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Pathophysiology of perinatal brain damage

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Abstract

Perinatal brain damage in the mature fetus is usually brought about by severe intrauterine asphyxia following an acute reduction of the uterine or umbilical circulation. The areas most heavily affected are the parasagittal region of the cerebral cortex and the basal ganglia. The fetus reacts to a severe lack of oxygen with activation of the sympathetic–adrenergic nervous system and a redistribution of cardiac output in favour of the central organs (brain, heart and adrenals). If the asphyxic insult persists, the fetus is unable to maintain circulatory centralisation, and the cardiac output and extent of cerebral perfusion fall. Owing to the acute reduction in oxygen supply, oxidative phosphorylation in the brain comes to a standstill. The Na^+/K^+ pump at the cell membrane has no more energy to maintain the ionic gradients. In the absence of a membrane potential, large amounts of calcium ions flow through the voltage-dependent ion channel, down an extreme extra-/intracellular concentration gradient, into the cell. Current research suggests that the excessive increase in levels of intracellular calcium, so-called calcium overload, leads to cell damage through the activation of proteases, lipases and endonucleases. During ischemia, besides the influx of calcium ions into the cells via voltage-dependent calcium channels, more calcium enters the cells through glutamate-regulated ion channels. Glutamate, an excitatory neurotransmitter, is released from presynaptic vesicles during ischemia following anoxic cell depolarisation. The acute lack of cellular energy arising during ischemia induces almost complete inhibition of cerebral protein biosynthesis. Once the ischemic period is over, protein biosynthesis returns to pre-ischemic levels in non-vulnerable regions of the brain, while in more vulnerable areas it remains inhibited. The inhibition of protein synthesis, therefore, appears to be an early indicator of subsequent neuronal cell death. A second wave of neuronal cell damage occurs during the reperfusion phase. This cell damage is thought to be caused by the post-ischemic release of oxygen radicals, synthesis of nitric oxide (NO), inflammatory reactions and an imbalance between the excitatory and inhibitory neurotransmitter systems. Part of the secondary neuronal cell damage may be caused by induction of a kind of cellular suicide programme known as apoptosis. Knowledge of these pathophysiological mechanisms has enabled scientists to develop new therapeutic strategies with successful results in animal experiments. The potential of such therapies is discussed here, particularly the promising effects of i.v. administration of magnesium or post-ischemic induction of cerebral hypothermia.

Keywords: Perinatal; Brain damage; Post-ischemic

Contents

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1. Introduction

Year after year, around a thousand children in Germany alone incur brain damage as a result of a perinatal hypoxic–ischemic insult ([249], Perinatal statistics for the Federal Republic of Germany). Depending on the extent and location of the insult these children can develop spastic paresis, choreo-athetosis, ataxia and disorders of sensomotor coordination (Fig. 1). Nor is it uncommon for damage to the auditory and visual systems and impairment of intellectual ability to develop later [339]. The resulting impact on the children affected and their families is considerable and their subsequent care demands a high level of commitment and co-operation between pediatricians, child neurologists, physio-, speech-, and psychotherapists and other specialists. Conservative estimates of the costs to society for treatment and care of such cases per birth year lie around 1 billion German marks. However, despite the severe clinical and socio-economic significance, no effective therapeutic strategies have yet been developed to counteract this condition; one possible explanation being that perinatal management up to now has focused on preventing hypoxic–ischemic brain damage altogether [339]. The pathophysiology of ischemic brain lesions has not been investigated in depth until recently. One of the most urgent tasks for obstetricians and neonatologists will now be to develop therapeutic strategies from these pathophysiological models and to test them in prospective clinical studies.

This review article presents our current understanding of the pathophysiology of hypoxic–ischemic brain damage in mature neonates. The situation in premature neonates is discussed separately wherever necessary. We first deal with the causes of ischemic brain lesion, especially intrauterine asphyxia of the fetus, and their effects on the cardiovascular system and cerebral perfusion. Next, the typical neuropathological findings arising from reduced perfusion of the fetal brain are described. Also of key importance are the cellular mechanisms that are triggered by an ischemic insult. These will be discussed in detail, with particular emphasis on alterations of energy metabolism, intracellular calcium accumulation, the release of excitatory amino acids and protein biosynthesis. A considerable portion of neuronal cell damage first occurs during the reperfusion phase following an ischemic insult. The formation of oxygen radicals, induction of the NO system, inflammatory reactions and apoptosis will therefore be discussed in depth in this context. Finally, therapeutic concepts will be presented that have developed out of our understanding of these pathophysiological processes and been tested in animal experiments. Of these, i.v. administration of magnesium and induction of cerebral hypothermia appear to be of the greatest clinical relevance.

2. Causes of hypoxic–ischemic brain lesions in neonates

With a few exceptions, acute hypoxic–ischemic brain lesions in neonates are caused by severe intrauterine asphyxia [339]. This is usually brought about by an acute reduction in the uterine or umbilical circulation (Review: Ref. [157]), which in turn can be caused by abruptio placentae, contracture of the uterus, vena cava occlusion syndrome, compression of the umbilical cord, etc. (Table 1).

3. Circulatory centralisation and cerebral perfusion

The fetus reacts to an oxygen deficit of this severity by activating the sympathetic–adrenergic system and redistributing the cardiac output in favour of the central organs

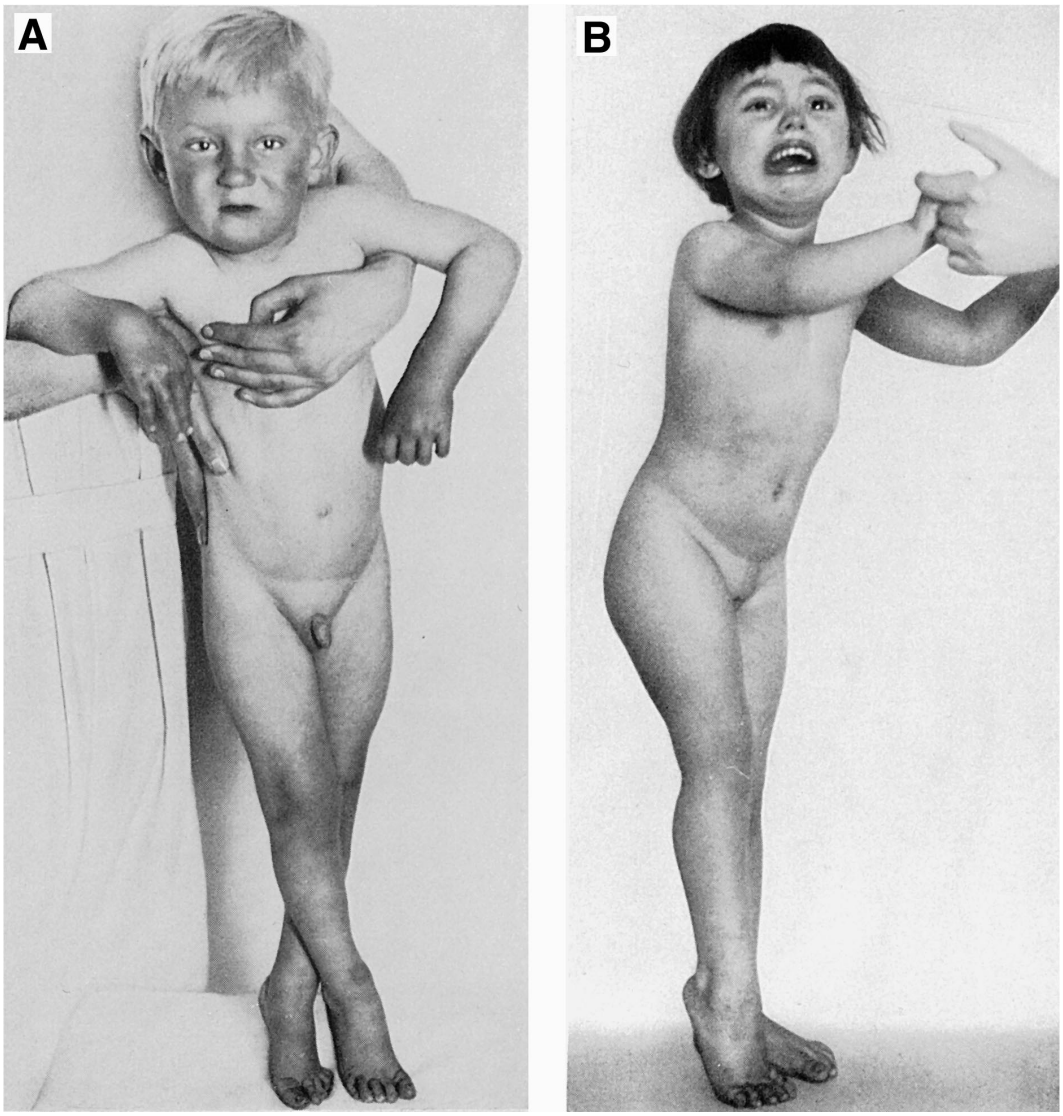

Fig. 1. Spastic diplegia in children with cerebral palsy [51].

(brain, heart and adrenals) (Review: Ref. [157]). The lowered oxygen and raised carbon dioxide partial pressures lead to vasodilatation of the cerebral vascular bed [163,172] causing cerebral hyperperfusion. This affects the brainstem in particular, while the blood flow to the white matter of the brain is hardly increased at all (Refs. [12,164,196], Review: Ref. [157]). Depending on the extent of the oxygen deficit and the maturity of the fetus, this cerebral hyperperfusion can reach two to three times the original rate of blood flow. Paradoxically, complete arrest of uterine perfusion is found to cause an initial reduction of blood flow to the brain [156]. If the oxygen deficit persists, the anaerobic energy reserves of the heart become exhausted. The cardiac output and the mean arterial blood pressure fall [289]. At mean arterial blood pressures of below 25–30 mmHg, there is an increasing loss of cerebral autoregulation, and a consequent reduction of the cerebral blood flow [195]. This affects the parasagittal region of the cerebrum [276] and the white matter [59,316] most of all. Immature fetuses seem to be particularly endangered by their limited ability to increase blood flow to the white matter through vasodilatation [316].

Table 1
Causes of severe intrauterine asphyxia

(I) Uteroplacental unit
- Contracture of the uterus
- Vena-cava-occlusion syndrome
- Hypotension
- Placenta praevia
- Abruptio placentae
(II) Umbilical vessels
- Compression of umbilical vessels
- Insertio velamentosa

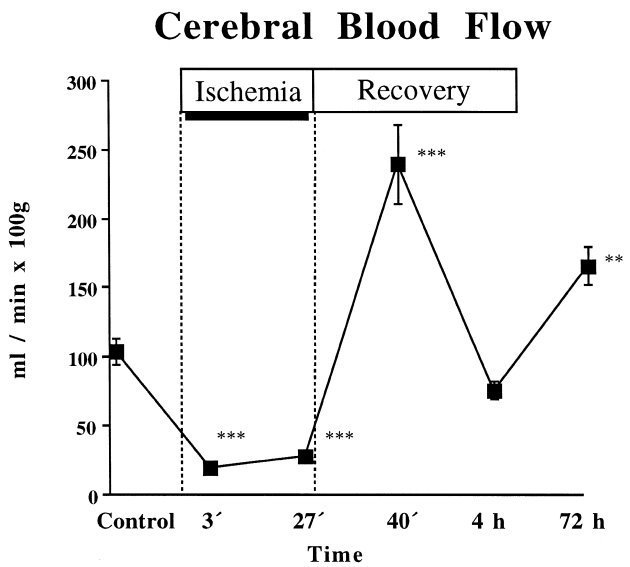

Fig. 2. Blood flow to the cerebrum ((ml/min)×100 g) in fetal sheep near term before, during, and after global cerebral ischemia of 30-min duration. Cerebral ischemia was induced by occluding both carotid arteries. Results are given as mean ± S.D. The data were analysed for intragroup differences by multivariate analysis of variance for repeated measures. Games–Howell-test was used as post-hoc testing procedure ($^{**}P < 0.01$, $^{***}P < 0.001$ (ischemia/recovery vs. control)) [33,39].

