Hypertonic induction of aquaporin-1 water channel independent of transcellular osmotic gradient

Fuminori Umenishi a,b,*, Takefumi Narikiyo a, Robert W. Schrier a

* Corresponding author. Fax: +1 303 315 4852.
E-mail address: Fuminori.Umenishi@UCHSC.edu (F. Umenishi).

a Department of Medicine, University of Colorado Health Sciences Center, Denver, CO 80262, USA
b Department of Physiology and Biophysics, University of Colorado Health Sciences Center, Denver, CO 80262, USA

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Abstract

Aquaporin-1 (AQP1) water channel plays a critical role for water reabsorption in the urinary concentrating mechanism. AQP1 expression in renal cells is upregulated by hypertonicity, but not urea, suggesting the requirement of an osmotic gradient. To investigate whether AQP1 expression is regulated by apical and/or basolateral hypertonicity, murine renal medullary mIMCD-K2 cells grown on permeable support were exposed to hypertonic medium. When the medium on the apical or basolateral membrane side was switched to hypertonic, the transcellular osmotic gradient was dissipated within 8 h. Basolateral hypertonicity increased AQP1 expression more than apical hypertonicity. Comparable apical and basolateral hypertonicity without a transcellular hypertonic gradient, however, increased AQP1 expression. Cell surface biotinylation experiments revealed that hypertonicity promoted AQP1 trafficking to both plasma cell membranes. These results indicate that AQP1 expression is predominantly mediated by basolateral hypertonicity but a transcellular osmotic gradient is not necessary for its induction.

Keywords: Aquaporin-1; Hypertonicity; Sodium chloride; Osmotic gradient; Trafficking; Kidney

The cellular and molecular mechanisms of osmoregulation of aquaporin-1 (AQP1) water channel have been studied by cultured murine renal epithelial cells. AQP1 expression was upregulated by hypertonicity and its induction was mediated by the activation of mitogen-activated protein kinase (MAPK) pathways and a hypertonicity response element in the AQP1 gene [1,2]. In the kidney, 80–90% of the filtered volume is reabsorbed in the proximal tubules and descending limbs of Henle’s loop. AQP1 is constitutively expressed in both the apical and basolateral membranes in these segments [3], indicating the facilitation of water transport. AQP1 osmoregulation is mediated by impermeable solutes such as sodium chloride (NaCl), but not by cell permeable urea. Therefore, it is suggested that extracellular hypertonicity and transcellular osmotic gradient may be necessary for the induction, regulation, and targeting of AQP1.

To determine whether a transcellular osmotic gradient is necessary for AQP1 induction by hypertonicity, AQP1 expression by hypertonicity was investigated by using polarized murine renal medullary epithelial mIMCD-K2 cells that were cultured on permeable support. In the present study we show that AQP1 upregulation by hypertonicity was significantly different when the apical or basolateral side was hypertonic by different concentrations of NaCl. Comparable degrees of hypertonicity on both the apical and basolateral sides were hypertonic by different concentrations of NaCl. Comparable degrees of hypertonicity on both the apical and basolateral sides without a transcellular osmotic gradient, however, upregulated AQP1 expression. We further show that hypertonicity mediated the insertion of AQP1 on both the apical and basolateral membranes.
Materials and methods

Cell line and culture condition. Murine renal medullary mIMCD-K2 cells [4] were cultured at 37 °C and 5% CO₂ in OPTI-MEM medium (GIBCO, Gaithersburg, MD) supplemented with 10% fetal bovine serum. For all experiments, cells were seeded on 6-well plates with polyester membrane filter (Transwell, Corning, Acton, MA). After confluent, culture medium was replaced by hypertonic solutions with different concentrations of NaCl on the apical or basolateral side, or both sides. Cells were incubated for 24 h to induce AQP1 expression.

