

Resuscitation of the newborn – with or without supplemental oxygen?

An experimental study in newborn piglets

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Effects of recombinant human superoxide dismutase during reoxygenation with 21% or 100% oxygen after cerebral asphyxia in newborn piglets. *J Matern Fetal Neonatal Med.* 2003; 14:96-101.

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Comparison of short and long duration oxygen treatment following cerebral asphyxia in newborn piglets. *Pediatr. Res.* 2004; 56:125-31.

Abbreviations

CaO₂, blood oxygen content

CBF, cerebral blood flow

CMRO₂, cerebral metabolic rate of oxygen

CO₂, carbon dioxide

EAA, excitatory amino acids

H₂O₂, hydrogen peroxide

HI, hypoxemia-ischemia

HIE, hypoxic ischemic encephalopathy

HIH, hypoxemia-ischemia-hypercapnia

L-NAME, N-nitro-*L*-arginine methyl ester

MABP, mean arterial blood pressure

NF-κB, nuclear factor-κB

NMDA, N-methyl-D-aspartate

NO, nitric oxide

NOS, nitric oxide synthase

O₂⁻ superoxide radical

OH·, hydroxyl radical

ROP, retinopathy of prematurity

ROS, reactive oxygen species

SOD, superoxide dismutase

Introduction

Birth asphyxia

Definition and incidence

Approximately 2-5% of babies born at term are in need of resuscitation, some of them as a result of birth asphyxia (1, 2). Each year more than 5 mill neonatal deaths occur. According to World Health Organization, 1 mill of these are caused by birth asphyxia (3). Birth asphyxia is often defined as a condition where an impaired perinatal placental or pulmonary gas exchange leads to progressive hypoxemia and hypercapnia followed by a significant respiratory and metabolic acidosis, and a variable degree of ischemia (4). It can be caused by a variety of conditions, for instance abruption or infarction of the placenta, fetal or maternal bleeding or anemia, compression of the umbilical cord or maternal or fetal cardiovascular disease. Unfortunately the condition does not have a worldwide uniform definition, and the incidence varies greatly between industrialized and developing countries. The incidence in industrialized countries is estimated to be between two and four cases per 1000 term deliveries (5), and much higher in developing countries (6, 7).

Cerebral hypoxemia-ischemia (HI) following birth asphyxia may lead to neurological injury, but this is only one of several possible causes of brain damage in the newborn. However, it is the single most important cause in term babies (8, 9).

Diagnosis and prognosis

Defining exact criteria for the diagnosis and prognosis of birth asphyxia has proven to be difficult. Several signs and symptoms are associated with asphyxia, but no single sign is specific. Fetal bradycardia, metabolic acidosis, meconium staining of the amniotic fluid and low Apgar score are not specific and have poor predictive value concerning neurological outcome. Apgar score is a widely used method of immediate evaluation of the newborn infant's condition (10, 11). However, low Apgar scores estimated during the first few minutes of life are not specific to any particular condition, and are poor predictors of brain injury (12-14). To date, birth asphyxia is probably best diagnosed and assessed by what it leads to,

hypoxic-ischemic encephalopathy (HIE). This condition develops within one or two days, and is classified in three clinical stages as first described by Sarnat and Sarnat (15, 16). Mild HIE (grade I) includes irritability, poor sucking and mild hypotonia, and have an almost uniform good outcome. Moderate HIE (grade II) includes lethargy, marked abnormalities of muscle tone, seizures and a need for tube feeding, and leads to severe handicap in approximately 20% of the cases. Severe HIE (grade III) includes coma, prolonged seizures, severe hypotension and respiratory failure, and leads to severe handicap or death in most cases (17). However, asphyxia is not the only possible cause for encephalopathy in newborn babies (18, 19), and the presence of HIE therefore needs to be accompanied by other signs of fetal distress such as umbilical artery acidosis ($\text{pH} < 7.00$), Apgar score ≤ 3 for more than 5 minutes and signs of multiorgan dysfunction in order to diagnose neurological injury as a result of birth asphyxia (20).

Special considerations concerning resuscitation of the newborn, some practical aspects

The resuscitation of babies at birth is somewhat different from the resuscitation of all other age groups and knowledge of the relevant physiology and pathophysiology is essential. In contrast to adults, their need for resuscitation is seldom caused by ventricular fibrillation or ventricular tachycardia. They usually have a beating heart although heart rate and cardiac output may be severely depressed.

The transition from placental gas exchange in a liquid-filled intrauterine environment to spontaneous breathing of air requires dramatic physiological changes in the infant within the first minutes to hours after birth. This change from fetal to extrauterine life is characterized by a series of unique events: the lungs change from fluid-filled to air-filled, pulmonary blood flow increases dramatically, and intra- and extra-cardiac shunts (foramen ovale and ductus arteriosus) initially reverse direction and subsequently close.

Birth asphyxia can eventually cause cardiac arrest. Prior to this, the infant will develop peripheral vasoconstriction, tissue hypoxia, acidosis, poor myocardial contractility and bradycardia. The key to successful neonatal resuscitation is establishment of adequate ventilation (21, 22), and the commonest reason for failure of the heart rate to respond is failure to achieve lung ventilation. Airways should be cleared and opened before ventilation is

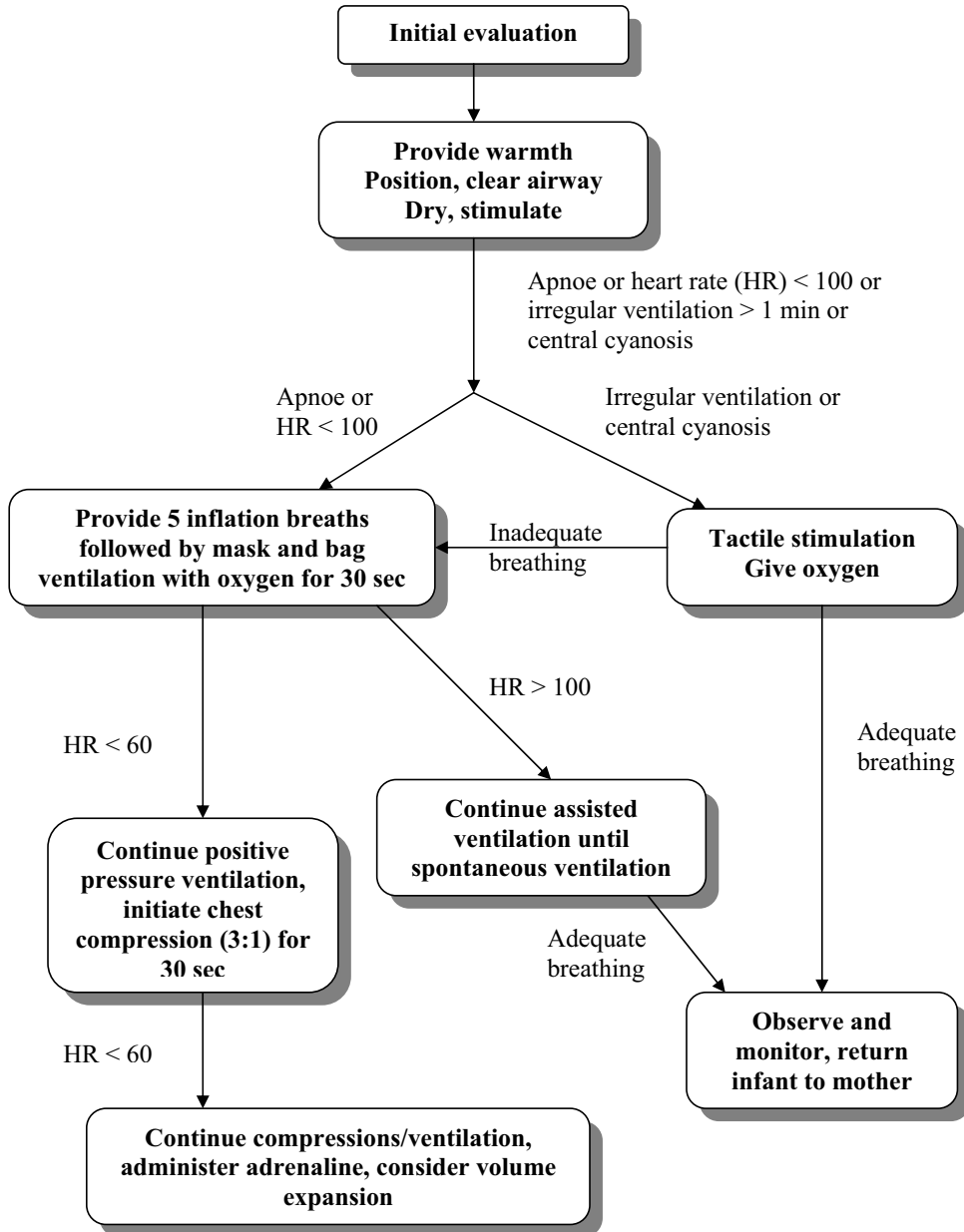
started. Reversal of hypoxia, acidosis and bradycardia depends on inflation of fluid-filled lungs with air or oxygen. Initially this may require higher ventilation pressures than are normally used in rescue breathing during infancy (23, 24). The first five breaths should be inflation breaths. Expansion of the lungs will establish functional residual capacity and increase alveolar oxygen tension which in turn decreases pulmonary vascular resistance and results in an increase in pulmonary blood flow after birth. Failure to do so may result in persistence of right-to-left intracardiac and extracardiac shunts (persistent pulmonary hypertension) and intrapulmonary shunting of blood with resultant hypoxemia.