If the supply of oxygen to the fetus can be improved, cerebral hyperperfusion is brought about by the progressive post-asphyxial increase in cardiac output (Ref. [282], Review: Ref. [157]). This hyperperfusion can also be demonstrated in experiments using animal models of isolated cerebral ischemia (Fig. 2) [33]. Vasodilatation induced by acidosis in cerebral tissues and a reduction of blood viscosity at higher rates of blood flow have been put forward as possible causes of such hyperperfusion [182,293,317]. The initial hyperperfusion of the brain is followed directly by a phase of hypoperfusion (Fig. 2) [33,283]. Surprisingly, the shorter the duration of ischemia, the more marked this hypoperfusion appears to be. Post-ischemic hypoperfusion is characterised by a dissociation of the disturbed CO_2-reactivity from autoregulation of the cerebral vascular bed which remains intact. This leads to vasoconstriction and an uncoupling of blood flow and metabolic activity (Review: Ref. [147,302]). Post-ischemic hypoperfusion may be caused by oxygen radicals formed during the reperfusion phase after ischemia. Rosenberg et al. demonstrated that this phenomenon can be prevented by inhibiting the synthesis of oxygen radicals after ischemia [283]. In addition, a so-called no-reflow phenomenon can be observed after severe cerebral ischemia [8]. This failure of reperfusion in various brain areas is a consequence of the greater viscosity of stagnant blood, compression of the smallest blood vessels through swelling of the perivascular glial cells, formation of endothelial microvilli, increased intracerebral pressure, post-ischemic arterial hypotension and increased intravascular coagulation. The extent of the no-reflow phenomenon depends on the duration and type of cerebral ischemia. It is most pronounced when the vessels are engorged with blood after venous congestion (Review: Ref. [147]). Directly after post-ischemic hypoperfusion, the cerebral blood flow recovers or overshoots into a second phase of hyperperfusion (Fig. 2) [33,271]. Since this hyperperfusion is often accompanied by an isoelectric encephalogram, it is regarded as an extremely unfavourable prognostic factor [271].

4. Neuropathology of hypoxic–ischemic brain lesions

There are essentially six forms of hypoxic–ischemic brain lesion (Table 2): selective neuronal cell damage, status marmoratus, parasagittal brain damage, periventricular leucomalacia, intraventricular or periventricular haemorrhage and focal or multifocal ischemic brain lesions (Table 2) [102,339].

In mature fetuses, selective neuronal cell damage is found most frequently in the cerebral cortex, hippocampus, cerebellum and the anterior horn cells of the spinal cord [96,179,241,279,310,339]. As shown in animal experiments, the damage occurs after ischemia of only 10 min [351]. Within the cortex, the border zones between the major cerebral arteries are the worst affected. The cell damage is mostly parasagittal and more marked in the sulci than in the gyri, i.e., the pattern of distribution is strongly dependent on perfusion. The neurons show the most damage while the oligodendrocytes, astroglia and microglia remain largely unscathed (Review: Ref. [339]).

Status marmoratus, which is observed in only 5% of children with hypoxic–ischemic brain lesions, chiefly affects the basal ganglia and the thalamus. The complete picture of the disease does not emerge until 8 months after birth although the insult begins to take effect during the perinatal period. Status marmoratus is characterised by loss

Table 2
Hypoxic–ischemic brain damage in the fetus and neonate

Neurologic lesion	Topographic localization
Selective neuronal necrosis	cortex cerebri
	cerebellum
	hippocampus
	anterior horn cells of the spinal cord
Status marmoratus	basal ganglia
	thalamus
Parasagittal cerebral injury	cortex cerebri and subcortical
	substantia alba
Periventricular leucomalacia	substantia alba
Intra-, periventricular hemorrhage	germinal matrix
	substantia alba
	ventricles
Focal/multifocal ischemic	cortex cerebri and subcortical
Brain damage	substantia alba

of neurones, gliosis and hypermyelination. The increased number of myelinated astrocytic cell processes and their abnormal distribution give the structures affected, especially the putamen, a marbled appearance [96,279,280].

Parasagittal brain damage caused by cerebral ischemia is mostly reported in mature neonates [96,179,241,279, 310,339] and affects the parietal and occipital regions in particular. The damage usually arises through insufficient perfusion of the border zones between the main cerebral arteries during cerebral ischemia. This form of damage has been reproduced in animal models (Fig. 3). The extent of the brain lesions was found to be closely dependent on the duration and severity of the cerebral ischemia [33,351]. Interestingly, in the cortex, sulci are more badly damaged than the gyri. This arises from the special way in which the blood vessels in the cortex and surrounding white matter develop. When the sulci take shape and deepen in mature neonates, the penetrating blood vessels branching out from the meningeal arteries are forced into a hairpin bend as they cross the border from grey matter into white matter [79]. This produces a triangular area within the white matter at the base of the sulci through which hardly any vessels pass. Thus, any reduction in the perfusion of this region causes most damage to the sulci of the cortex

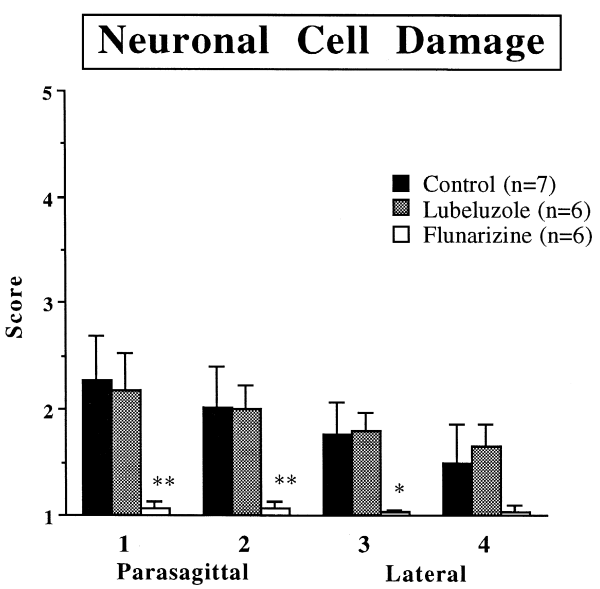

Fig. 3. Neuronal cell damage in the cerebrum of fetal sheep near term 72 h after induction of global cerebral ischemia of 30-min duration. Cerebral ischemia was induced by occluding both carotid arteries. Neuronal cell damage was quantified as follows: 0–5% damage (score 1), 5–50% damage (score 2), 50–95% 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 tremendous reduction in neuronal cell damage after pre-treatment with the calcium antagonist flunarizine (1 mg/kg estimated fetal body weight), whereas glutamate antagonist lubeluzole failed to protect the fetal brain. Values are given as mean ± 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)) [39,98].

[79,318]. This pattern of damage seems to correspond to that observed clinically in cases of subcortical leucomalacia [149,331].

Periventricular leucomalacia is characterised by damage to the white matter dorsal and lateral to the lateral ventricle [179,241]. It occurs most frequently in immature fetuses and chiefly affects the radiatio occipitalis at the trigonum of the lateral ventricle and the white matter around the foramen of Monro. At 6–12 h after an ischemic insult, necrotic foci can be observed in these areas [15]. These are characterised by swelling and rupture of neuronal axons. Necrotic oligodendrocytes are also found, especially ones undergoing differentiation or taking part in myelinisation. Over the next 24 to 48 h, activated microglia are seen more and more frequently. In 25% of cases, periventricular leucomalacia is accompanied by parenchymatous haemorrhaging [11,80,261]. As the disease progresses, small cysts develop out of the necrotic foci that can be identified by ultrasonography [81,144,261]. As gliosis progresses the cysts begin to constrict. The lack of myelinisation owing to the destruction of the oligodendrocytes and an enlargement of the lateral ventricle then become the most prominent features of the disease [75,280,318,319]. Periventricular leucomalacia around the radiatio occipitalis at the trigonum of the lateral ventricle and in the white matter around the foramen of Monro arises through vascular problems. Especially in immature fetuses, the ability to increase blood flow by vasodilatation during and after a period of arterial hypotension appears to be extremely limited in these brain areas [316]. After the 32nd week of pregnancy the vascularisation of these vulnerable areas is considerably increased and the incidence of periventricular leucomalacia thereby reduced.

Intra- or periventricular haemorrhage is another typical lesion of the immature neonate brain [339]. It originates in the vascular bed of the germinal matrix, a brain region that gradually shrinks until it has almost completely disappeared in the mature fetus [79,131,180,231,235,315]. Blood vessels in this brain region burst very easily [176,262]. Sub- and post-partum fluctuations in cerebral blood flow can therefore lead to rupture of these vessels causing intra- or periventricular haemorrhage [34,97,107,143,158, 206,221]. The bleeding is sometimes exacerbated by factors affecting the aggregation of thrombocytes or the coagulating process [7,198,299]. Possible consequences of a brain haemorrhage are destruction of the germinal matrix, a periventricular haemorrhagic infarction in the cerebral white matter or hydrocephalus (Review: Ref. [339]).

Focal or multifocal brain damage usually occurs within areas supplied by one or more of the main cerebral arteries. This form of insult is not normally observed before the 28th week of pregnancy. The incidence then rises with increasing maturity of the fetus [17]. Histologically, it is an infarct involving all types of cells (neurones, oligodendrocytes, astrocytes and endothelial cells). In the days following an insult, microglia and astrocytes migrate into the

Table 3
Concentrations of high-energy phosphates in the cerebral cortex of fetal guinea pigs near term during acute asphyxia caused by arrest of uterine blood flow [27,28]. Values are given as mean ± S.D.

	Brain metabolite [μmol/g]		
	Control	Asphyxia (2 min)	Asphyxia (4 min)
Adenosine triphosphate	2.59 ± 0.15	2.03 ± 0.21**	1.35 ± 0.32**
Adenosine diphosphate	0.37 ± 0.07	0.76 ± 0.13**	1.05 ± 0.15**
Adenosine monophosphate	0.04 ± 0.02	0.17 ± 0.09**	0.52 ± 0.21**

** $P < 0.01$ (asphyxia vs. control).

infarct zone [96,181,204,242,243,277]. The infarct is usually caused by arterial embolism or venous thrombosis. In 90% of the cases, the arterial occlusion is unilateral and mainly involves the left arteria cerebri media (Refs. [153,298,328], Review: Ref. [339]). Unlike the situation in the mature brain, this form of brain infarct leaves no scar tissue but often produces one or more cysts. They occur as a result of the high water content of the immature brain, an insufficient ability to myelinate and an inadequate astrocytic response to an ischemic insult. The scar-like structures running across the infarcted area are seldom pronounced in immature brain tissue. Thus, the morphological changes brought about by an ischemic insult also vary depending on the maturity of the brain [161,339]. Focal or multifocal brain lesions following infections, trauma or twin births, especially monochoriotic ones, are also relatively common [22,25,46,268,291,356]. It is thought that thromboplastic material or emboli from a miscarried cotwin sometimes occludes the cerebrovascular circulation of the living twin. Brain damage may also be caused by anemia or polycythemia and subsequent cardiac insufficiency and cerebral hypoperfusion arising from a feto-fetal transfusion. Alternatively, focal or multifocal brain damage can arise from systemic arterial hypotension, so that there is little distinction between this and other forms of brain damage such as selective neuronal cell damage, status marmoratus, parasagittal brain damage or periventricular leucomalacia (Review: Ref. [339]).

