Hypertonicity-induced expression of aquaporin 3 in MDCK cells

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Matsuzaki, Toshiyuki, Takeshi Suzuki, and Kuniaki Takata. Hypertonicity-induced expression of aquaporin 3 in MDCK cells. Am J Physiol Cell Physiol 281: C55–C63, 2001.—Aquaporins (AQPs) are water channel proteins that participate in water transport. In the principal cells of the kidney collecting duct, water reabsorption is mediated by the combined action of AQP2 in the apical membrane and both AQP3 and AQP4 in the basolateral membrane, and the expression of AQP2 and AQP3 is regulated by antidiuretic hormone and water restriction. The effect of hypertonicity on AQP3 expression in Madin-Darby canine kidney (MDCK) epithelial cells was investigated by exposing the cells to hypertonic medium containing raffinose or NaCl. Northern blot and immunoblot analyses revealed that the amounts of AQP3 mRNA and AQP3 protein, respectively, were markedly increased by exposure of cells to hypertonicity. These effects were maximal at 12 and 24 h, respectively. Immunofluorescence and immunoelectron microscopy also demonstrated that the abundance of AQP3 protein was increased in cells incubated in hypertonic medium and that the protein was localized at the basolateral plasma membrane. These results indicate that the expression of AQP3 is upregulated by hypertonicity.

immunohistochemistry; water channel; plasma membrane; Madin-Darby canine kidney cells

ALTHOUGH MOST CELL MEMBRANES exhibit some degree of water permeability as a result of simple diffusion through the lipid bilayer, the membranes of cells in certain organs, such as the kidney and salivary glands, manifest a much higher water permeability that underlies their physiological roles, water reabsorption in the kidney and salivary secretion. Such cells express specific water channel proteins, known as aquaporins (AQPs), that form aqueous pores across the plasma membrane. The first of these water channel proteins to be characterized, a channel-forming integral protein of 28 kDa (CHIP28), was purified from erythrocytes and kidney (1), and its cDNA was isolated from human bone marrow (25). CHIP28 is now referred to as aquaporin 1 (AQP1). To date, 10 members of the AQP family (AQP0–AQP9) have been identified in mammals (38).

AQP3 has been isolated from the kidney (4, 10, 16) and appears unique in that it confers permeability to glycerol and urea as well as to water (10, 41). AQP3 is localized to renal collecting duct cells in the kidney. In addition, many epithelial cells in the urinary, digestive, and respiratory tracts as well as epidermal cells in the skin express AQP3 (6, 7, 14, 20). In the renal collecting duct, AQP3 is localized in the basolateral membrane of principal cells (10) and, together with AQP2 present in the apical membrane (8), plays an important role in water reabsorption. Antidiuretic hormone (ADH) induces the rapid translocation of AQP2 from an intracellular vesicular storage compartment to the apical membrane (17, 23, 39). Although no evidence has been presented for such short-term regulation of basolateral AQP3, long-term ADH infusion or long-term water restriction markedly increases the abundance of AQP3 in rat kidney (3, 11, 35). On the basis of the fact that water restriction induces marked hypertonicity in the renal medulla, the expression of AQP3 could be upregulated by hypertonicity. We have now examined the effect of hypertonicity on AQP3 expression in Madin-Darby canine kidney (MDCK) cells, a renal epithelial cell line.

MATERIALS AND METHODS

Cell culture and media. MDCK II cells were maintained in DMEM containing 5 mM D-glucose, penicillin G (100 U/ml), streptomycin (100 μg/ml), and 5% FCS. Hypertonic (≈400 mosmol/kgH2O) medium was prepared by the addition of either raffinose or NaCl to the isotonic culture medium.

Exposure of cells to hypertonicity. For RT-PCR and Northern blot analyses, cells were seeded in 35-mm culture dishes at 5×10⁵ cells/dish. For immunoblot analysis and preparation of semithin frozen sections, cells were seeded in 100-mm culture dishes at 1×10⁶ cells/dish. For whole cell-mount observations, cells were seeded on poly-L-lysine-coated coverslips placed in 35-mm culture dishes at 5×10⁵ cells/dish. They were grown in isotonic DMEM at 37°C under 5% CO₂. When the cells reached subconfluency to confluency, the isotonic medium was replaced with hypertonic medium containing either raffinose or NaCl; the medium of control cells was replaced with fresh isotonic medium. Cells were then incubated in hypertonic medium and harvested at the indicated times for Northern blot and immunoblot analyses, cells were seeded in 35-mm culture dishes at 1×10⁶ cells/dish.