About 80% of newborns in need of resuscitation respond to mask ventilation only, whereas 20% need endotracheal intubation (1). Only a very small percentage (< 1-2%) will need chest compressions and adrenaline medication (25). If the heart rate remains low (less than 60 per minute) after 30 sec of adequate ventilation, cardiac compressions should be started. The most efficient way of doing this in the neonate is to encircle the chest with both hands with the fingers behind the baby and the thumbs apposed on the sternum just below the inter-nipple line. There should be a 3:1 ratio of compressions to ventilation, with 90 compressions and 30 breaths to achieve approximately 120 events per minute. Only if the heart rate has not responded after 30 sec of combined ventilation and compressions, drug therapy should be considered.

Volume expanders may be necessary if the baby is hypovolemic, and this should be suspected in any baby that is not responding to resuscitation. The fluid of choice is an isotonic crystalloid solution, and the initial dose is 10 mL/kg. O-negative blood may be indicated for replacement of large-volume blood loss. Heat loss should be prevented as cold stress increases oxygen consumption and impedes effective resuscitation (26, 27).

Often the first indication of success will be an increase in heart rate. Recovery of respiratory drive may be delayed. The more preterm a baby the less likely it is to establish adequate respiration, and preterm babies are likely to be deficient in surfactant. Discontinuation of resuscitation may be appropriate if there has been no spontaneous circulation after 15 minutes. The outcome in such cases is likely to be very poor (28, 29).

Neonatal resuscitation



Current guidelines for neonatal resuscitation, modified from ref. (30).

Mechanisms of perinatal hypoxic-ischemic encephalopathy

Brain injury secondary to hypoxic-ischemic disease is the predominant form of brain injury encountered in the perinatal period. Although other causes like hemorrhage, infection, neurologic disease and metabolic derangement are recognized, hypoxia-ischemia is the single most important cause in term babies (8, 9).

The way the infant responds to HI is maturity-dependent, the full-term infant being different from the premature infant. The location, extent and progression of brain damage are determined both by the severity and duration of the insult and the maturity of the brain, resulting in selective vulnerability to specific brain regions. In term infants, neuronal injury predominates, and in the premature infant, oligodendroglial/white matter injury predominates (8, 9). The areas most heavily affected in term infants are the parasagittal region of the cerebral cortex and the basal ganglia (31, 32). In preterm infants periventricular leucomalacia is more often seen (32, 33).

During the initial period of asphyxia, blood flow to the brain increases in order to maintain oxygen supply and brain metabolism. As the insult persists, cardiac depression with secondary bradycardia and systemic hypotension occurs. Insufficient supply of tissue oxygen and glucose develops, and oxidative metabolism shifts to anaerobic glycolysis with its inefficient generation of high-energy phosphate reserves leading to breakdown of cellular energy metabolism. As a consequence, cell membrane depolarization follows, accompanied by an increase of intracellular Na^+ and Ca^{2+} , and a decrease of intracellular K^+ . Large amounts of Ca^{2+} enters the cells both via voltage-dependent Ca^{2+} -channels and glutamate-regulated ion channels. This Ca^{2+} -overload leads to cell damage through activation of proteases, lipases and endonucleases (34).

The cerebral energy failure shows a biphasic pattern (35-37). The primary energy failure during the actual insult is followed by at least partial recovery soon after reoxygenation-reperfusion. However, after a latent period of hours to a few days, a secondary, non acidotic (35) phase of energy failure may develop, despite apparently adequate cerebral perfusion and oxygenation.

This biphasic pattern opens up a therapeutic window – between the primary and secondary energy failure - in which modulation of detrimental processes may be possible.

The events leading to brain injury following secondary energy failure, may be brought about or are at least modulated by reactive oxygen species (ROS), excitatory amino acids (EAA), nitric oxide (NO) and inflammatory reactions, finally triggering apoptosis and necrosis.

Reactive oxygen species

Free radicals are highly reactive chemical molecules containing one or more unpaired electrons. They donate or take electrons from other molecules in an attempt to generate a more stable species. Human cells generate oxygen-derived free radicals and oxidizing species, collectively termed reactive oxygen species (ROS), during normal respiratory and metabolic activities. ROS are redox intermediates produced during the sequential reduction of oxygen to water in the mitochondrial respiratory chain, as an integral part of the energy-regenerating process of oxidative phosphorylation. When molecular oxygen is reduced by one electron, the product is superoxide radical (O_2^-). The addition of a second electron to O_2^- gives rise to hydrogen peroxide (H_2O_2 , not a true radical). The one electron reduction of H_2O_2 yields water and in the presence of reduced transitional metals such as Fe^{2+} or Cu^{2+} , hydroxyl radical ($OH\cdot$), the strongest oxidant produced in biological systems. This highly reactive radical can damage all biological macromolecules including proteins, lipids, and nucleic acids directly or indirectly through the generation of secondary radicals, and consequently, cause considerable tissue damage (38).

To combat the cytotoxic actions of ROS, cells are equipped with a large variety of antioxidant defense that includes: 1) enzymes such as superoxide dismutase (SOD) which catalyze the dismutation of O_2^- to H_2O_2 ; 2) hydroperoxide scavenging enzymes such as catalase and glutathione peroxidase which convert H_2O_2 to water; 3) hydrophilic radical scavengers such as ascorbate, urate and glutathione; and 4) lipophilic radical scavengers such as tocopherols, flavonoids, carotenoids, and ubiquinol.

ROS are implicated in the pathogenesis of inflammatory, toxic, and metabolic insults, ischemia-reperfusion and hypoxia-reoxygenation injury, carcinogenesis, and atherosclerosis (39). Conditions that are known to increase production of ROS include HI and reperfusion

(40-42), exposure to supraphysiologic concentrations of oxygen (43, 44), influx of inflammatory cells (45, 46), and increased amounts of transitional metals (47).

Under physiological circumstances, there is a balance between ROS production and antioxidant defense mechanisms. Under pathological conditions, this balance is disturbed because of either excessive ROS generation or deficient antioxidant activity. Oxidative stress can be defined as generation of ROS in excess of the antioxidant defense mechanisms. Oxidative stress not only occurs during the initial asphyxic insult, but also during the recovery phase characterized by reoxygenation-reperfusion (48).

The brain, and especially the developing brain, is particularly susceptible to free radical injury because it is rich in polyunsaturated fatty acids, and the damaging effects of ROS on brain cells constitute one of the principal hypotheses put forward to explain brain injury following reoxygenation-reperfusion (48, 49). The primary site of ROS production within the brain is not known, as both endothelium, neurons and leukocytes may be important sources. The preterm infant is probably more vulnerable to oxygen radical attack than the term infant, as the antioxidant defense system is suggested to be better developed with increasing maturity (50, 51).

However, though ROS can be harmful in abundance, they also play important physiological roles which include signal transduction and regulation of cellular growth and differentiation (52), vasoactive control (53, 54), and they are involved in the body's defense against infection and in mediation of immune response (55).

Excitatory amino acids

As a result of hypoxemia-ischemia, excessive amounts of amino acids accumulate in the extracellular space of the brain (56-59).

The phenomenon of excitotoxicity was first described more than 30 years ago (60) when it was found that the excitatory amino acids (EAA) glutamate and aspartate could excite neurons to such an extent as to finally kill them.

Glutamate is the major EAA neurotransmitter that contributes to a number of developmental processes such as synaptogenesis, synaptic plasticity, learning and memory, as well as to neurodegeneration and hypoxic-ischemic brain injury (61, 62).

