Aquaporin-3 water channel localization and regulation in rat kidney

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Ecelbarger, Carolyn A., James Terris, Gustavo Frindt, Miriam Echevarria, David Marples, Soren Nielsen, and Mark A. Knepper. Aquaporin-3 water channel localization and regulation in rat kidney. Am. J. Physiol. 269 (Renal Fluid Electrolyte Physiol. 38): F663–F672, 1995.—The aquaporins are a family of water channels expressed in several water-transporting tissues, including the kidney. We have used a peptide-derived, affinity-purified polyclonal antibody to aquaporin-3 (AQP-3) to investigate its localization and regulation in the kidney. Immunoblotting experiments showed expression in both renal cortex and medulla, with greatest expression in the base of the inner medulla. Subcellular fractionation of membranes, using progressively higher centrifugation speeds, revealed that AQP-3 is present predominantly in the 4,000 and 17,000 g pellets and, in contrast to AQP-2, is virtually absent in the high-speed (200,000 g) pellet that contains small intracellular vesicles. Immunocytochemistry and immunofluorescence studies revealed that labeling is restricted to the cortical, outer medullary, and inner medullary collecting ducts. Within the collecting duct, principal cells were labeled, whereas intercalated cells were unlabelled. Consistent with previous immunofluorescence studies (K. Ishibashi, S. Sasaki, K. Fushimi, S. Uchida, M. Kuwahara, H. Saito, T. Furukawa, K. Nakajima, Y. Yamaguchi, T. Gojobori, and F. Marumo. Proc. Natl. Acad. Sci. USA 91: 6269–6273, 1994; T. Ma, A. Frigeri, H. Haegewa, and A. S. Verkman. J. Biol. Chem. 269: 21845–21849, 1994), the labeling was confined to the basolateral domain. Immunoelectron microscopy, using the immunogold technique in ultrathin cryosections, demonstrated a predominant labeling of the basolateral plasma membranes. In contrast to previous findings with AQP-2, there was only limited AQP-3 labeling of intracellular vesicles, suggesting that this water channel is not regulated acutely through vesicular trafficking. Immunoblotting studies revealed that thirsting of rats for 48 h approximately doubled the amount of AQP-3 protein in the inner medulla. These studies are consistent with a role for AQP-3 in osmotically driven water absorption across the collecting duct epithelium and suggest that the expression of AQP-3 is regulated on a long-term basis.

METHODS

Polyclonal Antibodies

To obtain a polyclonal antibody against AQP-3, a 26 amino acid synthetic peptide, corresponding to the carboxyl terminus of AQP-3 (with an added NH2-terminal cysteine), was produced by standard solid-phase, peptide-synthesis techniques (sequence: NH2-CHLEQPSTEAENVKLHMKHKEQI-COOH). The peptide was purified by high-performance liquid chromatography and was conjugated to maleimide-activated keyhole limpet hemocyanin via covalent linkage to the NH2-terminal cysteine. Two rabbits were immunized with this conjugate, using a combination of Freund’s incomplete adjuvants. One rabbit developed an enzyme-linked immunosorbent assay titer > 1:16,000 prior to exsanguinat...
With the "sequential fractionation protocol," spins were done injected to one of two subsequent fractionation protocols. Jolla, CA) with 1 pg/ml leupeptin (Bachem, Torrance, CA) and protocol produces an - lo-fold difference in urinary osmolality were initially given 600 mM sucrose as the sole drinking fluid a large-step change in water intake (14). Specifically, all rats were killed by decapitation, and both kidneys were removed. These rats were used in this study. These rats were maintained in Immunoblotting Studies

Animal treatments and kidney dissection. Pathogen-free male Sprague-Dawley rats (Taconic Farms, Germantown, NY) were used in this study. These rats were maintained in filter-top microisolator cages, with autoclaved food and bedding, and were allowed free access to drinking water. The rats were killed by decapitation, and both kidneys were removed. The kidneys were rapidly dissected to obtain separate tissue samples from the three major regions (cortex, outer medulla, and inner medulla) prior to homogenization (see below). In some cases, the inner medulla was further dissected into inner medullary base and true papilla.

