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PERIPHERAL NERVE INJURY INDUCES AQUAPORIN-4 EXPRESSION AND ASTROCYTIC ENLARGEMENT IN SPINAL CORD

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Abstract—Aquaporin-4 (AQP4), a water channel protein, is expressed mainly in the perivascular end-feet of astrocytes in the brain and spinal cord. Dysregulation of AQP4 is critically associated with abnormal water transport in the astrocytes. We aimed to examine whether peripheral nerve injury (PNI) could induce the changes of AQP4 expression and astrocytic morphology in the spinal cord. Two different PNI models [partial sciatic nerve transection (PST) and chronic constriction injury (CCI)] were established on the left sciatic nerve in Sprague–Dawley rats, which decreased the pain withdrawal threshold in the ipsilateral hind paws. Both PNI models were associated with a persistent up-regulation of AQP4 in the ipsilateral dorsal horn at the lower lumbar region over 3 weeks, despite an absence of direct injury to the spinal cord. Three-dimensional reconstruction of astrocytes was made and morphometric analysis was done. Up-regulation of AQP4 was accompanied by a significant increase in the length and volume of astrocytic processes and the number of branch points. The most prominent changes were present in the distal processes of the astrocytes and the changes were maintained throughout the whole experimental period. Extravasation of systemically administered tracers Evans Blue and sodium fluorescein was not seen in both models. Taken together, PNI was associated with a long-lasting AQP4 up-regulation and enlargement of astrocytic processes in the spinal cord in rats, both of which were not related to the disruption of blood–spinal cord barrier. The findings could provide novel insights on the understanding of pathophysiology of spinal cords after PNI.

Key words: aquaporin-4, astrocyte enlargement, morphometric analysis, peripheral nerve injury, spinal cord.

INTRODUCTION

Astrocytes, comprising of approximately four fifth of the cells in nervous system, participate in water transport and fluid homeostasis in the brain and spinal cord. In the brain, aquaporin-4 (AQP4) water channel protein is widely expressed at the end-feet of astrocytes surrounding the capillaries, the glia limitans, and the ependymal cells lining the ventricles (Rash et al., 1998; Furman et al., 2003; Vitellaro-Zuccarello et al., 2005). In the spinal cord, the expression pattern of AQP4 was similar to the one observed in the brain, especially in the neurovascular units, which are encircled by AQP4-enriched astrocytic foot processes. Consistent with this, we recently demonstrated that AQP4 was exclusively localized at the astrocytes in the rat spinal cord, however, its expression exhibited less polarized and more spatial distribution than that of brain astrocytes (Oklinski et al., 2014).

A number of previous studies demonstrated that osmotic regulation and water transport between the systemic circulation and the central nervous system (CNS) are largely dependent on the water transport across the plasma membrane of the astrocytes enriched in AQP4 expression (Amiry-Moghaddam et al., 2004). As water transport through AQP4 is bidirectional and driven by osmotic and hydrostatic gradient, altered expression of perivascular AQP4 could be critically associated with pathophysiological conditions, e.g., abnormal water transport and accumulation in the astrocytes (Amiry-Moghaddam and Ottersen, 2003). Thus, the perivascular end-feet of astrocytes, where AQP4 is abundantly expressed, plays a role as the primary route for fluid accumulation in the pathogenesis of cytotoxic edema (Manley et al., 2000; Yang et al., 2008).

In particular, AQP4 plays a critical role in the pathogenesis of the first build-up phase of brain edema (Amiry-Moghaddam et al., 2004; Papadopoulos and Verkman, 2005). Astroglial cells were not equally swollen. In the astrocytes, foot processes adjacent to blood vessels, where AQP4 is abundantly expressed, increase their volume more than cell bodies, whereas astrocytes not close to blood vessels did not show an increase in cell volume (Papadopoulos and Verkman, 2005; Nase et al., 2008). Therefore, AQP4 knockout mice had better survival and neurological outcome in an early phase of brain edema (Manley et al., 2000; Papadopoulos and Verkman, 2005). Similar observations were found in mice with α-syntrophin-null and dystrophin-null mice, where α-syntrophin is a member of dystrophin-associated

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Abbreviations: 3D, three-dimension; ACSF, artificial cerebrospinal fluid; AQP4, aquaporin-4; BSCB, blood–spinal cord barrier; CCI, chronic constriction injury; CNS, central nervous system; EAAT2, excitatory amino acid transporter 2; EB, Evans Blue; GFAP, glial fibrillary acidic protein; GS, glutamine synthetase; Na-Flu, sodium fluorescein; NF 200, Neurofilament 200; PBS, phosphate-buffered saline; PNI, peripheral nerve injury; PST, partial sciatic nerve transection.

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protein complex, essential for the localization of AQP4 in the astrocyte end-feet (Vajda et al., 2002; Amiry-Moghaddam et al., 2003). In contrast, AQP4 facilitates the absorption of excessive brain water in the resolution phase of brain edema, in which the excessive fluid is likely to be transported through AQP4 via transcellular pathway (Manley et al., 2004; Papadopoulos et al., 2004). Consequently, AQP4-null mice represented deteriorated neurological outcome, increased intracranial pressure and water accumulation, compared with wild type mice (Papadopoulos et al., 2004; Bloch et al., 2005; Tait et al., 2008, 2010).