5. Energy metabolism and calcium homeostasis

The normal functioning of the brain is not only dependent on an adequate oxygen supply but also requires sufficient glucose. Transmission of electric impulses and biosynthetic reactions within the neurones require a continuous source of energy which is produced by the breakdown of glucose. The most important metabolic pathway for glucose is aerobic glycolysis by which glucose is metabolised to pyruvate. Pyruvate is then metabolised further through the energy-producing citric acid cycle. The electrons thereby released yield energy as they pass down the respiratory chain in the mitochondria. The energy released on each transfer of electrons is incorporated into molecules of ATP, synthesized from the precursor ADP and high energy phosphate (Pi). ATP is the basic source of energy for all energy requiring reactions in the brain [337].

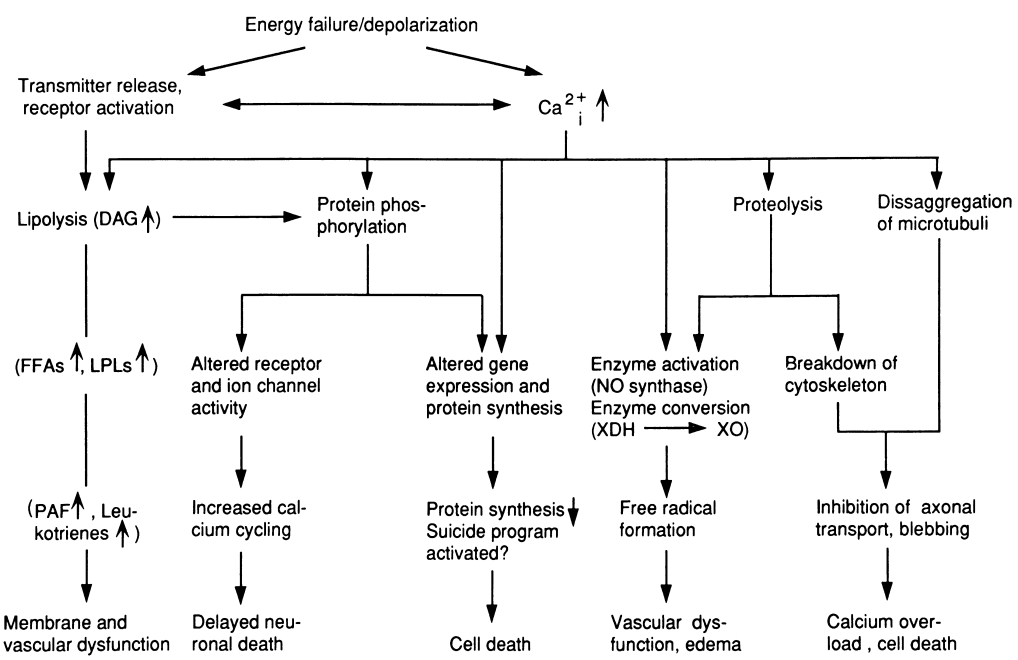

Fig. 4. Primary and secondary effects of the increased intracellular calcium concentration during and after cerebral ischemia [305]. XDH, xanthine dehydrogenase; XO, xanthine oxidase; PAF, platelet aggregating factor; FFAs, free fatty acids; DAG, diacylglyceride; LPL, lysophospholipids.

Whereas, during moderate hypoxemia, the fetus is able to maintain cerebral metabolism and adequate levels of ATP by speeding up the rate of anaerobic glycolysis [29,30,35], an acute reduction of the fetal oxygen supply will lead to a breakdown of energy metabolism in the cerebral cortex within a few minutes (Table 3) [27,28]. The ionic gradients for Na^+, K^+ and Ca^{2+} across the cell membranes can no longer be regulated since the Na^+/K^+-pump stops working through lack of energy. The membrane potential approaches 0 mV [133]. The energy depleted cell takes up Na^+, and the subsequent fall in membrane potential induces an influx of Cl^- ions. This intracellular accumulation of Na^+ and Cl^- ions leads to swelling of the cells as water flows in through osmosis. Cell oedema is therefore an inevitable consequence of cellular energy deficiency [305].

In addition, loss of membrane potential leads to a massive influx of calcium down the extreme extra-/intracellular concentration gradient. It is currently thought that the excessive increase in intracellular calcium levels, the so-called calcium-overload, leads to cell damage by activating proteases, lipases and endonucleases [305]. Some of the cellular mechanisms that are activated by the calcium influx occurring during ischemia are shown in Fig. 4.: alteration of the arachidonic acid cycle affecting prostaglandin synthesis, disturbances of gene expression and protein synthesis and increased production of free radicals and obstruction of the axonal transport system through disaggregation of microtubuli.

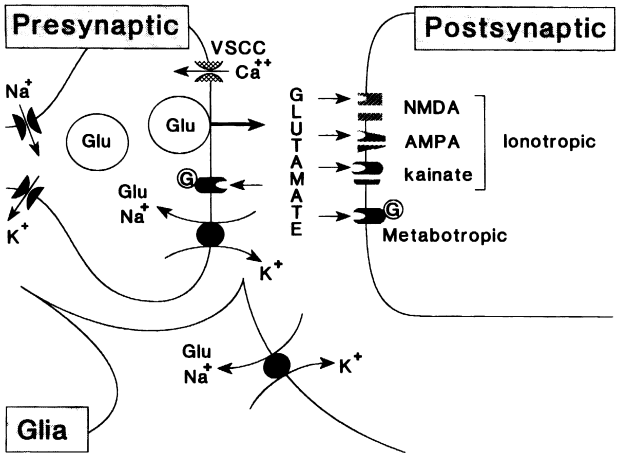

Fig. 5. Regulation of glutamate-mediated synaptic transmission. After depolarization of the presynaptic neuron vesicular glutamate is released by exocytosis into the synaptic cleft. Released glutamate activates postsynaptic ionotropic (NMDA, AMPA, Kainate) receptors and pre- or postsynaptic metabotropic (G-protein coupled) receptors. Glutamate action is terminated by Na^+-dependent uptake in the presynaptic neuron as well as in glial cells [248].

6. Excitatory neurotransmitters

As early as 1969, Olney succeeded in demonstrating that neuronal cell death could be induced by the exogenous application of glutamate, an excitatory neurotransmitter [252]. In subsequent years, this observation was confirmed in both immature and adult animals of various species including primates [253]. In 1984, Rothman showed that glutamate antagonists could prevent anoxic cell death in hippocampal tissue cultures [285]. That same year, Benveniste et al. reported an excessive release of glutamate into the extracellular space during cerebral ischemia in vivo [24], from which they concluded that glutamate might play an important role in neuronal cell death following ischemia [256,285–287].

Glutamate activates postsynaptic receptors, consisting of five subunits, that form ionic channels permeable to cations (Fig. 5) [294]. Three classes of ionotropic glutamate receptors have been identified on the basis of their pharmacological response to specific agonists such as amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA),kainate (KA) and *N*-methyl-D-aspartate (NMDA). These are referred to as the AMPA-, KA- and NMDA-receptors [230]. The corresponding channels are permeable to Na^+ and K^+ ions, while those of the NMDA-receptor also exhibit Ca^{2+}-permeability. Glutamate also activates the metabotropic receptors that regulate intracellular G-protein signal cascades [239]. The best characterised receptor in this family is the quisqualate receptor that mediates the hydrolysis of phosphatidylinositol-4,5 biphosphonate (PIP_2) into the messenger molecules 1,4,5-triphosphate (IP_3) and diacyl-glycerol. The activation of each of these receptors leads to an increase in the levels of free calcium in the cell cytoplasm. The NMDA-receptor regulates a calcium channel, the metabotropic receptors induce an emptying of intracellular calcium stores while the AMPA/KA receptors open a voltage-dependent calcium channel by membrane depolarisation. The increase in free calcium within the cell activates proteases, lipases and endonucleases that then initiate processes leading to cell death [62,303,304].

There is no longer any doubt that glutamate release plays a critical role in neuronal cell death after focal cerebral ischemia such as that caused by an arterial embolus [217]. Glutamate antagonists have been shown to exert a strong neuroprotective effect against hypoxic–ischemic brain damage in adult [174,263,336] and even in neonatal animals [10,93,101,138,212,246,254]. In neonatal rats, it was shown that glutamate release during and after an hypoxic–ischemic insult could evoke epileptogenic activity and that this effect was dependent on the maturity of the brain. In rats, the most marked effect was observed 10 to 12 days after birth (Fig. 6) [159]. The reason for this seems to be a developmental change in the subunits of the glutamate receptor which increases the neurone's permeability to calcium [160,161]. Furthermore, the levels of GABA, one of the most important inhibitory neurotrans-

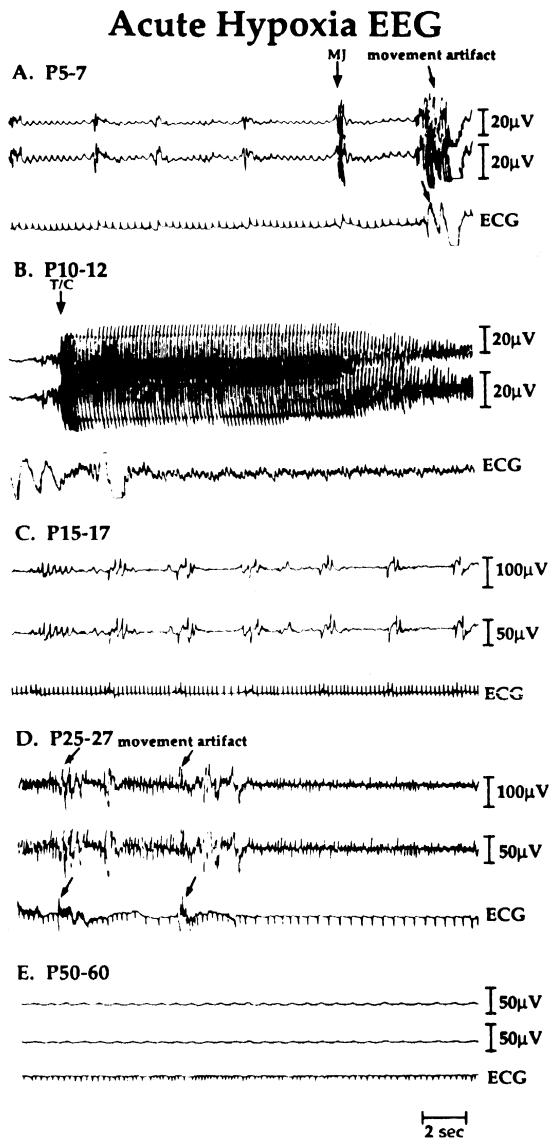

Fig. 6. EEG-recording during acute hypoxia (3% O_2) in rats of different post-gestational ages. Epileptogenic activity is registered in 10–12-day-old rats (P10–12) during hypoxia, whereas in the older animals (P50–60) isoelectric EEG-activity is registered [159,161].

mitters in neuronal tissue, are very low at this stage of development [70,255,313].

As shown in adult animals, epileptogenic impulses in the vicinity of a brain infarct cause a considerable rise in metabolic activity. In an inadequately perfused section of brain tissue such as the penumbra surrounding an infarct, this can rapidly lead to an imbalance between cell metabolism and blood circulation, resulting in brain damage [148]. In addition, the formation of LTPs (long-term potentials), that play an important role in synaptic plasticity and hence, in learning processes, may be disturbed by the induced epileptogenic activity [42]. Long-term neurological damage is the inevitable consequence in the children affected.