To test for effects of water restriction on AQP-3 expression, some animals were treated with a protocol designed to produce a large-step change in water intake (14). Specifically, all rats were initially given 600 mM sucrose as the sole drinking fluid for an initial 48-h equilibration period. The animals drank large amounts of this fluid, establishing a moderate water diuresis (14), which was the control state for all animals. Then, half the animals were deprived of water for the next 48 h, while the controls continued to receive the sucrose water. This protocol produces an ~ 10-fold difference in urinary osmolality (14). The rats were killed, and kidneys were dissected as described above.

Preparation of membrane fractions. The tissue was homogenized using a tissue homogenizer (Omni 1000, fitted with a microsawtooth generator) in ice-cold isolation solution containing 250 mM sucrose-10 mM triethanolamine (Calbiochem, La Jolla, CA) with 1 mg/ml leupeptin (Bachem, Torrance, CA) and 0.1 mg/ml phenylmethylsulfonyl fluoride (US Biochemical, Toledo, OH). Various membrane fractions were prepared by centrifuging the homogenate at appropriate speeds. Initially, homogenates were spun at low speeds (1,000 g) for 10 min to remove nuclei and incompletely homogenized membrane fragments. To increase yields, this pellet was rehomogenized and was spun again at 1,000 g for 10 min. The supernatants from the two 1,000 g centrifugations were pooled and were subjected to one of two subsequent fractionation protocols. 1) With the "sequential fractionation protocol," spins were done in series at 4,000, 17,000, and 200,000 g, keeping the pellet from each spin while utilizing the supernatant for the next spin. This provides a means of separating cellular membrane components into progressively smaller or less-dense membrane fragments, including small intracellular vesicles (microsomes), which pellet with the high-speed (200,000 g) spin, and plasma membranes, which generally pellet at lower speeds (19). 2) With the "single-speed fractionation protocol," only one of the above centrifugations was carried out after the initial 1,000 g spins to obtain a single membrane fraction. The 1,000, 4,000, and 17,000 g centrifugations were accomplished using a Sorvall RC2-B refrigerated centrifuge with an SS-34 rotor or a Tommy MTX-ISO refrigerated centrifuge with a TMA-3 rotor. The 200,000 g spin was carried out with a Beckman L8-M ultracentrifuge fitted with a type 80Ti rotor. The total protein concentration in membrane fractions was measured using the Pierce BCA Protein Assay reagent kit (Pierce).

Electrophoresis and immunoblotting of membranes. The membrane fractions were solubilized at 60°C for 15 min in Laemmli sample buffer, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was done on minigels of 12% polyacrylamide. The proteins were transferred from the gels electrophoretically to nitrocellulose membranes. After being blocked with 5 g/dl nonfat dry milk, membranes were probed with the affinity-purified polyclonal antibody to the AQP-3 water channel at IgG concentrations in the range of 0.15 to 0.46 μg/ml in an antibody-dilution buffer solution containing 150 mM NaCl, 50 mM sodium phosphate, 10 mg/dl sodium azide, 50 mg/dl Tween-20, and 1 g/dl bovine serum albumin (pH 7.5). The secondary antibody was donkey anti-rabbit IgG conjugated to horseradish peroxidase (Pierce no. 31458) used at a concentration of 0.16 μg/ml. Sites of antibody-antigen reaction were visualized using luminol-based enhanced chemiluminescence (LumiGLO, Kirkegaard & Perry, Gaithersburg, MD) before exposure to X-ray film (Kodak no. 165-1379 Scientific Imaging Film). Controls were carried out, as described in RESULTS, utilizing the IgG fraction of the preimmune serum and affinity-purified antiserum preadsorbed with the immunizing peptide. Where appropriate, relative quantification of the resulting band densities was carried out by densitometry using a laser densitometer (LKB Ultrascan XL).