Glutamate activates postsynaptic receptors consisting of five subunits that form ionic channels permeable to cations. Three classes of ionotropic glutamate receptors have been identified and named on the basis of their pharmacological response to specific agonists such as amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA-receptor), kainate (KA-receptor) and N-methyl-D-aspartate (NMDA-receptor).

The activation of each of these receptors leads to an increase in the levels of free Ca^{2+} in the cells. This increase in free Ca^{2+} can activate proteases, lipases and endonucleases that again initiate processes leading to cell death (34). Most of the damage caused by glutamate following hypoxemia ischemia appears to be mediated by the NMDA-receptor. Several animal studies have shown that blocking of the NMDA-receptors ameliorates brain injury following hypoxemia-ischemia (63-65).

Although it is extremely likely that HIE and brain injury in human neonates are triggered by excessive stimulation of NMDA-receptors as they are in animals, the experimental evidence is less complete for humans. However, significantly higher levels of EAA has been found in the cerebrospinal fluid of asphyxiated newborn infants compared with controls (66), and high levels of glutamate in the basal ganglia of neonates with severe HIE have been demonstrated by proton magnetic resonance spectroscopy (67). Possible sources of increased EAA are release of the transmitter pool due to depolarization, reversal of the cellular reuptake systems, nonspecific leakage from injured cells, and leakage via a disrupted blood-brain barrier. The quantitative contribution from the different sources probably varies.

Evidence from experimental models and clinical investigation indicates that HIE is triggered by a profound disruption in the function of glutamate synapses so that re-uptake of glutamate from the synapse is impaired and post-synaptic membranes containing glutamate receptors are depolarized. Severe hypoxia preferentially depolarizes neuronal membranes, while ischemia probably has greater impact on the activity of glial glutamate re-uptake (68). In animal models of focal ischemia extracellular glutamate concentrations between 30 and 90 μM (4-6 times baseline) have been reported (69, 70), and in global ischemia extracellular glutamate levels may reach 16-30 μM (5-8 times baseline) (71-73). After a few minutes of reoxygenation-reperfusion levels return to baseline (57, 74), but during reperfusion, a secondary elevation of glutamate levels occur (75). In a newborn rat model, it has been shown that extracellular levels of EAA during the hypoxic ischemic insult does not correlate to the degree of brain injury, whereas the EAA concentrations during reflow were related to the

extent of infarction (56). Together, severe hypoxemia and ischemia trigger a delayed cascade of events that may result in cell death by apoptosis and/or necrosis.

The important role that NMDA-receptors play during development makes them special targets for neonatal hypoxemia ischemia (76, 77). Not only have high densities of NMDA-like receptors been found in the brain of the human fetus and infant (78, 79), NMDA-receptors expressed during development have subunit compositions that allow them to open more easily in response to glutamate and flux more Ca^{2+} than more mature receptors (80). Therefore, vulnerability to NMDA stimulation is not merely a function of NMDA-receptor density, but also depends on subunit composition and the developmental stage of synaptogenesis (81).

Though experiments with NMDA- and non-NMDA- receptor blockers have been successful in many animal and cell culture models, this has not been demonstrated in humans. There are many uncertainties and concerns in introducing NMDA-receptor blockers for the treatment of hypoxic-ischemic injury in the newborn. In the perinatal period neurons may be particularly sensitive to NMDA blockers, and this may interfere with functions like transmission, neuronal survival, differentiation and learning (82).

Studies in cultured neurons and in newborn piglets suggest that mild hypothermia may exert some of its neuroprotective effects by inhibiting glutamate release from synaptic nerve endings (83, 84). The neuroprotective effect of free radical scavengers may be exerted partially at the level of glutamate release because free radicals increase neuronal glutamate release in some models (85), and may inhibit glutamate uptake transporters.

Because Mg^{2+} blocks the Ca^{2+} -influx which is necessary for glutamate release from presynaptic nerve endings (86), several studies using MgSO_4 to prevent brain injury have been conducted, but results are conflicting (87-89). A recent multicenter study has shown that MgSO_4 given to women immediately before very preterm birth may improve pediatric outcome in terms of substantial gross motor dysfunction without serious harmful side effects (90). However, this could perhaps be explained by vasodilatation and improved uteroplacental blood flow and fetal perfusion.

Nitric oxide

The role of nitric oxide (NO) in hypoxic-ischemic brain injury, both *in vitro* and *in vivo*, has remained controversial (91-96). NO is a free radical synthesized from L-arginine and oxygen by the enzyme NO-synthase (NOS) in endothelial cells and selected neurons in response to rises in levels of intracellular Ca^{2+} . Three major isoforms have been identified: constitutive neuronal (nNOS), constitutive endothelial (eNOS), and inducible macrophage (iNOS) isoform. Part of the problem has been that the complex biochemical characteristics of NO suggest that this agent can be either detrimental or beneficial to the injured brain. Although under normal conditions NO physiologically mediates vasodilatation by activation of guanylate cyclase (97) and inhibits platelet aggregation and leukocyte adhesion preventing microvascular plugging (98), several studies have shown that NO reacts with O_2^- to form the highly toxic peroxynitrite radical which may initiate lipid peroxidation and disrupt mitochondrial function and thereby cause neurotoxicity (99-103).

The observation that activation of the NMDA-receptor generates NO in a Ca^{2+} -dependent manner raised the hypothesis that NO participates in glutamate excitotoxicity (104-106). Until recently, mainly nonspecific NOS inhibitors were used in studies. There are however, several NO-producing systems in the brain, some being activated immediately upon HI and some responding in a delayed fashion. Induction of iNOS after HI is delayed whereas an upregulation of nNOS and eNOS occurs early. During ischemia, blocking of nNOS and iNOS has been shown to be neuroprotective (107, 108). Studies in knockout mice have supported the theory that the cytotoxic effects are largely confined to the nNOS and iNOS, and the protective effects are mainly due to the action of eNOS (98, 109).

Studies examining the effect of NO-producing compounds have shown a protective effect only during the very early stages of cerebral ischemia. Therefore, the role of NO on hypoxic ischemic brain injury seems to be protective or destructive depending on the stage of evolution of the process, and on the cellular compartment producing NO.

Inflammatory response

HI triggers inflammatory responses in the central nervous system that may modulate neuronal damage (48, 110). Following HI, expression of adhesion molecules is enhanced and this facilitates the invasion of leukocytes which may release inflammatory mediators and impair

blood flow. In a rat model of neonatal HI, neutrophil depletion has been found to reduce brain swelling (111).

HI and reperfusion also activates the transcriptional factor nuclear factor- κ B (NF- κ B). NF- κ B is a family of DNA-binding protein factors that are required for maximal transcription of many proinflammatory molecules that are thought to be important in the generation of inflammation, including adhesion molecules (intercellular adhesion molecule 1), critical enzymes (inducible nitric oxide synthase, cyclo-oxygenase-2), most cytokines (interleukin-1 β , tumor necrosis factor- α , IL-6), and chemokines (IL-8). NF- κ B is activated by hyperoxia, ROS (H₂O₂), and glutamate through NMDA receptors, and it is inhibited by antioxidants (112-116). The role of NF- κ B in the brain is still somewhat unclear, but it has been shown to be activated in neurons after focal cerebral ischemia in rats, and activation was blocked in the presence of an antioxidant (111). However, the precise role of ROS in NF- κ B regulation is uncertain.

As the inflammatory response following HI develops over an extended period of time, modulation of this response may be possible and could therefore represent an opportunity for neuroprotective treatment.

Apoptosis and necrosis

Cell death occurs by both apoptosis and necrosis following hypoxic-ischemic injury. Apoptosis is an active, energy-dependent, programmed cell death characterized by cell dissolution with nuclear condensation and fragmentation (117). In necrosis, cell death is triggered by an overwhelming external insult. Cells swell as membrane pumps fail to maintain ionic homeostasis, resulting in membrane disruption and spilling of cytoplasmic content into the surroundings, triggering an inflammatory response. However, the distinction between apoptosis and necrosis is not always clear, and the two modes of cell death can often be regarded as a continuum of a single cell fate following injury. Immediate cell death following HI has been considered to be mainly necrotic, and delayed cell death mainly apoptotic. However, in a piglet model of HI, apoptosis and necrosis occurred in adjacent population of neurons and glial cells, and the numbers of both apoptotic and necrotic cells were found to be linearly related to the severity of the insult (118).

