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Perinatal brain damage—from pathophysiology to prevention

Arne Jensen*, Yves Garnier, Johannes Middelanis, Richard Berger

Department of Obstetrics and Gynecology, Universitätsfrauenklinik Bochum, Knappschaftskrankenhaus, In der Schornau 23-25, 44982 Bochum, Germany

Abstract

Children undergoing perinatal brain injury often suffer from the dramatic consequences of this misfortune for the rest of their lives. Despite the severe clinical and socio-economic significance, no effective clinical strategies have yet been developed to counteract this condition. This review describes the pathophysiological mechanisms that are implicated in perinatal brain injury. These include the acute breakdown of neuronal membrane potential followed by the release of excitatory amino acids such as glutamate and aspartate. Glutamate binds to postsynaptically located glutamate receptors that regulate calcium channels. The resulting calcium influx activates proteases, lipases and endonucleases which in turn destroy the cellular skeleton. The acute lack of cellular energy during ischemia induces almost complete inhibition of cerebral protein biosynthesis. Once the ischemic period is over, protein biosynthesis returns to preischemic levels in non-vulnerable regions of the brain, while in more vulnerable areas it remains inhibited. A second wave of neuronal cell damage occurs during the reperfusion phase induced by the postischemic release of oxygen radicals, synthesis of nitric oxide (NO), inflammatory reactions and an imbalance between the excitatory and inhibitory neurotransmitter systems. Clinical studies have shown that intrauterine infection increases the risk of periventricular white matter damage especially in the immature fetus. This damage may be mediated by cardiovascular effects of endotoxins leading to cerebral hypoperfusion and by activation of apoptotic pathways in oligodendrocyte progenitors through the release of pro-inflammatory cytokines. Knowledge of these pathophysiological mechanisms has enabled scientists to develop new therapeutic strategies which have been shown to be neuroprotective in animal experiments. The potential of such therapies is discussed here, particularly the promising effects of postischemic induction of mild cerebral hypothermia, the application of the calcium-antagonist flunarizine and the administration of magnesium.

Keywords: Perinatal neuroprotection; Asphyxia; Brain damage; Fetus; Intrauterine infection; Periventricular leukomalacia

1. Introduction

Perinatal brain injury is a major contributor to perinatal morbidity and mortality [1]. Thus, in Germany alone approximately 1000 children per year experience brain damage from perinatal hypoxic-ischemic insults. A considerable number of these children will develop cerebral palsy (Fig. 1) [1,2]. The resulting impact on the children affected and their families is considerable and their subsequent care requires a high level of commitment and co-operation between pediatricians, child neurologists, physio-, speech-, and psychotherapists, and other specialists. Despite the severe clinical and socio-economic significance, the efficacy of present clinical strategies is too low to reduce the incidence of perinatal hypoxic-ischemic brain damage significantly. However, an increasing number of experimental studies describe the pathophysiological mechanisms that are involved in perinatal brain injury. Based on these pathophysiological mechanisms a variety of excellent neuroprotective strategies have been developed in various animal models. Obstetricians and neonatologists now face the important task of testing these neuroprotective strategies in clinical trials.

2. Pathophysiology

Perinatal brain injury is usually caused by cerebral ischemia, cerebral hemorrhage or an ascending intrauterine infection as shown by recent clinical studies. This review will focus on the pathophysiological pathways that are activated by cerebral ischemia or inflammatory processes, and will describe the mechanisms that are involved in cerebral hemorrhage.

3. Cerebral ischemia

3.1. Acute energy breakdown and calcium-overload

The normal functioning of the brain is inevitably dependent on adequate oxygen and glucose supply. Acute

* Corresponding author. Tel.: +49-234-2993300; fax: +49-234-2993309.
E-mail address: arne.jensen@ruhr-uni-bochum.de (A. Jensen).

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Fig. 1. Spastic diplegia in children with cerebral palsy [2].

reduction in cerebral oxygen delivery will lead to a breakdown of neuronal energy metabolism within a few minutes [3]. The Na^+/K^+-pump then stops working and the energy-depleted cell takes up Na^+, Ca^{2+} and Cl^- ions. The so-called calcium-overload activates lipases, proteases and endonucleases, which in turn destroy the cellular skeleton [4].

3.2. Glutamate release

Neuronal depolarization induces presynaptic release of glutamate, an excitatory neurotransmitter, which binds to postsynaptically located glutamate receptors. Three classes of ionotropic glutamate receptors have been identified on the basis of their pharmacological response (NMDA-, AMPA- and kainate-receptors) [5]. In addition, glutamate also activates metabotropic receptors that regulate intracellular G-protein signal cascades. The activation of each of these receptors leads to an increase in the level of free intracellular calcium. The NMDA-receptors regulate calcium channels, and the metabotropic receptors induce a depletion of intracellular calcium stores while the AMPA/KA-receptors open a voltage-dependent calcium channel by membrane depolarization [6]. As already mentioned, the cytosolic increase in free calcium activates proteases, lipases and endonucleases which in turn initiate processes leading to cell death [4]. There is no doubt that glutamate release plays a critical role in neuronal cell death after focal cerebral ischemia. Here, glutamate antagonists have been shown to exert a strong neuroprotective effect against hypoxic-ischemic brain damage in adult as well as in neonatal animals ([7,8]; for review: [9]).

3.3. Inhibition of protein synthesis

Acute breakdown of energy metabolism causes disturbances in protein synthesis. As shown in animal experiments, protein synthesis is reduced both during ischemia and in the early postischemic phase in vulnerable and non-vulnerable brain areas [10]. At the end of the ischemic period, protein synthesis in non-vulnerable regions recovers to preischemic levels, while in vulnerable regions it remains inhibited [11]. Similar findings have been reported in connection with developmental variations in the response of the brain to ischemic insults: Protein synthesis in the fetal brain was found to recover much faster from ischemic insults than that it did in adult brains [12]. The prolonged inhibition of protein synthesis is therefore an early indicator and possibly also one of the causes of neuronal cell damage arising after ischemia.

Electron microscopic and biochemical studies have shown that postischemic inhibition of protein synthesis is accompanied by a disaggregation of the polyribosomes [10]. This disaggregation seems to occur not during but after ischemia, and involves a dissociation of the monoribosomes into their smaller and larger subunits [10]. Ribosomes disaggregate when the starting of a new polypeptide chain takes longer than chain extension or termination. The disaggregation of the polyribosomes cannot occur during ischemia, because the breakdown of energy metabolism affects all stages of protein synthesis (initiation, elongation and termination). However, after ischemia, the recovered energy metabolism reactivates only the chain elongation and termination stages of protein synthesis, and not initiation. This leads inevitably to a disaggregation of the polyribosomes and a sustained inhibition of protein biosynthesis [13].