Immunocytochemistry

Fixation and preparation of tissue for immunocytochemistry. Male Wistar rats, weighing 250–300 g, were obtained from Mollegaard Breeding Center. The rats had free access to water and standard rat chow. Rats were anesthetized with intraperitoneal pentobarbital sodium and were perfused retrogradely via the aorta with ice-cold phosphate-buffered saline (in mM) 7.2 Na2HPO4, 2.8 NaH2PO4, 150 NaCl, pH 7.4). After 2 min, the perfusion was changed to ice-cold fixative (8% paraformaldehyde in 0.1 M sodium cacodylate buffer, pH 7.4) and continued for 3 min more. The kidneys were then removed and prepared for immunocytochemistry. Tissue blocks were prepared from 1) cortex, 2) outer stripe of the outer medulla, 3) inner stripe of the outer medulla, and 4) four levels of the inner medulla. The blocks were postfixed for 2 h in 8% paraformaldehyde and infiltrated with 2.3 M sucrose-2% paraformaldehyde for 30 min. The blocks were then mounted on holders and rapidly frozen in liquid nitrogen.

Sectioning and immunolabeling. Thin (0.85 μm) and ultrathin (80 nm) cryosections, cut on a Reichert Ultracut FCS cryoultramicrotome, were incubated with the affinity-purified antibodies against AQP-3 (3–7 μg IgG/ml) described above. The labeling was visualized as previously described (5, 16, 17) using horseradish peroxidase-conjugated secondary antibodies (Dako P448, 1:100; Dako, Glostrup, Denmark) for light microscopy. For double-labeling experiments, a mononuclear antibody against the proton pump was used (E11, diluted 1:10–1:40, kindly given by Dr. Steven Gluck). E11 was visualized using fluorescein-conjugated secondary antibodies (Dako F479, 1:40), whereas anti-AQP-3 was visualized with rhodamine-conjugated secondary antibodies (Dako R156, 1:40) for fluorescence microscopy. For immunoelectron microscopy, AQP-3 labeling was visualized in ultrathin sections with goat anti-rabbit gold (10-nm particles, EM.GAR10, 1:50; BioCell, Cardiff, UK). Controls using preimmune serum, nonimmun IgG, or omission of primary or secondary antibody revealed no labeling.
AQUAPORIN-3 LOCALIZATION IN KIDNEY

Fig. 1. Immunoblot of outer medullary membrane proteins (17,000 g pellet, 5 μg total protein per lane) probed with either affinity-purified anti-aquaporin (AQP)-3 [1:1,000 dilution; immunoglobulin G (IgG) concentration, 0.46 μg/ml, right lane] or same antiserum preadsorbed with 2 mg of immunizing peptide (left lane). Preadsorption completely ablated both predominant bands, 27 and 35 kDa. In addition, both of these bands were absent when preimmune IgG (0.42 μg/ml) was utilized in place of primary antibody or when primary antibody was omitted (results not shown).

Quantitation of AQP-3 immunogold labeling in inner medullary collecting duct cells. To determine quantitatively the fraction of AQP-3 labeling associated with basolateral plasma membrane, apical plasma membrane, and intracellular vesicles in collecting duct principal cells, electron micrographs were taken on Philips CM100 or Philips EM208 electron microscopes, covering the entire area of seven inner medullary collecting duct principal cells. The micrographs were taken at a primary magnification of ×18,000 and printed at a final magnification of ×48,000. A computerized digitizing tablet (resolution <4 nm) was used to measure the cell area in square micrometers (excluding nuclei). The numbers of gold particles associated with the apical plasma membrane, the basolateral plasma membranes, and intracellular vesicles were determined. AQP-3 labeling of each compartment was corrected for background labeling of the same specific compartment. The background was determined by use of nonimmune IgG as primary antibody, used at the same concentration as the specific affinity-purified anti-AQP-3 antibody. Background labeling represented <8% of the specific labeling. Total cell area examined (excluding nuclei) was 168 μm². The total number of gold particles counted was 1,159.