In global ischemia, such as that caused by cardiac insufficiency, the situation is quite different to that in focal ischemia. As shown in adult animals, it is far less clear whether glutamate is directly involved in neuronal cell death [2,4,50,183,187,326,344]. As Hossmannn points out in his 1994 review article, a number of observations argue

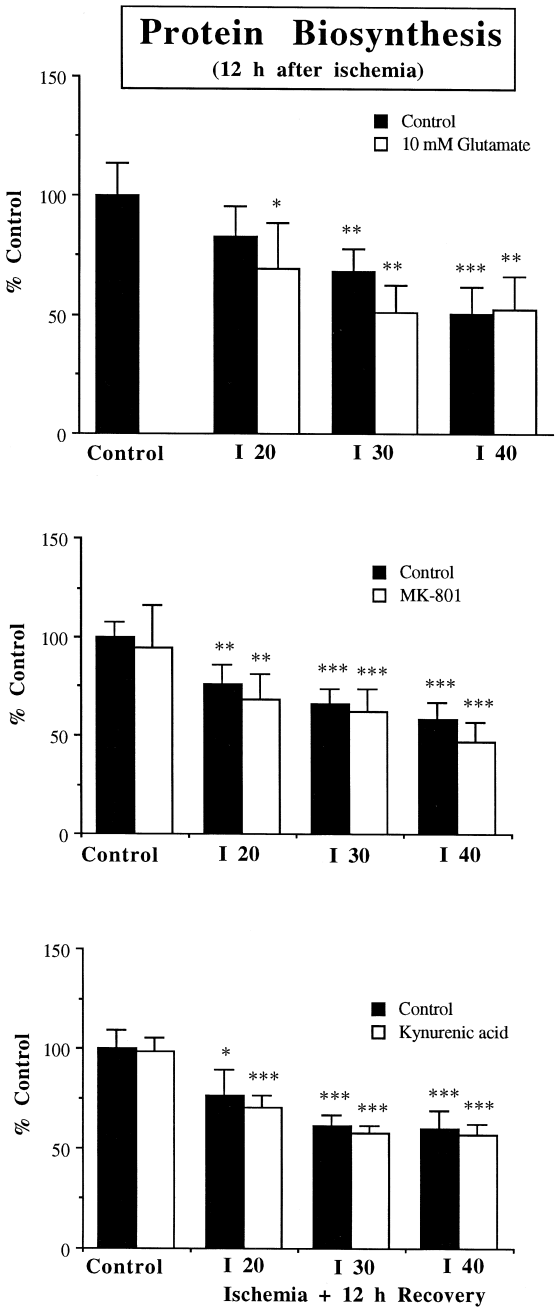

Fig. 7. Protein synthesis rate in hippocampal slices from mature fetal guinea pigs 12 h after in vitro ischemia. The ischemic period lasted between 20 and 40 min (I 20, I 30, I 40). Protein synthesis rate was not affected neither by application of glutamate nor by glutamate antagonists (MK-801 [100 µM], kynurenic acid [500 µM]). Values are given as mean ± S.D. Statistical analysis was performed by ANOVA followed by Scheffé's *F*-test ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ [ischemia vs. control]) [36].

against any major involvement of glutamate in processes leading to neuronal cell death after global ischemia [148]. (1) Neither the pattern of glutamate release during ischemia nor the cerebral distribution of glutamate receptors matches the regional manifestation of brain damage after global ischemia [68,105,208,225]. (2) Glutamate toxicity in cell cultures from vulnerable brain areas was found to be no higher than in cultures from non-vulnerable regions [87,148]. (3) In contrast to the effects of in vitro ischemia, application of glutamate to cell cultures or hippocampal tissue slices caused no prolonged inhibition of protein synthesis [58,84,87].

Since then, the possibility of glutamate playing a key role in the induction of brain damage either during or directly after global ischemia, even in the immature brain, has been effectively excluded by the following observations: Application of glutamate or glutamate antagonists to hippocampal slices from guinea pig fetuses did not affect post-ischemic protein biosynthesis, a parameter used as an early marker of neuronal cell death (Fig. 7) [36]. Furthermore, the glutamate antagonist lubeluzole was found to have no neuroprotective effect in a model of cerebral ischemia in mature sheep fetuses (Fig. 3, Ref. [98]). However, it is possible that later, during the reperfusion phase after cerebral ischemia, glutamate-induced epileptogenic activity does cause brain damage. This possibility will be discussed further on.

7. Protein biosynthesis

As animal experiments show, inhibition of protein synthesis plays a key role in the post-ischemic processes leading to neuronal cell damage [146]. Protein synthesis is reduced both during ischemia and in the early post-ischemic phase in vulnerable and non-vulnerable brain areas [171]. At the end of the ischemic period, protein synthesis in non-vulnerable regions recovers to pre-ischemic levels, while in vulnerable regions it remains inhibited [45, 324,347]. Thus, the inhibition of protein synthesis appears to be an early indicator of subsequent neuronal cell death [146]. This observation ties in with the results of experiments demonstrating the neuroprotective effect of hypothermia or barbiturates after cerebral ischemia [348,352]. Shortly after cerebral ischemia, the usual inhibition of protein synthesis set in, however, the recovery phase in the normally vulnerable areas was now much shorter (Fig. 8), and was accompanied by far less pronounced neuronal cell damage. Similar findings were 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 in adult brains [31]. 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 [146].

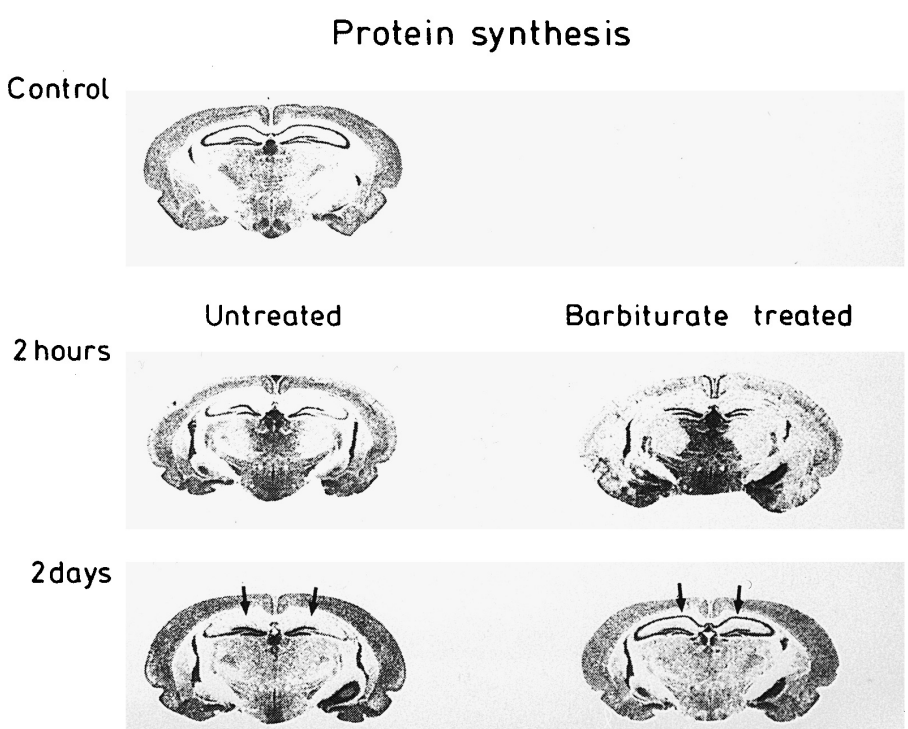

Fig. 8. Autoradiographic evaluation of protein synthesis before (control) and at two recirculation times (2 h and 2 days) after 5 min bilateral carotid artery occlusion in gerbil. Left: untreated animals. Right: treated animals (50 mg/kg pentobarbital i.p., shortly after ischemia). Note similar reduction of protein synthesis after 2 h of recirculation but recovery in all regions including CA1 sector in the barbiturate-treated animals after 2 days recovery (arrows) [146].

Electron microscopic and biochemical studies have shown that post-ischemic inhibition of protein synthesis is accompanied by a disaggregation of the polyribosomes [135,170,171]. This disaggregation seems to occur not during but after ischemia, and involves a dissociation of the monoribosomes into their smaller and larger subunits [171]. Ribosomes disaggregate when starting 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 hinders all stages of protein synthesis (initiation, elongation and termination) [145]. However, after ischemia, the regenerated 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 [146]. Recent research suggests that the post-ischemic inhibition of protein synthesis is based on a disturbance of calcium homeostasis in the endoplasmic reticulum [265,266].

Finally, post-ischemic protein synthesis seems to be involved in the cellular suicide programm known as apoptosis. This view is supported by studies showing that apoptotic cell death could be prevented by application of the protein synthesis inhibitor, cycloheximide [109].

8. Secondary cell damage during reperfusion

In cerebral tissue capable of regeneration after an ischemic insult, energy metabolism can be seen to recover rapidly [31,146]. A few hours later, however, the energy status is diminished once again in the affected tissue [43,269]. Simultaneously, a secondary cell oedema develops, followed a little later by epileptogenic activity that can be monitored on EEG. These events are quite probably brought about or modulated by oxygen radicals, nitric oxide (NO), inflammatory reactions and excitatory amino acids, particularly glutamate.

8.1. Oxygen radicals

During cerebral ischemia, the cut back in oxidative phosphorylation rapidly diminishes reserves of high-energy phosphates. Within a few minutes, considerable amounts of adenosine and hypoxanthine accumulate. During reperfusion these metabolic products are metabolised further by xanthine oxidase to produce xanthine and uric acid [211]. The activity of xanthine oxidase in the resting brain is very low [3], but during cerebral ischemia a massive conversion of xanthine dehydrogenase to xanthine oxidase takes place, regulated by the calcium-dependent protease calpain [169,211]. The breakdown of hypoxanthine by xanthine oxidase in the presence of oxygen, produces a flood of superoxide radicals. These are then converted by superoxide dismutase to hydrogen peroxide [94,95]. By the Haber–Weiss reaction shown below, hydrogen peroxide and tissue iron can then combine to form hydroxyl radicals.

$$\text{hypoxanthine} \Rightarrow \text{xanthine} + H_2O$$

$$\text{xanthine} + NAD^+ \overset{\text{xanthine-dehydrogenase}}{\Rightarrow} \text{uric acid} + NADH + H^+$$

$$\text{xanthine} + \cdot O_{2^-} \overset{\text{xanthine-oxidase}}{\Rightarrow} \text{uric acid} + 2 \cdot O_2^- + 2H^+$$

$$\cdot O_2^- + \cdot O_2^- + 4H^+ \overset{\text{superoxide-dismutase}}{\Rightarrow} 2H_2O_2$$

$$\cdot O_2^- + H_2O_2 \overset{\text{Haber–Weiss-reaction}}{\Rightarrow} O_2 + OH^- + \cdot OH^-$$

The so-called oxygen radicals then cause various forms of tissue damage [76,77,127,218,322,342]. Similarly, the increased rate of arachidonic acid metabolism in brain tissue or activated leucocytes after ischemia can also produce large amounts of oxygen radicals (Review: Ref. [139]).