3.4. Secondary neuronal cell death

In cerebral tissue capable of recovery after an ischemic insult, energy metabolism is restored very rapidly [12,13]. A few hours later, however, the energy status is diminished once again in the affected tissue. Simultaneously, a secondary cell edema develops, followed by epileptogenic activity that can be monitored on EEG. These events are likely to be caused or at least modulated by oxygen radicals, nitric oxide (NO), inflammatory reactions and excitatory amino acids, particularly glutamate (for review: [9]).

3.5. Oxygen radicals

After cerebral ischemia oxygen radicals are produced through activation of a variety of pathways involving xanthine oxidase, superoxide dismutase and the Haber–Weiss reaction. These radicals then destroy cellular membranes and cause various forms of tissue damage [14]. Similarly, the increased rate of arachidonic acid metabolism in brain tissue or activated leucocytes after ischemia can also produce large amounts of oxygen radicals (for review: [15]). The significance of oxygen radicals for the development of secondary neuronal cell loss after cerebral ischemia has been demonstrated by investigations studying the neuroprotective effect of xanthine oxidase inhibitors such as allopurinol.

Thus, in a neonatal rat model of perinatal hypoxic-ischemic brain damage, allopurinol completely protected the animals of the study group from severe neuronal cell loss [16].

3.6. Nitric oxide

Another radical that contributes to secondary brain injury is nitric oxide. During cerebral ischemia, a massive influx of intracellular calcium takes place through various channels, regulated, among other things, by the neurotransmitter glutamate [17]. The rise in intracellular calcium activates NO-synthase, which produces NO, citrulline and water from arginine, NADPH and oxygen [18]. NO then combines with superoxide radicals to produce peroxynitrite that spontaneously decomposes to form hydroxyl radicals, nitrogen dioxide and NO^{2+} [19]. Thus NO, like free iron, can raise the toxicity of superoxide radicals significantly by converting them into highly potent radicals which in turn cause neuronal cell damage. The neuronal toxicity of NO after cerebral ischemia was elegantly demonstrated by Hamada et al. [20]. They applied an inhibitor of the NO-synthase to neonatal rats 1.5 h before an hypoxic-ischemic insult, a procedure that had a highly neuroprotective effect. In neonatal nNOS deficient mice similar results were observed following cerebral ischemia, with a reduction in hippocampal and cortical damage compared to the wild type [21].

3.7. Epileptogenic activity

Epileptogenic activity arising several hours after an ischemic insult also seems to aggravate brain injury as shown by Gluckman and co-workers. They observed epileptiform activity in mature sheep fetuses about 8 h after 30 min of global cerebral ischemia, which reached a peak 10 h after the ischemic period [22]. This epileptiform activity could be completely inhibited by application of the glutamate antagonist MK-801. Interestingly, the suppression of the epileptiform activity was accompanied by a marked reduction in brain damage in the treated animals [23]. This suggests that a secondary wave of glutamate release or an imbalance between excitatory and inhibitory neurotransmitters during reperfusion may induce epileptiform bursts of neuronal activity that can lead to an uncoupling of cell metabolism and blood flow. This would automatically impair pathways of energy metabolism and cause secondary neuronal cell damage.

3.8. Apoptosis

Over the last few years an increasing body of evidence has accumulated showing that after cerebral ischemia neuronal cell damage occurs not only through necrotic but also through apoptotic processes. In necrosis, cell death is triggered by an overwhelming external insult damaging cellular organelles such as mitochondria. This results in the loss of membrane integrity and the leakage of cytoplasmic contents into the extracellular matrix. In contrast, cells dying by apoptosis undergo a well conserved and highly regulated genetic programme of cell death. They do not lose membrane integrity and the organelles remain largely intact. In the final stages, cell fragments are 'shrink-wrapped' in the contracting plasma membrane and bud off as apoptotic bodies which are subsequently phagocytosed by healthy neighbouring cells [24]. This process largely circumvents inflammatory reactions. Apoptosis is a biochemically and genetically programmed cell death that requires time, energy and, to a certain extent, new gene transcription and translation (for review: [25]). One pathway that is implicated in apoptotic cell death is the release of cytochrome *c* from the mitochondria through the permeability transition pore. This pore is controlled by the anti-apoptotic Bcl-2 proteins. Once in the cytosol, cytochrome *c* can form a complex with Apaf-1 and caspase-9. This results in the activation of caspase-9 which, in turn, proteolytically activates caspase-3. Caspase-3 is a protease that cleaves vital proteins and triggers apoptotic execution by activating downstream caspases and endonucleases. Many of these processes have been shown to be significantly involved in secondary neuronal cell death after perinatal hypoxic-ischemic insults [25].

4. Infection related cerebral injury

As demonstrated by a variety of recent studies, inflammatory reactions not only aggravate secondary neuronal damage after cerebral ischemia, but may also affect the immature brain directly. Thus, after chorioamnionitis the incidence of immature babies suffering from periventricular leucomalacia and peri- or intraventricular hemorrhage (PIVH) is significantly increased [26–29]. However, it remains unclear whether fetal brain damage following endotoxemia is the result of cerebral hypoperfusion caused by circulatory decentralization or whether it is caused by a direct cytotoxic effect of endotoxins on cerebral tissue. To clarify this point, we performed several sets of in vitro and in vivo experiments.

First, we studied the effects of lipopolysaccharides (LPS) on circulatory responses in chronically instrumented immature fetal sheep before, during, and after 2 min of intrauterine asphyxia [30]. Within 1 h after i.v. injection of LPS (53 ± 3 μg/kg estimated fetal weight) there was a marked fall in arterial oxygen saturation and pH. Whereas blood flow to the placenta severely decreased, that to the carcass rose. Shortly after asphyxia oxygen delivery to the cerebrum was minimal (Fig. 2).

Secondly, we examined the effects the chronic intravenous exposure to very low doses of LPS (100 ng) on fetal cardiovascular function and brain pathology [31]. Intravenous application of LPS caused a substantial and longlasting decrease in umbilical blood flow resulting in sustained fetal hypoxemia without acidemia (Fig. 3). In the LPS treated

