Acta Medica et Biologica
Vol. 53, No. 1, 13-19, 2005

Expression and Localization of the Water Channel, Aquaporin, in the Rat Urinary Tract

Zhenyun QU, Goro NISHIMOTO, Jun ZOU, Huiping LI, Yutaka YOSHIDA, Eishin YAOITA and Tadashi YAMAMOTO

Department of Structural Pathology, Institute of Nephrology, Graduate School of Medical and Dental Sciences, Niigata University, Niigata, Japan

Received 10 January 2005; accepted 19 January 2005

Summary. **Water is filtered from the circulation into urine in the glomerulus and is reabsorbed mostly in the proximal tubules and descending loop of Henle and partially in the collecting ducts through several types of water channels, the aquaporin (AQP1, AQP2, AQP3, AQP4 and AQP7). Since AQP2, AQP3, and AQP4 have been identified in the collecting ducts, which originate from urinary buds during the development of the kidney as the ureter, bladder, and urethra, it is presumable for these aquaporins to locate in the urothelium covering the luminal surface of these tissues. However, it has been suggested that the urothelium is impermeable to water and/or solutes. Hence, we examined the expression and localization of aquaporin family members (AQP1, AQP2, AQP3 and AQP4) in the urinary tract in rats. As a consequence, the expression of AQP1 and AQP3 mRNA were demonstrated in the ureter, bladder, and urethra by ribonuclease protection assay. Western blotting showed distinct bands for both AQP1 and AQP3 in the ureter, bladder, and urethra. By immunohistochemistry, AQP1 was localized in the endothelial cells of blood vessels and AQP3 in intermediate and basal cell layers of the urothelium in the ureter, bladder and urethra. No AQP2 or AQP4 mRNA expression or immunolocalization of AQP2 and AQP4 were detected in these tissues. AQP2 expression was enhanced in the kidney after dehydration; however, the expression was unchanged in the ureter, bladder, and urethra. The current findings suggest that water crosses the urothelium by utilizing AQP3 water channels (transcellular pathway) in the ureter, bladder, and urethra, but the expression of AQP3 is not regulated by a dehydrated condition.**

Key words—aquaporin, bladder, ureter, urethra, urothelium.

INTRODUCTION

The physiological regulation of water and solutes is important for many basic cellular functions such as proliferation, excitability, secretion, and reabsorption[1]. The discovery of the aquaporin (AQP) water channels has led to an understanding of the molecular basis of membrane water permeability[2]. Recent studies have revealed several aspects of the physiological relevance of the AQP family[3]. So far 11 members of AQPs have been isolated and characterized. AQP1, AQP2, AQP3, AQP4, AQP6, and AQP7 were identified or localized in the kidney[1]. The localization of AQP1 was in both the apical and basal membrane of proximal tubule cells, epithelial cells of Henle's descending loop, and vascular endothelial cells, whereas AQP2 was localized in the apical surface and both AQP3 and AQP4 in the basolateral membrane of collecting duct cells[4]. Studies of AQP knockout mice have revealed the importance of the water channel family with respect to water homeostasis[5]. AQP1, AQP3, and AQP4 gene disruption cause urinary concentrating defects, and a mutation of the AQP2 gene represents nephrogenic diabetes insipidus in humans[5, 6, 7].

Correspondence: Tadashi Yamamoto, Department of Structural Pathology, Institute of Nephrology Graduate School of Medical and Dental Sciences, Niigata University Asahimachi-dori 1-757, Niigata 951-8510, Japan.

Abbreviations—WKY, Wistar Kyoto; AQP, aquaporin; GAPDH, glyceraldehyde-3- phosphate dehydrogenase.

The luminal surface of the urinary tract is coated by a specialized epithelium, urothelium, which is suggested to be impermeable to water and ions to function as a barrier.[8, 9]. The urothelium is layered with three cell types including umbrella cells, intermediate cells, and basal cells. On the other hand, a recent study implied the permeation of ions and proteins through the urothelium[10]. In addition, Specter et al. demonstrated AQP1, AQP2, and AQP3 expression in the rat ureter and bladder[11]. Since the regulation of AQP2 expression and localization by vasopressin have been demonstrated to be responsible for the reabsorption of bulk free water in collecting ducts, AQP2 expression in the urothelium was assumed to imply its functional significance, contrasting with the predicted barrier function of urothelium[12, 13]. Thus, we followed the same track to examine the expression of AQP1, AQP2, AQP3, and AQP4 in the urinary tract while comparing this with the expression in the kidney.

