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REVIEW

AQUAPORINS IN SPINAL CORD INJURY: THE JANUS FACE OF AQUAPORIN 4

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Abstract—Although malfunction of spinal cord water channels (aquaporins, AQP) likely contributes to severe disturbances in ion/water homeostasis after spinal cord injury (SCI), their roles are still poorly understood. Here we report and discuss the potential significance of changes in the AQP4 expression in human SCI that generates glial fibrillary acidic protein (GFAP)-labeled astrocytes devoid of AQP4, and GFAP-labeled astroglia that overexpress AQP4. We used a rat model of contusion SCI to study observed changes in human SCI. AQP4-negative astrocytes are likely generated during the process of SCI-induced replacement of lost astrocytes, but their origin and role in SCI remains to be investigated. We found that AQP4-overexpression is likely triggered by hypoxia. Our transcriptional profiling of injured rat cords suggests that elevated AQP4-mediated water influx accompanies increased uptake of chloride and potassium ions which represents a protective astrocytic reaction to hypoxia. However, unbalanced water intake also results in astrocytic swelling that can contribute to motor impairment, but likely only in milder injuries. In severe rat SCI, a low abundance of AQP4-overexpressing astrocytes was found during the motor recovery phase. Our results suggest that severe rat contusion SCI is a better model to analyze AQP4 functions after SCI. We found that AQP4 increases in the chronic post-injury phase are associated with the development of pain-like behavior in SCI rats, while possible mechanisms underlying pain development may involve astrocytic swelling-induced glutamate release. In contrast, the formation and size of fluid-filled cavities occurring later after SCI does not appear to be affected by the extent of increased AQP4 levels. Therefore, the effect of therapeutic interventions targeting AQP4 will depend not only on the time interval after SCI or animal models, but also on the balance between protective role of increased AQP4 in hypoxia and deleterious effects of ongoing astrocytic swelling. Published by Elsevier Ltd on behalf of IBRO.

Key words: human spinal cord injury, AQP4-negative astrocytes, AQP4-overexpressing astrocytes, bumetanide, pain, fluid-filled cavity.

Contents

WHY STUDY AQUAPORINS (AQPs) IN SPINAL CORD INJURY (SCI)?

It is estimated that there are 2.5 million people worldwide living with an SCI, and that 130,000 new injuries occur each year (Thuret et al., 2006). Devastating losses of motor, sensory and autonomic functions cannot be reversed, principally because of the lack of any effective therapeutic treatments.

Methylprednisolone is the only pharmacological agent that has been shown to produce a clinical improvement

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Abbreviations: ALS, amyotrophic lateral sclerosis; AQPs, aquaporins; AQP4, aquaporin 4; ARE, antioxidant-responsive element; BBB, Basso, Beattie, Bresnahan motor recovery score; BSCB, blood–spinal cord barrier; FDA, food and drug administration; GFAP, glial fibrillary acidic protein; HIF-1α, hypoxia induced factor; IL-1ra, interleukin-1 receptor antagonist; MS, multiple sclerosis; NMO, neuromyelitis optica; PTS, posttraumatic syringomyelia; SCI, spinal cord injury.

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after SCI (Cayli et al., 2004) in a randomized blinded research design, but it is not approved by the FDA for SCI treatment (Rozet, 2008), and the actual benefits are debated (Miller, 2008; Rozet, 2008). Therefore, effective therapeutic interventions after SCI are greatly needed.

A dramatic SCI-induced deregulation of ion/water homeostasis significantly contributes to the final functional impairments (LoPachin et al., 1999). Therefore, a better understanding of the roles that AQPs play in the regulation of ion/water homeostasis in spinal cords may give important insights into some important outcomes resulting from SCI, including edema, the formation of fluid-filled progressively enlarging syringes, and final motor or sensory impairments. Detailed descriptions of the mechanisms underlying control of the ion water/water transport by different AQPs in the CNS is presented in other articles in this special issue of Neuroscience, so this study is primarily focused on describing current findings that implicate AQPs in the subsequent pathophysiology of SCI.

