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The effect of hypoxic–ischemic brain injury in perinatal rats on the abundance and proteolysis of brevican and NG2

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Abstract

Oligodendrocyte (OL) progenitor cells are particularly susceptible to perinatal hypoxia/ischemia (H–I) resulting in decreased myelination and attenuated development of white matter fiber tracts. Brevican is an aggregating chondroitin sulfate proteoglycan (CSPG) secreted by OLs and their progenitors prior to and during active developmental myelination whereas neuron-glia antigen 2 (NG2) is a transmembrane CSPG produced by early OL progenitors. Although both proteoglycans are associated with maturation of OLs, it is not known if they are altered by H–I brain injury in the neonate. We have therefore examined the time course of changes in brevican and NG2 abundance and proteolysis in the neonatal rat hippocampus after H–I. In a standard H–I model of unilateral carotid artery ligation and exposure to hypoxia, a cavitary infarct involving the ipsilateral parietal and temporal regions of cerebral cortex, hippocampus, and striatum of most rat pups was clearly evident 4 days after H–I. The abundance of total extractable brevican was markedly reduced in the ipsilateral hippocampus at 1 and 14 days after H–I (relative to the contralateral side). At these times, the total G1 proteolytic fragment of brevican was lower in the ipsilateral hippocampus and the level of a protease-generated brevican fragment was significantly diminished in the OL-rich hippocampal fimbria. Hippocampal NG2 levels were also lower at 1 and 4 days after H–I, but were not different from the contralateral side at 14 days. Since brevican, brevican G1 fragment, and NG2 loss occur around the time of progressive cell death and the appearance of the infarct, it may be that H–I rapidly induces a cellular response that actively depletes these proteoglycans from the hippocampal matrix. While the mechanism of this loss is unclear, it would appear to be an early event in the process that could be involved in apoptotic cell death and/or tissue injury.

Keywords: NG2; Brevican; Synaptophysin; Hippocampus; Glial reactivity; Oligodendrocyte; Astrocyte; Microglia; Infarction

Introduction

A common neuropathological correlate for cerebral palsy is periventricular leukomalacia, a demyelinating syndrome with focal and diffuse lesions that is often caused by hypoxia/ischemia around the time of premature birth (Rezaie and Dean, 2002; Volpe, 2001). In this syndrome, there is a loss of neurons and other cell types in highly ischemic regions that causes a focal infarct. However, an additional, most important event is the broader, maturation-dependent, hypoxia-induced death of oligodendrocyte (OL) precursors resulting in the inability to myelinate axonal fiber systems in the central nervous system (CNS), particularly pathways adjacent to the lateral ventricles that carry fibers important for motor control. OL progenitors are particularly susceptible to hypoxic–ischemic (H–I) conditions during the period of 25–35 weeks of gestation, and these cells are thought to undergo apoptosis in a cellular environment that includes elevated levels of free radicals, increased glutamate concentrations, and/or increased levels of iron stimulated by inflammatory cytokines (Back et al., 1998, 2001, 2002; Volpe, 2001). Thus, therapies aimed at inhibiting the

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oxidative environment or providing more favorable conditions for the survival and growth of OL progenitors could protect against brain injury in certain premature births.

A rodent model originally developed for the adult by Levine (1960) was reprised by Rice et al. (1981) and Vannucci et al. (1999) for the neonate and is most commonly used today to mimic human perinatal H–I insult. This model entails unilateral ligation of the common carotid artery in an early postnatal rodent, followed by transient (hours) exposure to hypoxic conditions. The resulting infarct of the cerebral cortex, striatum, and hippocampus is often "cavitary", with shrinkage or practical elimination of structure (Towfighi et al., 1991). Although there is excitotoxic and free radical-induced neuronal death, there is also a selective vulnerability of OL precursors to H–I (Back et al., 2002; Skoff et al., 2001). Treatments aimed at reducing excitotoxicity (Follett et al., 2000; Hattori et al., 1989; McDonald et al., 1987), inhibiting the formation of reactive oxygen species (Ferriero et al., 1996), or apoptosis (Cheng et al., 1998), or stimulating trophic pathways (Almli et al., 2000) all improve the neurohistological outcome of H–I in the neonatal rodent.

