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EXPRESSION OF AQUAPORIN WATER CHANNELS IN MOUSE SPINAL CORD

K. OSHIO,[a] D. K. BINDER,[a] B. YANG,[b] S. SCHECTER,[a] A. S. VERKMAN[b] AND G. T. MANLEY[a]*

[a]*Department of Neurological Surgery, University of California, 1001 Potrero Avenue, Building 1, Room 101, San Francisco, CA 94110, USA*

[b]*Departments of Medicine and Physiology, Cardiovascular Research Institute, University of California, San Francisco, CA 94131, USA*

Abstract—Aquaporins (AQPs) are membrane proteins involved in water transport in many fluid-transporting tissues. Aquaporins AQP1, AQP4, and AQP9 have been identified in brain and hypothesized to participate in brain water homeostasis. Here we use reverse transcriptase-polymerase chain reaction (RT-PCR), Western blotting and immunohistochemistry to describe the expression and immunolocalization of AQPs in adult mouse spinal cord. AQP4 was expressed in glial cells, predominantly in gray matter, and in astrocytic end-feet surrounding capillaries in spinal cord white matter. AQP9 expression extensively co-localized with glial fibrillary acidic protein-immunoreactive astrocytes, located predominantly in the white matter. AQP5 was detected by RT-PCR but not by immunohistochemical analysis. Interestingly, AQP8 was detected primarily in ependymal cells lining the fluid-filled central canal. The aquaporin expression pattern in spinal cord suggests involvement in water homeostasis and diseases associated with abnormal water fluxes such as spinal cord injury and syringomyelia.

Key words: spinal cord, water transport, aquaporins, knockout mice.

The aquaporins (AQPs) are a family of hydrophobic intrinsic membrane proteins that function as "water channels" in many cell types involved in fluid transport. AQP-1 was first identified from red blood cells and renal proximal tubular epithelium (Denker et al., 1988). Recent results indicate multiple physiological roles for AQPs outside of the kidney (Verkman, 2000, 2002), and in particular a role for AQPs in the CNS (Venero et al., 2001; Badaut et al., 2002; Amiry-Moghaddam and Ottersen, 2004). For example, AQP-4 is expressed throughout the brain at brain–blood and brain–cerebrospinal fluid (CSF) interfaces where it is thought to play a role in edema formation and CSF absorption (Nielsen et al., 1997; Rash et al., 1998). Previously, we found that mice deficient in AQP4 (AQP4 −/−) had decreased cerebral edema and improved neurological outcome following water intoxication and focal cerebral ischemia (Manley et al., 2000). Thus, AQPs may have a structural and functional role in the adult CNS. The localization of other AQPs in the brain has also been described. AQP1 is expressed in the choroid plexus where it may play a role in CSF production (Nielsen et al., 1993). Like AQP4, AQP-9 is expressed in astrocytic cell bodies and processes (Badaut et al., 2002). Both AQP4 and AQP9 expression appear to be upregulated after ischemic insult and may play a role in brain edema associated with stroke (Taniguchi et al., 2000; Badaut et al., 2001).

Despite the above studies reporting AQP expression and function in brain, there is limited information regarding AQP expression and function in spinal cord. AQP4 expression studies suggest that it is expressed in spinal glial cells (Frigeri et al., 1995a; Rash et al., 1998). We also reported in a preliminary analysis that AQP1 was expressed in peripheral nerve fibers that project to the dorsal horn of the spinal cord (Solenov et al., 2002). In this study, a novel imaging method used to map changes in water content of spinal cord slices from wild-type and AQP knockout mice demonstrated that AQPs can facilitate osmotically induced water transport in the spinal cord (Solenov et al., 2002). Like the brain, the spinal cord responds to a variety of injuries and diseases with dramatic water fluxes and edema (Wang et al., 1993; Orr et al., 1994); thus, AQPs may play a role in spinal cord function and water homeostasis. The goal of this study is to provide a comprehensive description of expression and localization of AQP family members in adult mouse spinal cord.

EXPERIMENTAL PROCEDURES

All animal experiments were carried out with a protocol approved by the UCSF Committee on Animal Research and in accordance with the NIH Guide for the Care and Use of Laboratory Animals. Care was taken to minimize the number of animals used and their suffering.

Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis

Spinal cords were carefully microdissected from outbred CD1 wild-type mice ($n=4$) after euthanasia by 2,2,2-tribromoethanol (Aldrich Milwaukee, WI, USA) overdose. Cervical segments were immediately homogenized in Trizol reagent (Gibco BRL, Carlsbad, CA, USA) for total RNA isolation. After reverse transcription, PCR was carried out using gene-specific primers designed to amplify portions of the coding sequences of each of the mouse AQPs as described in Table 1. Control PCR reactions were done in parallel using as template a cDNA mixture prepared from brain, lung, kidney, and liver.

*Corresponding author. Tel: +1-415-206-4467; fax: +1-415-206-4466.
E-mail address: manley@itsa.ucsf.edu (G. T. Manley).
Abbreviations: AQP, aquaporin; CSF, cerebrospinal fluid; PBS, phosphate-buffered saline.