Numerous studies have shown that oxygen radicals play an important role in processes leading to neuronal cell damage (Ref. [329], Review: Ref. [128]). In adult animals, various degrees of neuroprotection against ischemic insults can be achieved through the inhibition of xanthine oxidase by application of oxygen radical scavengers and iron chelators [18,40,56,125,172,191,209,223,267]. Oxygen radicals also appear to be involved in mechanisms underlying neuronal cell death in immature animals. The rate of lipid peroxidation was found to be considerably increased after hypoxia in fetal guinea pigs and newborn lambs [1,108,224]. The longer the gestational age, the greater this increase was [224]. Furthermore, marked production of oxygen radicals was observed after hypoxia both in vitro, in cultures of fetal neurones, and in vivo, in neonatal mice [136,250]. There is also evidence that the infarct volume can be reduced in a model of focal ischemia in neonatal rats by application of allopurinol, an inhibitor of xanthine oxidase and oxygen radical scavengers [256].

8.2. NO

NO is a free radical synthesized by NO-synthase in endothelial cells and neurones in response to rises in levels of intracellular calcium. Beside this endothelial and neuronal form of NO-synthase, another form of the enzyme is found in neutrophil granulocytes and microglia. This isoform can be stimulated by cytokines released by activated macrophages. It is calcium-independent and can sustain NO production for several days [236]. Beckman et al., however, demonstrated that NO and superoxide radicals combine to produce peroxynitrite that spontaneously decomposes to form hydroxyl radicals, nitrogen dioxide and NO_2^+ [19,20]. Thus NO, like free iron, can raise the

toxicity of superoxide radicals significantly by converting them to highly potent radicals that cause considerable cell damage [154].

During cerebral ischemia, a massive influx of intracellular calcium takes place through various channels, regulated, among other things, by the neurotransmitter glutamate [63,304]. The rise in intracellular calcium activates NO-synthase [88,99], which produces NO, citrulline and water from arginine, NADPH and oxygen.

$$\text{Arginine} + \text{NADPH} + \text{H}^+ + \text{O}_2 \overset{\text{NO-synthase}}{\Rightarrow}$$

$$\text{NO} + \text{Citrulline} + \text{NADP}^+ + \text{H}_2\text{O}$$

There is also an accumulation of cGMP [21]. Since there is no oxygen available during ischemia, NO cannot be synthesized until the reperfusion phase [21]. Likewise, large numbers of superoxide radicals are produced by xanthine oxidase and via other pathways in the mitochondria during and, to an even greater extent, after ischemia [199]. During reperfusion, NO and superoxide radicals combine, as described above, to produce peroxynitrite, leading to the formation of more potent radicals. Destruction of the tissue is the inevitable result [21]. Investigations of the action of inhibitors of NO-synthase in models of cerebral ischemia in adult animals have yielded highly variable results [49,55,74,78,130,177,234,240,245,272, 297,357,358]. This can be explained by the fact that the neuroprotective effect of NO-synthase blockers after ischemia, that is brought about by a lowering of NO production and consequent reduction of the build-up of potent radicals, is counteracted by a marked vasoconstriction induced by the fall in NO concentration in endothelial cells [75]. Thus, Huang et al. found markedly smaller infarct loci after occlusion of the A. cerebri media in mice whose expression of the neuronal form of NO-synthase had been blocked than in the wild type of the animal [151]. The same group was also able to protect the brain from ischemic insults by application of selective blockers of neuronal NO-synthase [75].

To date, hardly any studies have investigated the importance of NO in neuronal cell death in neonates or fetuses. After a hypoxic–ischemic insult in neonatal rats, a greater number of neurones were found to contain NO-synthase [141]. The activity of this NO-synthase, however, appeared to be diminished [162]. Furthermore, two peaks of NO production were detected in this animal model: one during hypoxia and the other during the reoxygenation period. The neuronal and the inducible form of NO-synthase seems to be differently involved in this process [142]. Some authors succeeded in preventing ischemic lesions in the brains of immature animals through application of NO-blockers [13,129,330], while other research teams were unable to achieve this effect or observed, instead, a worsening of the damage [205,309]. As already mentioned, this discrepancy may have arisen from the different effects of NO-blockers on vascular endotheli and neurones. In our investigations of the effect of blocking NO-synthase we therefore by-passed the cardiovascular system, by carrying out experiments on hippocampal slices [38]. Although post-ischemic NO-production could be completely blocked with NO-inhibitors, this intervention had no influence on the post-ischemic inhibition of protein biosynthesis, a parameter used as an early indicator of neuronal cell death

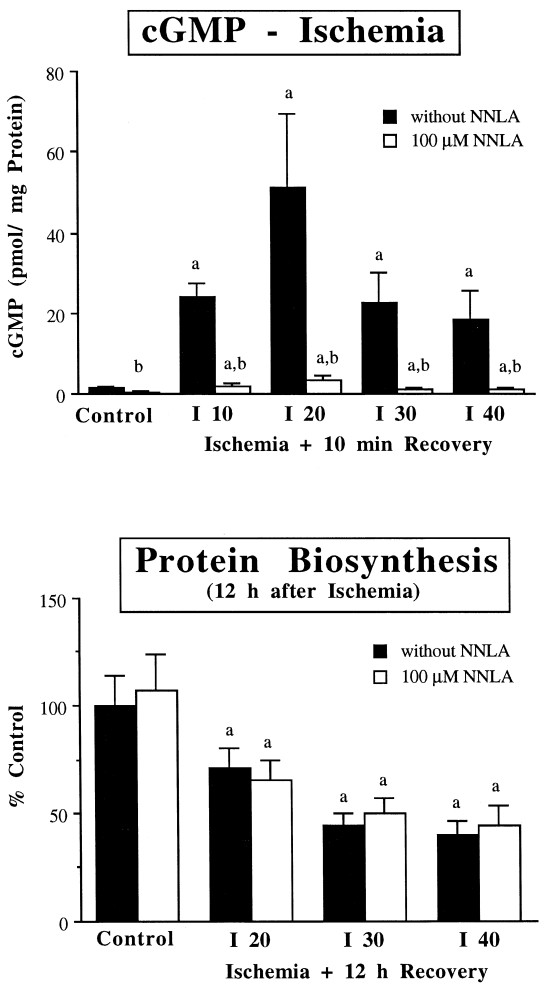

Fig. 9. (Top panel) cGMP concentrations in hippocampal slices from mature fetal guinea pigs after different durations of in vitro ischemia (10–40 min). A portion of the tissue slices was incubated for 30 min, before, during and 10 min after ischemia, in 100 µM *N*-nitro-L-arginine (NNLA). After 10 min recovery from 10 to 40 min of ischemia, a marked rise in cGMP levels was observed in tissue slices that had not been incubated in NNLA. Note that application of NNLA blocked the ischemia-induced elevation of cGMP almost completely. (Bottom panel) Protein synthesis rate in hippocampal slices from mature fetal guinea pigs after different durations of in vitro ischemia (20–40 min) and a recovery period of 12 h. A portion of the tissue slices was incubated in 100 µM NNLA for 30 min before, during and 12 h after ischemia. Protein synthesis rate was reduced to 50% of initial levels after 40 min ischemia. Note that blocking of NO-synthase with NNLA did not improve the post-ischemic recovery of protein synthesis. The statistical significance of differences between groups was assessed by ANOVA and the Scheffé post-hoc test (Top panel: $P < 0.05$ (ischemia vs. control), Bottom panel: $P < 0.05$ (NNLA vs. without NNLA)) [38].

(Fig. 9). Whether or not NO is directly involved in the pathogenesis of neuronal cell death following ischemia in fetuses therefore remains an open question.

8.3. Inflammatory reactions

As various studies have shown, ischemia and subsequent reperfusion can set off an inflammatory reaction in the brain (Fig. 10) [91,288]. Expression of a wide variety of cytokines, e.g., IL-1, IL-6, transforming growth factor-β, and fibroblast growth factor, was observed. In rats, mRNA of IL-1 was expressed within 15 min of global cerebral ischemia [222]. Cytokines appear to be formed in activated microglia [100,215,232]. They are thought to mediate the migration of inflammatory cells within the reperfused tissue.

Through increased expression of the adhesion molecules P- and E-selectin and ICAM-1 on the endothelial cells and of integrins on leukocytes, granulocytes become attached to the endothelium, migrate through the vessel wall and accumulate in the interstitium [90,110,132,210,251,259, 340]. There, after further activation by cytokines, they synthesize oxygen radicals, especially superoxide radicals that proceed to damage neuronal tissue. The role of inflammatory cells in the pathogenesis of secondary cell damage was further elucidated in reperfusion experiments using blood lacking granulocytes, or antibodies to adhesion molecules and trials on transgenic mice [126,140,152, 200,278,338,355]. Especially in the brain of immature fetuses exposed to a severe intrauterine infection such pathophysiological mechanisms appear to play a critical role [113,354].

8.4. Glutamate

Williams et al. observed epileptiform activity in mature sheep fetuses about 8 h after 30 min of global cerebral ischemia that reached a peak 10 h after the ischemic period [350]. They were able to completely inhibit this epileptiform activity by application of the glutamate antagonist MK-801, and show that the resulting brain damage was markedly reduced in the treated animals (Fig. 11) [320]. 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 a secondary wave of cell damage [148].

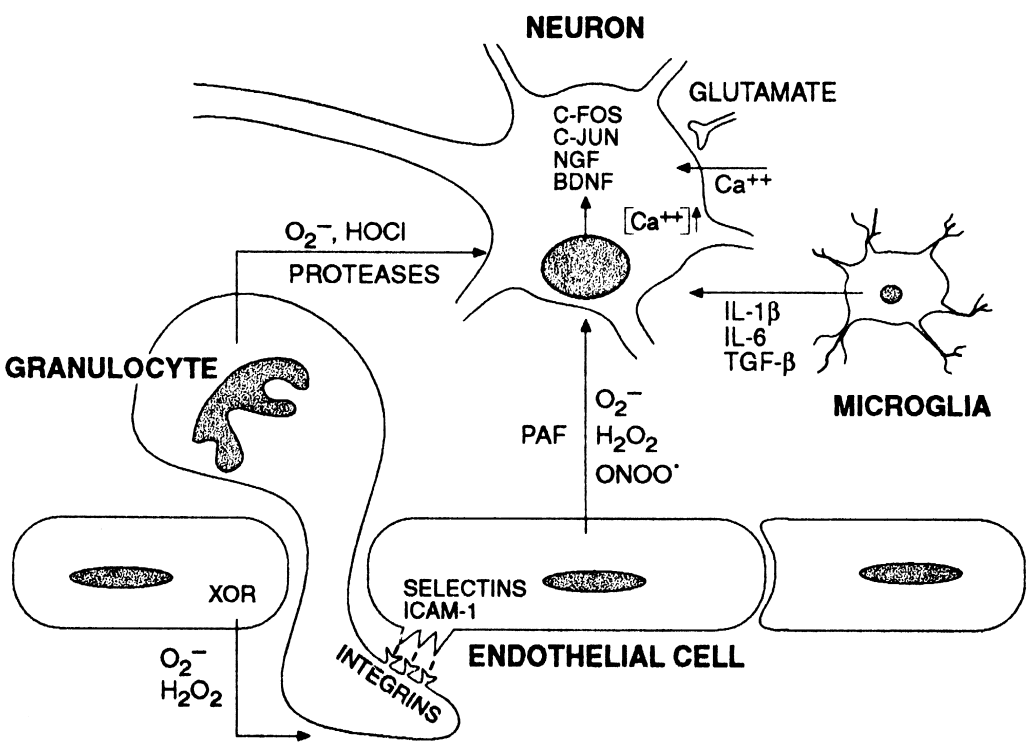

Fig. 10. Mechanisms of recirculatory induced brain damage. Ischemia and recirculation are possible inductors of gene expression and formation of oxygen radicals. Endothelium-derived oxygen radicals induce expression of adhesion molecules to allow granulocytes crossing the blood-brain barrier. The formation of oxygen radicals, glutamate-induced excitotoxicity, and cytokines produced by activated microglia are damaging neuronal cells. NGF, nerve growth factor; BDNF, brain-derived neurotrophic factor; TGF, transforming growth factor; PAF, platelet-aggregating factor; ICAM-1, intercellular adhesion molecule 1; IL, interleukin; $ONOO^-$, Peroxynitrite [91].