MATERIALS AND METHODS

Animals and tissues

Adult WKY (Wistar Kyoto) rats were fed on a standard rat chow with free access to water. To dehydrate the rats, water intake was restricted for 48 hours. The kidney (cortex and medulla), ureter, bladder, and urethra (1/3 part proximal to bladder) were removed to examine the expression and localization of AQP family members.

Ribonuclease protection assay

The tissues were homogenized and total cellular RNA was isolated by the acid guanidium thiocyanate-phenol-chloroform method. Ribonuclease protection assay was done as reported previously[1]. ^{32}P-labeled antisense cRNA probes for rat AQP1, AQP2, AQP3, AQP4 Mrna, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA were synthesized by in vitro transcription using linearized plasmids inserted with AQP1, AQP2, AQP3, AQP4, and GAPDH cDNAs[1].

Ten micrograms of RNA samples were hybridized with the mixture of cRNA probes ($1x10^5$ cpm each) overnight at 45° C, and unhybridized probes were digested with ribonuclease A and ribonuclease T1. The samples were applied for electrophoresis on 6% polyacrylamide gels, and the probes protected from the ribonuclease treatment were either visualized as bands after exposure of the gels on X-ray films or were quantitated by a phospher imaging operating system (Molecular Imager, Bio-Rad Japan, Tokyo).

Western blotting

Tissues were homogenized in a lysis buffer (8M urea, 0.8% Triton X-100, 0.2% sodium dodecyl sulfate, 3% 2-mercaptoethanol) using a Potter type homogenizer. Ten micrograms of total protein obtained from the kidney medulla, ureter, and bladder, or 40 μg from the urethra were loaded into each lane and separated by gel electrophoresis on 12% polyacrylamide slab gels containing SDS. The separated bands were blotted to nitrocellulose membranes. The membranes were incubated with 5% milk in PBS-T (80 mM Na_2HPO_4, 20mM NaH_2PO_4, 100mM NaCl, 0.1% Tween-20, pH 7.4) for 1 h and incubated with rabbit anti-rat AQP1 (CHEMICON International, Inc, Temecula, CA), AQP2 (a kind gift from Dr. Sei Sasaki, Tokyo Medical and Dental University, Tokyo), AQP3 (also a kind gift from Dr. Sasaki), and AQP4 (CHEMICON International, Inc) antibodies at 4° C for overnight. The membranes were washed three times for 30 min each in PBS-T and incubated with a horseradish peroxidase-labeled second antibody (1:1000, EnVision, DAKO, Japan) at room temperature for 60 min. The immunoreactivity was visualized by a chemiluminescence detection system (ECL plus, Amersham Pharmacia Biotech, Japan). The visualized membranes were stained by Coomassie blue in order to confirm equal loading of the samples.

Immunohistochemistry

Tissues were fixed in a methyl-Carnoy fixative, dehydrated in ethanol, and embedded in paraffin. They were sectioned at a thickness of 4 μm, and the sections were dewaxed and rehydrated. They were incubated with a rabbit anti-rat AQP1 (0.25 μg/ml), AQP2 (1:4000), AQP3 (1:1000), or AQP4 (1.0 μg/ml) antibody or normal rabbit serum (1:4000) for 60 min, and then reacted with a horseradish peroxidase-conjugated goat-anti-rabbit IgG antibody (1:5 EnVision; DAKO, Japan) for 30 min. The immunoreaction was detected by peroxidase reaction using 3′diaminobenzidine tetrahydrochloride and hydrogen peroxide. The sections were counterstained with hematoxyline.