Several different factors influence whether a cell will undergo apoptosis or necrosis. The stage of development, cell type, severity of mitochondrial injury and the availability of ATP for apoptotic execution is of importance. *In vitro* studies have shown that the same cell type can be triggered to undergo apoptosis following mild injury but necrosis if the damage is severe (118).

Apoptosis is far more prominent in the neonate than in the adult and activation of cysteine proteases such as caspase 3 is a very important pathway in exitotoxic neonatal injury.

The NMDA-receptor ion-channel mediated increase in intracellular Ca^{2+} may affect nuclear functions, including the expression of apoptotic and antiapoptotic genes that lead to activation of caspases and result in cell death (119, 120).

Under normal physiological conditions, the mitochondrial inner membrane is impermeable to all but a few selected metabolites and ions. ROS, increased levels of Ca^{2+} and neurotoxins may cause formation of a non-specific pore in the mitochondrial inner membrane (121, 122). This phenomenon is known as the mitochondrial permeability transition, and causes the release of molecules less than 1500 Daltons from the mitochondria, including cytochrome c (123). The mitochondrial dysfunction may have far reaching effects, leading not only to energy depletion and further ROS-production, but possibly also to the direct activation of caspases and the apoptotic execution of the cell.

Cerebral hemodynamics

Total and regional cerebral blood flow (CBF) increase with postnatal age. CBF in premature infants is between 10 and 20 mL/100g/min, approximately 20% of adult value. The CBF in term infants is double that of preterm infants (124). Total and regional CBF is constantly changing to maintain the brain metabolic demands for oxygen and glucose. The developing brain has lower metabolic rate and increased glycogen reserves. Also, CBF is influenced by metabolic by-products, which are of lower levels in neonates than adults, due to decreased neuronal activity. Under hypoxic conditions, inhibitory neurotransmitters such as adenosine (125), and opiates (126) are released mediating the suppression of electrical activity and reduction in oxygen consumption. Thus, the fetus and neonate are more resistant to hypoxic-ischemic cerebral insults than adults.

Hypoxemia is a potent stimulus for arterial dilatation, CBF beginning to increase at an arterial oxygen tension level of approximately 7 kPa in adult animals and humans (127, 128).

Animal studies have shown that the fetus reacts to an asphyxic insult by increasing CBF, especially to the brainstem (129, 130), while the blood flow to the white matter of the brain is hardly increased at all (129-131). This regional pattern of preferential cerebral perfusion is present in human as well as in other species (132).

Depending on the extent of the oxygen deficit and the maturity of the fetus, this cerebral hyperperfusion can reach two to three times baseline levels. As the insult persists, a reduction in CBF finally occurs (130) as the cardiac output and mean arterial blood pressure fall. This affects the parasagittal region of the cerebrum and the white matter (133, 134). Immature fetuses seem to be particularly endangered by their limited ability to increase blood flow to the white matter through vasodilatation (134). After the insult, a transient hyperperfusion may be seen before a period of hypoperfusion (135). If the ischemic insult is severe, the so called no-reflow phenomenon may occur with failure of reperfusion in various areas of the brain (136).

CBF is a result of the perfusion pressure (arterial blood pressure minus intracranial pressure) and the cerebrovascular resistance. The cerebrovascular resistance is determined, in part, by the degree of contraction of the precapillary arterioles and the prearteriolar arteries (137). Autoregulation refers to maintenance of a constant CBF over a broad range of arterial blood pressures. Although fully developed in the premature and term newborn (138), the range of CBF autoregulation is lower than in adults. The upper limit of CBF autoregulation in term neonates ranges from 70 – 100 mmHg (mean arterial blood pressure, MABP) and the lower limit is below 30 mmHg (138-142). Cerebral autoregulation is vulnerable to insults, such as severe asphyxia (132, 143), prolonged hypoxia (144), cerebral ischemia (145), metabolic acidosis (146), and intraventricular hemorrhage (142). When autoregulation is lost, CBF becomes pressure passive. The precise mechanism of autoregulation is not fully understood, but metabolic, myogenic and neurogenic mechanisms are all clearly involved. Carbon dioxide (CO₂) is a strong determinant of CBF, and term infants have a strong cerebrovascular sensitivity to changes in arterial CO₂ tension (138, 147). Studies in term infants have shown that autoregulation is lost before CO₂ reactivity following severe asphyxia (143). The CO₂-reactivity increases with gestational age (147).

The response of CBF to hyperoxemia (cerebral oxygen vasoreactivity) is less well defined. Clinical studies in both neonatal and adult humans (148, 149) have shown a variable degree of

reduction in CBF as a result of hyperoxemia. The reason for hyperoxic vasoconstriction is less obvious than the physiological hypoxemic vasodilatation. One hypothesis suggests that it is a mechanism by which the brain protects itself against high partial pressures of oxygen, perhaps to limit the production of ROS. This oxygen reactivity may be reduced if tissue is at risk of ischemia (150).

Oxygen in sickness and health (or what is wrong with oxygen?)

*From the greater strength and vivacity of the flame of a candle, in this pure air, it may be conjectured; that it might be peculiarly salutary to the lungs in certain morbid cases....pure dephlogisticated air (oxygen) might be very useful as a **medicine**. But, perhaps, we may also infer from these experiments...., it might not be so proper for us in the usual healthy state of the body; for as a candle burns out much faster in oxygen than common air, so we might, as may be said, live out too fast, and the animal powers be too soon exhausted in this pure kind of air. A moralist, at least, may say, that the air which nature has provided for us is as good as we deserve (Joseph Priestly, 1790).*

Oxygen is described as a necessary but dangerous substance, essential for multicellular life. It was not recognized as a separate gas until the late 18th century when it was discovered independently by the Swedish apothecary Karl W. Scheele, in 1772, and by the English chemist Joseph Priestly, in August 1774. Even though it was soon applied for medical purposes, its use was for many years sporadic, sometimes erratic, often controversial and only occasionally beneficial.

This powerful gas.....it inspires cheerfulness, renders the patient happy, and in desperate cases, it is certainly a most precious remedy, which can spread flowers on the borders of the tomb, and prepare us in the gentlest manner for the last dreadful effort of nature (Benjamin Silliman, 1830).

Experiments of oxygen administration to asphyxiated newborns are said to have been made by Chaussier in 1783, but it was not until the 20th century that oxygen therapy was used on a rational, scientific basis. During the first two decades, numerous papers on intra-abdominal intravenous, rectal, and subcutaneous oxygen treatment were published. In 1917 and 1922 Haldane described diffusion impairment and ventilation-perfusion mismatch as a cause of hypoxemia (151, 152). After this, administration of oxygen to hospitalized patients with acute and chronic lung disease became routine, observing that supplemental oxygen relieved dyspnea, improved function, and resolved peripheral edema.

Soon after its discovery, it was recognized that oxygen could be poisonous, and though oxygen was considered “a good thing”, it was quite possible to have “too much of a good thing”. The clinical importance of oxygen toxicity was however, not fully appreciated until an epidemic of retinopathy of prematurity (ROP) occurred in the 1940s and -50s (153, 154). Since that time, considerable information has been generated in the investigation of the physiology of oxygen transport, the mechanism of hypoxia, the effects oxygen therapy, and oxygen toxicity. Oxygen has been demonstrated to exert toxic effects on bacteria, fungi, plants, animals, and humans. Toxicity is mainly believed to result from the formation of ROS and activation of NF- κ B. Oxygen toxicity can result from either high concentrations of oxygen, hyperbaric oxygen, or reperfusion of ischemic tissue. Prolonged exposure to hyperbaric oxygen causes central nervous system and pulmonary toxicity, which results in seizures, atelectasis, and pulmonary edema. Lung damage and development of ROP may occur as a result of normobaric hyperoxia (155). In adults, hyperbaric oxygen is used as treatment for CO poisoning, air embolism or decompression sickness, radiation damage, and wound healing (156). In infants and children, hyperbaric oxygen has been a successful treatment for radiation induced bone and soft tissue complications, and CO poisoning (157, 158).

Age, species, nutritional status, presence of underlying diseases, and certain drugs may influence the development of oxygen toxicity (155). The toxic threshold (length of exposure and level) is still debated. In animals, oxygen resistance or tolerance has been obtained with intraperitoneal, intravenous and intratracheal endotoxin or cytokines administration (159-162). Previous exposure to high oxygen concentration has also been reported to increase survival rates and decrease pulmonary lesions in animal models. Exposure to 85% oxygen for 3 days protected rats against a secondary exposure to 100% oxygen while no protection occurred with 60% (163). Protection may rely on antioxidant enzymes synthesis, NO production, neutrophils recruitment and modulation of alveolar macrophages activity. In rats inhaled NO has been reported to prevent or limit pulmonary lesions induced by oxygen exposure or ischemic reperfusion (164). In animals, protection is mainly attributed to the induction of antioxidant enzymes. However, protection has not always been demonstrated after repeated exposure (165, 166). In humans, tolerance or resistance to high oxygen pressure is more difficult to demonstrate.