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Table 1. Mouse aquaporin primers

Gene	Primer	Product size base pair
AQP1	5′-TGCGTTCTGGCCACCACTGAC-3′	327
	5′-GATGTCGTCAGCATCCAGGTC-3′	
AQP2	5′-GCCATCCTCCATGAGATTACC-3′	305
	5′-ACCCAGTGATCATCAAACTTG-3′	
AQP3	5′-CTGGACGCTTTCACTGTGGGC-3′	309
	5′-GATCTGCTCCTTGTGTTTCATG-3′	
AQP4	5′-CTGGAGCCAGCATGAATCCAG-3′	310
	5′-TTCTTCTCTTCTCCACGGTCA-3′	
AQP5	5′-CTCTGCATCTTCTCCTCCACG-3′	335
	5′-TCCTCTCTATGATCTTCCCAG-3′	
AQP6	5′-TCTGTTCTGCCCTGGCCTGTG-3′	305
	5′-ACCGCCTGGCCAGTTGATGTG-3′	
AQP7	5′-GAGTCGCTAGGCATGAACTCC-3′	302
	5′-AGAGGCACAGAGCCACTTATG-3′	
AQP8	5′-CAGCCTTTGCCATCGTCCAGG-3′	311
	5′-CCTAATGAGCAGTCCTACAAAG-3′	
AQP9	5′-CCTTCTGAGAAGGACCGAGCC-3′	300
	5′-CTTGAACCACTCCATCCTTCC-3′	

Western blot analysis

Western blot analysis was performed on cervical spinal cord from wild-type, AQP4 −/− and AQP5 −/− mice from an outbred CD1 genetic background (n=3, each group). Control protein prepared from brain, lung, kidney, liver, and testis was processed in parallel. Samples were homogenized in 250 mM sucrose, 10 mM Tris–HCl and 20 μg/ml PMSF, pH 8.0, and centrifuged at 1000×g for 10 min at 4 °C. Protein concentration was determined by Bradford assay (Bio-Rad, Hercules, CA, USA). Protein samples were electrophoresed on 12% Tris-glycine gels (Invitrogen, Carlsbad, CA, USA) and electrotransferred to a PVDF membrane (Amersham, Piscataway, NJ, USA). The membrane-blotted proteins were blocked with 3% nonfat milk for 30 min and incubated with primary antibodies for 2 h. Primary antibodies were affinity-purified polyclonal rabbit anti-rat AQPs (Chemicon, Temecula, CA, USA) with the following dilutions: anti-AQP4 1:1000, anti-AQP5 1:2000, anti-AQP8 1:4000, anti-AQP9 1:10,000. After rinsing, membranes were incubated in 1:5000 peroxidase-linked donkey anti-rabbit IgG (Amersham) for 30 min, and detected by chemiluminescence (ECL Plus; Amersham).

Immunohistochemistry

For immunohistochemistry, the same affinity-purified polyclonal rabbit anti-rat AQPs antibodies (Chemicon) were used. AQP4- and AQP5-deficient mice were used to control for the specificity of the AQP immunolabeling (Ma et al., 1997, 2000; Ishibashi et al., 1998; Verkman, 2000). Optimal antibody dilutions were determined by lack of any immunohistochemical signal in appropriate knockout mouse tissues. Because AQP8- and AQP9-deficient mice are not yet available, working dilutions of anti-AQP8 and anti-AQP9 antibodies were decided by using peptide pre-incubation. Antibody dilutions for peroxidase immunohistochemistry were: anti-AQP4 1:500, anti-AQP5 1:2000, anti-AQP8 1:4000, and anti-AQP9 1:1000. Optimal dilutions for fluorescence immunohistochemistry were: anti-AQP4 1:200, anti-AQP8 1:1000, and anti-AQP9 1:200. For preparation spinal cord sections, mice were anesthetized with 2,2,2-tribromoethanol (0.5 mg/g BW i.p.), perfused with 2% paraformaldehyde (pH 7.4) in phosphate-buffered saline (PBS). Spinal cords (n=6) were post-fixed in perfusion buffer for 6 h and cryoprotected in 20% sucrose/PBS. Fourteen micrometer coronal cryostat sections were prepared from cervical cord segments. For immunoperoxidase labeling, endogenous peroxidase activity was quenched with 3% H_2O_2/methanol, and slides were blocked in 5% normal goat serum (Vector, Burlingame, CA, USA). Primary antibodies were incubated overnight at 4 °C (dilutions above). After extensive washing, tissues were incubated in 1:500 biotinylated goat anti-rabbit IgG (Vector) followed by 1:100 ABC reagent (Vector), developed with diaminobenzidine (Sigma, St. Louis, MO, USA). For double-labeling immunofluorescence, slides were blocked in 5% bovine serum albumin (Sigma) followed by incubation in primary antibody overnight at 4 °C. Slides were then incubated with the following secondary antibodies diluted 1:200: Texas-Red-conjugated goat anti-rabbit IgG (Vector), and Alexa Fluor 488-labeled goat anti-rabbit IgG antibodies (Molecular Probes, Eugene, OR, USA). For glial fibrillary acidic protein (GFAP) immunohistochemistry, a mouse monoclonal anti-GFAP antibody (Sigma) diluted 1:500 was co-incubated with secondary antibodies for 2 h. Mouse anti-NeuN monoclonal antibody (1:500; Chemicon) was used as a neuronal marker. Slides were coverslipped and then examined using an Olympus microscope equipped with bright field and fluorescence optics.