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incubated for 1, 12, 24, or 48 h before being subjected to the various assays described below; the culture medium was replaced with fresh medium after 24 h.

**Isolation of total RNA and poly(A)+ RNA.** For RT-PCR and Northern blot analyses, total RNA was isolated from MDCK cells and rat kidney medulla by acid guanidinium thiocyanate-phenol-chloroform extraction with the use of the TRIzol reagent (Life Technologies, Grand Island, NY). For Northern blot analysis, poly(A)+ RNA was isolated from total RNA using Oligotex-dT30 Super mRNA purification kit (TaKaRa, Tokyo, Japan).

**RT-PCR.** Total RNA (3 μg) was reverse-transcribed and amplified by PCR. Primers for AQP3 were designed as follows on the basis of the sequences of human, rat, mouse, and *Xenopus* AQP3 cDNAs, given that the sequence of the canine AQP3 cDNA is unknown: AQP3 sense primer, 5'-GGACCCTCATCCTNGTGATGT-3', and AQP3 antisense primer, 5'-AGCCNATCATNAGCTGGTACAC-3', corresponding to nucleotides 83–104 bp and 790–769 bp of rat AQP3 cDNA (GenBank accession no. D17695), respectively. PCR was performed for 30 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 2 min, and the resulting products were separated by electrophoresis on a 1.3% agarose gel. The PCR product was ligated into the pGEM-T Easy vector (Promega, Madison, WI), a positive clone was isolated, and its nucleotide sequence was analyzed with an automated sequencer (SEQ4×4 personal sequencing system; Amersham Pharmacia Biotech, Amersham, UK).

**Northern blot analysis.** Poly(A)+ RNA (3 μg) from MDCK cells was fractionated by electrophoresis on a 1% agarose gel containing formaldehyde and MOPS. The separated RNA molecules were transferred to a nylon membrane (BIODYNE B; Pall BioSupport, East Hills, NY) in the presence of 20% formamide and MOPS. The separated RNA was then fixed by baking the membrane at 80°C for 30 min. A rat AQP3 cDNA probe (10) that contains the entire open reading frame failed to detect canine AQP3 mRNA. We therefore used a cloned RT-PCR product prepared as described in **RT-PCR** from MDCK cells as the probe for canine AQP3 mRNA. The probe was labeled with [32P]dCTP (NEN, Wilmington, DE) by random priming. The membrane was incubated overnight at 42°C in a solution containing 50% formamide, 6× SSC, 5× Denhardt’s reagent, 0.5% SDS, and herring sperm DNA (100 μg/ml) and was then subjected to hybridization with the probe overnight at 42°C in a solution containing 50% formamide, 6× SSC, 10% dextran sulfate, 0.5% SDS, and herring sperm DNA (100 μg/ml). The membrane was washed twice for 15 min at 68°C in 2× SSC containing 0.1% SDS and then twice for 15 min at 68°C in 0.1× SSC containing 0.1% SDS. The membrane was exposed to an imaging plate (Fuji Film, Tokyo, Japan), and signals were scanned with a BAS2000 bioimaging analyzer. The signal intensities of AQP3 mRNA were normalized for those of GAPDH mRNA.

**Immunoblot analysis.** MDCK cells were washed with PBS, detached from the culture dish, frozen with liquid nitrogen, and stored at −80°C until analysis. Kidney medulla was rapidly removed from rats after death and was also stored at −80°C until analysis. Frozen specimens were homogenized with a glass homogenizer in 5–10 volumes of an ice-cold solution comprising PBS supplemented with pepstatin A (2 μg/ml), leupeptin (2 μg/ml), aprotinin (100 kIU/ml), and 2 mM phenylmethylsulfonyl fluoride. After denaturation by incubation at 37°C for 30 min in the presence of 2% SDS, 25 mM Tris-HCl (pH 7.5), 25% glycerol, 0.005% bromphenol blue, dithiothreitol (23 mg/ml), DNase I (300 μg/ml), and 4 mM MgCl2, samples were subjected to SDS-PAGE and immunoblot analysis with rabbit polyclonal antibodies to rat AQP3 (1:2,000 dilution) (20) and [125I]-labeled protein A (NEN). As a control, the primary antibodies (1:2,000 dilution) were preincubated with the COOH-terminal AQP3 peptide (25 μg/ml) used as the immunogen. Signals were scanned with a BAS2000 bioimaging analyzer. Equal application of protein samples was verified by staining with Coomassie brilliant blue.