The lung is the organ exposed to the highest partial pressure of inspired oxygen, and also the organ most severely damaged by exposure to hyperoxia. Disruption of the endothelium is a critical aspect of oxygen-induced lung injury. Interestingly, endothelial damage occurs earlier than epithelial damage, probably secondary to its exposure to ROS from a number of sources (167). Endothelial cells may also have less antioxidant defense capacity than epithelial cells. Such injury results in loss of microvascular integrity, with development of a protein-rich, hemorrhagic pulmonary edema. Exposure to hyperoxia results in activation of NF- κ B in several cell types, including human pulmonary artery endothelial and rat lung alveolar epithelial cells (168, 169). The effects of hyperbaric oxygen on the lungs are essentially those of normobaric oxygen, but with an accelerated onset. The unique problem of hyperbaric oxygen exposure is the effect on the central nervous system with generalized seizure activity at pressures above 3 ATM.

In neonates, oxidative stress has been hypothesized to play a causal role in the development of bronchopulmonary dysplasia (170, 171), ROP (172), necrotizing enterocolitis (173), intraventricular hemorrhage, and periventricular leucomalacia (174, 175). In 1988, Saugstad introduced the term “oxygen radical disease in neonatology”, suggesting an important role of ROS in a wide range of neonatal morbidities (176). Several studies in human neonates indicate that ROS production increases with higher levels of inspired oxygen (177) and increasing immaturity (178). The transition from intrauterine to extrauterine life in it self represents oxidative stress to the newborn.

Aims of the present study

The optimum way to reoxygenate asphyxiated newborns is still not known. Infants in need of resuscitation are often exposed to high concentrations of oxygen. But the role of supplementary oxygen given during resuscitation is under debate (179, 180). The possibility of increasing the load of toxic ROS by giving additional oxygen is of major concern.

In previous animal studies from our group, reoxygenation with 21% oxygen following global hypoxemia in newborn piglets have been extensively studied and found to be as efficient in normalizing MABP, base excess, CBF, plasma and cerebral hypoxanthine concentrations, cerebral EAA concentrations, and sensory evoked potentials, as reoxygenation with 100% oxygen (59, 181-184). In the present study, we wanted to shed further light on any possible differences between the two reoxygenation regimes (21% vs 100% oxygen) by changing our model of experimental asphyxia in newborn piglets from global hypoxemia to combined HI (Paper I), and then to combined hypoxemia-ischemia-hypercapnia (HIH) (Paper II-IV), asking the following questions:

1. Is reoxygenation with room air as efficient as with pure oxygen in normalizing MABP, cerebral cortical microcirculation and biochemical markers of asphyxia following combined cerebral HI (Paper I)?
2. Is reoxygenation with room air as efficient as with pure oxygen in normalizing MABP, cerebral cortical microcirculation and biochemical markers of asphyxia following combined cerebral HI when hypercapnia is added during the insult (Paper II)?
3. Can a single intravenous dose of the antioxidant enzyme SOD following an insult of combined cerebral HIH improve cerebral microcirculation and biochemical markers of asphyxia during reoxygenation with 21% or 100% oxygen (Paper III)?
4. If pure oxygen is used during reoxygenation following an insult of combined HIH, are there any differences between short and long duration oxygen treatment (100%

oxygen for 5 min vs 20 min) compared with 21% oxygen on cerebral microcirculation, oxygen metabolism and biochemical markers of asphyxia (Paper IV)?

Methodological considerations

Animal model

The underlying pathophysiology of neonatal asphyxia is difficult to study in the human. Neonatal hypoxic-ischemic brain injury has therefore been studied in a variety of animal models. Over the last few decades, a large body of data has been gathered in the newborn piglet. The piglet size and body weight matches that of term newborns, making this an attractive and workable model – the same type of equipment usually used in the neonatal intensive care unit may be used during experiments. Their anatomy and physiology are in many areas close to that of humans (185). The level of CNS maturity is of great importance when choosing an animal model. The histological maturation of the brain of a newborn piglet is considered to be approximately like that of a human infant of 36-38 weeks of gestation (186, 187), but physiologically it is probably more mature. Newborn piglets also have higher rates of cerebral metabolism and CBF than humans, and normal CBF has been found to be highest in neonatal pigs and decreasing with age (188), which is in contrast to humans. However attractive, the newborn piglet model shows large inter-individual variability. This makes it difficult to obtain large enough series for satisfactory statistical analyses. The most widely used animal model in asphyxia research is probably the Levine model in the 7-day old rat (189). This consists of unilateral ligation of the common carotid artery followed by inhalation of 8% oxygen. Rats are allowed to breathe spontaneously, and physiological variables are seldom monitored or controlled. However, rats are less expensive and easy to handle, allowing for a larger number of animals in each investigated group.

In the present work, two principally different asphyxia models were applied; combined cerebral HI (Paper I) and combined cerebral HIH (Papers II-IV). Although data from animal models of asphyxia do not necessarily truly mimic human perinatal conditions, they can hopefully provide us with important understanding of the general mechanisms of injury and possible neuroprotection following asphyxia and reoxygenation-reperfusion.

Anesthesia

All experiments in the present work were for obvious reasons conducted under general anesthesia. Though often an absolute necessity, the use and choice of anesthetics in animal

studies will represent a problem as the drugs may act as confounders. They may complicate interpretation of results and make comparison with previous experiments difficult. However, the wellbeing of the animals must always be our first priority.

Anesthesia was induced by halothane mixed with oxygen. An ear vein was cannulated, halothane was discontinued, and the piglets were given pentobarbital sodium and fentanyl as an intravenous bolus injection. Further anesthesia was maintained with a continuous infusion of either pentobarbital sodium and fentanyl (Paper I), or midazolam and fentanyl (Paper II-IV). Local infiltration of lidocain was used as an adjunct before skin incisions. The depth of anesthesia was monitored by response to painful stimuli elicited by pinching of the nasal septum in addition to standard monitoring of heart rate and blood pressure. Additional fentanyl was given if necessary. After completed surgical preparation, pancuronium bromide was given hourly to avoid shiverings which may occur in piglets even if anesthesia is sufficiently deep.

Halothane depresses cardiovascular function and modifies autoregulation. CBF increases, and cerebral metabolic rate of oxygen ($CMRO_2$) decreases (190). However, in the present study, halothane was used for a few minutes only until an intravenous line was established.

Pentobarbital sodium is classified as an intermediate-acting barbiturate, and is probably the most widely used intravenous anesthetic drug in animal research, often as a sole anesthetic. This practice should however be avoided, as the drug has little analgesic effect. In the presence of pain, barbiturates can even induce hyperalgesia (190, 191). They should therefore always be combined with an analgesic during painful procedures.

Barbiturates have a dose-dependent cardiovascular depressant effect (190, 191), but are generally well tolerated in pigs (192). They reduce CBF, intracranial pressure and $CMRO_2$ (193, 194), and they have anticonvulsant effects (191). Animal studies have shown protective effects of barbiturates against hypoxic-ischemic brain damage (195-197). The underlying mechanisms for this has not been finally settled, but has mainly been attributed to a reduction in cerebral metabolic rate. Other possible mechanisms proposed is free radical scavenging and reduction of glutamate release. *In vitro* studies in cell cultures have shown that barbiturates differ markedly in their neuroprotective effects with thiopental being the most effective, and that different barbiturates have different antioxidant effects (198).

Fentanyl and midazolam can cause a moderate reduction in CBF and CMRO₂, but a continuous fentanyl infusion in pigs have been shown to produce stable cerebral hemodynamic and metabolic conditions (199). However, both fentanyl and midazolam have been shown to increase cerebral fractional oxygen extraction in newborn piglets (200, 201), and these changes could reflect decreased brain perfusion and metabolism. It has also been shown that midazolam may inhibit glutamate release *in vitro* (202).

The nondepolarizing muscle relaxant pancuronium has little or no direct effect on the cerebral vasculature, but can cause an increase in MABP (190). The use of pancuronium prevented increased oxygen consumption as a result of shivering.