RESULTS

The ribonuclease protection assay demonstrated AQP1 and AQP3 mRNA expression in the ureter, bladder, and urethra, whereas neither AQP2 nor AQP4 were detected in these tissues (Fig. 1). The expression of AQP1 mRNA was much higher in the kidney cortex and medulla than the ureter, bladder, or urethra. On the other hand, AQP3 mRNA expression in the ureter, bladder, and urethra was

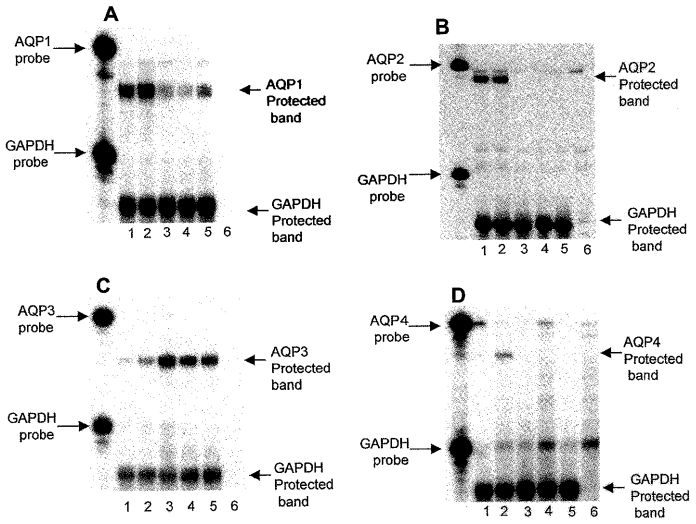

Fig. 1. Expression of aquaporin(AQP) mRNA in the rat urinary tract examined by ribonuclease protection assay.
A. AQP1. **B.** AQP2. **C.** AQP3. **D.** AQP4. *Lane 1*, renal cortex; *Lane 2*, renal medulla; *Lane 3*, ureter; *Lane 4*, bladder; *Lane 5*, urethra; *Lane 6*, transfer RNA(as a negative control).

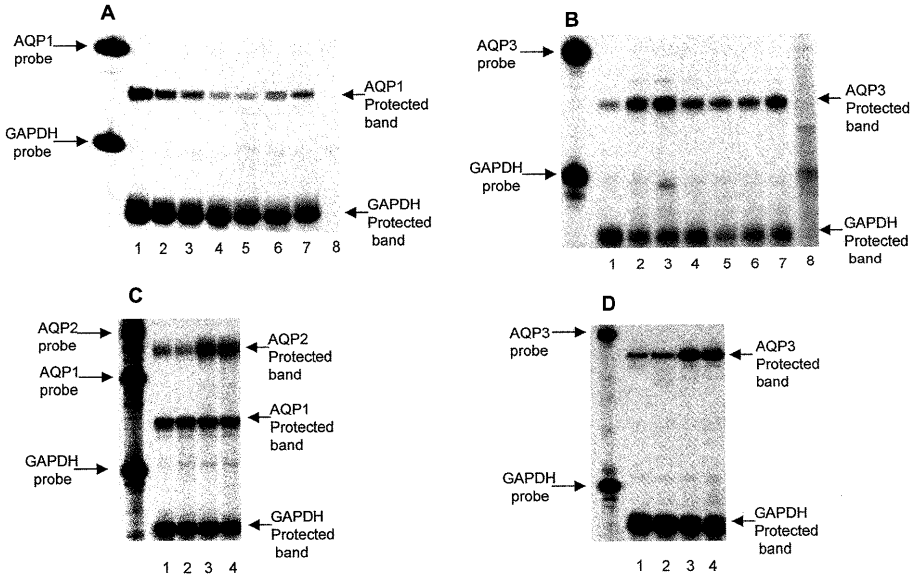

Fig. 2. Ribonuclease protection assay showing the mRNA expression in urinary tract of AQP1, AQP2, and AQP3 in hydrated and dehydrated rats.
A. AQP1. **B.** AQP3. *Lane 1*, kidney medulla (hydrated); *Lane 2*, ureter (hydrated); *Lane 3*, ureter (dehydrated); *Lane 4*, bladder (hydrated); *Lane 5*, bladder (dehydrated); *Lane 6*, urethra (hydrated); *Lane 7*, urethra (dehydrated); *Lane 8*, transfer RNA (as a negative control). **C.** AQP1, AQP2. **D.** AQP3. *Lane 1*, kidney cortex (hydrated); *Lane 2*, medulla (hydrated); *Lane 3*, cortex (dehydrated), and *Lane 4*, medulla (dehydrated).

more intense than in the kidney (Fig. 1).