AQPs IN SPINAL CORDS

Rodent spinal cords express transcripts for several AQPs: AQP4, AQP5, AQP8 and AQP9 (Oshio et al., 2004). With the exception of AQP5, expression of all other AQPs in rodent spinal cords has also been confirmed at the protein level. AQP4 protein is exclusively expressed in spinal astrocytes (Oshio et al., 2004; Nesic et al., 2006), while AQP9 proteins are found both in astrocytes and in neurons in the brain (Badaut, in press). AQP8 is found in ependymal cells around the central canal (Oshio et al., 2004), but detailed characterization of AQP8 and AQP9 in rodent spinal cords is lacking. Expression of AQP1 protein in sensory axons within dorsal horns has been confirmed in several studies on rodent spinal cords (Oshio et al., 2004; Shields et al., 2007; and Nesic et al., 2008), but the role of AQP1 in spinal cords remains unknown. AQP1 mRNA is not expected within spinal cords, since the axonal AQP1 protein in spinal cords originate in dorsal root ganglia neurons (Shields et al., 2007). With the exception of AQP4, none of the other AQPs found in rodent spinal cords has been confirmed in human spinal cords, so the challenging and intriguing study of AQPs' roles in different spinal pathologies has only just begun.

AQP4

Widely distributed AQP4 expression in white and gray matter spinal cord astrocytes is found in both rodents (mice, Oshio et al., 2004; and rats, Nesic et al., 2006, Fig. 2A) and in humans (Misu et al., 2007; Fig. 1A). There is little doubt that AQP4's normal function in astrocytes is to enable fast water influx or efflux, driven by osmotic or hydrostatic pressures (Solenov et al., 2002; Yang et al., 2008). It has been reported that rat spinal cord tissue has the highest expression levels of AQP4 among all AQP4-expressing organs, including several different brain regions as well as various muscles including heart tissue (Shibuya et al., 2008). Widespread and high expression levels of AQP4 in mammalian spinal cords suggest the importance of potentially altered AQP4 functions in different pathological spinal conditions. AQP4 function has been implicated in spinal cord trauma (Nesic et al., 2006; Saddoun et al., 2008), spinal cord ischemia (Xu et al., 2008); neuromyelitis optica (NMO) and multiple sclerosis (MS; Hinson et al., 2010; Misu et al., 2007), and amyotrophic lateral sclerosis (ALS; Nicaise et al., 2009). AQP4 expression changes are found in all those pathological conditions, including decreases in AQP4 levels (NMO), increases (ALS), and time-dependent combinations of both elevation and reduction (spinal cord trauma and ischemia). Fig. 1A shows typical AQP4 immunolabeling (brown) in white and gray matter astrocytes in uninjured human spinal cords (astrocytes are labeled for GFAP; pink). The same AQP4 expression pattern was found in cervical, thoracic and lumbar regions (not shown).

HUMAN SCI: AQP4-NEGATIVE AND AQP4-OVEREXPRESSING ASTROCYTES

AQP4 changes in human SCI have not been previously reported. We analyzed cords from three SCI patients suffering from trauma to the cervical segments at C5/6 (survival time 1 year), C2 (survival time 1 year) and C8 (survival time 2 years). Detailed histological analysis of these spinal cords is described in Guest et al. (2005); as patients No. 29, 28 and 59, respectively.

Immunohistochemical analysis of AQP4 labeling in all three injured cords at the lesion site showed the presence of GFAP-labeled astrocytes with and without AQP4. In contrast, GFAP-labeled astrocytes devoid of AQP4 have not been found in adult uninjured spinal cords. We and others have previously reported AQP4-labeled astrocytic processes that are not co-labeled with GFAP, reflecting extensive arborization of astrocytic processes not recognized by GFAP labeling (Nesic et al., 2006; Simard et al., 2003). However, GFAP-labeled processes without AQP4 have not been reported. We found AQP4-negative astrocytes at the lesion epicenter in all analyzed samples from three SCI patients, who differed in age, gender and the extent of SCI (complete vs. incomplete). We cannot rule out that causes other than SCI contributed to the AQP4 changes reported here; however, it is unlikely that three very different pathologies diagnosed as cause of death (e.g. pneumonia in patient #28, heart attack in patient #29 and sepsis in patient #59) all affected spinal cord astrocytes at the lesion site in the same manner, that is, by inhibiting AQP4 expression. Similarly, it is doubtful that all three SCI patients suffered from the same undiagnosed disease that affected their spinal cord AQP4 in the same way. Furthermore, co-morbidity or deterioration of human spinal cord samples before or after autopsy, as possible causes of AQP4 absence, are also very unlikely because AQP4-negative astrocytes were also found in injured rat spinal cords (as shown in Fig. 2C).

A representative example of AQP4 labeling in human SCI (Fig. 1B, Patient No. 29; Guest et al., 2005) showed typical lesion morphology with a cavity at the epicenter—the cell/tissue-free center of the cord (cells were labeled