Though all the anesthetics used in the present work could influence CBF and cerebral metabolism to different degrees, this can hardly explain the differences found between the groups, as all the piglets that were directly compared received the same drugs. However, the differences in choice of anesthetics in Paper I compared to Paper II-IV, makes comparisons between the different studies more complicated.

Laser Doppler flowmetry

Techniques for measuring microvascular blood flow includes injections of microspheres, intravital video microscopy, perfusion sensitive magnetic resonance imaging, high frequency Doppler ultrasound, and laser Doppler flowmetry.

In the present study changes in microcirculation was measured by laser Doppler flowmetry. The technique measures the Doppler shift of light scattered back from red blood cells as they flow through the microvasculature of the sample volume of tissue independent of the flow direction (203). The strength of the signal is proportional to the number and velocity of red blood cells (i.e. blood perfusion) through the illuminated area of the tissue. Blood flow is computed by determining the product of blood volume and velocity, and gives only a relative value expressed in arbitrary units. The light penetration depth may vary somewhat from area to area and one cannot distinguish whether the sample volume includes only capillaries, or terminal arterioles and collecting venules as well. The reference illuminated area for a needle probe is small, approximately 1.0-3.5 mm³, and dependent on fibre diameter and geometry (204).

Laser Doppler flowmetry has a time resolution in the millisecond range and gives instantaneous, continuous, and real time measurements of microcirculation as well as accurate assessment of relative changes in regional microcirculation. The major drawback of this technique is its inability to provide CBF values in absolute flow values (e.g. ml/100g/min). The clinical acceptance of this technique has been low. Recently, a new thermo-dilution flowmetry microprobe has been developed (205) with a high temporal resolution and sensitivity for flow changes comparable to those of laser Doppler. The advantage of this method is the assessment of regional CBF in absolute flow values.

As a result of the methodology employed in the present work, there is a lack of quantitative results on which conclusions are based upon. It must be remembered that laser Doppler flow measurements give relative, not absolute values. Great care is therefore needed when results and calculated variables (changes in oxygen delivery and $CMRO_2$) are interpreted. For further technical details of the probes used, see *Methods* section in Papers I-IV.

Microdialysis

Microdialysis is a technique to monitor the chemistry of the extracellular space *in vivo*. The technique was developed during the 1970s, and the sampling principle is based on diffusion across a semi permeable membrane continuously perfused with an artificial extracellular fluid that does not contain the substance of interest. The microdialysis probe (or catheter) mimics the function of a capillary vessel where substances of interest may diffuse over the membrane into the carrier solution, which is then collected for analysis (206). Microdialysis can be performed in almost any organ and tissue. Initially used in animal studies, the technique is now being applied in humans, especially in neurosurgical patients (150, 207), and in some neurointensive care units as a routine method of monitoring brain chemistry (208).

Microdialysis usually measures only a fraction of the actual concentration of the substance in question. As the probe is constantly perfused, the dialysate is removed and a concentration gradient is created from the extracellular fluid to the dialysate. Therefore, the concentration of the substance collected is always lower in the dialysate than in the extracellular fluid. The dialysate/extracellular concentration ratio expressed as percentage is called the microdialysis extraction fraction or *in vivo* recovery. The concentration measured depends on perfusion

flow rate, surface area of the probe, molecular cutoff of the membrane, and diffusion characteristics of the substance collected. Tissue temperature, blood flow and metabolism of the substance collected may also influence *in vivo* recovery. The absolute extracellular concentration of a substance is therefore not easy to determine, but changes in extracellular levels of a substance can be monitored.

Main results of the study

Paper I

Cerebral hypoxemia-ischemia and reoxygenation with 21% or 100% oxygen in newborn piglets: Effects on extracellular levels of excitatory amino acids and microcirculation.

Two groups of 1-3-day-old piglets were reoxygenated with either 21% oxygen (HI 21% group, $n = 12$), or 100% oxygen (HI 100% group, $n = 12$) following combined cerebral HI. HI was achieved by normoventilation with 8% oxygen and temporary occlusion of both common carotid arteries. After 20 min, reoxygenation-reperfusion was started with either 21% oxygen or 100% oxygen for 30 min. All piglets were observed for 2 hours. During the reoxygenation-reperfusion period, extracellular concentrations of amino acids in the striatum were significantly higher in the HI 21% group compared with the HI 100% group (glutamate, $p = 0.02$; aspartate, $p = 0.03$). Mean arterial blood pressure was significantly lower in the HI 21% group ($p = 0.04$). Microcirculation in cerebral cortex decreased to $< 10\%$ of baseline during the insult, and normalized during reoxygenation-reperfusion in the HI 100% group, but remained at a significantly lower level in the HI 21% group ($p = 0.03$). There were no significant differences in concentrations of hypoxanthine in plasma or cerebral cortex between the groups. These results suggested a less favorable outcome in the group reoxygenated with room air.

Paper II

Reoxygenation with 100 or 21% oxygen after cerebral hypoxemia-ischemia-hypercapnia in newborn piglets.

Twenty-eight 1-3-day-old piglets were reoxygenated with either 100% or 21% oxygen following combined cerebral HIH. HIH was induced by ventilation with 8% oxygen, temporary occlusion of both common carotid arteries, and adding of CO₂ to the insiratory gas. After 20 min, reoxygenation-reperfusion was started with 21% oxygen (HIH 21% group, $n =$

13) or 100% oxygen (HIH 100% group, n = 11) for 30 min. All piglets were observed for 2 hours. During the reoxygenation-reperfusion period, mean arterial blood pressure was significantly higher in the HIH 100% group compared with the HIH 21% group (MABP, $p = 0.008$) and microcirculation in the cerebral cortex was approximately 20 % higher in the HIH 100% group ($p = 0.004$). However, there were no differences in either levels of amino acids in the striatum or hypoxanthine in the cerebral cortex between the two groups, and all values had returned to baseline at the end of the observation period. These results suggested that the brain tolerated reoxygenation with 21% as well as 100% oxygen in this model of combined HIH in spite of the differences in mean arterial blood pressure and cerebral microcirculation. We speculated that the added hypercapnia during the insult served to protect the brain.

Paper III

Effects of recombinant human superoxide dismutase during reoxygenation with 21% or 100% oxygen after cerebral asphyxia in newborn piglets.

Forthy-three 1-3-day-old piglets were randomized to asphyxia (n = 40) or control (n = 3). Asphyxia was induced by ventilation with 8% oxygen, temporary occlusion of both common carotid arteries, and adding of CO₂ to the inspiratory gas. After 20 min, 16 piglets received rhSOD 5 mg/kg intravenously and reoxygenation with either 21% oxygen (rhSOD 21%, n = 8) or 100% oxygen (rhSOD 100%, n = 8), and 24 piglets received saline and reoxygenation with either 21% oxygen (21%, n = 13) or 100% oxygen (100%, n = 11). SOD is an antioxidant enzyme, which scavenges superoxide radicals that are generated during reoxygenation following asphyxia. rhSOD peaked in plasma after 5 min. No rhSOD was detected in brain tissue. There were no significant differences in cerebral cortical microcirculation or extracellular levels of glutamate in the striatum or hypoxanthine in the cortex between the rhSOD and non-rhSOD groups. In conclusion, rhSOD given as a single intravenous dose after asphyxia in newborn piglets did not significantly influence changes in brain microcirculation or biochemical markers of asphyxia.

Paper IV

Comparison of short and long duration oxygen treatment following cerebral asphyxia in newborn piglets.

Forty-one 1-3-day-old piglets were randomized to cerebral HIH or control ($n = 5$). HIH was achieved by ventilation with 8% oxygen, temporary occlusion of the common carotid arteries, and adding of CO₂ to the inspiratory gas. After 25 min, reoxygenation-reperfusion was started with 100% oxygen for 20 min (group 1, $n = 12$), 100% oxygen for 5 min (group 2, $n = 12$) or 21% oxygen (group 3, $n = 12$). All piglets were observed for 2 hours. Significantly higher mean arterial blood pressure and more complete restoration of microcirculation in the cerebral cortex were found during reoxygenation-reperfusion in both groups reoxygenated with 100% oxygen compared with 21% oxygen (regional cerebral blood flow $\geq 100\%$ vs. 70% of baseline, $p = 0.04$). Oxygen delivery in cortex was significantly higher in group 1 and 2 compared with group 3 ($p = 0.03$), but there were no significant differences in CMRO₂. In the striatum no significant differences were found in flow or biochemical markers of asphyxia between the three groups. We concluded that following experimental asphyxia, newborn piglets can be reoxygenated as efficiently with 100% oxygen for only 5 min as 100% oxygen for 20 min compared with room air, and that exposure to additional oxygen during resuscitation at least can be limited in time in this model.