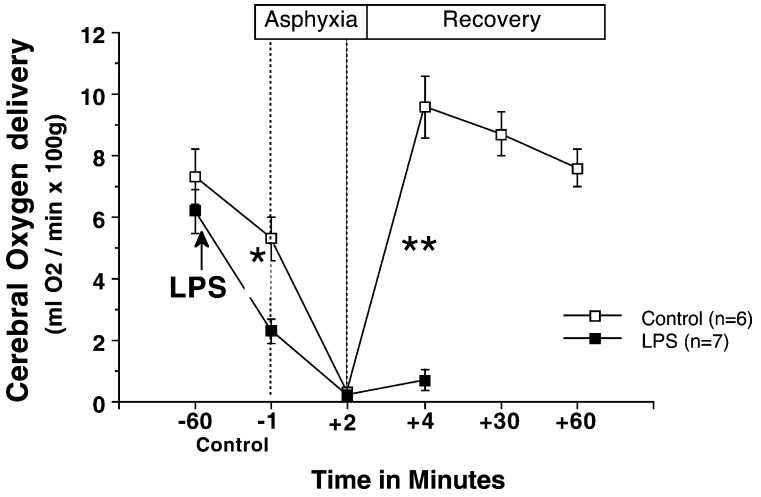

Fig. 2. Oxygen delivery to the cerebrum (ml O_2/min × 100 g) in control ($n = 6$) and LPS treated ($n = 7$) immature fetal sheep before, during and after arrest of uterine blood flow for 2 min. During the immediate recovery period there was still a severe cut back in delivery of oxygen in fetuses of the study group [30]. Values are given as means ± S.E.M. The data were analysed within and between groups using a two-way ANOVA followed by Games–Howell post-hoc test ($^{*}P < 0.05$; $^{**}P < 0.01$).

fetuses perivascular accumulation of polymorphonuclear cells was detected in the periventricular white matter. On electronmicroscopical evaluation these cells appeared to be activated microglia. Furthermore, LPS increased the rate of apoptotic cells in the periventricular region [31].

In a further experimental design we studied the effects of LPS on hippocampal slices from mature fetal guinea pigs [32]. The slices were incubated in artificial cerebrospinal fluid and gassed with carbogen (95% O_2/5% CO_2). After applying LPS (4 mg/l) to the incubation medium the release of TNF-α significantly rose over the following 12 h. Although energy metabolism and protein synthesis were not disturbed, it was thought the released TNF-α might induce apoptotic processes in oligodendrocytes and their progenitors, the cell line which is mainly affected in periventricular leucomalacia. To elucidate this point we established a primary culture of oligodendrocyte progenitors. Applying TNF-α to the incubation medium for 48 h resulted in a significant

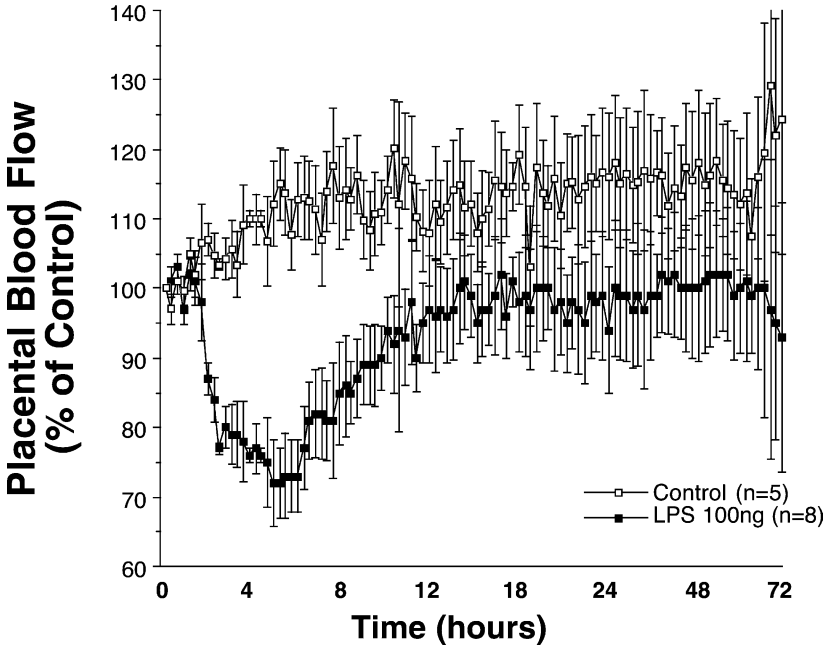

Fig. 3. Placental blood flow after chronic exposure to endotoxin (lipopolysaccharides, LPS) in chronically catheterized immature fetal sheep (0.75 of gestation) [31]. Measurements were performed with a transonic flow probe placed around the common umbilical artery. Placental blood flow began to fall 1 h after LPS infusion and was minimal (−30%, $P < 0.01$) at 4–5 h after LPS. Thereafter, placental blood flow slowly returned to control values at 10–12 h after LPS. Data were analyzed by two-way ANOVA and a Games–Howell post-hoc test.