RESULTS

Antibody Characterization

Figure 1 shows results from immunoblots, using a crude membrane preparation (17,000 g pellet) from the renal outer medulla of rat probed with the affinity-purified anti-AQP-3 antibody. A band was seen at 27 kDa, and a broader band was seen at 33–40 kDa (Fig. 1, right). This pattern is similar to that seen with anti-AQP-2 (17) and anti-AQP-1 (18). As with these previously characterized water channels, the upper band is likely to represent the glycosylated form of AQP-3, and the lower band is likely to represent the nonglycosylated form. When the anti-AQP-3 antibody was preadsorbed with the immunizing peptide, these bands were ablated. In addition, no bands were seen when the IgG fraction of the preimmune serum was used in lieu of the primary antibody (not shown) or when the primary antibody was omitted (not shown).

Regional Localization of AQP-3

Figure 2 shows the regional distribution of AQP-3 in the rat kidney as determined by immunoblotting of crude membrane preparations (17,000 g pellet) from cortex, outer medulla, and inner medulla. Figure 2, left, shows significant AQP-3 expression in all three regions, although the expression was greater in the medulla than in the cortex. In several such experiments, the upper (glycosylated) band in Fig. 2 consistently ran slightly higher using membranes from the inner medulla than with membranes from the outer medulla. Figure 2, right, shows data from a different rat comparing expression levels in the tip of the inner medulla (i.e., the papilla) and the base of the inner medulla. The expression of AQP-3 was much greater in the base than in the tip. An additional band at ~70 kDa, seen when larger amounts of protein were loaded, appears to ablate after
preadsorption with the immunizing peptide (Fig. 1) and may represent an undissociated AQP-3 complex.

**Immunohistochemical Localization of AQP-3**

Figure 3 shows localization of AQP-3 at a light microscopic level in different parts of the rat kidney using immunohistochemical methods. Anti-AQP-3 exclusively labeled collecting ducts, as previously reported (11). In the cortex, extensive labeling is seen of basolateral plasma membrane domains, with labeling extending from the basolateral infoldings to the lateral plasma membrane (Fig. 3b). Several collecting duct cells revealed an absence of labeling, presumably corresponding to intercalated cells (Fig. 3b, arrows). To test this, we used an antibody against the proton pump adenosinetriphosphatase, which has previously been shown in the collecting duct to be exclusively localized to intercalated cells (2, 3). Double-immunofluorescence microscopy revealed that cells that labeled for proton pump were not labeled for AQP-3 (Fig. 3, c and c'), consistent with AQP-3 being exclusively present in principal cells. Similar observations were made in numerous fields from several sections taken from cortex and outer medulla. Outer medullary collecting duct principal cells were also heavily labeled for AQP-3, whereas intercalated cells showed no labeling (Fig. 3, d and e). Both immunoperoxidase and immunofluorescence demonstrate extensive labeling of basolateral infoldings and of lateral plasma membranes with labeling extending toward the tight junction (Fig. 3, d and e). Within the inner medulla, the collecting duct labeling was very pronounced in the outer half of the inner medulla but was much weaker in the papillary tip. As shown in Fig. 3f (low magnification) and Fig. 3g (higher magnification), sections from the outer 25% of the inner medulla, basolateral plasma membrane domains of principal cells were heavily labeled, whereas intercalated cells, as well as nephron and vascular structures, were unlabeled. In contrast, in sections obtained from the deepest 25% of the inner medulla, a considerably weaker labeling is seen of collecting duct cells (Fig. 3h), and, in the most terminal portions of the collecting duct, AQP-3 labeling is virtually absent (not shown). Immunolabeling controls of sections obtained from outer and inner medulla (Fig. 3, i and j) revealed no labeling.

**Distribution of AQP-3 among subcellular membrane fractions.** The vasopressin-regulated water channel, AQP-2, is found in large quantities both in the apical plasma membrane of collecting duct cells and in intracellular vesicles, which act as a reservoir for regulated insertion of AQP-2 into the plasma membrane (16, 17). We have recently shown that portions of AQP-2 associated with plasma membranes and intracellular vesicles can be partially separated by sequential centrifugation at 17,000 g, which selectively pellets the plasma membranes, and at 200,000 g, which selectively pellets the membranes associated with small intracellular vesicles (1, Marples, M. Knepper, E. Christensen, and S. Nielsen; unpublished observations). Here, we have carried out similar studies using rat outer medullary homogenates to determine whether AQP-3 is distributed among subcellular fractions in a manner similar to AQP-2 (Fig. 4). Figure 4 compares the distribution of AQP-3 among pellets obtained by centrifugations at progressively higher speeds with the distributions of AQP-1 (CHIP28) and AQP-2 (AQP-CD). Unlike AQP-2, little or no AQP-3 is seen in the pellet from the high-speed (200,000 g) centrifugation, enriched in small intracellular vesicles. In contrast, AQP-3, like AQP-1, is found predominantly in lower-speed fractions that contain plasma membranes.