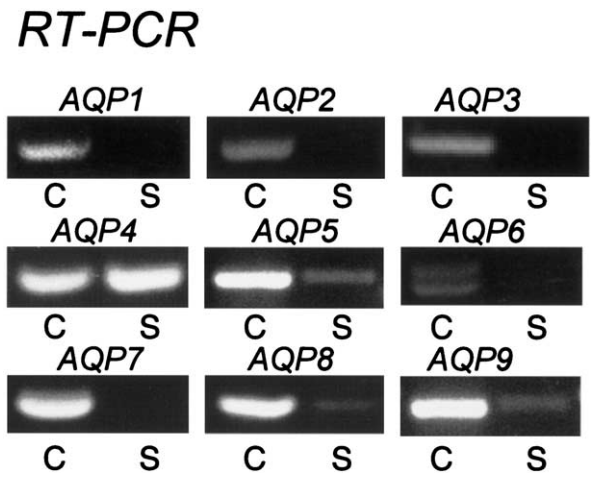

Fig. 1. RT-PCR analysis of AQPs in spinal cord. Mouse spinal cord RNA was reverse-transcribed and gene-specific primers for each of the nine AQPs were used for PCR amplification cDNA. Control lanes (C) represent amplification of a mixture of cDNAs from brain, lung, liver, kidney and testis, which contain all known mouse AQPs. Lanes marked S correspond to amplifications done using spinal cord cDNA as template. Expression of AQP4, AQP5, AQP8 and AQP9 was identified. There was no expression of AQP1, AQP2, AQP3, AQP6 and AQP7.

RESULTS

RT-PCR analysis

RT-PCR was carried out to identify transcripts encoding mammalian AQPs in mouse spinal cord (Fig. 1). Reverse-transcribed cDNA prepared from microdissected cervical spinal cord was PCR-amplified using specific primers for mouse AQP1-9. Although AQP10 has been reported in human (Ishibashi et al., 2002), we did not amplify the mouse AQP10 gene, as it has been recently shown to be a pseudogene (Morinaga et al., 2002). Fig. 1 shows a strongly amplified AQP4 fragment and weakly amplified bands for AQP5, AQP8, and AQP9. Amplified fragments for AQP1, AQP2, AQP3, AQP6, and AQP7 were not seen despite appropriate positive controls. Similar results were