**Immunofluorescence microscopy.** For whole-cell-mount observations, cells were cultured on coverslips placed in 35-mm culture dishes. They were washed with PBS, fixed on ice for 30 min with 1% acetic acid in ethanol, and washed again with PBS. For preparation of semithin frozen sections, cells were grown in 100-mm culture dishes and fixed for 1 h at room temperature with 3% formaldehyde in 0.1 M sodium phosphate buffer (pH 7.4). They were then washed with PBS, detached from the dish, embedded in 10% gelatin, and soaked overnight in 2.3 M sucrose (32). Semithin frozen sections were cut, mounted on poly-l-lysine-coated glass slides, immersed in ethanol at −20°C for 30 min, and rinsed with PBS. Indirect immunofluorescence staining was performed essentially as described previously (19, 30) with rabbit antibodies.
to AQP3 (1:500 dilution) and Rhodamine Red X-conjugated donkey antibodies to rabbit IgG (Jackson Immunoresearch, West Grove, PA). Both antibody preparations were diluted with 5% normal goat serum in PBS. For nuclear counterstaining, either 4',6-diamidino-2-phenylindole or SYBR Green I (Molecular Probes, Eugene, OR) (18, 29) was included in the secondary antibody solution. In some instances, actin filaments were simultaneously stained with fluorescein phalloidin (Molecular Probes) to facilitate the identification of plasma membrane domains. The specificity of immunostaining was verified by performing the following controls: 1) incubation with normal rabbit serum (1:500 dilution) instead of AQP3 antisera (Fig. 1).

Fig. 2. Northern blot analysis of the effect of hypertonicity on the abundance of AQP3 mRNA in MDCK cells. A: a blot of 3 μg of poly(A)⁺ RNA isolated from cells exposed to hypertonic medium containing raffinose for 12 h with a 32P-labeled AQP3 cDNA probe. A band of ~1.9 kb is shown. B and C: changes in the amount of AQP3 mRNA. Cells were maintained in isotonic medium (control) or exposed to hypertonic medium containing either raffinose (B) or NaCl (C) for the indicated times. Poly(A)⁺ RNA (3 μg) was subjected to Northern blot analysis with a 32P-labeled AQP3 cDNA probe (top blots) or a 32P-labeled glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA probe (bottom blots). Signal intensities of AQP3 mRNA were normalized for those of GAPDH mRNA. Relative intensities of the signals of AQP3 to those of control at time 0 are shown at right.

Fig. 3. Immunoblot analysis of the effect of hypertonicity on the abundance of AQP3 protein in MDCK cells. Cells were maintained in isotonic medium (control) or exposed to hypertonic medium containing either raffinose (A) or NaCl (B) for the indicated times. They were subsequently homogenized, and total homogenates (10 μg of protein) were subjected to immunoblot analysis with rabbit polyclonal antibodies to rat AQP3 (left). Total homogenate prepared from rat kidney medulla (3 μg of protein) was analyzed simultaneously. Single and double arrowheads indicate nonglycosylated and glycosylated forms of AQP3, respectively. As a control, immunoblot analysis was also performed with primary antibodies that had been preincubated with the peptide immunogen (right). The positions of molecular size standards (in kDa) are indicated.
of antibodies to AQP3 and 2) preincubation of the rabbit antibodies to AQP3 (1:500 dilution) with the AQP3 peptide (10 μg/ml) used as the immunogen. Specimens were examined with a BX-50 or an AX-70 microscope equipped with Nomarski differential interference-contrast and epifluorescence optics (Olympus, Tokyo, Japan).

Measurement of fluorescence intensity and statistical analysis. For the measurement of fluorescence intensity of whole cell-mount specimens, microscopic images were recorded with a cooled charge-coupled device (CCD) camera (PXL 1400; Photometrics, Tucson, AZ). Exposure time was adjusted and fixed so that the fluorescence intensity of each pixel was within the range of 4,096 gray scales. IPLab Spectrum software (Signal Analytics, Vienna, VA) was used to analyze fluorescence images. Five to twenty fields were recorded from each sample. After background subtraction, the sum of all pixel intensities for each field was calculated. All data are expressed as means ± SD, and values for cells exposed to hypertonic medium were compared with those for corresponding controls with Welch’s t-test. A P value <0.05 was considered statistically significant.