General discussion

Perinatal asphyxia accounts for a large proportion of infant mortality and disabilities in the survivors. Defining the optimum technique for neonatal resuscitation is therefore of great importance. One of the key questions yet to be answered is how to reoxygenate the infants – with or without supplemental oxygen?

Asphyxia and its consequences is a result of oxygen deprivation, and the efficiency of cellular energy production is highly dependent on the presence of oxygen. It therefore seems almost self-evident that it would be beneficial to give asphyxiated newborns supplemental oxygen during resuscitation. However, during the insult, purine derivatives (hypoxanthine, adenosine) accumulate, and during recovery, these metabolites are oxidized to uric acid in the presence of xanthine oxidase, using excess oxygen as substrate. As a result, a burst of ROS are produced (50, 176). The damaging effects of ROS on brain cells constitute one of the principal hypotheses put forward to explain brain damage during the asphyxia-reoxygenation-reperfusion process (49, 209, 210). ROS are produced both during the initial asphyxic insult and during the recovery phase (See *Reactive Oxygen Species*). Due to short half-life, ROS are not easy to measure directly, and evidence for the role of ROS in reperfusion injury is therefore mainly indirect, based on prevention of damage by the use of antioxidants. The production of ROS may be proportional to oxygen tension during reoxygenation, but results of *in vivo* experiments are still conflicting (43, 211-213). It is possible that even normoxic reoxygenation gives a high production of ROS, and *in vitro* studies have indicated a non-linear relationship between oxygen tension and ROS production (214, 215).

Over the past few years, data from several animal and human studies have accumulated, indicating that asphyxiated newborns may be reoxygenated as efficiently with 21% as 100% oxygen (59, 181-184, 216-224). Some of the studies have even suggested a better outcome in the groups reoxygenated with room air. In the clinical studies, time to first breath and cry and established normal breathing was found to be significantly shorter in the room air group compared to the 100% oxygen group (221, 223, 224). Biochemical markers of oxidative stress (reduced/oxidized glutathione ratio, increased SOD and catalase activity in whole blood) were also found to be significantly higher in the 100% oxygen groups, not only 72 hours after birth, but also 4 weeks later (222). The clinical studies have, however, been criticized for not being

properly randomized and blinded, the number of neonates included being limited, having to short follow-up, the neonates not being severely asphyxiated, and the significance of measuring biochemical markers of asphyxia in whole blood has been questioned (179, 225, 226). A recent Cochrane Review (227) concluded that even though a reduction in mortality has been seen in infants resuscitated with 21% O₂ (pooled estimate of effect), and no harmful effects have been demonstrated, there is still insufficient evidence to recommend a policy of using room air over 100% oxygen for newborn resuscitation.

Oxygen supply to the tissue is mainly determined by blood flow, blood oxygen content (CaO₂), and the affinity of hemoglobin for oxygen, i.e. the shape of the oxyhemoglobin dissociation curve. CaO₂ is calculated as the sum of the oxygen bound by hemoglobin and the oxygen dissolved in plasma. Under normal conditions, hemoglobin is almost fully saturated (97%) breathing room air, and physically dissolved oxygen contributes only about 1.5% to CaO₂. The amount of dissolved oxygen is small, but linearly dependent on oxygen tension. If there is a right shift of the oxyhemoglobin curve, as in acidosis secondary to asphyxia, hemoglobin becomes less saturated, and an increased inspired oxygen concentration can significantly increase CaO₂ and thereby tissue oxygen tension. This could be of value if blood flow is compromised. On the other hand, an increase in CaO₂ might result in tissue oxygen tension values above normal if blood flow is normal or increased (as in reactive hyperemia), with the possible risk of increasing the load of toxic ROS to the tissue. During exposure to high oxygen concentrations, only the lungs and vascular endothelium are exposed to very high oxygen tensions. Brain tissue oxygen tension will increase, but to a much more moderate degree (228, 229). In rabbits, brain tissue oxygen tension increases almost linearly with arterial oxygen tension under baseline conditions (230). The question is, are there data to indicate that hyperoxia can improve outcome following states of cerebral hypoxemia-ischemia? Treatment with hyperbaric oxygen has been shown to increase survival after carotid ligation in gerbils (197), and to reduce brain injury after transient focal ischemia in adult rats (231) and after HI in neonatal rats (232). Treatment with 100% oxygen has also been shown to mitigate brain injury following transient focal ischemia adult rats (233). In humans, hyperoxia was found to reduce brain lactate levels following traumatic brain injury (150). However, so far no study has shown enhanced oxidative energy metabolism with adenosine triphosphate generation as a result of increased arterial oxygen tension.

In the human neonate, considerable variations in the degree and nature of the asphyxic insult are associated with differences in neurological outcome. Survival is unlikely after severe and prolonged insults, whereas intact survival may occur after severe but brief insults. Death or morbidity has been reported with less severe, repetitive insults. Animal experiments have shown that to cause brain injury, the insult may need to be prolonged and nearly fatal (234, 235). This is in accordance with human epidemiological studies showing that babies who are asphyxiated usually either die or survive intact (236).

Differences in models such as species, age, duration of the insult, hypoxia versus ischemia, hypoxia and ischemia, with or without hypercapnia, global versus focal ischemia, permanent versus transient ischemia, different organs and areas studied etc., may explain differences in results obtained. In the present study, two principally different asphyxia models were applied. In Paper I, asphyxia was induced by ventilation with 8% oxygen and temporary occlusion of the common carotid arteries. In Paper II, III and IV CO₂ was added to the inspiratory gas during the insult. It has been questioned whether hypoxemia alone is capable of inducing brain damage without superimposed cerebral ischemia (233, 237). Temporary occlusion of the carotid arteries was therefore added to the global hypoxemia in Papers I-IV. In this model of combined HI, a significant and consistent difference in MABP and cerebral cortical microcirculation during reoxygenation-reperfusion was found throughout the study, with higher MABP and better restoration of microcirculation in the groups reoxygenated with 100% oxygen compared with 21% oxygen (Papers I, II and IV). In Paper I significantly higher levels of extracellular amino acids in the striatum were also found in the room air group. This is in contrast to previous findings in the global hypoxemia model (59, 181-183) even though a nonsignificant trend towards higher cortical microflow in the animals reoxygenated with 100% oxygen had been demonstrated earlier (94). Making the ischemic component more pronounced by temporary occlusion of the carotid arteries seemed to change the situation (substantially). The differences in cortical microcirculation and amino acids could be secondary to differences in MABP in the two groups, as asphyxia may lead to impaired pressure-flow autoregulation, rendering CBF pressure passive (238). Cortical blood flow could thus be lower as a result of lower perfusion pressure, and inadequate CBF could in turn lead to impaired energy metabolism. However, other mechanisms could possibly explain the differences found. Following ischemia, perfusion abnormalities and microcirculatory disturbances play an important role in reperfusion injury. If the ischemic insult is severe, the so-called no-reflow phenomenon may occur with failure of reperfusion in various areas of the

brain (136). Under such conditions, an increase in arterial oxygen content may be of benefit to borderline perfused areas (197, 239), with better restoration of microcirculation as a result. In addition, ROS are known to have vasoactive properties, and can act as vasodilators on cerebral arterioles and arteries (53, 136). Assuming that more ROS are produced during reoxygenation with 100% compared with 21% oxygen, such a mechanism could perhaps also contribute in normalizing microcirculation. However, the production of ROS was not measured in this study, and effects of topically applied ROS alone may be very different from the situation of reoxygenation-reperfusion following asphyxia where both contracting and relaxing factors (i.e. production and action of ROS, NO, peroxynitrite etc.) will act together.

In an attempt to mimic the clinical situation of asphyxia more closely, a moderate hypercapnia was added during the insult in Papers II-IV. This seemed to result in a less severe insult (higher MABP and somewhat less reduction in microcirculation at the end of HI/start of reoxygenation-reperfusion). Again significantly higher MABP and restoration of cortical microcirculation were found in the 100% oxygen groups, without significant differences in biochemical markers. However, the differences in MABP and cortical microcirculation were less marked (Paper II, IV). Even though groups with and without hypercapnia were not directly compared, we speculate that the moderate hypercapnia during the insult served to protect the brain. It has been shown that CO₂ can protect the neonatal brain from hypoxic-ischemic damage. In a rat model, mild hypercapnia during the insult was found to be protective compared to normocapnia (240). Hypercapnia can also influence cerebral metabolism in a favorable way (241, 242). Furthermore, CO₂ is a strong determinant of CBF, and both animal and human studies have shown that term infants have a strong cerebrovascular sensitivity to changes in CO₂ tension levels (129, 143, 147). In addition, anesthesia was altered from a combination of continuous pentobarbital and fentanyl infusion in Paper I to continuous midazolam and fentanyl infusion in Papers II-IV, a change which also could influence outcome.