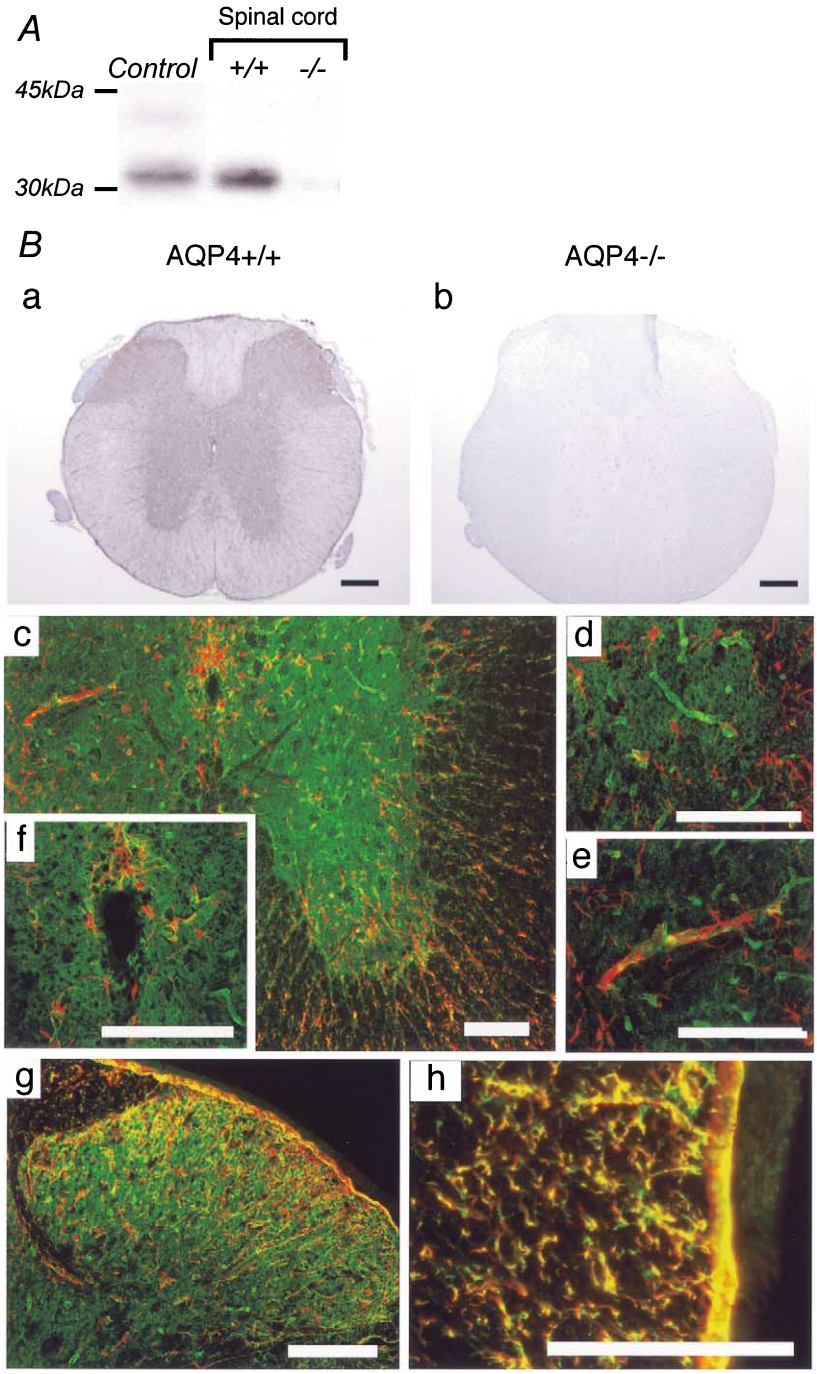

Fig. 2. AQP4 expression in mouse spinal cord. (A) Western blot analysis demonstrates an approximately 32 kDa band in control cerebral cortex (Control) and wild-type spinal cord (+/+). No expression was detected in tissue from AQP4-deficient mice (−/−). (B) Immunohistochemistry for AQP4 reveals extensive expression in gray and white matter in cervical spinal cord from wild-type mice (+/+) (a). No specific immunostaining was found in spinal cord from AQP4 −/− mice (−/−) (b). Dense AQP4 staining (green) appeared extensively in gray matter (c), especially in astrocytic end-feet surrounding capillaries (d). GFAP-immunopositive (red) glial processes extending to capillaries were observed (e). Faint AQP4 staining was detected in ependymal cells lining the central canal (f). GFAP and AQP4 were co-localized in fibrous thin astrocytes in the superficial dorsal horn (g). In white matter, AQP4 was co-expressed prominently with GFAP-immunoreactive radial fibrous glial processes surrounding the blood vessels and the glia limitans (h). (c–h; Red: GFAP, Green: AQP4.) Black scale bar=0.2 mm. White scale bar=0.1 mm.

obtained in thoracic and lumbar spinal cord (data not shown). Based on these results, Western blotting and immunohistochemistry were done for AQP4, AQP5, AQP8 and AQP9.

AQP4 expression

Western blot analysis for AQP4 demonstrated a strong 32 kDa band in spinal cord wild-type mice (Fig. 2A). This band was absent in spinal cord from AQP4 −/− mice. Immuno-

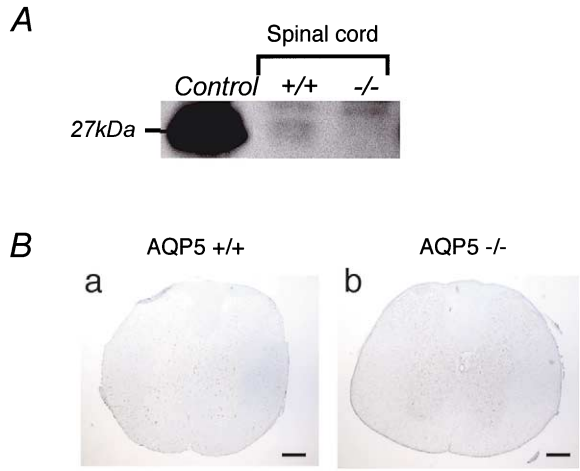

Fig. 3. AQP5 expression in mouse spinal cord. (A) Western blot analysis for AQP5 demonstrates an approximately 27 kDa band in control lung tissue (Control) and a much weaker band in spinal cord from wild-type mice (+/+). No expression was detected in tissue from AQP5-deficient mice (−/−). (B) No specific spinal cord immunoreactivity was found for AQP5 in wild-type (+/+) (a) and AQP5-deficient (−/−) (b) mice. Scale bar=0.2 mm.

histochemical analysis demonstrated that AQP4 was expressed in both gray and white matter in spinal cord from wild-type mice (Fig. 2B-a). As expected, there was no immunostaining in spinal cord from AQP4 −/− mice (Fig. 2B-b). Intense AQP4 staining was found throughout the gray matter, especially in the astrocytic end-feet surrounding the capillaries (Fig. 2B-c, d). Large motor neurons in the anterior horn did not express AQP4. The morphology of the AQP4-positive cells suggests that they are protoplasmic astrocytes. Consistent with this, there was limited co-localization with GFAP which is typically more abundant in fibrous astrocytes (Fig. 2B-d). The GFAP-immunopositive glial processes extended to capillaries associated with AQP4-positive astrocytic end-feet (Fig. 2B-e). Faint AQP4 staining was also detected in glial cell processes adjacent to the ependymal cells lining the central canal (Fig. 2B-f). In the superficial layers of the dorsal horn (substantia gelatinosa), thin fibrous astrocytes co-expressed AQP4 and GFAP (Fig. 2B-g). A similar pattern of AQP4 and GFAP co-expression was found in the dorsal root entry zone (data not shown). In white matter, AQP4 was expressed in fibrous glial processes surrounding the blood vessels. AQP4 was also strongly co-expressed with GFAP in the glia limitans (Fig. 2B-h).

