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Expression and localization of aquaporin-4 in sensory ganglia

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ABSTRACT

Aquaporin-4 (AQP4) is a water channel protein that is predominantly expressed in astrocytes in the CNS. The rapid water flux through AQP4 may contribute to electrolyte/water homeostasis and may support neuronal activities in the CNS. On the other hand, little is known about the expression of AQP4 in the peripheral nervous system (PNS). Using AQP4$^{-/-}$ mice as a negative control, we demonstrated that AQP4 is also expressed in sensory ganglia, such as trigeminal ganglia and dorsal root ganglia in the PNS. Immunohistochemistry revealed that AQP4 is exclusively localized to satellite glial cells (SGCs) surrounding the cell bodies of the primary afferent sensory neurons in the sensory ganglia. Biochemical analyses revealed that the expression levels of AQP4 in sensory ganglia were considerably lower than those in astrocytes in the CNS. Consistently, behavioral analyses did not show any significant difference in terms of mechanical and cold sensitivity between wild type and AQP4$^{-/-}$ mice. Overall, although the pathophysiological relevance of AQP4 in somatosensory perception remains unclear, our findings provide new insight into the involvement of water homeostasis in the peripheral sensory system.

1. Introduction

Aquaporin-4 (AQP4), a water channel protein, is widely expressed in various organs including the central nervous system (CNS), kidney, intestine, skeletal muscle, lung and secretory glands [1–3]. AQP4 forms stable tetramers in plasma membranes; in these tetramers, each monomer contains six transmembrane helical domains with two shorter helical segments, forming a narrow water-selective pore [4]. Structural studies and molecular dynamic simulations suggest that steric and electrostatic factors contribute to AQP4's highly efficient and selective ability to transport water [5,6].

In the CNS, AQP4 is exclusively expressed in astrocytes. AQP4 localizes specifically at foot processes extending toward brain–water interfaces, such as vascular endfeet and the glia limitans [7,8]. Given this polarization, AQP4 is thought to play an important role in brain–water homeostasis. Moreover, recent studies have shown the involvement of water flux through AQP4 in neuronal activities in the CNS. AQP4$^{-/-}$ mice exhibit abnormalities in the electrophysiological activities of their neurons, such as altered sensory-evoked potentials and increased seizure duration [9–12]. Although the underlying mechanism remains unclear, AQP4-dependent water permeation is thought to play an important role in K^+ reuptake by astrocytes following neuronal excitation, contributing to electrolyte/water homeostasis in the extracellular space for the securement of normal neuronal activities. Therefore, AQP4 is now considered to be a potential therapeutic target for various neurological disorders that involve the abnormal water/electrolyte homeostasis, such as brain edema and epilepsy. Previously, we demonstrated that propofol, a general anesthetic drug, profoundly inhibits the osmotic water permeability of AQP4 in the presence of Zn^{2+} [13].

On the other hand, little attention has been given to the expression and function of AQP4 in the peripheral nervous system (PNS). Descriptions of AQP4 expression in the PNS have only been made for neurons in the enteric nerve plexus [14,15]. Sensory ganglia, which consist of small nodular structures connecting to the spinal cord and brain, contain the cell bodies of primary afferent neurons that convey sensory input from the periphery to the CNS [16]. In sensory ganglia, the neuronal cell bodies are thoroughly wrapped by non-neuronal cells called satellite glial cells (SGCs). Similar to astrocytes in the CNS, SGCs also express various kinds of ion channels and transporters and contribute to the regulation of the

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extracellular space (ECS) around the neurons, which is the reason why SGCs are often described as peripheral astrocytes [17]. Given the great functional similarities between astrocytes and SGCs, it seems reasonable that SGCs might also express AQP4.

In this study, we examined the expression of AQP4 in sensory ganglia, including dorsal root ganglia (DRG) and trigeminal ganglia (TG), using AQP4$^{-/-}$ mice as negative controls and sought to explore the potential roles of AQP4 in peripheral somatosensory perception.

2. Materials and methods

2.1. Animals

All animal procedures were performed with an approved protocol from the Animal Care Committee of Keio University School of Medicine, in accordance with the Guidelines for Proper Conduct of Animal Experiment and Related Academic Research Institutions under the jurisdiction of the MEXT (Notice No.71, 2006, MEXT, Japan). AQP4 null mice were generated as described previously (acc. No. CDB0758 K: http://www.cdb.riken.jp/arg/mutant%20mice%20list.html) [18]. For experiments in an inbred strain, the AQP4 null genotype was transferred to the Balb/cAJcl (CLEA Japan. Inc.) by >12 back-crosses.

