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BRAIN RESEARCH

Research Report

Elevated pressure induced astrocyte damage in the optic nerve

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ARTICLE INFO

Article history:
Accepted 10 September 2008
Available online 24 September 2008

Keywords:
Astrocyte
Intraocular pressure
Glaucoma
Optic nerve head
Lamina cribrosa

ABSTRACT

Astrocytes maintain an intimate relationship with central nervous system (CNS) neurons and play a crucial role in regulating their biochemical environment. A rise in neural tissue pressure in the CNS is known to lead to axonal degeneration however the response of astrocytes during the early stages of neural injury has not been studied in great detail. The optic nerve is a readily accessible model in which to study CNS axonal injury. Previous work from our laboratory has shown that an acute increase in intraocular pressure (IOP) results in axonal cytoskeleton changes and axonal transport retardation within the optic nerve head. Axonal changes occurred in a time-dependent manner with the magnitude of change being proportional to the duration of the IOP rise. Using glial fibrillary acidic protein (GFAP) as a marker of astrocytes we have now studied pressure induced changes in astrocyte structure in the optic nerve head. Using confocal microscopy we found that an increase in IOP resulted in morphological changes in the astrocytes that were consistent with previous reports of swelling. In addition there was also a decrease in GFAP intensity within these astrocytes. These changes occurred in a time-dependent manner with the chronology of change coinciding with that of axonal change. There was no evidence of apoptosis in regions where astrocyte changes were found. The present results provide evidence that in the early stages of neural tissue pressure rise there are both astrocyte and axonal injury.

1. Introduction

Astrocytes are the most abundant cell type within the central nervous system (CNS) and play a crucial role in maintaining neuronal well being (De Keyser et al., 2008). Although their primary role in a physiological environment is to support neuronal function, significant aberrations in cellular homeostasis can result in activation of astrocytes causing them to become the primary mediators of neuronal damage (Neufeld and Liu, 2003). Glial fibrillary acidic protein (GFAP) is a type III intermediate filament which is a major constituent of astrocytes and a marker commonly used to study the distribution and morphology of these cells (Eng et al., 2000). Within the CNS, astrocytes typically increase GFAP expression in an activated state and decrease GFAP expression in conditions of ischemia and injury (Norenberg, 1994).

A rise in cerebrospinal fluid (CSF) pressure within the brain occurs in congenital hydrocephalus but also in many acquired conditions such as cerebral contusion, haemorrhage and infection (Rangel-Castillo and Robertson, 2006). A rise in CSF pressure causes a proportional increase in neuronal tissue pressure which over time has been shown to result in both

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doi:10.1016/j.brainres.2008.09.044

axonal and dendritic degeneration (Penn et al., 2005; McAllister et al., 1985). This is one pathway believed to cause cognitive impairment in patients with hydrocephalus. CNS damage following a rise in intracranial pressure (ICP) is not localised, but instead involves diffuse regions of cortical and white matter tissue within the brain (Del Bigio, 1993; Clark and Milhorat, 1970). Consequently, experimental and histological studies that are aimed at investigating the interaction between astrocytes and neurons within the CNS in intracranial hypertension are complicated with inherent experimental difficulties.

The optic nerve is a direct extension of the CNS and has been used in previous studies to simulate models of axonal injury in the brain (Gennarelli et al., 1989). The accessibility of the optic nerve and the uni-directional projection of retinal ganglion cell (RGC) axons within the nerve make it an ideal structure for use in experimental studies. The optic nerve acquires a myelin sheath behind the lamina cribrosa and by doing so shares a similarity to many axon pathways within the brain. RGC axons commence in the eye, which like the brain is also a rigid chamber with unique compartmental pressure properties. In the same manner that parenchymal tissue pressure within the brain is raised following a rise in ICP, we have previously demonstrated that optic nerve tissue pressure within the eye is elevated following an increase in intraocular pressure (IOP) (Morgan et al., 1995).

A rise in IOP is the most significant risk factor for glaucoma which is a disease characterised by the gradual loss of RGC axons over time (Quigley, 1996). The site of damage to RGC axons in glaucoma occurs at the optic nerve head resulting in posterior optic disk displacement and distortion (Quigley et al., 1983; Quigley and Green, 1979). Histologically, the human optic nerve head is divided into pre-laminar, lamina cribrosa and post-laminar regions with each of these regions having distinct structural and functional properties (Anderson, 1969). Astrocytes are the predominant cell type in the optic nerve head and in addition to providing homeostatic support to axons they also play an important role in controlling the mechanical properties of the lamina cribrosa (Pena et al., 2001).