No significant changes in AQP1 and AQP3 mRNA expression were demonstrated in the ureter, bladder, or urethra of dehydrated rats although both AQP2 and AQP3 mRNA expressions were increased in the kidney medulla after dehydration (Fig. 2), while AQP1 mRNA expression was unchanged.

Western blotting using the anti-AQP1 antibody showed two bands of ~28 kDa and ~35 kDa, respectively corresponding to non-glycosylated and glycosylated forms in the ureter, urethra, and kidney medulla. A single band of ~28 kDa was demonstrated in the

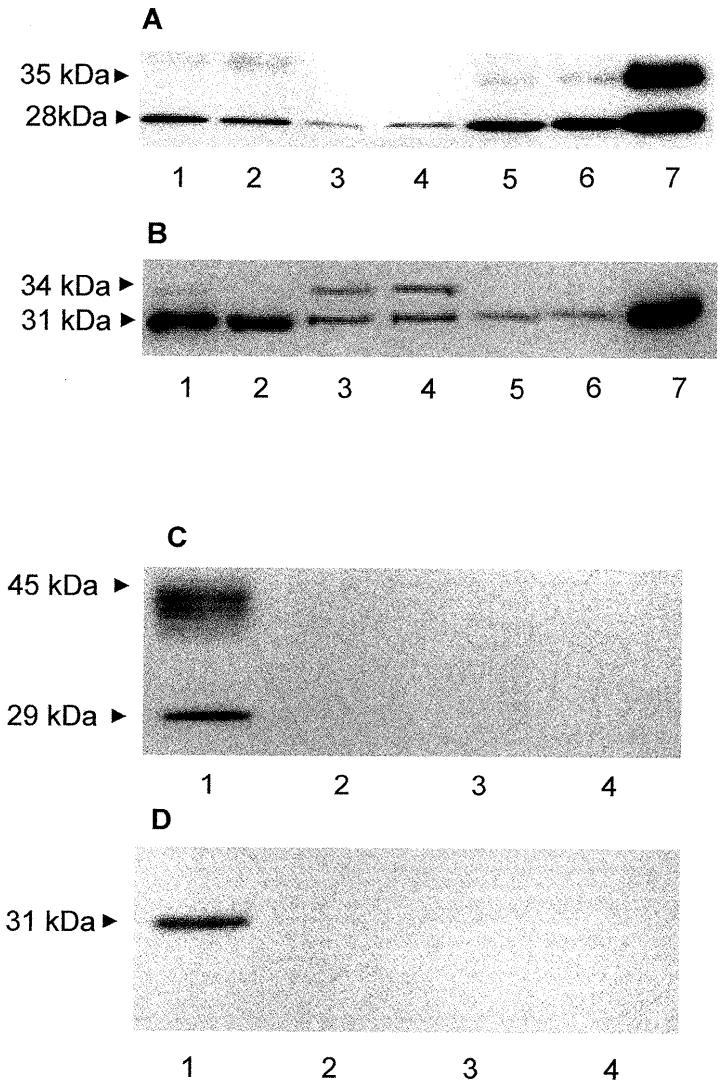

Fig. 3. Western blot analysis in the urinary tract.
A. AQP1. **B.** AQP3. Lanes 1, ureter (hydrated); Lane 2, ureter (dehydrated); Lane 3, bladder (hydrated); Lane 4, bladder (dehydrated); Lane 5, urethra (hydrated); Lane 6, urethra (dehydrated); Lane 7, kidney medulla (hydrated). **C.** AQP2. **D.** AQP4. Lanes 1, kidney medulla; Lane 2, ureter; Lane 3, bladder; Lane 4, urethra. Ten micrograms of total protein obtained from renal medulla, ureter, and bladder, and 40 μg of total protein from the urethra were loaded into the gel lanes.

bladder, however. (Fig. 3). The anti-AQP3 antibody immunoblotted two bands of ~31 kDa and ~34 kDa, respectively corresponding to non-glycosylated and glycosylated forms of AQP3 in the ureter and bladder, and single bands of ~31 kDa in the urethra and kidney (Fig. 3).

No significant differences in amounts of AQP1 and AQP3 proteins in the ureter, bladder, or urethra were detected between the hydrated and the dehydrated rats (Fig. 3). No bands for AQP2 and AQP4 were detected in the ureter, bladder, or urethra, while AQP2 bands of both non-glycosylated ~29 kDa and glycosylated ~38 kDa, and an AQP4 band of ~31kDa were observed in the kidney medulla by Western blot analysis (Fig. 3).

