Mini-Review

Rat Model of Perinatal Hypoxic-Ischemic Brain Damage

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To gain insights into the pathogenesis and management of perinatal hypoxic-ischemic brain damage, the authors have used an immature rat model which they developed many years ago. The model entails ligation of one common carotid artery followed thereafter by systemic hypoxia. The insult produces permanent hypoxic-ischemic brain damage limited to the cerebral hemisphere ipsilateral to the carotid artery occlusion. The mini-review describes recently completed research pertaining to the use of the immature rat model, specifically, investigations involving energy metabolism, glucose transporter proteins, free radical injury, and seizures superimposed upon cerebral hypoxia-ischemia. Future research will focus on molecular mechanisms of neuronal injury with a continuing focus on therapeutic strategies to prevent or minimize hypoxic-ischemic brain damage. J. Neurosci. Res. 55:158–163, 1999. © 1999 Wiley-Liss, Inc.

INTRODUCTION

Perinatal hypoxic-ischemic brain damage remains a major cause of acute mortality and chronic neurologic morbidity in infants and children. Statistics suggest an incidence of systemic asphyxia in 2–4/1,000 full-term infants and an incidence that approaches 60% in low birth weight (premature) newborns (1). Between 20% and 50% of asphyxiated newborn infants who exhibit hypoxic-ischemic encephalopathy expire during the newborn period. Of the survivors, up to 25% exhibit permanent neuropsychologic handicaps in the form of mental retardation, cerebral palsy, learning disability, or epilepsy. Given the magnitude of the problem, it is appropriate that health professionals provide high priority to the fetus and newborn infant at risk for cerebral hypoxia-ischemia. Animal studies also are important in providing important information regarding underlying mechanisms of perinatal hypoxic-ischemic brain damage and how tissue injury can be prevented or minimized through therapeutic intervention.

Over the past several years, we have investigated a model of hypoxic-ischemic brain damage in the immature rat (2). The 7-day postnatal rat was originally chosen for study because at this stage of development the animal's brain is histologically similar to that of a 32- to 34-week gestation human fetus or newborn infant; i.e., cerebral cortical neuronal layering is complete, the germinal matrix is involuting, and white matter as yet has undergone little myelination. We have also investigated hypoxic-ischemic responses in more mature rats to ascertain maturational differences in the nature and extent of histopathologic injury. In this regard, the brain of the 12- to 13-day postnatal rat is roughly equivalent to that of the full-term newborn human infant. The immature rat model has proved useful for numerous studies of perinatal hypoxic-ischemic brain damage and presently is utilized by many investigators throughout the United States and abroad.

The method to produce hypoxic-ischemic brain damage in the immature rat is based on the Levine preparation in the adult rat (3) and consists of unilateral common carotid artery ligation followed by systemic hypoxia produced by the inhalation of 8% oxygen-balance nitrogen. The rat pups are capable of surviving this severity of hypoxia for 3 or more hours before an appreciable mortality occurs. Measurements of systemic physiologic variables during the course of hypoxia reveal hypoxemia combined with hypocapnia produced by hyperventilation (4). The hypocapnia compensates for the

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metabolic acidosis caused by lactacidaemia, such that systemic pH does not change from the control value. Mean systemic blood pressure decreases by 25–30% during the course of the systemic hypoxia.

Hypoxic-ischemic brain damage is a near universal finding in those immature rats surviving 2–3 h of systemic hypoxia. Damage, largely restricted to the cerebral hemisphere ipsilateral to the common carotid artery occlusion, is observed in cerebral cortex, subcortical and periventricular white matter, striatum (basal ganglia), and hippocampus. Tissue injury takes the form of either selective neuronal death, infarction, or a combination thereof. Milder cortical lesions consist of varying combinations of columns of dead neurons oriented at a right angle to the pial surface, as well as laminar neuronal death involving layers 3 and 5 + 6. The columnar pattern of damage has been described in premature infants suffering hypoxia-acidosis with hypotension and is proposed to be the early pathologic lesion of uleugryia. In the rat pups, there is also necrosis of subcortical and periventricular white matter. Thus, at least in the immature rat, hypoxic-ischemic brain damage involving cerebral cortex, white matter, and deep gray matter structures can coexist in the same animal and, of necessity, results from the same hypoxic-ischemic stress (2,5,6).