**Immunoelectron Microscopy**

Figure 5 shows the distribution of AQP-3 labeling within collecting duct principal cells from kidney inner medulla (proximal 25% of inner medullary collecting duct) using the immunogold technique. The basolateral plasma membranes were heavily labeled. The labeling of the basolateral plasma membrane was predominantly localized to the lateral plasma membrane (Fig. 5A) and to the basolateral infoldings (Fig. 5B), whereas the basal plasma membrane adjacent to the basement membrane exhibited little labeling (Fig. 5D). The apical plasma membrane exhibited virtually no labeling (Fig. 5A), whereas moderate labeling of small intracellular vesicles was observed. The labeled intracellular vesicles were...
The detection limit (lo/o) was 0.01%, and there was no labeling in excess. Corrected for background using nonimmune IgG revealed an absence of labeling (Fig. 5B). Little labeling; inset: small intracellular vesicles also exhibit labeling in region near Golgi apparatus (G, arrowheads). Labeling was localized to lateral plasma membranes (L, arrows) forming intercellular space. Small intracellular vesicles, distinct bands were seen for AQP-1 and AQP-2, while only an extremely faint band was present for AQP-3.

To determine quantitatively the subcellular distribution of AQP-3 in collecting duct principal cells, immunogold particles associated with basolateral plasma membrane, intracellular vesicles, and apical plasma membrane were counted. The quantitation was performed on electron micrographs covering the entire cell area (excluding nuclei) of seven principal cells from the base of the kidney inner medulla. Particle counts were corrected for background using nonimmune IgG as substitute for specific antibody. Out of 1,159 gold particles, 80% were associated with the basolateral plasma membrane, and 19% were associated with intracellular vesicles. Labeling of the apical plasma membrane was at detection limit (1%), and there was no labeling in excess of background of other cellular components. Thus the labeling is predominantly associated with the basolateral plasma membrane; however, a distinct fraction of the labeling was also associated with intracellular vesicles.

Regulation of AQP-3 Expression by Water Restriction

Figure 6 shows immunoblotting results from experiments designed to test whether the expression level of AQP-3 in the rat inner medulla is altered as a result of changes in water intake or the associated changes in urinary concentration. In Fig. 6, both nonglycosylated and glycosylated bands exhibited a marked increase in density in rats thirsted for 48 h, relative to the control (water-loaded) state. Densitometry of the lower band revealed a 2.4-fold increase from 0.20 ± 0.02 in water-loaded rats to 0.48 ± 0.09 arbitrary units in thirsted rats (P < 0.05, unpaired t-test). Densitometry of the upper band revealed a 1.5-fold increase from 1.56 ± 0.11 in water-loaded rats to 2.27 ± 0.10 arbitrary units in thirsted rats (P < 0.05, unpaired t-test).

DISCUSSION

In this study, we fully characterize the regional, cellular, and subcellular distribution of AQP-3 water channel protein in rat kidney and provide definite evidence for long-term regulation of AQP-3 expression in the renal medulla. To pursue these goals, we have raised a polyclonal antibody to AQP-3 by immunizing rabbits with a synthetic peptide corresponding to the COOH-terminal 25 amino acids of the predicted polypeptide sequence. Among the known aquaporins, the sequence of this peptide is unique to AQP-3. Furthermore, a comparison of this sequence to the GenBank database using BLAST analysis revealed no significant overlap with other known eukaryotic proteins. This suggests that the resulting antibody is likely to be specific for AQP-3. Indeed, the immunocytochemical localization of AQP-3 in the kidney using this antibody differed from that seen previously for AQP-1 (18, 23), AQP-2 (8, 17), and AQP-4 (23a). Furthermore, controls using preimmune serum or antibody that was preadsorbed with the immunizing peptide showed no labeling on either immunoblots or tissue sections.