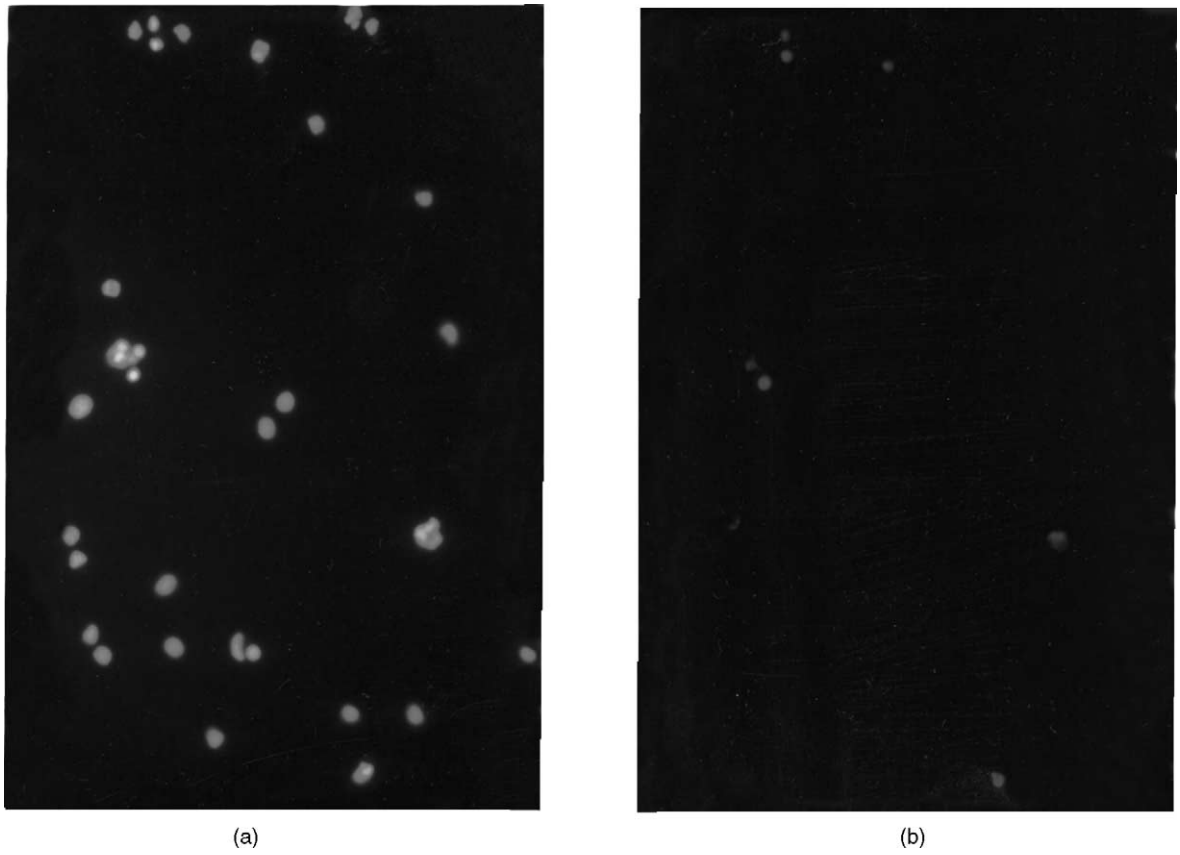

Fig. 4. Oligodendrocyte progenitor cells separated from mixed glial cells cultures were cultured in neurobasal-medium (B27-supplement + 10 ng/ml PDGF + 10 ng/ml bFGF). After 24 h 20 ng/ml TNF-α was applied to the incubation medium and the cells were fixed with 4% paraformaldehyde 48 h later. Cellular nuclei were visualized with Hoechst dye (a). Apoptotic cells were identified by TUNEL-staining (TRITC) (b) [33].

rise in the rate of apoptotic cells (Fig. 4) [33]. In a final study, we set out to clarify whether LPS might aggravate an hypoxic-ischemic injury in the immature brain. For this, we subjected neonatal rats to an hypoxic-ischemic insult using a combination of a common carotid artery ligation and hypoxic exposure for 60 min. One hour before the insult the rats received either NaCl or 5 μg LPS into the cisterna magna. Interestingly, the resulting neuronal cell damage in the cerebral cortex was significantly larger in the animals of the study group than in those of the control group [34].

From these experiments we conclude that an ascending intrauterine infection may affect the immature brain in two ways. First, lipopolysaccharides induce the release of cytokines such as TNF-α from astrocytes and microglia which will damage oligodendrocyte progenitors and cause periventricular leucomalacia. Second, the infection may cause severe circulatory decentralization accompanied by cerebral hypoperfusion which then in turn results in a hypoxic-ischemic brain damage.

5. Cerebral hemorrhage

Peri- or intraventricular hemorrhage is a typical lesion of the immature neonate brain [1]. It originates in the vascular bed of the germinal matrix, a brain region that gradually disappears during development and does not exist in the mature fetus [35]. Blood vessels in this brain region burst easily. Fluctuations in cerebral blood flow during and after delivery can therefore lead to rupture of these vessels causing intra- or periventricular hemorrhage [26,28,36]. The bleeding is sometimes aggravated by factors affecting the aggregation of thrombocytes or the coagulating process

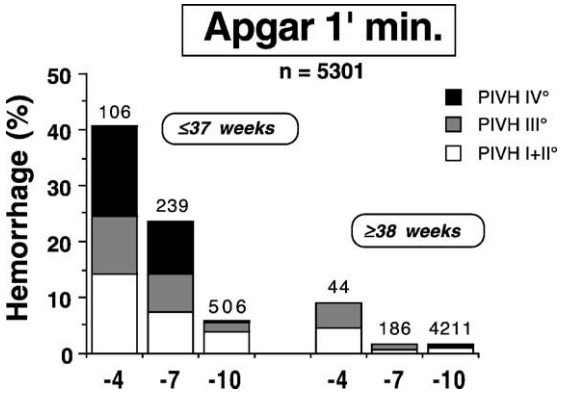

Fig. 5. Incidence of peri- or intraventricular hemorrhage in neonates in relation to the 1 min Apgar score [27,28].