Clinically, spinal cord injury is an important insult of the CNS and several lines of evidence have demonstrated both beneficial and detrimental involvement of aquaporin water channels in the pathogenesis of spinal cord injury. For instance, AQP4 up-regulation was known to be related to the neuropathy and persistent pain progression (Nesic et al., 2005, 2010). While many previous studies exploited an animal model where injury was directly applied to the spinal cord itself, in this study we aimed to examine the changes of AQP4 expression and astrocytic morphometry in the spinal cord of rats, which were subjected to peripheral nerve injury (PNI). Since the sciatic nerve was directly and selectively injured, the observed pathophysiological changes in the spinal cord could be the response of spinal cord to PNI. We demonstrated the time course of changes over 3 weeks in AQP4 expression and the morphological changes of astrocytes in rat spinal cord in response to PNI.

EXPERIMENTAL PROCEDURES

Animal models of PNI

The animal protocols were approved by the Animal Care and Use Committee of the Kyungpook National University, Korea, in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23). Six week-old male Sprague–Dawley rats (200–220 g, Charles River, Seongnam, Korea) were housed during whole experimental period under the 12-h light–dark cycle with free access to food and water intake. Rats were anesthetized under isoflurane inhalation and all surgical procedures [partial sciatic nerve transection (PST) and chronic constriction injury (CCI)] were done on the left sciatic nerve. Sham operation was performed in the same way, where the sciatic nerve was exposed but not ligated or transected. *Protocol 1:* Four rats in each time point were included in each group and their spinal cords were collected for protein isolation (12 h, 3-, 7-, 14- and 21-days after injury) for immunoblotting [sham (n = 20), PST (n = 20) and CCI (n = 20) groups: total 60 rats]. *Protocol 2:* Four rats in each time point were included in each group. Their spinal cords were collected and the lower lumbar level (L4–L5) of the spinal cords was divided into ipsilateral dorsal, contralateral dorsal, ipsilateral ventral and contralateral ventral part for protein isolation (12 h, 7- and 21-days after injury) for immunoblotting [sham (n = 12), PST (n = 12) and CCI (n = 12) groups: total 36 rats]. *Protocol 3:* Three rats in each time point were subjected to immunohistochemistry (12 h, 7-, and 21-days after injury) [sham (n = 9), PST (n = 9) and CCI (n = 9) groups: total 27 rats]. *Protocol 4:* Three rats in each time point were subjected to blood–spinal cord barrier (BSCB) disruption assessment, exploiting sodium fluorescein (Na-Flu), 12 h, 3-, 7-, and 21-days after injury [sham (n = 12), PST (n = 12) and CCI (n = 12) groups: total 36 rats] or Evans Blue (EB), 12 h and 3 days after injury [PST (n = 6) and CCI (n = 6) groups: total 12 rats; rats from Na-Flu study were used as controls].

PST

The PST model was established according to the methods previously proposed by Lindenlaub and Sommer (2000). Rats were anesthetized and the hair on the left side of the hind limb, lower and upper thigh was shaved. Skin was sterilized with 70% ethyl alcohol and a single longitudinal incision (~12–15 mm in length) was made at mid-thigh level through biceps femoris. The wound was spread out by wound retractor and left sciatic nerve was exposed. The nerve was lifted and a 6-0 non-absorbed silk ligature was applied through the middle part of the nerve, and the part of the sciatic nerve branching to the peroneal and tibial nerve was transected with fine scissors. Then, ligature was removed from the nerve. The length of partial transection was about half nerve diameter. The skin was closed with wound clamps.

CCI

The surgical procedure was identical to the PST, however, the nerve was constricted according to the methods proposed by Bennett and Xie (1988). After an exposure of nerve, the nerve was gently elevated for an application of four silk ligatures (1–2 mm apart) with 4-0 non-absorbed silk. The ligatures were made around the nerve above the point of trifurcation (branching to peroneal, tibial, and sural nerve). The ligatures were gently tied for preventing necrosis and transection. The skin was closed with wound clamps.

Pain withdrawal threshold measurements

The pain withdrawal threshold was measured by Dynamic Plantar Aesthsiometer (Ugo Basile, cat. no 37400-001, Italy). The rats were placed in plastic cages with mesh wire bottom surface and acclimatized for about 15 min. A moveable force actuator was positioned below the plantar surface of the paw. A force needed to evoke the paw withdrawal reflex was recorded. In each rat, ten measurements were taken from one paw. All measurements were taken shortly before the injury, and in the subsequent 12 h, 3-, 7-, 10-, 14-, 17-, and 21-days after injury.

Spinal cord isolation and sample collection

All spinal cords were isolated by the hydraulic extraction method after a brief perfusion, as we described previously (Oklinski et al., 2014). Perfusion with 0.01 M