Some of the methods by which astrocytes regulate neuronal function in the CNS are through the buffering of the neuronal extracellular environment, the regulation of ionic homeostasis, physiological control of the nodes of Ranvier and maintenance of the blood-brain barrier (Ransom and Orkand, 1996). In the brain, a chronic rise in ICP has been shown to alter astrocyte metabolism resulting in swollen and oedematous astrocytes with impaired function (Hasan and Glees, 1990; Kondziella et al., 2003). In the eye, a chronic rise in IOP has demonstrated a change in GFAP expression and morphology of astrocytes in the various laminar regions (Varela and Hernandez, 1997). Astrocytes have the ability to undergo apoptosis, necrosis, activation or proliferation following prolonged periods of IOP or ICP elevation with all these responses being described to various extents by previous reports that have examined glaucomatous eyes and hydrocephalic brains. Although a large body of evidence suggests that astrocytes contribute to axonal injury in chronic states of neural tissue pressure elevation, the acuity of the astrocyte response following brief periods of IOP or ICP rise has not been investigated in the same level of detail. Furthermore, there have only been a few studies that have concurrently examined astrocyte and neuronal changes in the same region of injured CNS tissue following alterations in tissue pressure.

Axonal cytoskeleton disruption is a hallmark of neuronal injury and has been demonstrated in hydrocephalic dog brains (Aoyama et al., 2006). We have previously shown that a rise in IOP causes disruption of the RGC axonal cytoskeleton within the optic nerve head. Additionally, we have also shown that regions of cytoskeleton disruption in the optic nerve head corresponded closely to areas of axonal transport inhibition (Balaratnasingam et al., 2007; Balaratnasingam et al., 2008). Both cytoskeleton protein and axonal transport change were affected in a time-dependent manner, with the degree of change being proportional to the duration of IOP elevation (Balaratnasingam et al., 2008). The purpose of this work was to investigate if there was an acute astrocyte response to the raised IOP. In order to determine this we studied astrocyte change in the optic nerve heads of animals that had been used for cytoskeleton protein and axonal transport studies. Using GFAP as our astrocyte marker we used highly magnified images to study changes in astrocyte morphology and GFAP immunostaining in control and high-IOP eyes after selected periods of IOP elevation. Using TUNEL stain we also looked for evidence of apoptosis within the optic nerve head of these eyes. By doing so we were able to determine if astrocyte changes in the optic nerve head preceded, occurred concurrently with, or followed changes to RGC axons. Our experimental design also allowed us to examine the effects of IOP, CSF pressure and the translaminar pressure gradient on astrocyte morphology in each of the laminar regions. This has not previously been studied and would provide valuable information regarding the pathogenic role of astrocytes in optic nerve head changes in the early stages of IOP elevation. Using the optic nerve as a model of CNS injury the results from this work can also be extrapolated to the brain to help understand the relationship between astrocytes and axons in neuronal damage secondary to intracranial hypertension.

2. Results

2.1. *General*

The mean systolic blood pressure for all 25 pigs was 85.6±1.4 mm Hg. Average arterial pO_2 was 99.3±2.1 mm Hg, pCO_2 was 38.0±0.7 mm Hg, and pH was 7.5±0.0 on blood gas analysis. The mean CSFp was 6.3±0.6 mm Hg. The average left- and right-eye IOP was 43.1±0.3 and 13.0±0.3 mm Hg respectively. The average differences between the IOP and CSFp in the left and right eyes were 38.4±0.8 and 8.3±0.7 mm Hg respectively. There were no differences in blood pressure, CSFp, left-eye IOP, right-eye IOP, pO_2, pCO_2 and pH between the 3, 6, 9 and 12 hour time groups (all $P>0.183$).

2.2. *Neuronal, nuclei and astrocyte distribution within porcine optic nerve heads*

Like the human eye, the pig has a well defined lamina cribrosa with the collagen plates that constitute this structure being clearly evident on Van Gieson stained specimens (Fig. 1A).