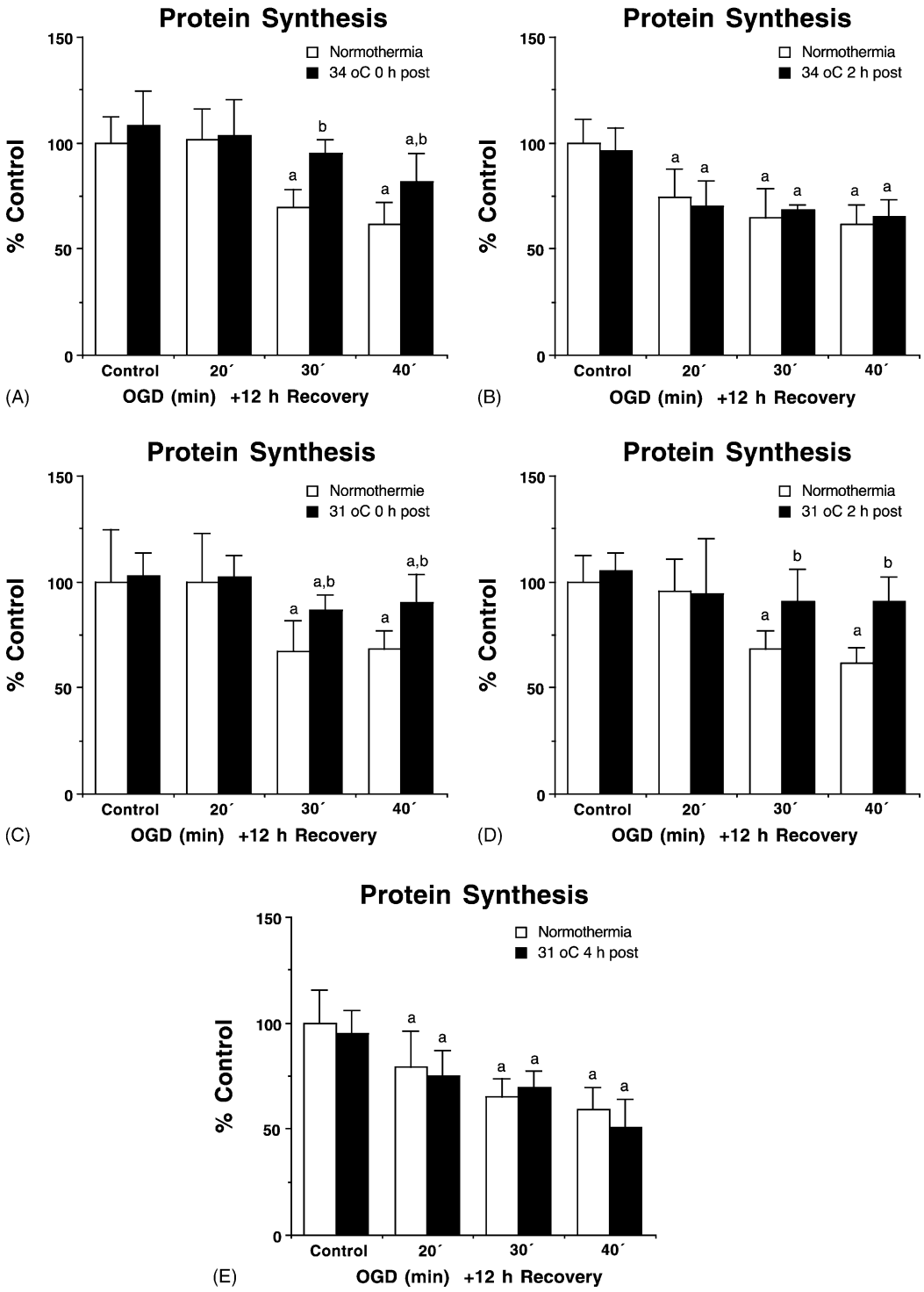

Fig. 6. Protein synthesis rate (PSR) (% of control) in hippocampal slices of mature guinea pig fetuses 20–40 min after oxygen–glucose deprivation (OGD) followed by 12 h of hypothermia [44]. Incubation temperature was reduced from 37 to 34 °C or 31 °C immediately [A (34 °C); C (31 °C)], 2 h [B (34 °C); D (31 °C)] or 4 h [E (31 °C)] after OGD. PSR in the five untreated control groups averaged 124 ± 47 dpm/30 min/μg protein. Values are given as means ± S.D. Intra- and intergroup differences were analysed by a two-way analysis of variance followed by Scheffé F-test (a: $P < 0.05$ OGD vs. control; b: $P < 0.05$ hypothermia vs. normothermia).

[37]. Possible consequences of a brain hemorrhage are destruction of the germinal matrix, periventricular haemorrhagic infarction in the cerebral white matter or hydrocephalus (for review: [1]).

Microscopic studies of the periventricular hemorrhagic necrosis indicate that the lesion is often a hemorrhagic infarction [38]. There is increasing evidence from recent clinical and experimental data that the hemorrhagic

component of the infarction tends to be most concentrated near the ventricular angle where the medullary veins draining the cerebral white matter become confluent and ultimately join the terminal vein in the subependymal region. Thus, it appears likely that periventricular hemorrhagic necrosis occurring in association with large IVH is, in fact, a venous infarction (for review: [38]).

To evaluate the relations between PIVH and various perinatal risk factors we launched a prospective cranial ultrasound study [26,28]. For this purpose newborns were enrolled 5–8 days after birth and screened by ultrasound for cerebral abnormalities. In immature neonates (<37 weeks of gestation) there was a close inverse relationship between Apgar score at 1, 5 and 10 min and both incidence and severity of PIVH (Fig. 5). This was in contrast to findings in mature neonates (>38 weeks of gestation) where a marked increase in the incidence of PIVH was found only with Apgar scores as low as 0–4 points. The relation between the incidence of PIVH and both cardiotocography and arterial cord blood pH was poor regardless of gestational age. From a physiological point of view, the close correlation between Apgar scores and the incidence of PIVH in preterm neonates is not surprising, since a lower Apgar score denotes a condition of circulatory shock [39]. The brain of preterm babies is extremely vulnerable and the ability of these infants to effectively centralize their circulation is limited, because their sympathetic nervous system is not fully developed. When oxygen deficiency arises they are, therefore, more prone to a severe reduction in oxygen transport to the brain than mature neonates. Furthermore, the loss of autoregulation in preterm fetuses may lead either to rupture of the fragile cerebral vessels when the arterial blood pressure is high or ischemic lesions when the pressure is too low. In contrast, babies born at term have fully developed circulatory protective mechanisms. Hence, their brain is more resistant as evidenced by a marked tolerance to asphyxia and a lower incidence and severity of PIVH. The overall incidence of PIVH in mature neonates born with Apgar scores of 5–7 points is, therefore, as low as in those born with Apgar scores of 8–10. However, the incidence of PIVH rises in those neonates born with Apgar values below 4 points. A further interesting result of this study was the increased incidence of PIVH in babies born to mothers suffering from fever >38 °C during delivery. This relation between ascending intrauterine infection and associated perinatal brain damage has already be discussed above.

6. Neuroprotection

Despite the critical clinical and socio-economic consequences of perinatal brain damage, no effective therapeutic strategies have yet been developed to prevent its causes. However, as already mentioned, some promising possibilities have been revealed through animal experiments that could be developed and tested in clinical studies (for review: [9]). Since a significant proportion of neuronal cell damage is brought about by pathophysiological processes that first begin several hours or even days after an ischemic insult (see secondary cell damage and apoptosis), the setting up of a therapeutic window would be feasible. In the following passages, current therapeutic concepts will be described by which neuroprotection has been achieved in animal models.

6.1. Mild hypothermia

The induction of mild hypothermia has raised interesting possibilities for neuroprotection from cerebral ischemia (for review: [40]). Over the last few years, the effects of mild hypothermia in protecting the brain from ischemically induced damage have been examined. Experimental studies on adult animals have shown that lowering of the brain temperature by 3–4 °C during global cerebral ischemia reduces neuronal cell damage dramatically [41]. Furthermore, treated animals were found to perform better than controls in subsequent learning and behavioural tests [42].

We were also able to demonstrate a neuroprotective effect of mild hypothermia in fetal brain tissue subjected to ischemic insults. Thus the recovery of protein synthesis and energy metabolism in hippocampal slices from mature guinea pig fetuses was found to be considerably improved, in comparison to controls, by induction of mild hypothermia during and after oxygen–glucose deprivation (OGD) [43]. Furthermore, we studied the exact relationship between the postischemic time delay and the degree of mild hypothermia

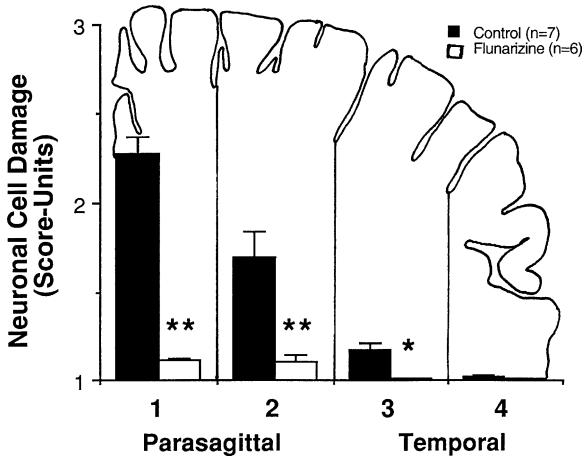

Fig. 7. Neuronal cell damage in the cerebrum of term fetal sheep 72 h after 30 min of ischemia [49]. Cerebral ischemia was induced by occluding both carotid arteries. Neuronal cell damage was quantified as follows: 0–5% damage (score 1), 5–50% damage (score 2), 50–95% damage (score 3), 95–99% damage (score 4), and 100% damage (score 5). Neuronal cell damage was most pronounced in the parasagittal regions, whereas in the more lateral part of the cortex only minor neuronal damage occurred. There was a significant reduction in neuronal cell damage after pretreatment with the calcium-antagonist flunarizine (1 mg/kg estimated fetal body weight). Values are given as means ± S.D. The data were analysed within and between groups using a two-way ANOVA followed by Games–Howell post-hoc test (*$P < 0.05$; **$P < 0.01$ (treated vs. untreated)).