2.2. Immunohistochemistry

Mice were anesthetized with isoflurane, and then perfused with saline followed by 4% paraformaldehyde in PBS. After mice were killed, TGs and DRGs were harvested, postfixed, and cryoprotected with 20% sucrose in PBS. Tissue sections were cryocut and incubated with 5% normal goat serum in 0.2% Triton X-100/PBS to block nonspecific binding. The sections were then incubated overnight with primary antibodies; anti-AQP4 (1:1000, made in rabbit, Sigma, MO, USA), anti-Kir4.1 (1:1000, made in rabbit, Alomone labs, Jerusalem, Israel), anti-AQP1 (1:1000, made in rabbit, Alpha Diagnostic, TX, USA), and Alexa 488- conjugated anti-NeuN (1:200, made in mouse, Millipore, CA, USA). The immunoreactivity was visualized using secondary antibodies conjugated to Alexa 488 or Alexa 555 (each 1:500; Invitrogen, CA, USA). To visualize nuclei, a mounting medium containing an instant-blue nuclear probe fluorescing compound (SouthernBiotech, AL, USA) was applied. Images were acquired using an inverted fluorescence microscope (Olympus, Tokyo, Japan). For some experiments, immunostained slices were observed with a confocal microscope (Olympus). DAB immunostaining was performed using EnVision systems (DAKO, CA, USA) as described previously [19]. Tissues were fixed with 5% paraformaldehyde, paraffin-embedded and sliced at 5 μm thickness. The sections were deparaffinized in xylene, rised in 100% and 70% ethanol, and blocked with 10% goat serum for 15 min at room temperature. The sections were then incubated with anti-AQP4 antibody (1:200, made in rabbit, Santa Cruz, Texas, USA) for 16 h at 4 °C. HRP-conjugated anti-rabbit IgG antibody was applied as a secondary antibody. The sections were counterstained with haematoxylin.

2.3. Western blotting

Two TGs, 20–30 DRGs from thoracic and lumber spinal cord and cerebellum from each mouse were homogenized in a homogenizing buffer [0.32 M sucrose, 10 mM HEPES (pH 7.4), 2 mM EDTA, protease inhibitors (cOmplete ULTRA Tablets, Mini, EDTA-free EASYpack, Roche, Mannheim, Germany), centrifuged at 1000×*g* for 15 min, and the supernatant was ultracentrifuged at 200,000×*g* for 1 h at 4 °C (Type SW 60 Ti rotor, Beckman Coulter, CA, USA) to obtain crude membrane pellets. Pellets were lysed in RIPA buffer [150 mM NaCl, 1.0% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris (pH 8.0), and protease inhibitors (Roche)]. Total protein was measured using a BCA protein assay reagent kit (Pierce, IL, USA). Samples were incubated at 37 °C for 15 min in 5X Lammli sample buffer. The protein samples (50 μg for TG and DRG samples, 2 μg for cerebellum samples) were separated on a 12% SDS–PAGE and transferred onto a PVDF membrane, blocked with 5% skim milk in PBS, and incubated with a primary antibody against AQP4 (1:2000, made in rabbit, Sigma) overnight at 4 °C followed by the incubation with an HRP-conjugated secondary antibody (1:2000, Sigma) for 1 h at room temperature. Signals were visualized with a chemiluminescence (Immobilon western, Millipore) and detected with Image Quant LAS system (GE healthcare, WI, USA).

2.4. RT-PCR analysis

Cerebellum, spinal cord, DRGs and TGs were harvested from wild type and AQP4$^{-/-}$ mice after euthanasia and immediately homogenized in Isogen reagent (Wako, Osaka, Japan) for the isolation of total RNA. Reverse transcription reaction was performed using SuperScript® VILO™ Master Mix (Invitrogen). The resulting cDNA products were amplified by PCR using GoTaq® Green Master Mix (Promega, WI, USA). For PCR, we performed 30 cycles using the following conditions: 1 min at 94 °C, 1 min at 59 °C and 30 s at 72 °C. β-Actin was used as an internal control. The sequences of primers used in RT-PCR were as follows: for mouse AQP4: forward: 5′-CTTTCAAAGGAGTCTGGACTCAGGC-3′; reverse: 5′-CTTTCAAAGGAGTCTGGACTCAGGC-3′. For β-actin: forward: 5′-TGTATGCCTCTGGTCGTACC-3′; reverse: 5′-CAGGTCCAGACGCAGGATG-3′. PCR products (10 μl) were separated on 1% agarose gels with ethidium bromide and photographed under an ultraviolet illuminator.

2.5. Surgery

Spared nerve injury surgery was performed as described previously [20] under 3–5% sevoflurane (Maruishi, Osaka, Japan) anesthesia. The three branches of the sciatic nerve were exposed and both the tibial and common peroneal nerves were ligated and transected together, while the sural nerve was carefully preserved. The muscles and skin were then closed with a suture. The animals were recovered from the general anesthesia in a pre-warmed chamber, and housed as usual.

2.6. Assessment of mechanical and cold hypersensitivity

Each mouse was individually placed in an acryl cylinder on a wire mesh floor and habituated to the environment for 30 min prior to the testing. Mechanical sensitivity was measured with von Frey monofilaments perpendicularly applied to the lateral side of the plantar surface of the hindpaw. A positive response was noted if the paw was promptly withdrawn, flinched or licked. The 50% threshold of mechanical sensitivity was measured and calculated based on a up-and-down paradigm described as previously [21]. Sensitivity to cold stimuli was assessed with the acetone drop test [22]. One droplet of acetone was applied to the lateral side of the plantar surface of the hindpaw, and the duration of the nocifensive response (biting, licking, or shaking of paw) was measured over 30 s. The test was repeated 3 times to calculate the averages.

3. Results

To examine whether AQP4 is expressed in sensory ganglia, we first performed immunohistochemistry (IHC) for DRG and TG specimens from wild-type mice, using specimens from AQP4$^{-/-}$ mice as