Chondroitin sulfate proteoglycans (CSPGs) appear to play a role in the development, survival, and differentiation of OLs (Levine et al., 2001). NG2, a transmembrane and extracellular matrix-associated CSPG, is expressed in two distinct populations of cells in the developing CNS, one is the immediate precursor of premyelinating, proteolipid protein-expressing OLs and the other is an $NG2^+$ population of "glia" that is also present in adult brain (Mallon et al., 2002). In the adult, it is generally agreed that $NG2^+$ cells differentiate into mature, myelinating OLs after demyelinating injury (Dawson et al., 2000), although other evidence disputes this (Greenwood and Butt, 2003). It is also clear that OL progenitors are vulnerable to H–I insult in the neonate. A second CSPG, brevican, is produced and secreted by OLs in developing hippocampal fimbria and other white matter. Brevican is not synthesized by mature OLs, it is produced by astrocytes in the mature adult (Ogawa et al., 2001). Interestingly, increases in the proteinase matrix metalloproteinase-9 (MMP-9) accompany developmental myelination (Uhm et al., 1998) and pharmacological inhibition of MMP-9 expression in OL culture or OLs cultured from matrix metalloproteinase-9 (MMP-9) null mice show reduced process extension suggesting a role for matrix proteolysis in OL maturation (Oh et al., 1999). The major families of proteases involved in the cleavage and turnover of CSPGs are the MMPs and a disintegrin and metalloproteinase with thrombospondin repeats (ADAMTSs). Since little is known about how the two proteoglycans, brevican and NG2, or their proteolytic products are altered in response to H–I, we set out to examine brevican and NG2 abundance and turnover at various times in postnatal day 7 rats undergoing H–I. Specifically, changes in the hippocampus (HP) were examined, a region of the brain that is located in deep tissue, and is a well-delineated region for dissection, even after injury due to H–I insult.

Materials and methods

Hypoxic–ischemic insult

All animal procedures were approved by the institutional IACUC review panel prior to their being carried out. Postnatal day 7 rat pups were removed one at a time from the mother and prepared for surgery. The pups were exposed to 2.5% halothane until anesthesia of surgical depth and then reduced to 1%. The right common carotid was surgically exposed, permanently ligated with 6 × 0 nylon suture, the musculature and skin were sutured in sequence, and each animal was placed on a heating pad until awakening. The pups were returned to the dam for 2 h after surgery for recovery and feeding. The pups were then carefully placed in the bottom of 500 ml polycarbonate centrifuge bottles (Nalgene) and the cap replaced. Caps of the bottle had holes drilled that fit Tygon tubing attached to a certified tank of 8% oxygen, remainder, nitrogen. Two animals were placed in each bottle and six bottles were attached via the tubing to the tank. The bottles were positioned into a custom-made, form-fitted box and lowered into a 37°C water bath. Flow of water-saturated gas was begun and continued for 120 min. At the end of exposure to hypoxia, the pups were returned to the dam for 1, 4, 14, or 30 days. For collection of tissue for biochemistry, animals that were 8, 11, 21, or 37 days old (1, 4, 14, and 30 days after H–I) were exposed to excess CO_2 until death, and decapitated. Brain tissues were rapidly dissected, rinsed, and snap frozen on dry ice and frozen for the long term at −80°C. Brain tissues were thawed and homogenized by hand with 10 strokes using a teflon-glass homogenizer in a buffer containing 20 mM Tris–HCl, pH 7.6, and 1.0% Triton X-100, and proteinase inhibitor cocktail III (Calbiochem-Novabiochem Co., La Jolla, CA).

Perfusion and immunohistochemistry

Rats that were 8, 11, 21, or 37 days old and had undergone H–I at P7 were anesthetized with sodium pentobarbital (Nembutal, 60 mg/kg), their rib cage opened, and the left ventricle/left ascending aorta perfused with cold phosphate-buffered saline, pH 7.4, followed by 4% paraformaldehyde buffered with 0.1 M sodium phosphate, pH 7.4. The brain was carefully removed from the skull and postfixed in 4% paraformaldehyde for 16 h at 4°C. The brains were then cryoprotected with 15% and then 30% sucrose in saline. The brains were frozen to −19°C and 30-μm free-floating cryostat sections were cut rostrally from the largest region of the lateral ventricles through the most caudal aspect of the HP. Sections were stored in saline or in "anti-freeze" until stained with cresyl violet or were used for immunohistochemistry. Sections were stained with cresyl violet and the immunohistochemistry procedure performed as described (Yuan et al., 2002; Zhang et al., 1998). For cresyl violet staining, sections were rinsed in 20 mM Tris