by which neuroprotective effects on ischemic insults of different severity can be achieved in this animal model. Hypothermia initiated directly after OGD significantly improved the recovery of energy metabolism and protein synthesis. If there was a time delay of 2 h before the onset of hypothermia, neuroprotection depended on the degree of hypothermia (Fig. 6) [44]. Reduction of the incubation temperature to 31 °C diminished the disturbances of energy metabolism and protein synthesis, whereas lowering the bath temperature to only 34 °C was not effective. Inducing hypothermia 4 h after OGD did not have any influence on the recovery of energy metabolism and protein synthesis. These results tie in with studies performed in fetal sheep. Extending the hypothermic period to 72 h, Gunn et al. [45] were able to alleviate ischemic brain injury even when cerebral temperature was not lowered until 5.5 h after the insult. Based on these results, many authors now consider the induction of hypothermia during and particularly after a hypoxic-ischemic insult to be an effective therapeutic strategy [46]. In fact, recent clinical safety studies have demonstrated that induction of mild hypothermia in newborn infants after perinatal asphyxia has no harmful side-effects [47,48].

6.2. Pharmacological intervention

Now that the pathophysiological mechanisms underlying neuronal cell damage are better understood, diverse possibilities present themselves for pharmacological intervention. Interest is currently focused on the administration of oxygen radical scavengers, NO inhibitors, glutamate antagonists, growth factors and anti-cytokines. Given the key significance of the so-called calcium-overload for the development of neuronal cell death we tested the neuroprotective effect of flunarizine, an antagonist of voltage-dependent

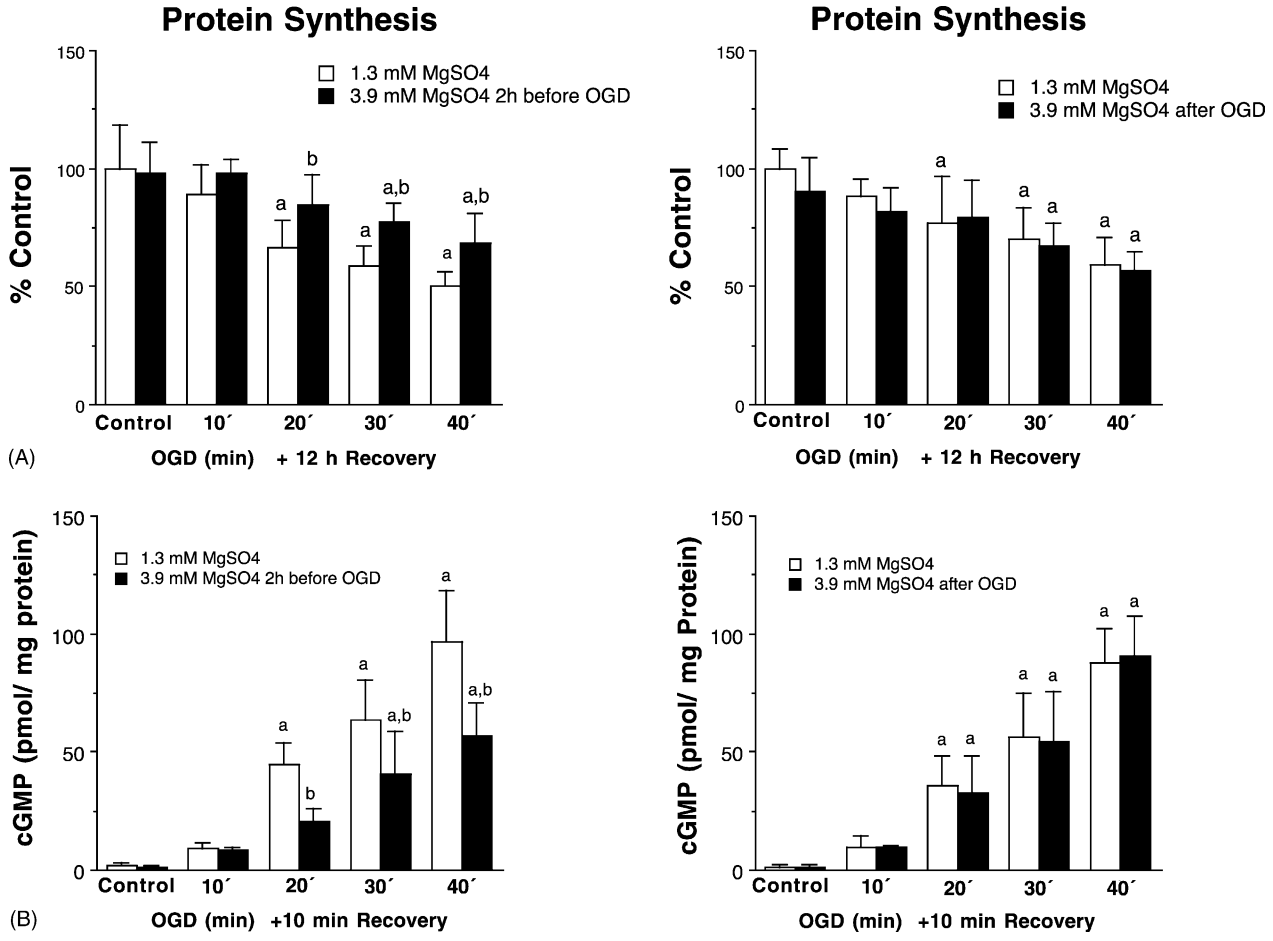

Fig. 8. (A) Protein synthesis rate (PSR) (% of control) in hippocampal slices of mature guinea pig fetuses 12 h after oxygen–glucose deprivation (OGD). OGD lasted between 10 and 40 min. PSR in the two untreated control groups averaged 124 ± 23 and 125 ± 11 dpm/30 min/μg protein, respectively. Magnesium concentration in the aCSF of the study groups was increased from 1.3 to 3.9 mM either 2 h before or immediately after OGD [53]. (B) Concentration of cGMP in hippocampal slices of mature guinea pig fetuses 10 min after OGD. As shown previously, the increase of cGMP tissue concentrations after OGD is an indirect measure of NO-production in this model. Magnesium concentration in the aCSF of the study groups was increased from 1.3 to 3.9 mM either 2 h before or immediately after OGD. cGMP concentrations increased significantly in hippocampal slices 10 min after OGD. When the magnesium concentration in the aCSF was increased 2 h before OGD the rise in tissue levels of cGMP was considerably reduced, while treatment after OGD had no effect. Values are given as means ± S.D. Intra- and intergroup differences were measured by a two-way analysis of variance followed by Scheffé F-test (a: $P < 0.05$ OGD vs. control; b: $P < 0.05$ $MgSO_4$ vs. control).