AQP5 expression

Consistent with RT-PCR results, the AQP5 Western blot analysis revealed a 27 kDa band in spinal cord from wild-type mice that was very faint in comparison to the positive control (Fig. 3A). This faint band was absent in spinal cord from AQP5 −/− mice. AQP5 −/− mice were employed to optimize AQP5 immunohistochemical analysis and reduce non-specific staining using a wide range of antibody concentrations and fixation methods. Using these optimized conditions, faint staining of the pia and some large neurons was seen in wild-type mice; however, a similar non-specific staining pattern was also observed in AQP5 knock −/− mice (Fig. 3B-a, b). Thus, no specific staining for AQP5 in mouse spinal cord was observed.

AQP8 expression

Western blot analysis of AQP8 expression revealed an approximately 28 kDa band in spinal cord, liver and testis (Fig. 4A). A higher molecular weight band was also observed in testis and spinal cord, presumably corresponding to the 32–40 kDa N-glycosylated form of AQP8 previously described (Calamita et al., 2001). Immunohistochemical analysis demonstrated strong AQP8 staining predominantly in the ependymal cells lining the central canal (Fig. 4B-a, b, d). There was also faint staining in some surrounding cells indicating a small amount of AQP8 expression in astrocytes or a low level of non-specific staining. It appeared that AQP8 staining was more intense in the apical membrane facing the central canal. This signal disappeared following pre-absorption with immunizing peptide (Fig. 4B-c).

AQP9 expression

Western blot analysis demonstrated an approximately 32 kDa band for AQP9 in control liver and testis tissue, and a much weaker band in spinal cord from wild-type mice. This band disappeared after pre-adsorption with immunizing peptide (Fig. 5A).

Immunohistochemical analysis demonstrated robust AQP9 expression in spinal cord white matter (Fig. 5B-a). This signal also disappeared after pre-adsorption with immunizing peptide (Fig. 5B-b). In white matter, AQP9 and GFAP were extensively co-localized in radially oriented glial processes (Fig. 5B-c, d). AQP9 was also strongly co-expressed with GFAP in the glia limitans (Fig. 5B-c, d). While the majority of AQP9 positive processes were in the white matter, rare AQP9-immunoreactive cells were found in the gray matter near the central canal (Fig. 5B-e). Some of these cells were noted to have a single process that extended to the central canal, passing between two adjacent ependymal cells. Slightly more AQP9 immunoreactivity was observed in thin fibrous GFAP-positive astrocytes seen in the substantia gelatinosa of dorsal horn (Fig. 5B-f).

DISCUSSION

In this study, we used RT-PCR, Western blotting and immunohistochemistry to describe the expression and immunolocalization of AQPs in adult mouse spinal cord. Prior studies have demonstrated AQP4 expression in multiple regions of the brain (Hasegawa et al., 1994; Frigeri et al., 1995b; Nielsen et al., 1997; Verbavatz et al., 1997; Venero et al., 2001). Similarly, AQP4 in spinal cord has been shown to be expressed in glial cells throughout the gray matter and glial foot processes adjacent to the spinal capillary endothelium (Frigeri et al., 1995a,b; Rash et al., 1998); however, no detailed neuroanatomical localization studies have been performed. The present data demonstrate that AQP4 is expressed throughout the spinal cord,