Using the immature rat model of perinatal hypoxic-ischemic brain damage, numerous investigators throughout the United States and abroad have studied mechanisms of brain damage, brain plasticity, and therapeutic interventions in relation to perinatal cerebral hypoxia-ischemia. These investigations have provided major insights into the manner in which hypoxia-ischemia damages the perinatal brain and why the distribution of injury differs from that of the adult. Studies have focused on neurotransmitter status and glutamate neurotoxicity during hypoxia-ischemia (7–9), nitric oxide neurotoxicity during hypoxia-ischemia (10,11), brain plasticity during development following cerebral hypoxia-ischemia (12–17), immediate early gene induction by cerebral hypoxia-ischemia (18–20), and the evolution of magnetic resonance imaging abnormalities following hypoxia-ischemia (21).

We and other investigators also have applied various physiologic and therapeutic manipulations to the immature rat model of perinatal hypoxic-ischemic brain damage. Physiologic manipulations known to protect the brain from hypoxic-ischemic damage include hyperglycemia, fasting hypoglycemia, mild hypothermia, mild hypercapnia, and hypoxic preconditioning (22–34). Pharmacologic interventions known to improve neuropathologic outcome include glucocorticosteroids, glutamate receptor antagonists, calcium channel blockers, free radical inhibitors and scavengers, nitric oxide inhibitors, nerve growth factor, platelet-activating factor antagonist, and 21-amino steroids (35–54).

Over the past several years, our research group has continued to use the immature rat model of hypoxic-ischemic brain damage to investigate basic mechanisms of tissue injury as well as to study specific therapeutic interventions. The paragraphs which follow highlight the most important aspects of our accomplishments over the past 3 years.

Clinical investigations have suggested that premature human infants who require mechanical ventilation for respiratory distress syndrome are at increased risk for the occurrence of periventricular leukomalacia if hypocapnia occurs during the respiratory management. The relationship between hypocapnia and hypoxic-ischemic brain damage in the human infant is reminiscent of the situation in our immature rat model of perinatal hypoxic-ischemic brain damage. During hypoxia-ischemia, the immature rat hyperventilates, which causes hypocapnia to an extent that blood pH remains normal despite lactacidaemia (4). Accordingly, we conducted an experiment to ascertain the influence of carbon dioxide (CO₂) on hypoxic-ischemic brain damage in our immature rat model (33). To do so, we exposed the immature rats to hypoxia-ischemia during which they inhaled 8% oxygen combined with either 0%, 3%, 6%, or 9% CO₂. CO₂ tensions averaged 26, 42, 54, and 71 mmHg in the 0%, 3%, 6%, and 9% CO₂-exposed animals, respectively, during hypoxia-ischemia. The neuropathologic results showed that those immature rats exposed to normocapnic cerebral hypoxia-ischemia sustained less brain damage than animals subjected to hypocapnic hypoxia-ischemia. The data also showed that the greatest reduction in brain damage occurred in those immature rats exposed to 6% CO₂ (mild hypocapnia) with slightly less protection at 9% CO₂ (moderate hypocapnia). The results indicate that in our immature rat model, normocapnic cerebral hypoxia-ischemia is associated with less severe brain damage than during hypocapnic hypoxia-ischemia and that mild hypercapnia is more protective than normocapnia. These findings in an experimental model merit further animal investigations as well as a clinical reappraisal of the ventilatory management of sick newborn human infants. Indeed, investigations are currently being conducted to ascertain the pulmonary and cerebral consequences of "permissive hypercapnia" in premature human infants suffering respiratory distress syndrome.

Continuing our investigations on glucose metabolism in the immature brain, we conducted experiments related to the effect of hypoxia-ischemia on glucose transporter proteins in the immature rat (55). The facilitative glucose transporter (GLUT) proteins mediate the transport of glucose across the blood–brain barrier (55 kDa GLUT1) and into neurons and glia (GLUT3 and 45
kDa GLUT1). Glucose uptake and utilization are low in the immature rat brain, as are also the levels of the glucose transporter proteins (56). Thereafter, we investigated the effect of cerebral hypoxia-ischemia in the immature rat on the expression of GLUT1 and GLUT3 in both the ipsilateral (damaged, hypoxic-ischemic) and contralateral (undamaged, hypoxic) hemispheres of immature rat brain. Early during recovery from hypoxia-ischemia, both cerebral hemispheres exhibited increased expression of blood–brain barrier GLUT1 as well as neuronal GLUT3. Also with recovery, there was a rapid rise in brain glucose concentrations in both cerebral hemispheres, such that at 4 hr of recovery, brain glucose and brain/plasma glucose ratios were ~160% of control. These findings suggest increased glucose transport into brain, which is likely being mediated by the increased GLUT1 in the cerebral microvessels. By 24 hr of recovery, the level of blood–brain barrier GLUT1 had normalized in the contralateral cerebral hemisphere but was increased even further in the ipsilateral hemisphere. That these changes were confined to the blood–brain barrier was confirmed in isolated microvessels. There was also an acute induction of neuronal GLUT3, which at 4 hours of recovery was equivalent in both cerebral hemispheres relative to control. This early increase in GLUT3 levels was essentially resolved by 24 hr of recovery in the contralateral hemisphere and was further reduced in the ipsilateral hemisphere, consistent with the onset of neuronal necrosis. Glucose utilization, as well as GLUT3 levels, were somewhat increased in the contralateral cerebral hemisphere at 24 hr of recovery, presumably reflecting an increased demand for glucose disposal, possibly coupled with repair, occurring at this interval. By 72 hr of recovery, brain glucose levels had normalized in both hemispheres, and the sole enduring difference in the expression of the glucose transporter proteins was a reduction in the concentration of GLUT3 in the area of infarction, commensurate with neuronal loss.