Immunoblot analysis. mIMCD-K2 cells grown on Transwell chambers were washed with ice-cold PBS, suspended with 20 mM Tris (pH 7.5) containing 150 mM NaCl and 1% Triton X-100, and incubated for 30 min on ice. After centrifuged at 14,000g for 10 min at 4 °C, the supernatant was collected, and protein concentration was measured using the Bradford protein assay method (Bio-Rad Protein Assay Kit; Bio-Rad, Hercules, CA). Protein (30 μg) was resolved on a 12% SDS–polyacrylamide gel and transferred to a PVDF membrane. The membrane was incubated with polyclonal anti-AQP1 antibody. After washing, the membrane was incubated with anti-rabbit IgG horseradish peroxidase secondary antibody (Amersham, Piscataway, NJ). The immunoreactive bands were visualized by enhanced chemiluminescence method (NEN Life Science, Boston, MA). The bands on the film were scanned and analyzed by using the NIH image software. To confirm that equal amounts of protein samples were loaded, duplicate gels were stained with Coomassie brilliant blue.

Cell surface biotinylation and immunoprecipitation. Cells were grown on Transwell chambers. After confluent, culture medium was replaced by hypertonic solution adding 200 mM NaCl on the basolateral side of the chamber. After 24 h incubation, cell surface biotinylation on the apical and basolateral sides was performed by using the Bradford protein assay method (Bio-Rad Protein Assay Kit; Bio-Rad, Hercules, CA). Protein (30 μg) was resolved on a 12% SDS–polyacrylamide gel and transferred to a PVDF membrane. The membrane was incubated with polyclonal anti-AQP1 antibody. After washing, the membrane was incubated with anti-rabbit IgG horseradish peroxidase secondary antibody (Amersham, Piscataway, NJ). The immunoreactive bands were visualized by enhanced chemiluminescence method (NEN Life Science, Boston, MA). The bands on the film were scanned and analyzed by using the NIH image software. To confirm that equal amounts of protein samples were loaded, duplicate gels were stained with Coomassie brilliant blue.

Results

A previous report suggested that a hypertonic stimulus was required for AQP1 induction in murine renal medullary cells [1]. To elucidate it, mIMCD-K2 cells were cultured on permeable supports. After confluent, culture medium was replaced by hypertonic on the apical or basolateral side, or both sides. When cells were exposed to hypertonic medium adding 150 mM NaCl on the apical or basolateral side, the transcellular osmotic gradient was dissipated within 8 h (Fig. 1). The difference of the final medium osmolality in both conditions is due to the volume of the medium on the apical and basolateral sides (see Fig. 3).

To investigate AQP1 expression by a hypertonic gradient, cells were incubated with hypertonic medium by the addition of NaCl (100–250 mM) on the apical or basolateral side for 24 h. After the treatment, cell extract was isolated and immunoblot analysis was performed. As shown in Fig. 2, AQP1 expression was strongly induced by the addition of NaCl on the basolateral side. The maximal induction of AQP1 was led by the addition of 200 mM NaCl. However, the expression was diminished by the addition of 250 mM NaCl because of the significant decrease of cell viability. On the contrary, when the cells were exposed to hypertonic medium on the apical side, AQP1 expression was gradually increased in a dose dependent manner although the increase by apical hypertonicity was much smaller than that by basolateral hypertonicity. Further, comparable degrees of hypertonicity by the addition of 100 or 150 mM NaCl on both the apical and basolateral sides, i.e., without a transcellular osmotic gradient, upregulated AQP1 expression.

![Fig. 1. Change of medium osmolality by the treatment with hypertonic medium on the apical or basolateral membrane side. Cells grown on permeable supports were incubated in hypertonic medium adding 150 mM NaCl on the apical or basolateral membrane side. The medium on the (A) apical and (B) basolateral sides was harvested at specific times (0, 0.5, 1, 1.5, 2, 3, 4, 6, and 8 h) and the medium osmolality was measured by osmometer. Each value is an average of three independent experiments.](Image 147x105 to 459x244)
These results suggest that AQP1 induction by hypertonicity may be due to a tonicity rather than a hypertonic gradient and a transcellular osmotic gradient is not necessary for its induction.

Since the volume of the medium on the apical side is smaller than that on the basolateral side (1.5 ml versus 2.5 ml), the final medium osmolality after replacing hypertonic medium on the apical or basolateral side is not equal as demonstrated in Fig. 1. Therefore, the designated hypertonic medium was added to the apical (200, 250, 300, 350, or 400 mM NaCl) or basolateral side (120, 150, 180, 210, or 240 mM NaCl). The final medium osmolality on both the sides in these different hypertonic conditions was virtually equal after 24 h incubation (Table 1). In these conditions AQP1 expression was compared between apical and basolateral hypertonicity.