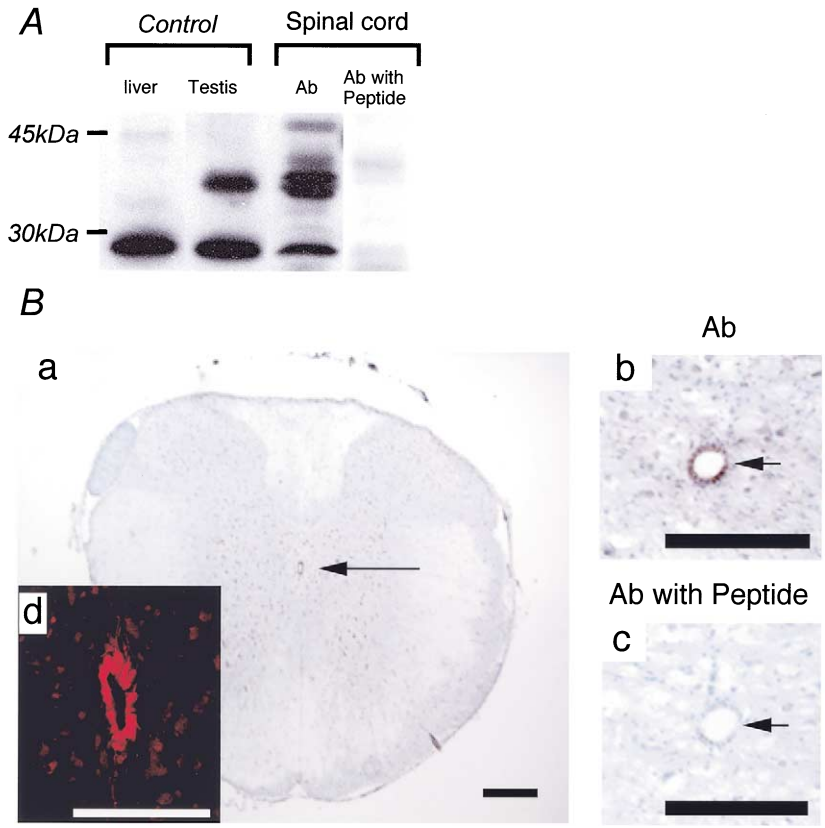

Fig. 4. AQP8 expression in mouse spinal cord. (A) Western blot analysis demonstrates an approximately 28 kDa band and also a 40 kDa band corresponding to the N-glycosylated form of AQP8 in control testis (Control) and spinal cord from wild type mice (Ab). These bands were not detected following pre-adsorption with immunizing peptide (Ab with peptide). (B) Immunohistochemical analysis demonstrates AQP8 immunoreactivity in ependymal cells lining the central canal (arrow; a, b, d). This labeling disappeared following pre-adsorption with immunizing peptide (c; arrow). Scale bar=0.2 mm.

predominantly in the gray matter. The principal components of the gray matter are cell bodies of the motor neurons, local circuit interneurons, projection neurons, capillaries, and astrocytes. Protoplasmic astrocytes are most common in the gray matter and have dense thin processes containing few GFAP-positive fiber bundles. The morphology of the AQP4-positive cells in the gray matter, along with their faint GFAP immunoreactivity, indicates that they are likely protoplasmic astrocytes. Much of the dense AQP4 staining in the gray matter also results from its expression in astrocytic foot processes surrounding capillaries. Similar staining of foot processes has been previously reported in the CNS and confirmed by immunogold techniques (Nielsen et al., 1997; Rash et al., 1998). It is thought that these end-feet induce changes in the vascular endothelium to form a barrier between blood and the CNS (Tout et al., 1993; Nico et al., 2001).

The dense localization of AQP4 in the gray matter suggests a role for AQP4 in spinal cord water balance. In support of this hypothesis, our group has recently demonstrated that rate of osmotic swelling is significantly reduced in the deeper lamina of the dorsal horn in AQP4 knockout mice (Solenov et al., 2002). Traumatic injury to the spinal cord is often associated with edema. Interestingly, the edema is most prominent in the gray matter of the cord as evidenced by MRI studies of acutely injured patients (Shepard and Bracken, 1999). Thus, the pattern of AQP4 expression coincides with the location of the spinal cord edema. We previously demonstrated that mice deficient in AQP4 had decreased edema and improved outcome following water intoxication and focal ischemic stroke (Manley et al., 2000). It will be of interest to determine whether mice deficient in AQP4 will also have reduced edema and improved outcome following spinal cord injury.

AQP4 was also expressed at other tissue-fluid interfaces in the spinal cord. There was abundant AQP4 expression by glial cells lining the ependymal and pial surfaces in contact with the CSF of the central canal and subarachnoid space, respectively. There was faint staining of the ependymal cells that appeared to be mostly in the basolateral membrane. This finding is in keeping with an earlier study by Rash and colleagues (1998) describing immunogold labeling of AQP4 in ependymal cells of the rat spinal cord. Unlike other epithelia, ependymal cells do not have a basement membrane. Instead, the glia limitans of the underlying glial cells forms the basal membrane. A similar anatomical relationship is found at the junction of the glia limitans and the pia on the surface of the spinal cord. These anatomical features suggest that AQP4 may