In an additional investigation, the role of iron (Fe) metabolism was ascertained in perinatal hypoxic-ischemic brain damage (57). The immature rat model of unilateral hypoxic-ischemic brain injury was used, whereupon the animals were histopathologically examined at 0, 1, 4, 8, and 24 hr, and 1 and 2 weeks of recovery. Frozen sections of brain were prepared and stained for Fe. It was apparent from the sections that Fe accumulated within the neuropil as well as the cytoplasm of injured cells as early as 4 hr of recovery from hypoxia-ischemia. Fe accumulation within cells increased rapidly over the first 24 hr of recovery and eventually included the nucleus. All damaged regions became intensely Fe-stained. At 1 week of recovery, Fe staining in cerebral cortex formed radial columns which were perpendicular to the pial surface and which followed the course of penetrating blood vessels. Many reactive glial cells were also Fe-positive. Accordingly, there is a dramatic increase in Fe accumulation within brain occurring shortly following a hypoxic-ischemic insult. The early enhancement of Fe histochemistry supports a role for Fe in the progression of post-hypoxic-ischemic (reperfusion) brain injury.

In adult animals, neutrophils are known to contribute to ischemic brain damage. The role of neutrophils in perinatal hypoxic-ischemic brain damage is unknown, and these cellular elements are typically not seen in the brains of immature rats following a hypoxic-ischemic insult (see below). Accordingly, an investigation was designed to investigate whether and how neutrophils contribute to perinatal hypoxic-ischemic brain damage and whether or not neutropenia is neuroprotective (48). Unilateral cerebral hypoxia-ischemia was produced in 7-day postnatal rats. Half of the rats were rendered neutropenic with an anti-neutrophil serum. At 15 min of recovery from hypoxia-ischemia, half of the neutropenic and non-neutropenic rats received the oxygen free radical inhibitor/scavenger allopurinol. Thereafter, the protective effect of the four treatment groups was determined on brain swelling, as a reflection of tissue injury, at 42 hr of recovery. Neutropenia alone reduced brain swelling by 70%. Allopurinol alone produced similar protection. There was no apparent additive effect of combined neutropenia and allopurinol. Neutrophil accumulation in brain was measured by myeloperoxidase (MPO) activity assay and by neutrophil counts in brain sections stained with MPO and anti-neutrophil serum immunostaining. MPO activity peaked between 4 and 8 hr of recovery in both cerebral hemispheres. Hemispheric neutrophil counts peak at the end of the hypoxic-ischemic insult and again at 18 hours of recovery. Neutrophils were stained within blood vessels and did not infiltrate into the injured brain before infarction had occurred. The findings support the concept that neutrophils contribute to perinatal hypoxic-ischemic brain damage and that neutrophil depletion is neuroprotective.