The localization of AQP1, AQP2, AQP3, and AQP4 in the urinary tract was examined by immunohistochemistry. AQP1 was found in vascular endothelial cells of capillaries and small arteries in the ureter, bladder, and urethra (Fig. 4). In addition, an immunoreactivity for AQP1 was present in the circular smooth muscle layer in the ureter. In the urethra, AQP1 was intensely demonstrated in the endothelium of the cavernae corporum urethrae (Fig. 4).

AQP3 was detected in the cytoplasmic membrane of epithelial cells, especially the intermediate and basal

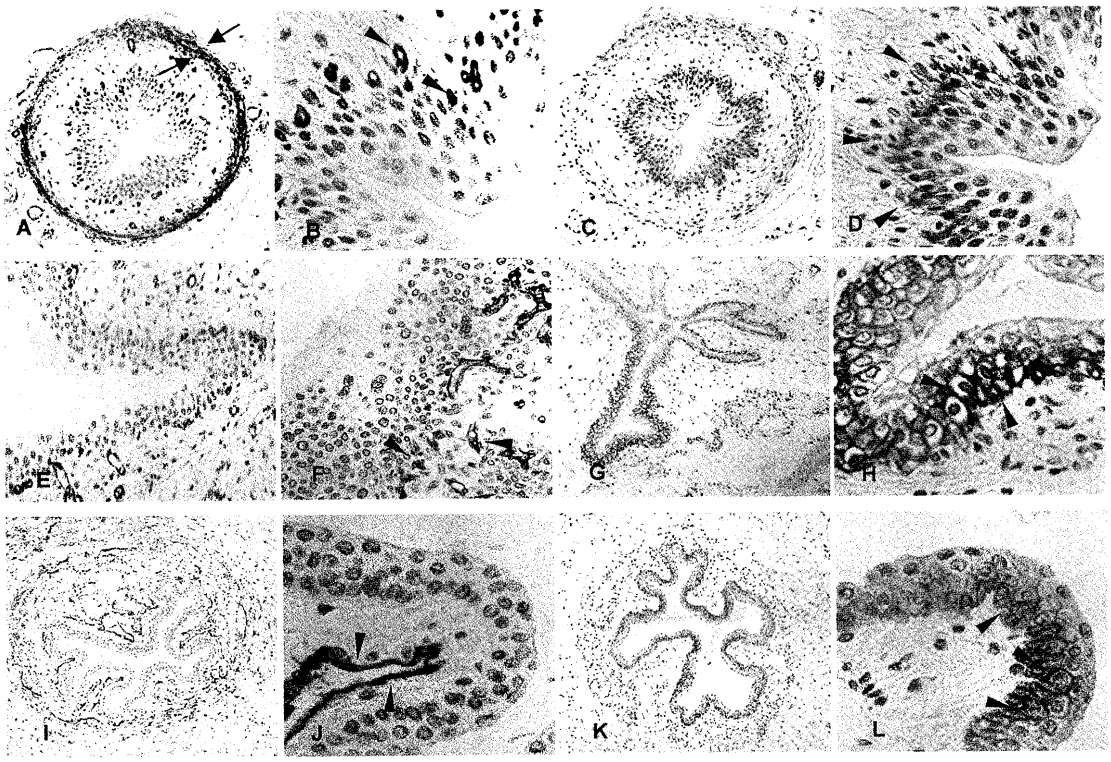

Fig. 4. Localization of AQP1 and AQP3 protein examined by immunohistochemistry in the ureter, bladder, and urethra. AQP1 is present in the endothelium of capillaries and small arteries of the ureter (**A,** high power; **B**) and bladder (**E,** high power; **F**). AQP1 staining is visible in the adventitia adjacent to the circular muscle layer (**A,** *arrows*) and tunica externa including blood vesssels, and endothelium of cavernae corporum urethrae in the urethra (**I;** high power, **J**). AQP3 is demonstrated in plasma membranes of intermediate and basal cells in the ureter (**C,** high power, **D**), bladder (**G,** high power; **H**) and urethra (**K,** high power; **L**). Note that the superficial umbrella cells are not stained.