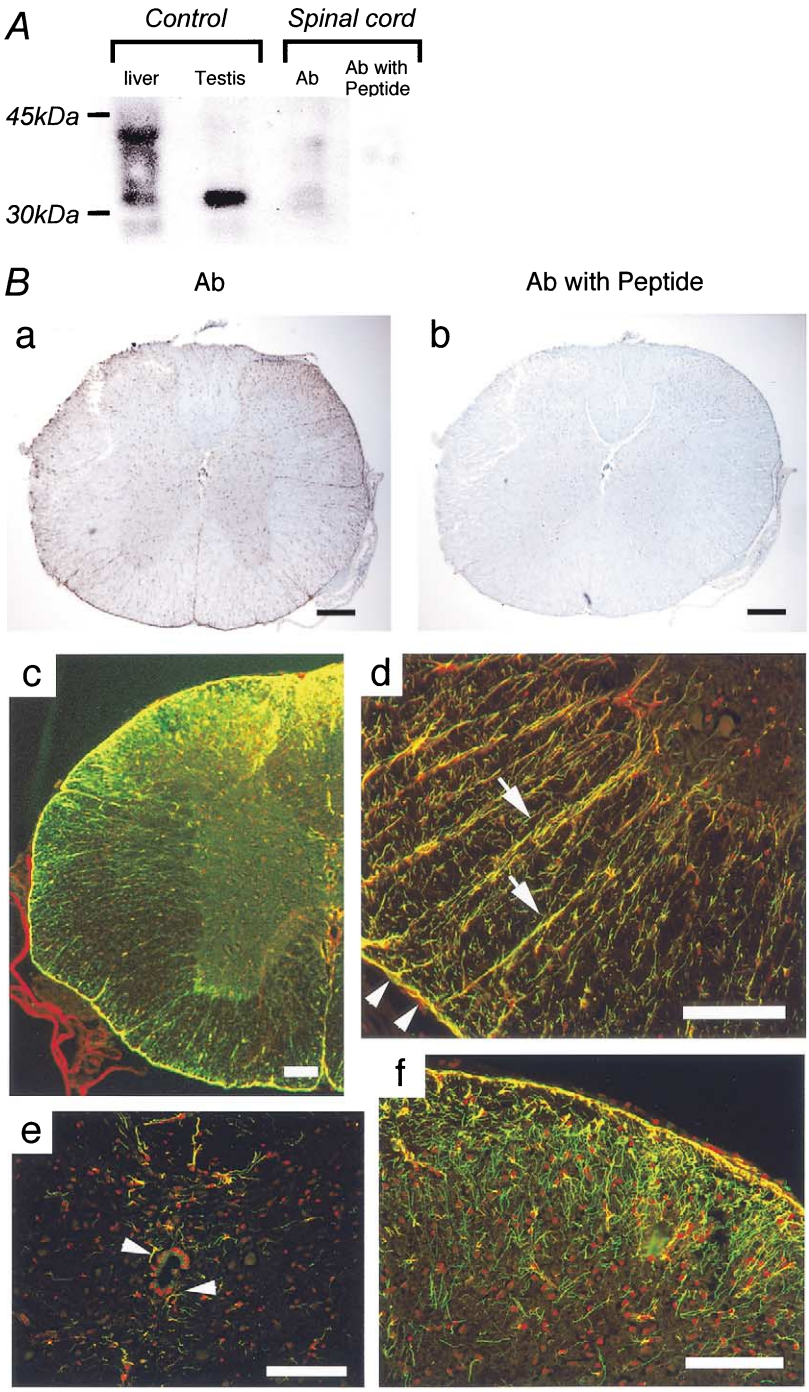

Fig. 5. AQP9 expression in mouse spinal cord. (A) Western blot analysis demonstrates an approximately 32 kDa band in control liver and testis tissue (Control) and a much weaker band in spinal cord from wild-type mice (Ab). This band disappeared after pre-adsorption with immunizing peptide (Ab with Peptide). (B) Immunohistochemical analysis demonstrates broad expression in cervical spinal cord white matter (a, c). This signal disappeared after pre-adsorption with immunizing peptide (b). In white matter, AQP9 and GFAP double-labeling demonstrates co-localization in radial glial processes (arrow) and the glia limitans (arrowhead) (d). In gray matter, those few AQP9-immunoreactive cells that were seen co-localized with GFAP immunoreactivity. Ependymal cells were not immunoreactive for AQP9 protein (e). GFAP and AQP9 were co-expressed in fibrous thin astrocytes seen in the superficial dorsal horn (f). (c–f; Red: GFAP, Green: AQP9.) Black scale bar=0.2 mm. White scale bar=0.1 mm.

also have a role in regulating fluid transport between the spinal cord and surrounding CSF.

Although AQP3 expression has been reported on meningeal cells covering the rat brain (Frigeri et al., 1995b), we were unable to demonstrate AQP3 expression in the mouse spinal cord by RT-PCR. For AQP5, whereas we found a small amount of expression in spinal cord by RT-PCR, we were unable to detect any specific immuno-

histochemical staining. Western blot analysis indicated that the antibody was specific for the AQP5 protein; however, the signal from spinal cord was significantly less than control lung tissue, indicating a very low level of expression. Additional amplification of signal may necessary to detect AQP5 in individual cells. *In situ* hybridization studies might also help to localize AQP5 expression in the spinal cord.

AQP8 expression was initially described in the testis, pancreas, placenta and liver (Ishibashi et al., 1997). By Northern blot analysis, there is no AQP8 RNA expression in the brain. Our results indicate that AQP8 is expressed in the spinal cord, predominantly in the ependymal cells lining the central canal. There was also some faint staining in cells surrounding the canal suggesting a small amount of AQP8 expression in astrocytes or a low level of non-specific staining. The specificity of this faint staining will likely be resolved once AQP8-deficient mice are available. The ependymal cells form a sheet of cuboidal cells in direct contact with the CSF (Del Bigio, 1995). Structurally, ependymal cells are bound to each other by prominent desmosomal junctions, which prevent back diffusion of transported molecules. The central canal was long thought to be a developmental remnant in mammals, filled with motionless CSF. However, the ependyma of the central canal are ciliated similar to ventricular ependymal cells, suggesting a potential for CSF flow. Indeed, it has been demonstrated that fluid is capable of moving rapidly from the spinal subarachnoid space, into the perivascular space, across the interstitium, and into the central canal (Milhorat et al., 1993; Stoodley et al., 1996). The expression pattern of AQP8 suggests that it may play a role in concert with AQP4 and AQP9 in this water transport process. It is possible that trans-pial water movement is mediated by AQP4 and AQP9 expressed in the glia limitans. The AQP4 expressed in the perivascular foot processes may participate in transport across the perivascular space. AQP8, in turn, could then facilitate transport into the central canal.

