birth, respectively.

Conclusion: In the healthy newborn infant, expression of alpha- and gamma-ENaC remain constant over the first 48 postnatal hours, whereas there is a significant decrease in the expression of beta-ENaC. Beta-ENaC has been considered to have a regulatory function in the ENaC complex. We conclude that the expression of beta-ENaC may play a role in the postnatal pulmonary adaptation of the healthy newborn infant.

109

EPISODIC ALCOHOL CONSUMPTION DURING PREGNANCY: MECHANISMS OF FETAL BRAIN INJURY

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Background: Exposure to alcohol during fetal life has been associated with poor neurodevelopmental outcome; episodic (binge) exposure is considered to particularly harmful. However, the mechanisms leading to altered neurological development remain to be fully elucidated. **Objective:** The aim of these on-going studies is to identify mechanisms of altered fetal brain development following repeated, "binge" exposure to ethanol (EtOH).

Methods: EtOH (1g/kg of maternal weight) was administered to 7 twin-bearing, chronically catheterized pregnant ewes over 1 hour on 3 consecutive days at 0.7 of gestation. We measured fetal and maternal blood alcohol concentrations (BAC), as well as fetal blood gases, glucose and lactate concentrations, mean arterial pressure (MAP) and heart rate. Fetal brains were subsequently analysed histologically. Data were analysed by ANOVA and are presented as mean ±SEM.

Results: BAC in the mother and fetus reached maximal values of 0.11±0.01 g/dL (~20 mmol/L) 1h after the start of EtOH infusions. Following the EtOH infusions, fetal pH significantly increased to reach a maximum at 4h (7.37±0.004) and was still elevated at 6h relative to pre-EtOH values of 7.34±0.003. Fetal PaCO2 decreased significantly from pre-EtOH (48.7±0.7 mmHg) to reach minimum values at 4h (43.2±0.9 mmHg) and it remained decreased at 6h. Fetal PaO2 increased significantly after EtOH, relative to pre-EtOH values (17.8±0.7 mmHg) to reach peak values at 2h (20.5±0.3 mmHg) and 4h (20.4±0.6 mmHg) and it remained elevated at 6h. Fetal blood cortisol levels did not change. No significant changes were observed in maternal blood gases or glucose levels; however maternal blood lactate significantly increased from pre-EtOH values (0.5±0.07 mmol/L) to reach a maximum value at 2h (1.7±0.14 mmol/L) and it remained elevated at 6h. Histological analysis revealed white matter gliosis in 3 of 8 EtOH treated fetuses studied, white matter damage in the cerebellum of 2 of 8 EtOH fetuses and no hippocampal damage in any fetus.

Conclusions: Alterations in fetal blood gases and glucose levels following EtOH are suggestive of reduced metabolic activity by the fetus. Fetuses were not hypoxemic; therefore gliosis noted in some fetal brains induced by EtOH are most likely to be a result of mechanisms other than cerebral hypoxia.

110

REDUCED EXPRESSION OF MICROSOMAL PROSTAGLANDIN SYNTHASE 1 ATTENUATES VENTILATORY EFFECTS OF INTERLEUKIN-1A IN NEONATAL DBA/1LACJ MICE

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Background: Infection and apnea represent major medical concerns in the neonatal population, and the pro-inflammatory cytokine interleukin-1Â (IL-1Â) may serve as a critical link between these events. We recently showed that IL-1Â depresses respiration and anoxic survival via a prostaglandin-dependent mechanism (Olsson et al, 2003). In brain, microsomal prostaglandin E synthase 1 (mPGES-1) has been demonstrated crucial for the development of endotoxin-induced fever and is widely expressed in endothelial cells of the blood-brain-barrier after IL-1Â provocation. These same cells also express the IL-1 receptor as well as cyclooxygenase-2, which catalyzes the formation of prostaglandin H2 (PGH2) from arachidonic acid. mPGES-1 subsequently synthesizes prostaglandin E2 (PGE2) from PGH2. This study tested the hypothesis that IL-1Â alters respiratory control in newborn mice via a PGE2-mediated pathway.

Methods: Respiratory behavior was examined using flow plethysmography in 9 day-old DBA/1lacJ wildtype mice and heterozygote knockout mice for mPGES-1. At 70 min after an intraperitoneal injection of either IL-1Â or saline, mice were placed unrestrained in the plethysmograph chamber. Basal respiration and the ventilatory response to anoxia (100% N2) and hyperoxia (100% O2) were then examined. Genotyping was performed after experimentation in all animals.

Results: During normoxia, wildtype mice given IL-1Â exhibited a reduced respiratory frequency and tidal volume compared to control animals. Heterozygote knockout mice for mPGES-1 experienced less depression of basal respiration by IL-1Â. In response to anoxia, the IL-1Â-treated wildtype mice displayed fewer gasps and were unable to sustain gasping efforts for as long as control animals. They also were less able to autoresuscitate and survive following severe hypoxic apnea compared to control animals. These deleterious effects of IL-1Â were prevented in mice with a reduced expression of mPGES-1. Skin temperature was similar between groups prior to and following experimentation, suggesting that the results observed here are not due to confounding systemic changes induced by IL-1Â.

Conclusion: This study indicates that IL-1Â adversely affects respiration and autoresuscitation in newborn mice and that these changes are mediated by PGE2. These findings may have clinical implications for the screening and treatment of neonatal apnea associated with infection.