cells of the ureter, bladder, and urethra (Fig. 5). In the kidney, AQP1 was present in the cytoplasmic membranes of proximal tubules, descending loop of Henle, and vascular endothelium. In the proximal tubules, the immunolabeling for AQP1 was much stronger in the apical membrane than in the basal membrane (Fig. 5). AQP2 was intensely immunolocalized in the apical sites of collecting duct cells, while both AQP3 and AQP4 were localized in the basolateral membrane of collecting duct epithelial cells in the kidney. Neither AQP2 nor AQP4 were detected in the ureter, bladder, or urethra by immunohistochemical study (Fig. 5).

DISCUSSION

In this study, two isoforms of the AQP family, AQP1 and AQP3, were identified in the ureter, bladder, and urethra at both mRNA and protein levels. AQP1 mRNA expression in the ureter, bladder, and urethra was much lower than in the kidney cortex and medulla. Immunoreactive AQP1 was localized in the vascular endothelial cells of these tissues, suggesting a role for AQP1 in water movement between the interstitium and blood. AQP1 immunoreactivity was also observed in the circular muscle layer of the ureter although we could not detect clearly whether the immunoreactivity was on the smooth muscular cells or other constitutes in the circular muscle layer such as capillaries or lymphatic vessels. In addition, AQP1 may be present in nerves of the muscular layer as was shown in sensory nerves[14]. AQP1 in the cavernae corporum urethrae of the urethra suggests functions for water movement between the interstitium and cavernae[15].

On the other hand, AQP3 mRNA expression in the ureter, bladder, and urethra was more intense than in the kidney. Immunohistochemistry demonstrated that AQP3 was localized in the transitional or multilayered epithelial cells of these tissues. Since the immunoreaction was intense in the plasma membrane of basal and