Immunogold electron microscopy. For the pre-embedding method, cryostat sections of 10–16 μm in thickness were cut from formaldehyde-fixed specimens of MDCK cells, mounted on poly-L-lysine-coated glass slides, immersed in ethanol at −20°C for 30 min, and rinsed with PBS. Nanogold labeling was performed essentially as described previously (20). After incubation with rabbit antibodies to AQP3 (1:500 dilution), the sections were exposed to Nanogold-conjugated goat antibodies to rabbit IgG (1:50 dilution; Nanoprobe, Stony Brook, NY), washed with PBS, and fixed for 10 min with 1% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.4). The sections were then rinsed with water, incubated in silver acetate solution for the appropriate time, rinsed quickly with water, and immersed in 0.05% sodium acetate for 1 min. After extensive rinsing with water, the sections were treated with 0.05% gold chloride for 2 min, again rinsed extensively with water, fixed for 30 min with 1% osmium tetroxide in 0.1 M sodium phosphate buffer (pH 7.4), dehydrated with ethanol, and embedded in Epon. Ultrathin sections were cut, stained with uranyl acetate and lead citrate, and observed with a JEM-1010 transmission electron microscope (JEOL, Tokyo, Japan). To verify the specificity of immunostaining, we preincubated the primary antibodies (1:500 dilution) with the immunogenic peptide (10 μg/ml).

For the ultracryotomy method, ultrathin frozen sections were cut from formaldehyde-fixed specimens, mounted on grids coated with Formvar and carbon, and labeled essentially as described previously (31). In brief, grids were floated for 10 min on PBS containing 5% normal goat serum and 1% gelatin and then incubated with rabbit antibodies to AQP3 (1:200 dilution), followed by affinity-purified goat antibodies to rabbit IgG (Jackson Immunoresearch) that were conjugated with colloidal gold (10-nm diameter) (27). They were subsequently washed with PBS, refixed with 2% glutaraldehyde, treated with uranyl acetate, and embedded in 1.8% methyl cellulose-0.5% uranyl acetate (15). Specimens were observed with a JEM-1010 transmission electron microscope. To verify the specificity of immunostaining, we preincubated the primary antibodies (1:200 dilution) with the immunogenic peptide (50 μg/ml).

RESULTS

RT-PCR analysis of AQP3 mRNA in MDCK cells. To check the presence of AQP3 mRNA in MDCK cells, we subjected total RNA isolated from these cells to RT-PCR analysis with AQP3-specific primers. As a control, total RNA from rat kidney medulla, previously shown to contain AQP3 mRNA (4, 10), was similarly analyzed. A single PCR product of the expected size (~700 bp) was amplified from rat kidney medulla as well as from MDCK cells that had been either maintained in isotonic medium or exposed to hypertonic medium containing raffinose or NaCl (Fig. 1). The clone was isolated from the PCR product from MDCK cells, and ~80% of a continuous nucleotide sequence was analyzed. AQP3 PCR product obtained from MDCK cells displayed a ~90%, ~87%, and ~88% homology with the comparable region in human, rat, and mouse AQP3.
cDNA, respectively. These results thus demonstrated the presence of AQP3 mRNA in MDCK cells.

Northern blot analysis of the effect of hypertonicity on the abundance of AQP3 mRNA in MDCK cells. To examine the effect of hypertonicity on the amount of AQP3 mRNA in MDCK cells, we performed Northern blot analysis with poly(A)^+ RNA isolated from cells at 1, 12, 24, or 48 h after exposure to hypertonic medium. Given that a rat AQP3 cDNA probe containing the entire open reading frame failed to detect AQP3 mRNA from canine MDCK cells, we used the cloned AQP3 RT-PCR product obtained from these cells, whose nucleotide sequence has been checked, as the probe. Northern blot analysis revealed an AQP3 mRNA of ~1.9 kb expressed in MDCK cells (Fig. 2A). Exposure of MDCK cells to hypertonic medium induced a marked time-dependent increase in the abundance of AQP3 mRNA compared with that in corresponding control cells maintained in isotonic medium (Fig. 2B and C). The time courses of the changes in the abundance of AQP3 mRNA were similar for cells exposed to hypertonic medium containing either raffinose (Fig. 2B) or NaCl (Fig. 2C), with the effects being maximal at 12 h and decreasing thereafter in magnitude. A small increase in the amount of AQP3 mRNA was also observed when cells were maintained in isotonic medium.