As previously seen for both AQP-1 (18) and AQP-2 (17), two predominant bands were seen on AQP-3 immunoblots prepared from kidney membranes, a broad upper band of 33 to 40 kDa and a lower band of 27 kDa. Based on prior observations with AQP-1 and AQP-2, the broad upper band is likely to be the glycosylated form of the protein, although we have not specifically tested this assumption in this study. In samples from inner medulla, the upper band consistently ran at slightly higher...
AQUAPORIN-3 LOCALIZATION IN KIDNEY

Water-Loaded

| 29 | 35 |

Thirsted

| 29 | 35 |

molecular weight than in samples from the outer medulla (Fig. 2), suggesting differences in posttranslational modifications of AQP-3 (e.g., glycosylation or phosphorylation). The lower band, presumably the nonglycosylated form, has an apparent molecular mass of 27 kDa. Interestingly, each of the three groups that have independently cloned the cDNA for AQP-3 assumed a different translation-initiation methionine in predicting the primary structure of the AQP-3 protein (7, 11, 15). This gave predicted molecular mass ranging from 29.8 to 31.4 kDa for the unglycosylated form. The finding of a lower apparent molecular mass (27 kDa) raises two alternative possibilities. 1) The translated protein may have a molecular mass in the range of 29.8 to 31.4 kDa as proposed but may run aberrantly rapidly on the gel due to its hydrophobic nature, as has been seen with AQP-5 (22). 2) The initiation of AQP-3 translation may occur further downstream than believed. For instance, if translation were initiated from the methionine that is 36 residues downstream from the initiating methionine assumed by Echevarria et al. (7), then a 256-residue product would be predicted. Consistent with the idea that the unmodified protein may be smaller than initially estimated or may run aberrantly on the gel, Echevarria et al. (6) found that, when AQP-3 was translated in vitro in a rabbit reticulocyte lysate system, the product had a molecular mass of ~25 kDa (as estimated by 12% SDS-PAGE).

We used our affinity-purified anti-AQP-3 antibody to determine the regional distribution of AQP-3 protein in rat kidney by immunoblotting (Fig. 2). These experiments demonstrated that AQP-3 is present in all three regions of the kidney, namely, cortex, outer medulla, and inner medulla (Fig. 2, left). When normalized by membrane protein, expression of AQP-3 appears greater in the medulla than in the cortex and greater in the base of the inner medulla than in the papillary portion of the inner medulla (Fig. 2). This regional localization corresponds well with immunohistochemical findings (Fig. 3), which demonstrate labeling of collecting ducts in all regions of the kidney, but with gradually decreasing labeling of collecting ducts in the papilla. The regional distribution of protein expression determined by immunoblotting also correlates well with the distribution of AQP-3 mRNA in the kidney as determined by Northern blotting (7, 11).

Immunohistochemistry (Fig. 3) revealed that AQP-3 labeling was restricted to the collecting ducts. This is congruent with the findings of in situ hybridization studies showing heavy labeling of collecting ducts (7, 15). Furthermore, labeling was restricted to the basolateral domain of principal cells in the cortex and outer medulla and the basolateral domain of inner medullary collecting duct cells, as indicated previously by immunofluorescence studies using cryostat sections and employing similarly derived anti-peptide antibodies (11, 15). Collecting duct intercalated cells, which are not believed to be involved in water transport across the collecting duct, were unlabeled (Fig. 3).