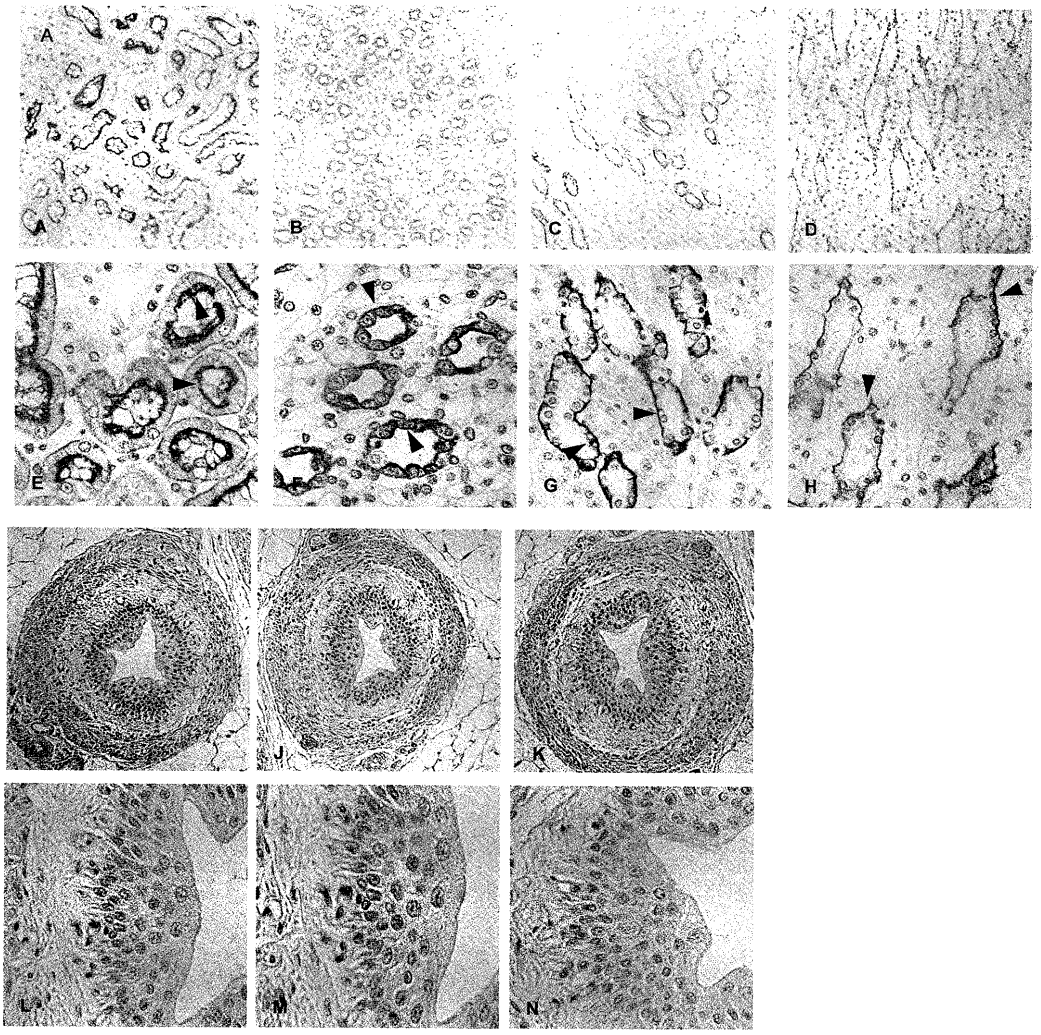

Fig. 5. Immunohistochemical localization of AQP1, AQP2, AQP3, AQP4 in the urinary tract. **A:** AQP1 is present in apical and basal membranes of proximal tubules (**A;** high power, **E**).AQP2 is found in the apical membrane of collecting ducts (**B;** high power, **F**). AQP3 (**C;** high power, **G**) and AQP4 (**D;** high power, **H**) are localized in the basolateral membrane of collecting ducts. **B:** AQP2 (**I;** high power, **L**) and AQP4 (**J;** high power, **M**) are not detected in the ureter. Normal rabbit serum (1:4000) shows faint immunolabeling at a background level (**K;** high power, **N**).

intermediate epithelial cells, AQP3 was presumed to transfer water from the interstitium to superficial epithelial cells or the epithelial cell surface. These findings were comparable to those demonstrated by Spector et. al.[11]. However, we examined the expression of AQP1 and AQP3 mRNA in these tissues by ribonuclease protection assay as a more quantitatively reliable method.

The lack of any immunoreactive AQP3 in umbrella cells despite AQP3 immunoreactivity being demonstrated in both intermediate and basal cell layers in the ureter, bladder, and urethra is of interest, because this layer compries the main barrier for the impermeability of water and ions[9, 10]. AQP3 expression in both intermediate and basal cells may constitute a novel urothelium function in that each cell layer may exert different levels of water permeability. Since AQP3 knockout mouse suffered defective skin hydration, elasticity, and barrier function, AQP3 is presumed to hydrate the multilayed epithelium in the body[16].

AQP2 was first isolated as a water channel, which was localized at collecting ducts in the kidney exclusively and was regulated by vasopressin[12, 17]. This water channel is a critical molecule for water reabsopition in the kidney, and approximately 20% of the water filtrated at the glomerulus was reabsorbed in the collecting ducts through AQP2. After the discovery of AQP2 in the kidney, it was demonstrated in other parts of organs and tissues in the body[18, 19]. Both ureter and collecting

duct epithelial cells originate from the same cell lineage, ureteric buds, during development of the kidney. Therefore, the AQP2 expression detected in the ureter by RT-PCR may be an intriguing finding[11]. However, the sites of the localization and its quantity are crucial for judging the role of AQP2 in the ureter and bladder. In the present study, we could not detect any expression of either AQP2 mRNA or protein in the ureter as well as in the bladder and urethra. These results may indicate that AQP2 is functionally negligible in the ureter, even if it might be expressed.

We further analyzed whether dehydration could affect the expression of AQP1 and AQP3 in the ureter, bladder, and urethra. However, neither AQP1 nor AQP3 expression was upregulated by dehydration as shown previously[20].

In conclusion, AQP1 and AQP3 were expressed in the ureter, bladder, and urethra. AQP1 expression was mainly detected in vascular endothelial cells, suggesting a contribution to water movement between the interstitium and blood vessels. AQP3 was present in both intermediate and basal cell layers in urothelia, indicating that there may be water movement within the urothelia. The functional regulation of AQP3 in the urothelium remains elusive.