9. Apoptosis and post-ischemic genome expression

It is still unclear whether secondary cell death after ischemia is necrotic or apoptotic. The latter condition is characterised by a shrinking of the cell, blessing of the cell membrane, condensation of chromatin and DNA fragmentation induced by a calcium-dependent endonuclease (Fig. 12) [327]. In DNA electrophoresis, this fragmentation can be recognised by a typical DNA ladder [178,192,214,327]. In neuronal cell cultures, apoptosis can be prevented by

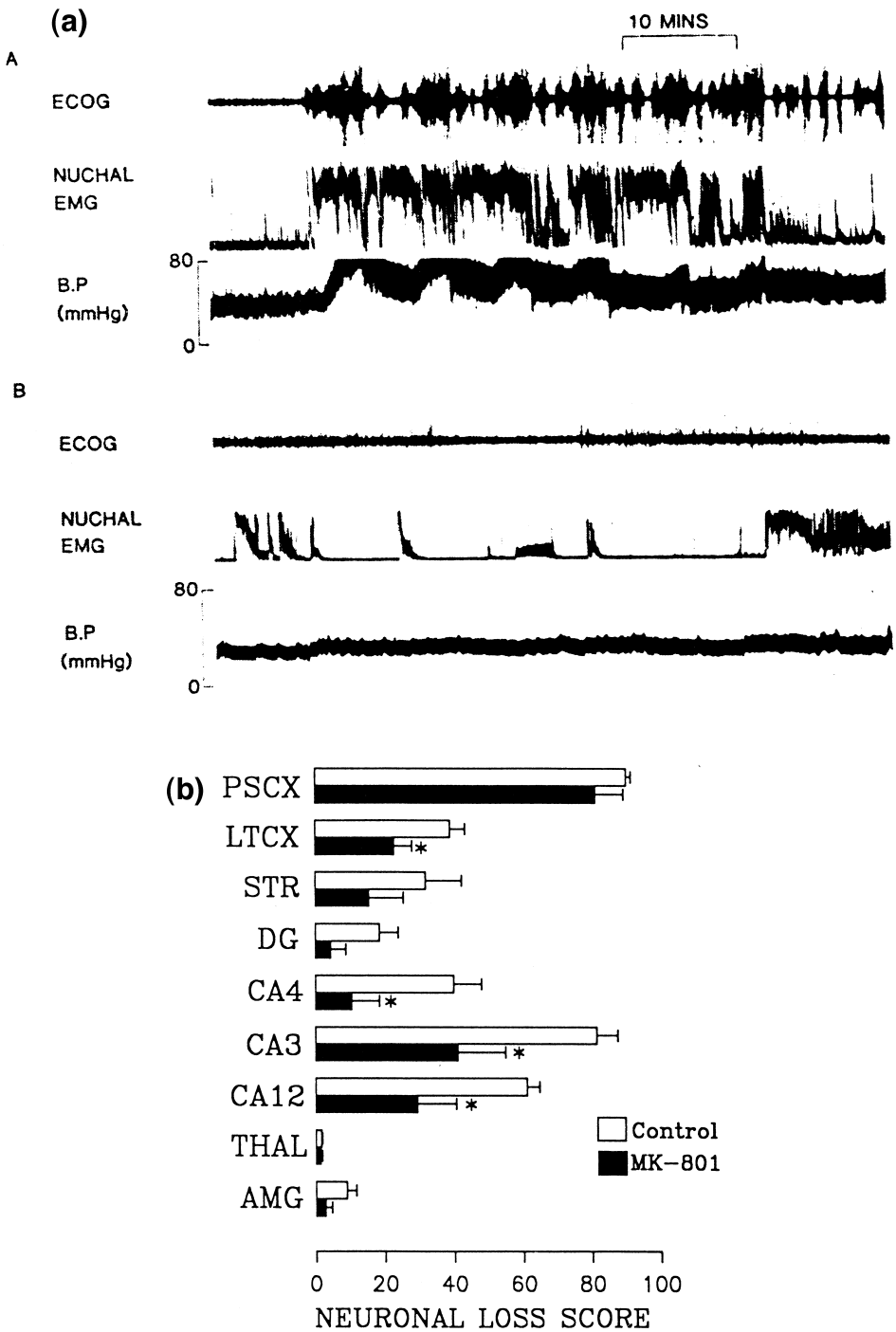

Fig. 11. (a) Registration of electrocortikogramm (ECOG), nuchal electromyogramm (nuchal EMG) and arterial blood pressure (BP) in term fetal sheep 9 h after 30 min of global cerebral ischemia. Note the absence of epileptogenic activity in treated (B; 0.3 mg/kg MK-801 over 36 h starting 6 h after ischemia) in contrast to untreated fetuses (A, B). (b) Neuronal cell damage 72 h after global cerebral ischemia of 30-min duration in term fetal sheep. Treated (0.3 mg/kg MK-801 over 36 h starting 6 h after ischemia) vs. untreated fetuses. $^{*}P < 0.05$; PSCX, parasagittal cortex; LTCX, lateral cortex; STR, striatum; DG, dentate gyrus; CA1, 2, 3, 4 hippocampal sectors; THAL, thalamus; AMG, amygdala [320].

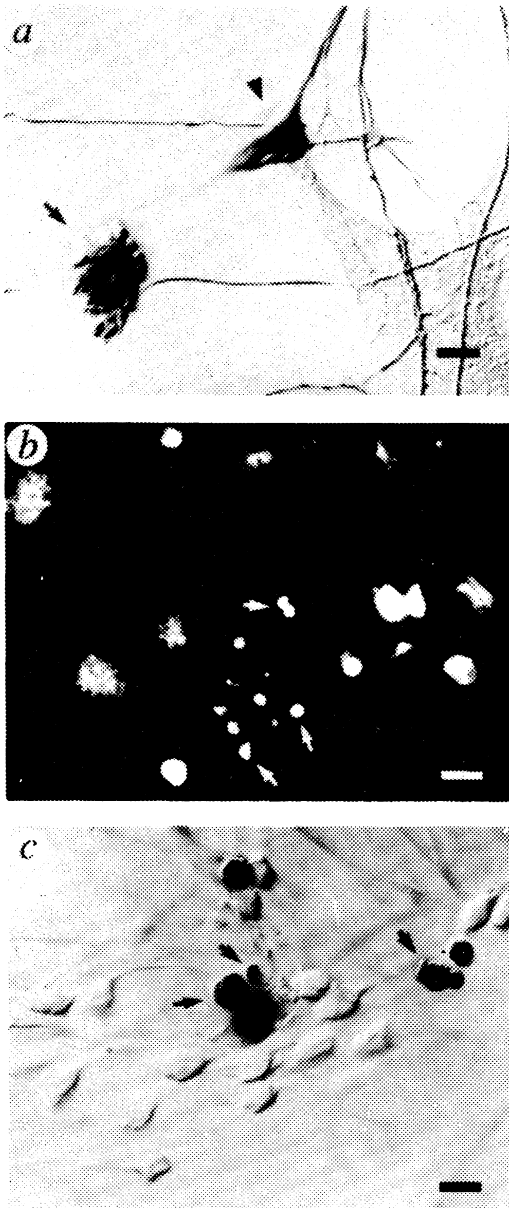

Fig. 12. Apoptosis in neuronal cell culture. (a) Illustration of an intact neuron (arrowhead) and an apoptotic neuron with typical intracytoplasmic vesicles (arrow). (b) Fluorescence staining of 10-day-old apoptotic neurons which shows fragmentation of nuclei and condensation of chromatin (arrows). (c) DNA-fragmentation in neurons illustrated by the TUNEL-method [52].

post-ischemic inhibition of protein synthesis using cycloheximide, or inhibition of RNA synthesis with actinomycin or through inhibition of endonuclease with aurin tricarboxylic acid. In addition, the amount of apoptotic cell death can also be reduced by inhibition of caspases in neonatal rats after a hypoxic–ischemic insult [61]. These findings all point towards the existence of a built-in cellular suicide programme [274,281]. It is also possible that the form of secondary cell death following ischemia is determined by the severity of the primary insult. Thus, Dragunow et al. were able to demonstrate that delayed cell death in immature rat brains subjected to a 15-min period of hypoxic–ischemia was of an apoptotic nature, while after a 60-min insult, the neuronal damage was predominantly necrotic [85]. Other investigators have also reported correlations between the severity of the insult and the extent of apoptotic cell death [190,216].

As has since been shown in numerous studies, including some on immature animals, cerebral ischemia can induce the expression of a whole series of proto-oncogenes [44,82,92,137,173,233,308]. Proto-oncogenes themselves code for proteins that act as transcription factors and regulate the expression of genes modulating cell growth and differentiation. They are also termed ‘immediate early genes’ since they are expressed within a few minutes of an insult.

These include c-fos, c-jun, jun-B, jun-D. The transcriptional activity of proteins of the fos-family is caused by a heterodimer formation with proteins of the jun-family [194]. Fos- and jun-proteins can also form dimers with proteins of the ATF- and CREB families and thereby increase their promotor affinity [122].

As already mentioned, transcription factors control the expression of genes participating in cell growth and differentiation. Depending on the severity of the insult, these factors are therefore capable of initiating processes leading to apoptotic cell death or triggering a recovery programme. Recent research findings have indicated that the proto-oncogenes and cell cycle-dependent proteins such as cyclin D1 [325,349], and tumor suppressor genes such as p53 are critically involved in this control function (Fig. 13). Thus, the expression of the p53 gene was demonstrated in focal cerebral ischemia or kainate-induced seizures causing neuronal DNA lesions in the rat [189,290]. Weaker expression of p53 in transgenic mice subjected to cerebral ischemia was accompanied by a milder degree of brain damage [73]. As we know from other organ systems, p53 protein recognizes and binds DNA-lesions, possibly straight onto its C-terminal [155,186]. Furthermore, it acts as a transcription factor, inducing the expression of p21 [89], that inhibits cyclin-dependent kinases [134]. p21, on the other hand, restricts the ability of PCNA (proliferating cell nuclear antigen) to activate DNA polymerase δ the principle replicative DNA polymerase [341]. In apparent contradiction with its role in suppressing cell proliferation via p21 expression, p53 also increases the mRNA and protein for cyclin D1. Cyclin D1 is a major effector of G1 phase entry supporting the contention that besides its role in the cell cycle, it may also be involved in p53-mediated apoptosis.