Immunoblot analysis of the effect of hypertonicity on the abundance of AQP3 protein in MDCK cells. Using immunoblot analysis, we next examined whether the hypertonicity-induced increase in the amount of AQP3 mRNA is accompanied by an increase in the abundance of AQP3 protein. As previously described (20), rabbit polyclonal antibodies to rat AQP3 recognized two protein species in a total homogenate prepared from rat kidney medulla (Fig. 3): one protein species, corresponding to the nonglycosylated form of AQP3, yielded a sharp band at ~26 kDa, whereas the other species, representing the glycosylated form of AQP3, yielded a broad band at ~30 to ~40 kDa (3). A similar pattern of immunoreactivity was observed with total homogenates prepared from MDCK cells, with the exception that the broad band migrated at a position slightly above that of the corresponding band of rat kidney medulla; this difference in mobility may be due to a difference in the extent of AQP3 glycosylation. These immunoreactive bands were not detected by primary antibodies that had been preincubated with the COOH-terminal peptide of AQP3 used as the immunogen. The rabbit antibodies to rat AQP3 thus recognized the canine AQP3 protein.

No specific immunoreactive band was detected in control cells at time 0. Exposure of MDCK cells to hypertonic medium resulted in a marked time-dependent increase in the abundance of both nonglycosylated and glycosylated AQP3 proteins compared with the amounts in corresponding control cells (Fig. 3). The amount of AQP3 was increased slightly at 12 h, was maximal at 24 h, and decreased thereafter in cells exposed to hypertonic medium containing either raffinose (Fig. 3A) or NaCl (Fig. 3B). The abundance of both nonglycosylated and glycosylated forms of AQP3 also increased slightly with time in cells maintained in isotonic medium.

Immunolocalization of AQP3 in MDCK cells. We examined the subcellular localization of AQP3 in MDCK cells exposed to hypertonic medium. Observation of whole cell-mount preparations by laser confocal microscopy (Fig. 4A) or semithin frozen sections by conventional immunofluorescence microscopy (Fig. 4B) revealed that AQP3 was localized at the basolateral...
Fig. 6. Changes in AQP3 immunofluorescence induced by exposure of MDCK cells to hypertonic medium containing either raffinose or NaCl. Cells were maintained in isotonic medium (control, left) or exposed to hypertonic medium containing either raffinose (middle) or NaCl (right) for the indicated times. Fluorescence images were recorded with a cooled charge-coupled-device (CCD) camera under identical conditions, and representative images are shown. Bar, 100 µm.
membrane, with no labeling apparent at the apical membrane, in MDCK cells exposed for 24 h to hypertonic medium containing raffinose. Immunoelectron microscopy with pre-embedding (Fig. 5A) or ultracyrotyom (Fig. 5B) methods also revealed that AQP3 was localized to the basolateral plasma membrane. Replacement of the antibodies to AQP3 with normal rabbit serum or preincubation of the antibodies to AQP3 with the peptide immunogen demonstrated the specificity of all staining (data not shown).

Immunofluorescence microscopic analysis of the kinetics of hypertonicity-induced changes in the abundance of AQP3 in MDCK cells. We also examined the time course of the hypotonicity-induced changes in the abundance of AQP3 by conventional immunofluorescence microscopy. MDCK cells cultured on coverslips were exposed to hypertonic medium containing either raffinose or NaCl and were fixed after incubation for 1, 12, 24, or 48 h. All samples were immunostained at the same time and under the same conditions, and fluorescence images of 5–20 fields from each sample were recorded with a cooled CCD camera, again under identical conditions (Fig. 6). Few AQP3-positive cells were apparent among control cells at time 0. However, exposure of cells to hypertonic medium induced marked increases in both the number of AQP3-positive cells and the fluorescence intensity of individual cells. The number of AQP3-positive cells among control cells maintained in isotonic medium showed only a small increase, with the fluorescence intensity of each cell being low. Similar results were obtained with raffinose (Fig. 6, middle) and NaCl (Fig. 6, right).