As shown in Fig. 3, the addition of hypertonic medium supplemented with 350 mM NaCl to the apical side and 210 mM NaCl to the basolateral side revealed the highest induction of AQP1, respectively. However, the increase in AQP1 expression by basolateral hypertonicity was significantly greater than apical hypertonicity. These results indicate that AQP1 induction is predominantly mediated by basolateral hypertonicity rather than apical hypertonicity.

AQP1 expression by basolateral hypertonicity was detected at 12 h by immunoblot and increased to 24 h in a time-dependent manner (Fig. 4A). Next, to examine whether AQP1 protein newly synthesized by basolateral hypertonicity is targeted to the apical and/or basolateral plasma membranes, the membrane insertion of AQP1 was investigated by cell surface biotinylation experiments (Fig. 4B). The biotinylated proteins on the apical and basolateral membranes were isolated by streptavidin–agarose beads and the biotinylated AQP1 protein was analyzed by immunoblot. In isotonic conditions, AQP1 protein was not detectable on either the apical or basolateral membrane. In contrast, basolateral hypertonicity mediated the targeting of AQP1 to both the apical and basolateral membranes. This result indicates that AQP1 protein is not targeted preferentially to either the apical or basolateral membrane.
Hypertonicity, which results from a high concentration of NaCl, provides a mechanical stress to shrink cells. Renal medullary cells adapt to hypertonicity by a variety of responses through an efflux of water, cell shrinkage by NaCl, chronic accumulation of organic osmolytes, and acute activation of immediate early genes. NaCl is one of the major solutes in the interstitial fluid of renal medulla, which differs from urea, because NaCl is impermeant to the plasma membrane. Chronic adaptation to hypertonicity increases expression of genes encoding for proteins involved in the accumulation of compatible organic osmolytes such as the sodium/myo-inositol cotransporter, sodium/chloride/betaine cotransporter, and aldose reductase. In addition, it has been reported that several aquaporin water channels (AQP1, 2, 3, 4, 5, and 9) are upregulated by hypertonicity. Thus, the upregulation of such transporters containing AQPs under hypertonic condition is essential for survival of these cells and keeping intracellular ion concentrations lower.

In the present study AQP1 expression by hypertonicity was investigated in cultured murine renal medullary cells. A previous study suggested that AQP1 induction by hypertonicity may require a hypertonic gradient. Therefore, we developed the simple method to examine AQP1 expression by hypertonicity using Transwell chamber with permeable support. This culture system provides epithelial cell polarity and allows study of transepithelial cell events. Using this system, we demonstrated that AQP1 expression was induced predominantly by basolateral hypertonicity. However, we also found that a transcellular osmotic gradient was not always necessary for AQP1 induction by hypertonicity. All data support that an increased tonicity is indispensable for AQP1 induction rather than a transepithelial osmotic gradient. Yamauchi et al. were reported previously that sodium/myo-inositol transporter was mainly induced by basolateral hypertonicity. Thus, some hypertonicity-induced transporters may be regulated by basolateral tonicity.

It was also demonstrated in the present study that AQP1 protein was targeted to both the apical and basolateral membranes by the stimulus of basolateral hypertonicity. This indicates that AQP1 protein induced by hypertonicity is not targeted preferentially to either the apical or basolateral membrane and the polarized event of cells may not be necessary for AQP1 induction by hypertonicity. This corresponds to in vivo circumstances in which AQP1 is constitutively expressed on both the apical and basolateral membranes in the proximal tubules and descending limbs of Henle's loop. It has also been reported that the pattern of both apical and basolateral membrane expression of AQP1 is present in AQP1-transfected MDCK and LLC-PK1 cells. This nonpolarized expression of AQP1 on the apical and basolateral membranes in renal cells may be essential for the maximal effect of water absorption.
ber is quite useful for the study of osmoregulation of aquaporin water channels and a number of transporters.

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References