If lesion-induced signal transformation pathways or the ectopic expression of growth factors, some of which are potent mitogens, induce the expression of cell cycle components in postmitotic neurons, the concomitant DNA damage-induced p53 may halt or antagonize this pathway leading to a possible conflict in decisions. p53-mediated halt in replication may be associated with a p53-dependent

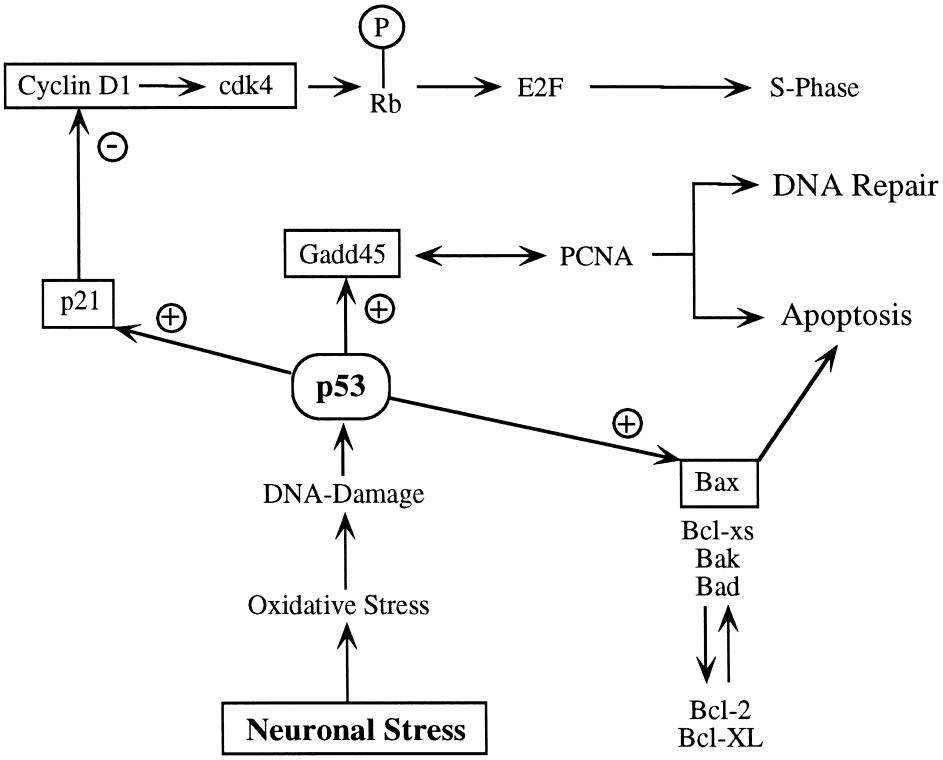

Fig. 13. Diagram illustrating the central role of p53 in mediating DNA repair or apoptosis in neurons during and after cerebral ischemia [168]. Gadd45, growth-arrest-and-DNA-damage-inducible; cdk4, cyclin D-dependent kinase; for further abbreviations see text.

transactivation of the ubiquitously expressed mammalian gene Gadd45 (growth-arrest-and-DNA-damage-inducible) [167]. Its product is involved in DNA repair and interacts with PCNA. PCNA is implicated in replication of cellular DNA, but is also required for DNA excision repair [300,307]. Besides p53, PCNA, Gadd45, and p21 are also induced in brain pathologies suggesting that some of the molecular mechanisms referred to in non-neural cells, may also hold true in the brain. Depending upon the developmental stage of the injured brain and the extent of cell damage on the one hand, and upon damage-induced p53 expression on the other, neurons may attempt cell cycle entry, a process that will involve a certain amount of DNA repair, or may only attempt transcription-coupled DNA repair. The cell death decision may result from the impossibility to proceed with both processes. Indeed, it has recently been shown in vitro that the p53 transcription factor, besides its role in halting replication while favoring repair, attenuates *Bcl*-2 expression, and is a direct transcriptional activator of the *Bax* gene, whose product is shown to induce apoptosis [9,168,227–229,275].

10. Therapeutic strategies

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. 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 Sections 8 and 9), 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.

10.1. Hypothermia

The induction of mild hypothermia has raised interesting possibilities for neuroprotection from cerebral ischemia (Review: Ref. [203]). Various publications dating back to the 1950s, have described the therapeutic benefits of hypothermia in brains subjected to a wide variety of insults including brain trauma [264,295], cerebral haemorrhage [150], cardiac arrest [26], carbon monoxide poisoning [71], neonatal asphyxia [346] and seizures [48]. Based on these findings, routine induction of hypothermia was introduced early on in heart and brain surgery to protect the brain in the event of iatrogenic intraoperative cardiac arrest [47,86,188,197,237]. Over the last few years, induction of mild hypothermia has been examined once again as a means of protecting the brain from ischemically induced damage. Experimental studies on adult animals have shown

Table 4
Protein synthesis rate as percentage of control in hippocampal slices from mature fetal guinea pigs 12 h after in vitro ischemia. Hypothermia was induced by lowering the incubation temperature from 37° to 33° [37]. Values are given as mean ± S.D.

	Percentage control	
	Normothermia	Hypothermia
Control	100.0 ± 16.5	100.0 ± 12.1
Ischemia 10 min	91.7 ± 12.6	114.8 ± 11.5[b]
Ischemia 20 min	67.8 ± 8.7[a]	95.1 ± 9.2[b]
Ischemia 30 min	61.5 ± 15.9[a]	85.8 ± 3.1[b]
Ischemia 40 min	50.8 ± 14.1[a]	78.2 ± 19.2[a,b]

[a] $P < 0.05$ (ischemia vs. control).
[b] $P < 0.05$ (normothermia vs. hypothermia).

that lowering of the brain temperature by 3–4°C during global cerebral ischemia reduces neuronal cell damage dramatically [53,67,111,345,348]. Furthermore, the treated animals were found to perform better than controls in subsequent learning and behavioural tests [111].

The author's research team was also able to demonstrate a neuroprotective effect of mild hypothermia in fetal brain tissue subjected to ischemic insults. They found that the post-ischemic recovery of protein synthesis and energy metabolism in hippocampal slices from mature guinea pig fetuses was considerably improved, in comparison to controls, by induction of mild hypothermia (Table 4) [32,37].

In a recently published study, Gunn et al. described the effects of moderate hypothermia in sheep fetuses subjected to severe global cerebral ischemia in utero [117]. Hypothermia was initiated during the reperfusion phase, 90 min after induction of 30 min of ischemia, in a four-vessel occlusion model, and maintained for 72 h. By this method, it was possible to reduce the extent of neuronal cell damage in areas of the cortex cerebri by up to 60% (Fig. 14) [117]. Even if hypothermia was started 5.5 h after ischemia, neuroprotection could be observed in this animal model [119]. 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 [54,117]. In fact, Gunn et al. demonstrated in a recent clinical study that selective head cooling in newborn infants after perinatal asphyxia is a safe and convenient method of quickly reducing brain temperature [118].

The mechanisms underlying the neuroprotective effect of mild hypothermia are still unclear (Review: Ref. [203]). For a long time, it was assumed that hypothermia exerted its effect by reducing cerebral oxygen consumption [23,41] and a delayed emptying of energy stores during ischemia [175,219,220,311,312]. However, this hypothesis could not be confirmed in experiments on hippocampal slices. The fall in ATP levels during ischemia did not correlate with the post-ischemic inhibition of protein synthesis, a parame-

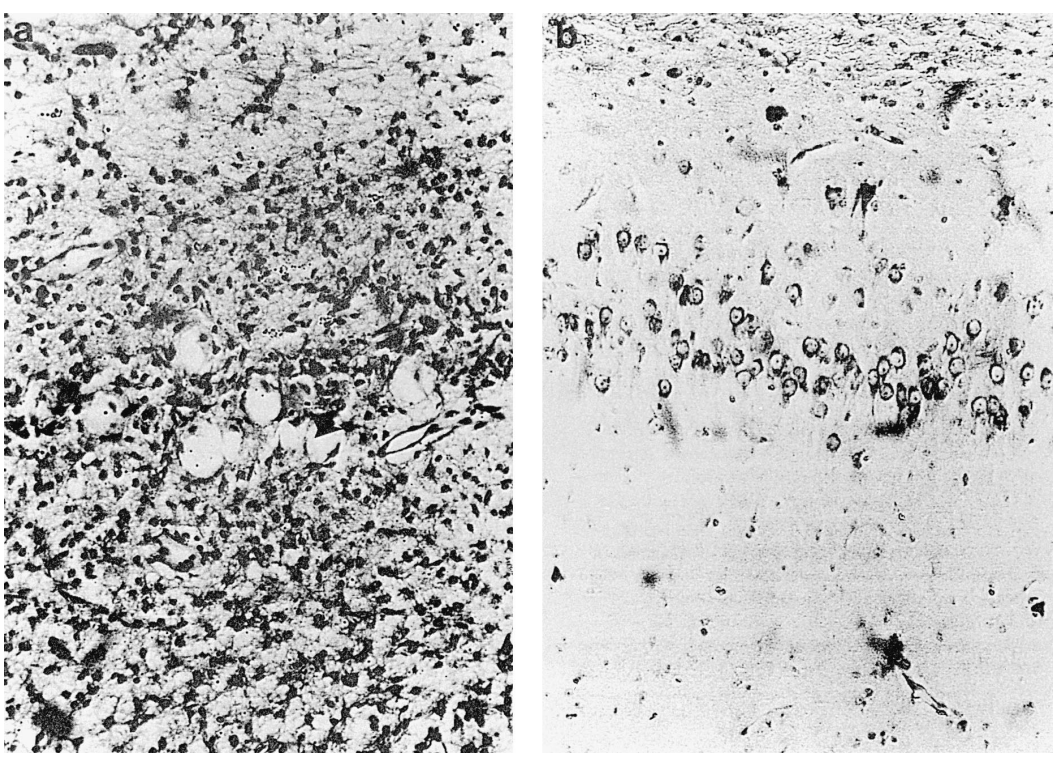

Fig. 14. (a,b) Section of the parasagittal cortex in (370-fold magnification) in term fetal sheep 5 days after 30 min of cerebral ischemia followed by normothermia (a) or mild hypothermia (b). (a) Complete neuronal necrosis (normothermic group). (b) Minor degree of neuronal cell damage (hypothermic group) [117].

ter taken as a measure of neuronal cell damage [37]. Whether the effect of mild hypothermia can be explained by an improved recovery of energy metabolism directly after ischemia is also debatable. Chopp et al. found only a minimal improvement in concentrations of creatine phosphate and ATP after induction of mild hypothermia in rats subjected to global cerebral ischemia [64]. Nor, as in vitro experiments have demonstrated, can modulation of cerebral flow after ischemia be the sole basis of the neuroprotective effect of hypothermia [37]. Although the release of excitatory amino acids both during and after ischemia is prevented by mild hypothermia [54,106], it remains unclear whether these findings are simply an epiphenomenon or the true basis of hypothermia's neuroprotective effect [37]. Other effects that appear to be associated with the therapeutic induction of mild hypothermia after cerebral ischemia are: reduced oxygen radical formation [60,104], a stabilisation of the blood-brain barrier [83] and a modification of enzyme activation [57,66,353], etc.

10.2. 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, calcium antagonists, growth factors and anticytokines. Table 5 presents all the potential neuroprotective substances currently under investigation (modified according to Ref. [335]).

10.3. Magnesium

The last interesting therapeutic approach to be discussed emerged from a retrospective analysis carried out by Nelson and Grether. 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 [238]. 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 (Fig. 15). The same effect could be observed in the children of patients not suffering from pre-eclampsia. The protective effect of magnesium was independent of variables such as the administration of tocolytic agents or drugs to accelerate fetal lung development or any other maternal or fetal risk factors. Almost identical results were recently obtained in a retrospective study carried out by Schendel et al. [292].