Recent investigations from our and other laboratories have shown that mild to moderate systemic hypothermia decreases the brain damage resulting from hypoxia-ischemia in the immature rat (28–31). To determine whether suppression of oxidative metabolism during hypoxia-ischemia is a critical mechanism for the neuroprotection of hypothermia, we used 31P NMR spectroscopy to measure high energy metabolites in immature rats under conditions of modest hypothermia during a hypoxic-ischemic insult (32). Seven-day postnatal rats underwent unilateral common carotid artery ligation followed by exposure to hypoxia in 8% oxygen for 3 hr. Environmental temperature was decreased by either 3°C or 6°C from the control temperature of 37°C; the latter reliably produces cerebral hemispheric damage in over 90% of
the rat pups. Metabolite variables and tissue swelling (edema) at 42 hr of recovery varied significantly with the three temperatures. Tissue swelling was 26.9%, 5.3%, and 0.3% greater than control at 37°C, 34°C, and 31°C, respectively. Multislice magnetic resonance (MR) imaging, histology, and triphenyltetrazolium chloride staining confirmed the fairly uniform damage, which was confined to the cerebral hemisphere ipsilateral to the carotid artery ligation. The MR metabolite levels were integrated over the last 2 hr of hypoxia-ischemia and were normalized to their baseline for all surviving animals. ATP was 47.9%, 69.0%, and 83.0% of control, and the estimation of the phosphorylation potential (phosphocreatine/inorganic phosphorus) was 16.9%, 27.8%, and 42.6% of control at 37°C, 34°C, and 31°C. There was a significant correlation of both phosphocreatine/inorganic phosphorus and ATP levels with brain swelling. Brain swelling, as a reflection of cerebral edema, and thus tissue damage, can be reliably predicted from a threshold of these metabolite levels. The data indicate that for all three temperatures, a large change in integrated high energy metabolism during hypoxia-ischemia is a prerequisite for brain damage to occur. With a moderate hypothermic change of 6°C, there is an insufficient change in the energy metabolites to cause subsequent hypoxic-ischemic brain damage. As a corollary, treatment for hypoxia-ischemia should be aimed at preserving energy metabolism during and following the insult.

Additional experiments have focused on the potential brain-damaging effect of chemically induced status epilepticus in immature rats with or without superimposed cerebral hypoxia-ischemia. The rationale for the conduct of these experiments relates to the fact that no clinical studies in human newborn infants have demonstrated conclusively that seizures per se are damaging to the brain whether or not that brain is previously injured by hypoxia-ischemia.

An experiment was designed to investigate chemically induced seizures in immature rats previously subjected to cerebral hypoxia-ischemia to determine whether or not either a single, prolonged seizure or repetitive seizures (status epilepticus) lead to permanent brain damage or accentuate the neuronal injury caused by the prior hypoxic-ischemic insult in these animals (58). Once again, we used our established model of perinatal hypoxic-ischemic brain damage. We also ascertained the contribution of hypoglycemia to mortality during status epilepticus and the extent to which glucose supplementation improves survival but prolongs the convulsive activity. Seven-day postnatal rats were subjected to unilateral cerebral hypoxia-ischemia for 2 hr. Thereafter, they received multiple subcutaneous injections of bicuculline (6 mg/kg body weight) adequate to produce behaviorally apparent seizures lasting greater than 1 hr (status epilepticus). Repeated episodes of status epilepticus at 2, 6, and 12 hr of recovery from hypoxia-ischemia produced a mortality rate of 53%. Among the survivors, there was no statistically significant difference in the extent of brain damage between convulsing and nonconvulsing hypoxic-ischemic control animals, analyzed neuropathologically at 30 days of postnatal age. Furthermore, histopathologic examination for acute lesions also indicated no difference in the severity of brain damage between dead and surviving rat pups subjected to status epilepticus, indicating that mortality was not related to the severity of the prior hypoxic-ischemic brain damage. Further experiments showed that those immature rats that died during status epilepticus exhibited substantially lower blood glucose concentrations (30 mg/dl) compared to surviving, convulsing animals (80 mg/dl). Glucose supplementation (0.1 ml of 50% glucose) early during status epilepticus improved survival and significantly prolonged seizure activity (90 min) compared to nonglucose-treated, convulsing littersmates (47 min). Glucose supplementation did not increase the extent of brain damage despite the improved survival and increased duration of seizure activity. Taken together, the findings of the investigation indicate that even repetitive episodes of status epilepticus in immature rats previously subjected to cerebral hypoxia-ischemia do not accentuate brain damage despite a substantial mortality. Hypoglycemia contributes to death arising from status epilepticus, and both survival and seizures can be prolonged by glucose supplementation without risk of increasing the severity of any existing brain damage.

In summary, our immature rat model of perinatal hypoxic-ischemic brain damage has proved useful in clarifying basic mechanisms underlying tissue injury as well as in testing potentially protective physiologic and pharmacologic interventions. Future research will focus on cellular/molecular mechanisms of neuronal injury with a continuing focus on therapeutic strategies to prevent or minimize hypoxic-ischemic brain damage.

REFERENCES