Optimal and critical values for regional CBF in asphyxiated newborns have not been established, and vary with gestational age (243). Obviously, severe impairment of blood flow will lead to damage, but even high values have been of concern. The so-called luxury perfusion phenomenon during reperfusion with uncoupling between blood flow and metabolic demands has been described (244, 245), and a state of post-asphyxic hyperperfusion and vasoparalysis has been found in severely brain damaged human newborns (143). In the

present study, a transient hyperperfusion was seen in several animals both in the cortex and in the striatum post asphyxia (Papers I-IV), especially in the oxygen treated groups. Recent positron emission tomography studies in both humans and animals have however, indicated that early hyperperfusion following ischemia is not necessarily detrimental (246). Throughout the present study, cerebral microcirculation was restored and biochemical markers of asphyxia were normalized during reoxygenation with 100% oxygen. In Paper I, a 30% restoration only of cortical microcirculation and failure to normalize extracellular levels of amino acids indicated severely compromised cerebral circulation in the room air group. In Paper II, a 70-80% restoration of cortical microcirculation in the room air group could indicate sufficient flow as biochemical markers both in the cortex and the striatum normalized during reoxygenation-reperfusion. In Paper IV, microcirculation in the striatum was restored in both the room air group and the two groups reoxygenated with 100% oxygen, which is in accordance with previous reports from a different group (217). No differences were found in biochemical markers in the striatum between the groups. Significantly higher MABP and more complete restoration of microcirculation and cortical oxygen delivery were found after reoxygenation with 100% oxygen for 5 or 20 min compared with 21% oxygen. Again, a 70-80% restoration of cortical microcirculation in the room air group could indicate borderline, but perhaps sufficient cortical blood flow as no significant differences in relative changes in $CMRO_2$ was found between the groups. A recent study in primates (247) has indicated that $CMRO_2$ is a better predictor of brain damage than CBF. Regional differences in mechanisms for regulation of CBF could perhaps explain the observed differences in response to 21% and 100% oxygen in cortex and striatum (248, 249).

The consistent findings of significantly higher MABP after reoxygenation with 100% compared with 21% oxygen in this model of combined HI with or without hypercapnia, have been somewhat unexpected and difficult to explain. Hyperoxia has been shown to cause increased systemic vascular resistance (250, 251), but no significant differences in MABP have been demonstrated in the numerous previous studies from our group using the model of global hypoxemia without clamping of cerebral vessels. Better myocardial function as a result of reoxygenation with 100% oxygen is less likely, as studies have demonstrated no significant differences in cardiac output, normalizing of pulmonary hypertension and cardiac troponin (219, 252) between room air and oxygen resuscitated groups following global hypoxemia. Piglets have a well-developed collateral circulation to the brain (253). Temporary occlusion of the common carotid arteries in combination with hypoxemia leads to a highly variable but

substantial reduction in CBF, especially to the forebrain. How this hypoxic-ischemic insult affects circulation to the brain stem with its vasomotor control area has not been settled, but a recent magnetic resonance image study has indicated a possible effect on brain stem regions as well (254). However, this remains speculations only.

In Paper III, groups reoxygenated with 100% or 21% oxygen were not directly compared. In this study, no effects of a single intravenous dose of recombinant human SOD on cortical microcirculation or cerebral extracellular levels of hypoxanthine and glutamate during reoxygenation-reperfusion with 100% or 21% oxygen were found. Assuming that more O_2^- would be generated during reoxygenation with 100% than with 21% oxygen, any effects of the antioxidant enzyme would perhaps most easily have been seen in the groups reoxygenated with 100% oxygen, but this could not be demonstrated. No recombinant human SOD was detected in brain tissue. Numerous studies on the possible protective effects of SOD on reoxygenation-reperfusion injuries have been carried out, and the results have been conflicting. There are many possible explanations for the lack of effect observed in this study. The most likely is that the recombinant human SOD simply did not cross the blood-brain barrier due to its large molecular weight and negative polarity, or that its short half-life made the exposure time too short (255, 256).

In most cases of asphyxia in newborn infants, the occurrence, duration, frequency of episodes, or severity of the insult is not known.

The tissues of fetal and newborn mammals have a remarkable but poorly understood resistance to hypoxia. The fetal circulation is designed to meet the needs of a rapidly growing organism existing in a state of relative hypoxia. The blood in the umbilical vein in humans is 80% saturated with oxygen (with an oxygen tension of 3.7 kPa), compared with 98% saturation in the arterial circulation of the adult. Two factors allow a sufficient oxygen supply to the fetus even when placental blood is not fully saturated: 1) fetal hemoglobin has a greater affinity for oxygen than adult hemoglobin and can be more fully saturated at the same oxygen tension; 2) whatever oxygen is supplied to the fetus is optimally distributed so that tissues with the greater need receive the greater amounts through shunting of the blood.

Nonfunctioning tissues such as the lungs and liver are largely bypassed. At birth, shunts are rapidly reversed and subsequently closed, and the newborn circulation adapts to extrauterine life within hours to days. At the same time, $CMRO_2$ and energy requirement increases.

However, in spite of the resistance to hypoxia, once the brain has become hypoxic, subsequent reoxygenation is not necessarily handled well.

The present work cannot answer the question whether newborn babies in general should be resuscitated with or without supplemental oxygen, or if subgroups of newborns should be treated differently. It has shown that in a model of combined cerebral HI in newborn piglets, exposure to 100% oxygen during initial resuscitation can be of benefit in restoring cerebral microcirculation and MABP, especially if the insult is severe. When hypercapnia is added to the insult, reoxygenation with room air might be sufficient, judged by the lack of differences in biochemical markers and CMRO₂. Exposure to additional oxygen during resuscitation can at least be limited in time in this model. It has to be kept in mind that the models used in the present work are simplified models of a very complex situation, and great care should be exercised in drawing clinical conclusions based upon experimental data. Piglets in the present study had healthy lungs and were already adapted to extrauterine life, and they were anesthetized, intubated and mechanical ventilated before the onset of experimental asphyxia. Furthermore, brain recovery was investigated during the first 2 hours of resuscitation only. It must also be kept in mind that only the two extremes were investigated; reoxygenation with 21% versus 100% oxygen. Only further clinical studies in humans can give us the full answer to our question. However, oxygen should in any clinical situation be regarded as a drug and be administered as such - on a positive indication and in a dose-response related manner.

Conclusions

1. In a model of combined normocapnic cerebral HI in newborn piglets, significantly higher levels of extracellular EAA in the striatum, significantly lower MABP and significantly decreased cortical microcirculation were found after reoxygenation with 21% oxygen compared with 100% oxygen. This suggested a less favorable outcome in the room air group.
2. When moderate hypercapnia was added during HI, significantly higher MABP and more complete restoration of cerebral cortical microcirculation were found after reoxygenation with 100% oxygen compared with 21% oxygen. However, no differences in biochemical markers were found between the groups. This indicated that the brain tolerated reoxygenation with 21% oxygen as well as 100% oxygen in this model in spite of the differences in MABP and cerebral microcirculation.
3. In the model of cerebral HIH in newborn piglets, no effects of a single intravenous dose of recombinant human SOD given after the insult could be demonstrated after reoxygenation with 100% or 21% oxygen on cortical microcirculation or extracellular levels of hypoxanthine and glutamate, and it probably did not cross the blood-brain barrier.
4. Following cerebral HIH in newborn piglets, significantly higher MABP and more complete restoration of microcirculation and oxygen delivery in the cerebral cortex were found after reoxygenation with 100% oxygen for 5 or 20 min compared with 21% oxygen. There were no differences in CMRO₂. No significant differences were found in microcirculation or biochemical markers in the striatum. Reoxygenation with 100% oxygen for 5 min was as efficient as 20 min; a finding which indicates that exposure to additional oxygen during resuscitation at least can be limited in time in this model.

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Errata

Paper IV

- *Discussion*, p130, first column, line 35-39 should read: “Somehow, a brief exposure to oxygen for only 5 min seems to trigger the same mechanisms that restore blood pressure, microcirculation, and oxygen metabolism in cortex more completely in piglets reoxygenated with 100% O₂ for 20 or 30 min (8) compared with room air.”