As numerous animal experiments, both in vivo and in vitro have shown, magnesium can reduce the extent of ischemically induced neuronal cell damage [165,166, 213,284,332]. This neuroprotective effect could be based on a number of pathophysiological mechanisms, one being the well-known vasodilatory properties of magnesium as a calcium antagonist [6,273,296,317]. It has also been established that hypoxic–ischemic brain damage is partly caused by the intracerebral release of excitatory amino acids [286]. Magnesium may protect neurones from anoxic damage by preventing the presynaptic release of these substances [166,284]. The massive intracellular influx of calcium that takes place during ischemia plays a key role in the development of neuronal cell damage [62,304]. Magnesium blocks the glutamate-controlled NMDA receptor [201,244] as well as voltage-dependent calcium channels, hindering the influx of extracellular calcium into the neurons. The activation of numerous calcium-dependent proteases, lipases and endonucleases is thereby counteracted. As already mentioned, the release of excitatory amino acids during and after cerebral ischemia in damaged brain regions can lead to epileptiform activity. This, in turn, can create an imbalance between blood flow and cell metabolism, causing brain damage [148]. Magnesium has proven anti-convulsive properties [292], that can diminish epileptiform activity and thus reduce the extent of possible brain damage. The lowering of the rate of cell metabolism has been put forward as another possible explanation of the neuroprotective effect of magnesium [166,314].

In the United States, magnesium has been administered for over 20 years for treatment of premature contractions and pre-eclampsia [69,301]. Magnesium crosses the placental barrier and enters the fetal blood plasma [72,112]. The concentration in the fetal blood plasma corresponds roughly to that in the maternal plasma [72,112]. Very high magnesium levels can lead to a temporary lowering of muscle tone, weakened reflexes and respiratory depression in the newborn. Serious complications in either the infant or mother are, however, extremely rare [193]. The Collaborative Eclampsia Trial showed that infants of mothers treated with magnesium for EPH gestosis are less frequently intubated or transferred to the children's hospital for intensive care than those whose mothers received phenytoin [323]. However, a recently published study described for the first time an increased child mortality after pregnancies in which expectant mothers were treated with i.v. magnesium [226]. However, a large percentage of these infants died after the neonatal period. Some of the deaths were caused by feto-fetal transfusion syndrome in twins or congenital abnormalities, making any causative link between the magnesium therapy given during pregnancy and these fatalities quite unlikely. This has been confirmed by a new retrospective analysis carried out by Grether et al. [114].

To date, research findings strongly suggest that administration of magnesium can lower the incidence of cerebral palsy in immature neonates weighing less than 1500 g. However, the consistency of this therapeutic effect still

Table 5
Pharmacological intervention on hypoxic/ischemic brain damage in various models of hypoxia/ischemia [335]
UCO: unilateral occlusion of carotid arteries, BCO: bilateral occlusion of carotid arteries, VOAO: occlusion of the vertebro-occipital anastomoses, BP: arterial blood pressure, bFGF: basic fibroblast growth factor.

Treatment class	Treatment details	Age/species	Hypoxic/ischemic insult	Time of treatment with respect to insult	Neuroprotection/ pathology	Refs.
VSCC's antagonists	Flunarizine (30 mg/kg)	7 days/rat	UCO + 2 h 8% O_2	pre	partial	[306]
	Flunarizine (30 mg/kg)	7 days/rat	UCO + 3 h 8% O_2	pre	partial	[65]
	Flunarizine (30 mg/kg)	21 days/rat	UCO + 2 h 8% O_2	pre	partial	[115]
	Flunarizine (9 mg/kg)	fetal sheep	30 min BCO (+ VOAO)	pre	partial	[116]
	Flunarizine (1 mg/kg)	fetal sheep	30 min BCO (+ VOAO)	pre	partial	[39]
	Nimodipine (70 μg/kg or 0.5 mg/kg)	7 days/rat	UCO + 3 h 8% O_2	pre	no effect	[65]
	Nimodipine (0.5 mg/kg)	0–3 days/pig	30 min BCO + hypotonia and 15 min 6% O_2	post	no effect	[185]
NMDA antagonists	MK-801 (10 mg/kg)	7 days/rat	BCO + 1 h 8% O_2	pre	total	[138]
	MK-801 (10 mg/kg)	7 days/rat	BCO + 1 h 8% O_2	post	partial	[138]
	MK-801 (1 mg/kg)	7 days/rat	UCO + 3 h 8% O_2	pre, intra	partial	[202]
	MK-801 (10 mg/kg)	7 days/rat	UCO + 2 h 8% O_2	pre, intra	partial	[93]
	MK-801 (0.3 bzw. 0.5 mg/kg)	7 days/rat	UCO + 1.5 h 7.6% O_2	post (0 h)	partial	[121]
	MK-801 (0.75 mg/kg)	7 days/rat	UCO + 1.5 h 7.6% O_2	post (0 h)	no effect	[121]
	MK-801 (3 mg/kg)	0–3 days/pig	30 min BCO + hypotonia and 15 min 6% O_2	post (0 h)	no effect	[184]
	MK-801 (0.3 mg/kg)	Schaffet	30 min global ischemia	post (6–36 h)	partial	[320]
	Felbamate (300 mg/kg)	7 days/rat	BCO + 1 h 6.5% O_2	post	partial	[343]
AMPA antagonist	NBQX (20 + 20 mg/kg)	7 days/rat	UCO + 1.5 h 7.6% O_2	post (0 + 1 h)	partial	[121]
Glutamate release inhibitor	BW1003C87 (10 mg/kg)	7 days/rat	UCO + 1.5 h 7.7% O_2	pre	partial	[101]
Nonspecific glutamate antagonist	Kynurenic acid (300 mg/kg)	7 days/rat	UCO + 2 h 7.7% O_2	post	partial	[5]
	Kynurenic acid (200–300 mg/kg)	7 days/rat	UCO + 1.5 h 8% O_2	pre (1 h)	partial	[246]
Antioxidant enzymes	PEG-SOD + PEG-Catalase (10.000 U/kg)	0–3 days/pig	30 min BCO + hypotonia u. 15 min 6% O_2	post	no effect	[185]
Iron chelator	Deferoxamine (100 mg/kg)	7 days/rat	UCO + 2.25 h 8% O_2	post (5 min)	partial	[258]
Free radical scavengers	Allopurinol (135 mg/kg)	7 days/rat	UCO + 3 h 8% O_2	pre or post (15 min)	partial	[256,257]
	U74006F (7.5 mg/kg)	7 days/rat	UCO + 2 h 7.7% O_2	post or pre and post, pre and post	partial	[14]
	U74689F (10 mg/kg)	7 days/rat	UCO + 3 h 8% O_2	pre and post	no effect	[65]
NO synthase inhibitors	Nitro-L-arginine (2 mg/kg)	7 days/rat	UCO + 2.5 h 8% O_2	pre and post	partial/no effect	[129]
	Nitro-L-arginine (50–100 mg/kg)	7 days/rat	UCO + 8% O_2	pre	partial	[330]
Glucocorticoids	Dexamethasone (0.01–0.5 mg kg^{-1} Tag^{-1})	7 days/rat	UCO + 3 h 8% O_2	pre	total	[16]
	Dexamethasone (0.1 mg/kg)	7 days/rat	UCO + 3 h 8% O_2	pre	total/no effect	[16]
					partial	[65]
	Methylprednisolone (0.7 mg/kg)	7 days/rat	UCO + 3 h 8% O_2	pre (24 h)	partial	[334]
	Corticosterone (40 mg/kg)	7 days/rat	UCO + 2 h 8% O_2	pre (24 + 5 h)	partial	[333]
	Dexamethasone (0.1 mg/kg)	14 days/rat	UCO + 1 h 8% O_2	pre (24 + 5 h)	partial	[334]
	Dexamethasone (0.1 mg/kg)	1 month/rat	UCO + 30 min 8% O_2	pre (24 + 5 h)	no effect	[334]
Antiinflammatory	Interleukin-1-receptor-antagonist (100 mg/kg)	7 days/rat	UCO + 2 h 7.5% O_2	pre and post	partial	[207]
	Osteogenic protein-1 (50 μg)	12 days/rat	BCO + 20 min 8% O_2	pre	partial	[270]
	Antineutrophil serum	7 days/rat	UCO + 2.25 h 8% O_2	pre	partial	[260]
Growth factor	bFGF (100 μg/kg)	7 days/rat	UCO + 1.5 h 8% O_2	pre (30 min)	partial	[247]
Gangliosides	GM1 (50 mg kg^{-1} Tag^{-1})	7 days/rat	2 h 7% O_2	pre and post	partial	[120]
	GM1 (30 mg kg^{-1} Tag^{-1})	fetal sheep	30 min ischemia	pre	partial	[321]
Anticonvulsants	Zonisamide (75 mg/kg)	7 days/rat	UCO + 2.5 h 8% O_2	pre	partial	[123]
	Phenytoin (50 mg/kg)	7 days/rat	UCO + 2.5 h 8% O_2	pre	partial	[124]
Inhibition of caspases	boc-aspartyl-fluoromethyl-ketone	7 days/rat	UCO + 2 h 7.5% O_2	post	partial	[61]

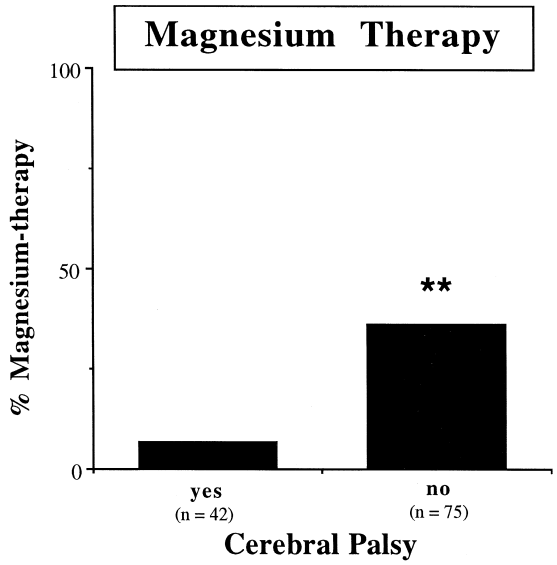

Fig. 15. Incidence of cerebral palsy in very low birth weight children (<1500 g) at the age of 3 years. Note that the incidence of cerebral palsy in children from women with ante-natal magnesium therapy was much lower than in those without magnesium therapy (7% vs. 36%; $^{**}P < 0.01$) [238].

needs to be demonstrated in multicentre randomised, double-blind studies.

11. Conclusion

Perinatal brain damage in the mature fetus is usually brought about by severe intrauterine asphyxia following an acute reduction of the uterine or umbilical circulation. Owing to the acute reduction in oxygen supply, oxidative phosphorylation in the brain comes to a standstill. The Na^+/K^+ pump at the cell membrane has no more energy to maintain the ionic gradients. In the absence of a membrane potential, large amounts of calcium ions flow through the voltage-dependent ion channel, down an extreme extra-/intracellular concentration gradient, into the cell. Additionally to the influx of calcium ions into the cells via voltage-dependent calcium channels, calcium also enters the cells through glutamate-regulated ion channels. Current research suggests that the excessive increase in levels of intracellular calcium, so-called calcium overload, leads to cell damage through the activation of proteases, lipases and endonucleases. A second wave of neuronal cell damage occurs during the reperfusion phase. This cell damage is thought to be caused by the post-ischemic inhibition of protein synthesis, release of oxygen radicals, synthesis of NO, inflammatory reactions and an imbalance between the excitatory and inhibitory neurotransmitter systems. Part of the secondary neuronal cell damage may be caused by induction of a kind of cellular suicide programme known as apoptosis. Knowledge of these pathophysiological mechanisms has enabled scientists to develop new therapeutic strategies with successful results in animal experiments. Of these i.v. administration of magnesium and post-ischemic induction of cerebral hypothermia may become clinical relevance over the next years.

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