calcium channels, in a fetal sheep model. Ischemic brain injury was caused by occlusion of both carotid arteries for 30 min. One hour before the insult flunarizine was applied at a concentration of 1 mg/kg body weight i.v. to the fetuses (Fig. 7). This regimen reduced neuronal cell damage significantly, especially in the parasagittal cortex [49]. Severe drug-related cardiovascular side-effects could be excluded [50]. Thus, flunarizine was neuroprotective without adverse effects on fetal circulatory centralization during acute asphyxia, a crucial mechanism that protects the fetal brain from neuronal injury by increasing cerebral perfusion when oxygen is in short supply.

6.3. Magnesium

A further interesting therapeutic approach emerged from a retrospective analysis carried out by Nelson and Grether [51]. Recently, in a population of 155,636 infants, these authors showed that ante-partum application of magnesium considerably lowered the incidence of cerebral palsy in newborns weighing less than 1500 g. The incidence of moderate to severe cerebral palsy was 4.8% in this group. Seventy-five matched pairs were compared with the 42 children suffering from cerebral palsy. In the control group 36% of the children had been treated with magnesium, whereas, in the group with cerebral palsy only 7% had been treated. This difference was statistically highly significant. Almost identical results were recently obtained in a retrospective study carried out by Schendel et al. [52].

The neuroprotective efficacy of magnesium has been attributed to a variety of effects of this molecule on pathophysiological mechanisms during and after cerebral ischemia, i.e. vasodilation, inhibition of the NMDA-receptor, anti-convulsant properties. Furthermore, magnesium also seems to block the activation of NO-synthase after cerebral ischemia (Fig. 8) [53]. On the strength of these results, several clinical studies have been conducted to test the effect of magnesium on the incidence of cerebral palsy in preterm fetuses.

References

[1] Volpe JJ. Neurology of the newborn. 3rd ed. Philadelphia: Saunders; 1995.

[2] Bumke O, Foerster O. Handbuch der neurologie. Band XVI. Berlin: Springer; 1936.

[3] Berger R, Jensen A, Krieglstein J, Steigelman JP. Effects of acute asphyxia on brain energy metabolism in fetal guinea pigs near term. J Dev Physiol 1991;16:9–11.

[4] Siesjö BK, Katsura K, Pahlmark K, Smith M-L. The multiple causes of ischemic brain damage: a speculative synthesis. In: Krieglstein J, Oberpichler-Schwenk H, editors. Pharmacology of cerebral ischemia. Stuttgart: Medpharm Scientific Publishers; 1992. p. 511–25.

[5] Monaghan DT, Bridges RJ, Cotman CW. The excitatory amino acid receptors: their classes, pharmacology, and distinct properties in the function of the central nervous system. Ann Rev Pharmacol Toxicol 1989;29:365–402.

[6] Choi DW. Calcium-mediated neurotoxicity: relationship to specific channel types and role in ischemic damage. TINS 1988;11:465–9.

[7] Andine P, Lehmann A, Ellren K, Wennberg E, Kjellmer I, Nielsen T, et al. The excitatory amino acid antagonist kynurenic acid administered after hypoxic ischemia in neonatal rat offers neuroprotection. Neurosci Lett 1988;90:208–12.

[8] Kochhar A, Zivin JA, Lyden PD, Mazzarella V. Glutamate antagonist therapy reduces neurologic deficits produced by focal central nervous system ischemia. Arch Neurol 1988;45:148–53.

[9] Berger R, Garnier Y. Pathophysiology of perinatal brain damage. Brain Res Brain Res Rev 1999;32:107–34.

[10] Kleihues P, Hossmann K-A, Pegg AE, Kobayashi K, Zimmermann V. Resuscitation of the monkey brain after 1 h of complete ischemia III. Indications of metabolic recovery. Brain Res 1975;95:61–73.

[11] Bodsch W, Takahashi K, Barbier A, Grosse Ophoff B, Hossmann K-A. Cerebral protein synthesis and ischemia. Prog Brain Res 1985;63: 197–210.

[12] Berger R, Djuricic B, Jensen A, Hossmann K-A, Paschen W. Ontogenetic differences in energy metabolism and inhibition of protein synthesis in hippocampal slices during in vitro ischemia and 24 h of recovery. Dev Brain Res 1996;91:281–91.

[13] Hossmann K-A, Widmann R, Wiessner Ch, Dux E, Djuricic B, Röhn G. Protein synthesis after global cerebral ischemia and selective vulnerability. In: Krieglstein J, Oberpichler-Schwenk H, editors. Pharmacology of cerebral ischemia. Stuttgart: Medpharm Scientific Publishers; 1992. p. 289–99.

[14] Davies KJA, Goldberg AL. Oxygen radicals stimulate intracellular proteolysis and lipid peroxidation by independent mechanisms in erythrocytes. J Biol Chem 1987;262:8220–6.

[15] Helfaer MA, Kirsch JR, Traystman RJ. Radical scavengers: penetration into brain following ischemia and reperfusion. In: Krieglstein J, Oberpichler-Schwenk H, editors. Pharmacology of cerebral ischemia. Stuttgart: Medpharm Scientific Publishers; 1994. p. 297–309.

[16] Palmer C, Towfighi J, Roberts RL, Heitjan DF. Allopurinol administered after inducing hypoxia/ischemia reduces brain injury in 7-day-old rats. Pediatr Res 1993;33:405–11.

[17] Choi DW. Excitotoxic cell-death. J Neurobiol 1992;23:1261–76.

[18] Garthwaite J. Glutamate, nitric oxide and cell–cell signaling in the nervous system. Trends Neurosci 1991;14:60–7.

[19] Beckman JS, Beckman TW, Chen J, Marshall PA, Freeman BA. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. Proc Natl Acad Sci 1990;87:1620–4.

[20] Hamada Y, Hayakawa T, Hattori H, Mikawa H. Inhibitor of nitric oxide synthesis reduces hypoxic/ischemic brain damage in the neonatal rat. Pediatr Res 1994;35:10–4.

[21] Ferriero DM, Holtzman DM, Black SM, Sheldon RA. Neonatal mice lacking neuronal nitric oxide synthase are less vulnerable to hypoxic-ischemic injury. Neurobiol Dis 1996;3:64–71.