The central canal of the spinal cord extends from the floor of the fourth ventricle in the brain to the end of the spinal cord. Under normal conditions, in humans the diameter of the central canal decreases significantly during the first few years of life. In some pathological states, however, there is cavitary enlargement of the central canal, referred to as syringomyelia. Although syringomyelia is associated with a wide variety of congenital and acquired disorders, the pathophysiological mechanism for excess fluid in the central canal remains unknown. It will be of interest to examine the development of syringomyelia in the well-established kaolin model (Yamada et al., 1996) using AQP4-deficient mice, as well as in AQP8- and AQP9-deficient mice once they are available.

AQP9, originally identified in human leukocytes (Ishibashi et al., 1998), is also expressed in liver, testis, and brain (Tsukaguchi et al., 1998). In the brain, AQP9 is expressed in a subset of GFAP-positive ependymal cells lacking cilia, called tanycytes. The tanycytes are found in circumventricular organs of the third ventricle lacking a blood–brain barrier, such as the mediobasal hypothalamus, subfornical organ, and pineal gland (Elkjaer et al., 2000; Nicchia et al., 2001). There are conflicting reports on whether AQP9 is expressed in the subset of ciliated ependymal cells (Elkjaer et al., 2000; Badaut et al., 2001). AQP9 is also expressed in astrocytes of the glia limitans and white matter tracts (Badaut et al., 2001). In contrast to AQP4, which is expressed primarily in the foot-processes of astrocytes, AQP9 is expressed throughout the astrocyte cell bodies and processes in the brain (Badaut et al., 2001). The current study shows that AQP9 is also expressed in the spinal cord. The cellular expression pattern was similar to previous reports, with heavy staining of the GFAP-positive cell bodies and processes (Badaut et al., 2001). Like the brain, we also found AQP9 staining in the glia limitans and a subset of cells in the white matter tracts of the spinal cord. AQP9 has been hypothesized to play a role in extracellular water homeostasis and edema formation similar to AQP4 (Badaut et al., 2001). AQP9 is also know to facilitate glycerol and monocarboxylate diffusion (Tsukaguchi et al., 1998; Carbrey et al., 2003). As previously described by Badaut et al., 2001 AQP9 could also play a role in clearing lactate from the extracellular space in pathological ischemic conditions such as stroke and spinal cord injury where lactic acidosis is common.

The present results suggest that AQP9 and AQP4 are expressed in different subsets of astrocytes in the gray matter and white matter and co-expressed in the glia limitans. In the gray matter there was limited AQP9 staining in astrocytes adjacent to the central canal with processes that extend between ependymal cells lining the central canal, raising the question as to whether these might be spinal cord tanycytes. Although little is known about the exact function of spinal tanycytes and ependyma, they may play a role in the proliferative capacity of the spinal cord (Rubinstein and Herman, 1989; Johansson et al., 1999; Shihabuddin et al., 2000). Future experiments examining the proliferation and distribution of these AQP9-positive cells following spinal cord injury would be of interest. In agreement with the results of Badaut et al. (2001) for AQP9 expression in the brain, AQP9 was not expressed in ciliated ependymal cells lining the central canal of the spinal cord. In the white matter, two morphologically distinct astrocytic subpopulations have been identified (Liuzzi and Miller, 1987). One of the populations has characteristics of fibrous astrocytes, whereas the other group is radially oriented, spanning the white matter from the gray–white interface to the pial surface (Liuzzi and Miller, 1987). The radially oriented glia cells are believed to be derivatives of the original radial glia that participate in the ontogenesis of the spinal cord. Due to the absence of specific markers for these two distinct glial populations, the relationship of the morphology to the function of these cells remains unknown. Our results indicate that AQP9 is expressed in the radially oriented astrocytes. The AQP9 staining pattern was identical to that previously described for radially oriented astrocytes with reactivity in GFAP-positive cells that extend from the gray–white junction of the spinal cord to the pial surface. The intense GFAP staining of these cells suggests that they are mature astrocytes and not radial glia. However, additional develop-

mental expression and co-expression studies will be needed to determine the significance of the present findings.

The discovery of AQPs has provided a molecular basis for understanding water transport in a number of tissues, including the brain (Manley et al., 2000; Badaut et al., 2002). Here, we have demonstrated that at least three of the known AQPs are expressed in the mouse spinal cord. The comprehensive description of expression of these AQPs in discrete areas and cell types of the spinal cord provides direction for future studies of the role for these water channels in spinal cord fluid transport and function.

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