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Original article

Excitatory amino acid release and electrocortical brain activity after hypoxemia in near-term lambs

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Abstract

Background: Energy failure due to insufficient cerebral O_2-supply leads to excess accumulation of calcium ions in presynaptic neurons, followed by excess release of excitatory amino acids (EAAs), which are potent neurotoxins, into the synaptic cleft. *Aim:* The aim of the present study was to determine whether extracellular EAAs release after prolonged hypoxemia affects electrocortical brain activity (ECBA), as a measure of brain cell function, in near-term born lambs. *Methods:* Ten near-term lambs (term: 147 days) were delivered at 131 days of gestation. After a stabilization period, prolonged hypoxemia (FiO_2: 0.10; duration 2.5 h) was induced. Mean values of physiologic variables, including ECBA, were calculated over the last 3 min of normoxemia as well as of hypoxemia. Cerebral arterial and venous blood gases were determined at the end of the normoxemic and hypoxemic periods. Cerebrospinal fluid (CSF) was obtained at the end of the hypoxemic period. CSF from six normoxemic sibs was used for comparison. HPLC was used to measure EAAs in the CSF. *Results:* During hypoxemia, aspartate and glutamate concentration increased significantly (4.8 and 6.0 times, respectively), while asparagine and glutamine did not. ECBA decreased to 30% of the normoxemic value. Glutamate was significantly higher in lambs with a flat cerebral function monitor (CFM) tracing than in lambs with a burst–suppression pattern. *Conclusions:* After prolonged hypoxemia aspartate and glutamate accumulated excessively in the CSF of near-term born lambs. Especially glutamate concentrations in CSF were related to the decline in brain cell function.

Keywords: Excitatory amino acids; EEG; Hypoxemia; Preterm

1. Introduction

Adequate oxygen availability and blood flow are prerequisites for normal brain function and growth. In preterm newborns, cerebral oxygenation and hemodynamics are easily disturbed because of the immaturity of various organ systems, e.g. pulmonary and cardiovascular systems. Long-term follow-up data of children born before 32 weeks of gestation show that 10% of them develop cerebral palsy and 50% exhibit cognitive and behavioral problems [1,2].

A major problem in neonatology is the rapid and reliable determination of the severity of a hypoxemic and/or ischemic insult so as to allow the appropriate management in order to prevent or reduce cerebral damage.

Glutamate is one of the principle excitatory amino acid (EAA) neurotransmitters in the mammalian brain. Glutamate is not degraded, but instead is removed from the synaptic cleft by energy-dependent neuronal and glial uptake transporters [3]. A decline in the cerebral arterial oxygen content (CaO_2) reduces the cerebral energy production. Neurons are very sensitive to a reduced supply of oxygen and glucose. Depletion of cellular energy stores

Abbreviations: $Ca(v)O_2$, cerebral arterial (venous) oxygen content; CBF, cerebral blood flow; CFM, cerebral function monitor; CSF, cerebrospinal fluid; EAA, excitatory amino acids; ECBA, electrocortical brain activity; EEG, electroencephalogram; MABP, mean arterial blood pressure; OD, outer diameter; PaO_2, arterial oxygen tension; $PaCO_2$, arterial carbon dioxide tension; Q_{car}, carotid artery blood flow; RMS, root mean square; $Sa(v)O_2$, arterial (venous) oxygen saturation.

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that accompanies prolonged hypoxia, results in depolarization of neurons and glia and the release of EAAs into the extracellular space. Energy-dependent re-uptake mechanisms become compromised. Imbalance between the mechanisms responsible for synaptic release and uptake of glutamate could cause glutamate accumulation in the synaptic cleft and excessive excitation of EAA receptors. Overexcitation of EAA receptors can initiate a cascade of events leading to excessive calcium entry, neuronal injury and death [4].