[22] Williams CE, Gunn A, Gluckman PD. Time course of intracellular edema and epileptiform activity following prenatal cerebral ischemia in sheep. Stroke 1991;22:516–21.

[23] Tan WKM, Williams CE, Gunn AJ, Mallard CE, Gluckman PD. Suppression of postischemic epileptiform activity with MK-801 improves neural outcome in fetal sheep. Ann Neurol 1992;32: 677–82.

[24] Tominaga T, Kure S, Narisawa K, Yoshimotot T. Endonuclease activation following focal ischemic injury in the rat brain. Brain Res 1993;608:21–6.

[25] Taylor CT, Colgan SP. Therapeutic targets for hypoxia-elicited pathways. Pharm Res 1999;16:1498–505.

[26] Dammann O, Leviton A. Maternal intrauterine infection, cytokines, and brain damage in the preterm infant. Pediatr Res 1997;42:1–8.

[27] Berger R, Bender S, Sefkow S, Klingmüller V, Künzel W, Jensen A. Peri/intraventricular haemorrhage: a cranial ultrasound study on 5286 neonates. Eur J Obstet Gynecol Reprod Biol 1997;75:191–203.

[28] Jensen A, Klingmüller V, Künzel W, Sefkow S. Das Hirnblutungsrisiko bei Früh- und Reifgeborenen. Geburtsh u Frauenheilk 1992;52: 6–20.
[29] Yoon H, Kim CJ, Romero R, Jun JK, Park KH, Choi ST, et al. Experimentally induced intrauterine infection causes fetal brain white matter lesions in rabbits. Am J Ostet Gynecol 1997;177: 797–802.
[30] Garnier Y, Coumans A, Jensen A, Berger R, Hasaart THM. Endotoxemia severely affects circulation during normoxia and asphyxia in immature fetal sheep. J Soc Gynecol Invest 2001;8: 134–42.
[31] Garnier Y, Coumans ABC, Vaihinger HM, von Duering M, Supçun S, Berger R, et al. Low dose endotoxin (LPS) results in substantial umbilico-placental vasoconstriction and discrete neuropathological changes in preterm sheep. J Soc Gynecol Invest 2002;9:72A–3A.
[32] Berger R, Garnier Y, Pfeiffer D, Jensen A. Lipopolysaccharides do not alter energy metabolism and protein synthesis in an in vitro model of fetal cerebral ischemia. Pediatr Res 2000;48:531–5.
[33] Feldhaus B, Dietzel ID, Berger R. TNF-α induces cell death in primary cultures of oligodendrocyte progenitors. J Soc Gynecol Invest 2001;8:248A.
[34] Coumans ABC, Middelanis J, Garnier Y, Leib SL, von Duering M, Hasaart THM, et al. Intracisternal application of endotoxin enhances the susceptibility to subsequent hypoxic-ischemic brain damage in neonatal rats. Pediatr Res 2003;53:770–5.
[35] Hambleton G, Wigglesworth JS. Origin of intraventricular haemorrhage in the preterm infant. Arch Dis Child 1976;51:651–9.
[36] Milligran DW. Failure of autoregulation and intraventricular haemorrhage in preterm infants. Lancet 1980;1:896–8.
[37] Amato M, Fauchere JC, Hermann Jr U. Coagulation abnormalities in low birth weight infants with peri-intraventricular hemorrhage. Neuropediatr 1988;19:154–7.
[38] Volpe JJ. Brain injury in the premature infant—from pathogenesis to prevention. Brain Dev 1997;19:519–34.
[39] Jensen A, Berger R. Fetal circulatory responses to oxygen lack. J Dev Physiol 1991;16:181–207.
[40] Maher J, Hachinski V. Hypothermia as a potential treatment for cerebral ischemia. Cerebrovasc Brain Metab Rev 1993;5:277–300.
[41] Busto R, Dietrich WD, Globus MY, Valdes I, Scheinberg P, Ginsberg MD. Small differences in intraischemic brain temperature critically determine the extent of ischemic neuronal injury. J Cereb Blood Flow Metab 1987;7:729–38.
[42] Green EJ, Dietrich WD, van Dijk F, Busto R, Markgraf CG, McCabe PM, et al. Protective effect of brain hypothermia on behavior and histopathology following global cerebral ischemia in rats. Brain Res 1992;580:197–204.
[43] Berger R, Jensen A, Hossmann K-A, Paschen W. Effect of mild hypothermia during and after transient in vitro ischemia on metabolic disturbances in hippocampal slices at different stages of development. Dev Brain Res 1998;105:67–77.
[44] Garnier Y, Pfeiffer D, Jensen A, Berger R. Effects of mild hypothermia on metabolic disturbances in fetal hippocampal slices after oxygen/glucose deprivation depend on depth and time delay of cooling. J Soc Gynecol Invest 2001;8:198–205.
[45] Gunn AJ, Gunn TR, Gunning MI, Williams CE, Gluckman PD. Neuroprotection with prolonged head cooling started before postischemic seizures in fetal sheep. Pediatrics 1998;102:1098–106.
[46] Gunn AJ, Gunn TR, de Haan HH, Williams CE, Gluckman PD. Dramatic neuronal rescue with prolonged selective head cooling after ischemia in fetal lambs. J Clin Invest 1997;99:248–56.
[47] Gunn AJ, Gluckman PD, Gunn TR. Selective head cooling in newborn infants after perinatal asphyxia: a safety study. Pediatrics 1998;102:885–92.
[48] Azzopardi D, Robertson NJ, Cowan FM, Rutherford MA, Rampling M, Edwards AD. Pilot study of treatment with whole body hypothermia for neonatal encephalopathy. Pediatrics 2000;106: 684–94.
[49] Berger R, Lehmann T, Karcher J, Garnier Y, Jensen A. Low dose flunarizine protects the fetal brain from ischemic injury in sheep. Pediatr Res 1998;44:1–6.
[50] Garnier Y, Berger R, Pfeiffer D, Jensen A. Low dose flunarizine does not affect short term fetal circulatory responses to acute asphyxia in sheep near term. Reprod Fertil Dev 1998;10:405–11.
[51] Nelson KB, Grether JK. Can magnesium sulfate reduce the risk of cerebral palsy in very low birthweight infants? Pediatrics 1995;95: 263–9.
[52] Schendel DE, Berg CJ, Yeargin-Allsopp M, Boyle CA, Decoufle P. Prenatal magnesium sulfate exposure and risk for cerebral palsy or mental retardation among very low-birth-weight children aged 3–5 years. JAMA 1996;276:1805–10.
[53] Garnier Y, Middelanis J, Jensen A, Berger R. Neuroprotective effects of magnesium on metabolic disturbances in fetal hippocampal slices after oxygen–glucose deprivation: mediation by nitric oxide system. J Soc Gynecol Invest 2002;9:86–92.