REFERENCES

1) Yamamoto T, Sasaki S: Aquaporins in the kidney: Emerging new aspects: *Kidney Int* **54**: 1041-1051, 1998.
2) Preston GM, Carrol TP, Guggino WB, Agre P: Appearance of water channels in Xenopus oocytes expressing red cell CHIP28 protein. *Science* **256**: 385-387, 1992.
3) Borgnia M, Nielsen S, Engel A, Agre P: Cellular and molecular biology of the aquaporin water channels. *Annu Rev Biochem* **68**: 425-458, 1999.
4) Nielsen S, Freklar J, Marples D, Kwon TH, Agre P, Knepper MA: Aquaporin in the kidney: From Molecules to Medicine. *Physiol Rev* **82**: 205-244, 2002.
5) Verkman AS: Lessons on renal physiology from transgenic mice lacking aquaporin water channels. *J Am Soc Nephrol* **10**: 1126-1135, 1999.
6) Ma S, Song Y, Yang B, Gillespie A, Carlson EJ, Epstein CJ, Verkman AS: Nephrogenic diabetes insipidus in mice lacking aquaporin-3 water channels. Proc *Natl Acad Sci USA* **97**: 4386-4391, 2000.
7) Lieburg AF, Verdijk MA, Knoers VV, Essen AJ, Proesmans W, Mallmann R, Monnens LA, Oost BA, Os CH, Deen PM: Patients with autosomal nephrogenic diabetes insipidus homozygous for mutations in the aquaporin 2 water-channel gene. *Am J Hum Genet* **55**: 648-652, 1994.
8) Hicks RM: The mammalian urinary bladder: an accommodating organ. *Biol Rev Camb Philos Soc* **50**: 215-246, 1975.
9) Lewis SA: Everything you wanted to know about the bladder epithelium but were afraid to ask. *Am J Physiol* **278**: 867-874, 2000.
10) Apodaca G: The uroepithelium: Not just a passive barrier. *Traffic* **5**: 117-128, 2004.
11) Spector D, James BW, Russell D, Deborah AS, Edward JW: Expression, localization, and regulation of aquaporin-1 to -3 in rat urothelia. *Am J Physiol* **262**: 1034-1042, 2002.
12) Nishimoto G, Zelenina M, Li D, Yasui M, Aperia A, Nielsen S, Nairn A: Arginine vasopressin stimulates phosphorylation of aquaporin-2 in rat renal tissue. *Am J Physiol* **276**: 254-259, 1999.
13) Yamamoto T, Sasaki S, Fushimi K, Ishibashi K, Yaoita E, Kawasaki K, Marumo F, Kihara I: Vasopressin increases AQP-CD water channel in apical membrane of collecting duct cells in Brattleboro rats. *Am J Physiol* **268**: 1546-1551, 1995.
14) Solenov EI, Vetrivel L, Oshio K, Manley GT, Verkman AS: Optical measurement of swelling and water transport in spinal cord slices from aquaporin null mice. *J Neurosci Methods* **113**: 85-90, 2002.
15) Creed KE, Carati CJ, Keogh EJ: The physiology of penile erection. *Oxf Rev Reprod Biol* **13**: 73-95, 1991.
16) Hara M, Verkman AS: Glycerol replacement corrects defective skin hydration, elasticity, and barrier function in aquaporin-3-deficient mice. *Proc Natl Acad Sci USA* **100**: 7360-7365, 2003.
17) Fushimi K, Uchida S, Hara Y, Hirata Y, Marumo F, Sasaki S: Cloning and expression of apical membrane water channel of rat kidney collecting tubule. *Nature* **361**: 549-552, 1993.
18) Gallardo P, Cid LP, Vio CP, Sepulveda FV: Aquaporin-2, a regulated water channel, is expressed in apical membranes of rat distal colon epithelium. *Am J Physiol* **281**: 856-863, 2001.
19) Stevens AL, Breton S, Gustafson CE, Bouley R, Nelson RD, Kohan DE, Brown D: Aquaporin 2 is a vasopressin-independent, constitutive apical membrane protein in rat vas deferens. *Am J Physiol* **278**: 791-802, 2000.
20) Terris J, Ecelbarger CA, Nielsen S, Knepper MA: Long-term regulation of four renal aquaporins in rats. *Am J Physiol* **271**: 414-422, 1996.