In normal conditions, glutamate, as a neurotransmitter, has a physiological role in electrical signaling, which can be monitored by EEG-like measurements. However, during conditions of compromised energy status of the brain, glutamate becomes an excitotoxic agent. Energy failure in the brain leads to a blockade of neuronal synaptic function and reduced electrical firing of neurons. This is reflected in recordings of electrocortical brain activity (ECBA), which can reveal a general picture of the functional state of the brain [5]. Integrated electroencephalogram (EEG)-signals have been found to correlate with the number of firing neurons [6]. A disadvantage of EEG is that it requires the presence of an expert interpreting the large data volumes. In an effort to solve this problem, various methods of compressing the EEG signal have been developed, the cerebral function monitor (CFM) being one of them. Non-invasive recording of electrocortical brain cell activity by means of EEG and CFM-like signals (e.g. ECBA) in the newborn period can be used as a measure for brain cell function [5]. Moreover, abnormal tracings are related to neonatal death and in the survivors to impaired neurodevelopmental outcome [7,8]. One of the major problems in the care for high risk neonates is to determine whether a baby's brain has suffered severe, irreversible damage [9]. The extent of the damage is important with respect to the reversibility of the compromised brain function.

Tan et al. [10] observed an on average two-fold increase in aspartate and glutamate concentrations in the cerebral extracellular space after 30 min of cerebral ischemia in near-term fetal lambs. Furthermore, cortical EEG activity appeared to be suppressed during that ischemic insult. However, no relationship between the extent of EEG suppression and increase in aspartate and glutamate concentrations was assessed.

The aim of the present study was to study the relationship between extracellular EAA concentrations and the suppression of electrocortical brain activity (ECBA) during prolonged hypoxemia in near-term born lambs.

2. Materials and methods

The study was approved by the Institutional Animal Care and Use Committee of the University of Nijmegen before implementation. The experiments were carried out in accordance with the European Communities Council Directive (86/609/EEC).

2.1. Animal preparation and instrumentation

Pregnant ewes of Dutch Texel breed were intubated, mechanically ventilated with a mixture of air and oxygen, and operated under general anesthesia with 3% isoflurane at 131 days of gestation (term 147 days). The ewes were placed in the left lateral tilt to reduce aortal compression by the uterine contents. After a polyvinyl catheter was inserted into the ewe's jugular vein, isoflurane anesthesia was replaced with infusion of 600 mg/h ketamine hydrochloride and 15 mg/h midazolam. An arterial catheter was placed into the left carotid artery of the ewe for measurement of arterial blood pressure and blood gas sampling. A pregnant horn of the uterus was exposed through a midline incision in the ewe's abdomen, and a uterine incision was made over the fetal head of one lamb only. Siblings were kept in utero and were not instrumented. They were used to determine (fetal) normoxemic values of extracellular EAA concentrations in the cerebrospinal fluid (CSF). The ewe was monitored throughout the experiment and was kept in an optimal ventilatory (PaO_2: 10–15 kPa, $PaCO_2$: 4–5 kPa, pH 7.3–7.4) and circulatory (mean arterial blood pressure (MABP): 100–120 mmHg) condition.

The fetus' head and right fore limb were exteriorized and an occluder was placed around the umbilical cord, but was not clamped yet. The fetal body weight was estimated for drug administration. A polyvinyl catheter [outer diameter (OD) 2.1 mm] was placed in the right brachial vein for administration of ketamine hydrochloride (10 mg/kg h), glucose 5% (2 ml/kg h) and antibiotics (amoxicilline and gentamicine). Furthermore, the right brachial artery (polyvinyl catheter, OD: 2.1 mm, with its catheter tip in the arcus aortae) and right jugular vein (polyurethane catheter, OD: 0.9 mm) were cannulated for measurement of the arterial blood pressure and arterial and venous blood gas sampling. The venous catheter was inserted in the cranial direction of the right jugular vein to obtain information on the venous cerebral compartment. Arterial and venous blood gases were analyzed with a blood gas analyzer (ABL 510, Radiometer Medical A/S, Copenhagen, Denmark). Oxygen saturation values were corrected for interspecies differences according to Nijland et al. [11]. Arterial and venous blood pressures were measured with disposable transducers (Edwards Life Sciences BV, Los Angeles, USA).

After exposing the left carotid artery, we applied an appropriately sized perivascular ultrasonic blood flow transducer (2SL, S or B, Transonic System Inc., New York, USA) to fit around the vessel in order to assess changes in carotid artery blood flow (Q_{car}). Changes in Q_{car} were used to assess changes in cerebral blood flow (CBF), since a close linear relationship between Q_{car} and CBF (determined with radioactive microspheres) was reported by van Bel et al. [12].