Immunoelectron microscopic studies, employing the immunogold technique in ultrathin cryosections, provided unique information on the subcellular distribution of the AQP-3 water channel (Fig. 5). In contrast to AQP-2, which was found in the apical plasma membrane and in intracellular vesicles (17), AQP-3 was identified chiefly in the basolateral plasma membrane, although a small fraction of the labeling was present in intracellular vesicles. Because AQP-3 and AQP-2 are differently targeted in the same cells, the amino acid sequences of these aquaporins presumably contain structural information that directs trafficking to different domains, although the principles involved have not yet been defined. Subcellular fractionation of outer medullary membranes (Fig. 4) gave results for cellular localization of AQP-3 that are consistent with the findings from immunoelectron microscopy. These experiments showed that virtually all of the immunodetectable AQP-3 in the outer medulla can be found in the pellets from low-speed centrifugation, which are enriched in plasma membranes. The presence of a small component of vesicular labeling raises the possibility that, like AQP-2 (16), AQP-3 might be regulated acutely via membrane trafficking between the intracellular vesicles and the plasma membrane. Recent findings by Yamamoto et al. (25) suggest that such trafficking may not occur. Indeed, although further studies are warranted, it appears more likely that the presence of AQP-3 labeling in intracellular vesicles represents newly synthesized AQP-3 in transit from the Golgi apparatus to the basolateral plasma membrane.

In contrast to the lack of evidence for short-term regulation of AQP-3, we clearly demonstrate here the long-term regulation of AQP-3 with a marked increase in AQP-3 expression in the rat inner medulla in response to restriction of water intake (Fig. 6). We previously demonstrated long-term regulation of AQP-2 protein expression in response to water restriction (17) and to vasopressin infusions (5) in rat renal medulla. The presence of a coordinate increase in AQP-2 and AQP-3 expression raises the possibility that the overall long-term regulation of collecting duct water permeability, previously demonstrated in isolated perfused tubule studies (5, 14), may result from parallel increases in apical and basolateral water permeability. The increase in AQP-3 protein in collecting ducts is presumably
secondary to increases in AQP-3 mRNA levels following water restriction (6). It has not been established, however, whether the increase in AQP-3 mRNA is a result of transcriptional regulation or an alteration in mRNA stability.

The physiological role of AQP-3 in the collecting duct has not yet been unequivocally defined. Ma et al. (15) have proposed that it functions predominantly as a glycerol transporter and may have little or no water-transporting capacity. Hence, they refer to the protein as glycerol-transporting integral protein. In contrast, both Ishibashi et al. (11) and Echevarria et al. (7) found that substantial water permeabilities are induced in Xenopus oocytes when AQP-3 cRNA is injected, although both also show permeation of small nonelectrolytes. Thus it appears quite possible that AQP-3 may constitute a major pathway for osmotically driven transport of water across the basolateral membrane of collecting duct cells. If so, AQP-3 may be considered a critical protein in the concentration of urine, since collecting duct water absorption depends on rapid water transport across both the apical and basolateral plasma membrane. In addition, unlike the other aquaporins, AQP-3 appears to be significantly permeable to urea (7, 11).

Based on this observation and the finding that urea permeation through AQP-3 is sensitive to phloretin, Ishibashi et al. (11) have proposed that AQP-3 may constitute the basolateral pathway for phloretin-sensitive urea transport across the inner medullary collecting duct. However, in studies of AQP-3 expressed in Xenopus oocytes, we have been unable to confirm the presence of a substantial degree of inhibition of urea permeation by phloretin (M. Echevarria, G. Frindt, and E. Windhager; unpublished observations). Nevertheless, given our demonstration here that AQP-3 is found in the basolateral membrane throughout the length of the collecting duct from cortex through inner medulla, the potential role of AQP-3 as a phloretin-sensitive basolateral urea transporter appears to be experimentally testable by measuring phloretin-sensitive basolateral urea transport across the basolateral membrane in the cortical or outer medullary collecting duct.

We thank Inger Kristoffersen and Trine Møller for expert technical assistance.

This work was supported by the intramural budget of the National Heart, Lung, and Blood Institute (C. Echevarria, J. Terris, and M. A. Knepper) and by National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Grant DK-11489 (M. Echevarria and G. Frindt), as well as by the Danish Research Academy, the Novo Nordic Foundation, the Danish Medical Council, the University of Aarhus Research Foundation, the Danish Foundation for the Advancement of Medical Science, the Biomembrane Research Center, and the John and Birthe Meyer Foundation (D. Marples and S. Nielsen). C. A. Echevarria was supported by NIDDK Research Service Award DK-08832.

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Received 20 March 1995; accepted in final form 19 May 1995.

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