The effect of hypertonicity on the abundance of AQP3 was assessed semiquantitatively by image analysis. The fluorescence intensity of AQP3 in cells exposed to hypertonic medium containing either raffinose (Fig. 7A) or NaCl (Fig. 7B) was significantly higher than that of AQP3 in control cells at 12, 24, and 48 h.}

DISCUSSION

The water channel protein AQP3 is expressed in the kidney and in many epithelial tissues (6, 7, 14, 20). In the kidney collecting duct, AQP3 (10) and AQP4 (34) are localized at the basolateral membrane of principal cells and, together with AQP2 present in the apical plasma membrane (8), play an important role in trans-epithelial water reabsorption. Regulation of the water permeability of collecting duct cells is mediated at the level of these water channel proteins. AQP2 is subject to both short-term and long-term regulation; ADH induces the rapid translocation of AQP2 from an intracellular vesicular storage compartment to the apical membrane (17, 23, 39), and long-term ADH infusion or long-term water restriction markedly increases AQP2 expression (2, 24, 35). AQP3 is present constitutively in the basolateral membrane, and its expression is increased by long-term ADH infusion or long-term water restriction (3, 11, 35). Terris et al. (35) suggested that these effects of water restriction and ADH infusion are mediated predominantly by a direct action of ADH on the collecting duct cells, rather than through changes in medullary hypertonicity, on the basis of the following observations: 1) the upregulatory response to water restriction was apparent not only in the medulla but also in the cortex, where interstitial osmolality is maintained at a level similar to that of the general circulation; and 2) the upregulatory response to ADH was not prevented by the collapse of the corticomедullary osmolality gradient induced by infusion of furosemide. However, these researchers did not rule out the possibility that changes in interstitial hypertonicity might have an independent effect on AQP3 expression. We have now demonstrated a direct effect of hypertonicity on AQP3 expression in MDCK epithelial cells. Hypertonicity induced increases in the abundance of both AQP3 mRNA and AQP3 protein in these cells. The amount of AQP3 mRNA was maximal 12 h after cells were exposed to hypertonic medium and decreased thereafter, whereas the amount of AQP3 protein was increased at 12 h, was maximal at 24 h, and decreased thereafter. These results suggest that AQP3 expres-
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Expression is regulated by osmolality at the level of transcription or of mRNA stability and that the response to hypertonicity is transient. Our data suggest that hypertonicity may directly control the expression of AQP3 in the principal cells of the kidney collecting duct as well as in cultured MDCK cells. Inase et al. (9) have isolated a human AQP3 gene and shown that the 5'-flanking region of the gene has a promoter activity and that its activity is upregulated by phorbol ester but not cAMP with luciferase reporter analysis. We examined whether these arginine vasopressin (AVP)-mediated signals induce the expression of AQP3 in MDCK cells by immunofluorescence microscopy. We examined the effect of AVP, the adenylate cyclase activator forskolin, and the protein kinase C activator phorbol 12-myristate 13-acetate. None of these induced the expression of AQP3 in MDCK cells (data not shown). It seems that AVP-mediated signals do not affect the expression of AQP3 in MDCK cells.

Our immunocytochemical analysis demonstrated the localization of AQP3 at the basolateral plasma membrane of hypertonicity-stimulated MDCK cells. This basolateral localization is identical to that in principal cells of the kidney collecting duct (3, 6, 10) as well as that in absorptive epithelial cells in the intestine and ciliated cells in the upper respiratory epithelium (20).

We have previously demonstrated the localization of AQP3 in rat epithelia (20). This protein is present in epithelia in the urinary, respiratory, and digestive tracts and in the skin that is exposed to the severe environment and susceptible to water loss. The expression of AQP3 in the epidermis begins in the late stage of fetal development. On the basis of these observations, we proposed that AQP3 might play a role in providing water to water-deprived cells to maintain intracellular osmolality and cell volume (20). It is now suggested that AQP3 expression in extrarenal organs as well as in the kidney might be regulated by toxicity. Tanaka et al. (33) showed that the expression of AQP3 is induced by dexamethasone in A549 human airway epithelial cells. These results suggest a possibility that the AQP3 expression is regulated by different factors among each organ and/or by many factors.

It is well known that hypertonicity increases the transcription of some genes such as the Na+-coupled myo-inositol cotransporter (40), aldose reductase (28), and the Na+- and Cl−-coupled betaine cotransporter (36), whose products catalyze cellular accumulation of compatible osmolytes. A toxicity-responsive enhancer (TonE), or osmotic responsive element (ORE), has been shown to regulate the expression of these genes (5, 13, 21, 26). Furthermore, a transcription factor that binds to TonE, known as TonE-binding protein (TonEBP), has been isolated and characterized (21, 22, 37). We have shown that the expression of AQP3 is also upregulated by hypertonicity in mIMCD-3 (mouse inner medullary collecting duct) cells. It remains to be determined whether the expression of the AQP3 gene and AQP1 gene are regulated in a similar manner to these hypertonicity-inducible genes in response to hypertonicity.

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