Long-term regulation of four renal aquaporins in rats

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Laboratory of Kidney and Electrolyte Metabolism, National Heart, Lung, and Blood Institute, Bethesda 20892; Department of Physiology, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814; and Department of Cell Biology, Institute of Anatomy, University of Aarhus, DK-8000 Aarhus, Denmark

Terris, James, Carolyn A. Ecelbarger, Soren Nielsen, and Mark A. Knepper. Long-term regulation of four renal aquaporins in rats. Am. J. Physiol. 271 (Renal Fluid Electrolyte Physiol. 40): F414–F422, 1996.—The aquaporins are molecular water channels expressed in the kidney and other organs. To investigate long-term regulation of renal expression of these water channels, we carried out immunoblotting studies using membrane fractions from rat renal cortex and medulla. Both 48-h water restriction in Sprague-Dawley rats and 5-day arginine vasopressin (AVP) infusion in Brattleboro rats caused significant increases in the expression levels of two aquaporins, aquaporin-2 and aquaporin-3, while the levels of aquaporin-1 and aquaporin-4 were unchanged. The increases in aquaporin-2 and aquaporin-3 expression were seen in inner and outer medulla as well as cortex. Ablation of the corticomedullary interstitial osmotic gradient with an infusion of furosemide did not eliminate the upregulatory response to AVP infusion in Brattleboro rats. Furthermore, 5-day furosemide infusion to Sprague-Dawley rats did not decrease expression levels of the collecting duct aquaporins, but rather increased them. We conclude that the expression of aquaporin-2 and aquaporin-3, but not aquaporin-1 or aquaporin-4, is increased in response to elevated circulating AVP. Because regulation of aquaporin-2 and aquaporin-3 levels was observed in the cortex and because osmotic gradient ablation did not abrogate the increase, we conclude that changes in interstitial osmolality are not necessary for the AVP-induced upregulation of aquaporin-2 and aquaporin-3 expression.

REGULATION OF OSMOTIC WATER transport across the renal collecting duct epithelium depends on at least two independent mechanisms that manifest themselves over different time scales. 1) Short-term regulation of collecting duct water permeability is a consequence of the rapid action of arginine vasopressin (AVP) to increase the water permeability of the apical plasma membrane of collecting duct cells by triggering the insertion of aquaporin-2 water channels from an intracellular vesicular pool (23, 24, 29, 36). This short-term response to AVP is complete within 30–40 min of exposure to the peptide (26, 35). The short-term effect of AVP may also be partially dependent on adenosine 3′,5′-cyclic monophosphate (cAMP)-dependent phosphorylation of aquaporin-2 (19). 2) Long-term regulation of collecting duct water permeability is manifested as a stable increase in collecting duct water permeability after a restriction in water intake for 24 h or more (8, 10, 20).

The primary goal of the present study is to investigate long-term regulation of expression of renal aquaporin water channels as a possible factor in the long-term regulation of collecting duct water permeability. Previous studies using immunoblotting revealed that the level of aquaporin-2 expression was markedly increased in the renal inner medulla in response to fluid restriction of rats (25). Immunocytochemical observations suggested that the increase in aquaporin-2 abundance occurred in both the plasma membrane and in intracellular vesicles. Studies using Northern blotting have demonstrated an associated increase in aquaporin-2 mRNA in the inner medullas of water-restricted rats (22, 37). These studies, therefore, provided strong evidence that the long-term upregulation of water permeability in the rat inner medullary collecting duct in response to water restriction was due, at least in part, to increased expression of the aquaporin-2 water channel. Subsequently, DiGiovanni et al. (4) demonstrated in the Brattleboro rat that infusion of AVP for 5 days induced a threefold increase in inner medullary aquaporin-2 expression associated with a proportionate threefold increase in osmotic water permeability of isolated perfused inner medullary collecting ducts. These results further supported the view that long-term regulation of collecting duct water permeability is due to changes in aquaporin-2 expression. Based on these studies, two possible mechanisms for upregulation of aquaporin-2 expression in response to increased circulating levels of AVP were proposed as follows. 1) AVP may act directly on the collecting duct cells to increase aquaporin-2 expression, possibly as a result of cAMP-induced increases in transcription of the aquaporin-2 gene. 2) The increase in aquaporin-2 expression could be a result of the high osmolality or ionic strength of the interstitial fluid during antidiuresis and thus could be an indirect response to high AVP levels. Obviously, these two possibilities are not mutually exclusive.

In addition to aquaporin-2, three additional members of the aquaporin family of water channels have been found to be expressed in the kidney, namely, aquaporin-1 (3, 27, 30), aquaporin-3 (5, 6, 14, 21), and aquaporin-4 (11, 16, 33). Among these, aquaporin-3 and aquaporin-4 have both been found to be present in the basolateral plasma membrane of collecting duct cells (5, 9, 14, 21, 33) and thus could conceivably be involved in the long-term regulation of collecting duct water permeability. In fact, we have recently demonstrated that the inner medullary abundance of aquaporin-3 (5), but not aquaporin-4 (33), increases in response to 48-h fluid restriction in rats. The remaining renal aquaporin, aquaporin-1 (or CHIP28), is expressed in the descending limb of Henle’s loop and in the proximal tubule (3, 27, 30) but not in the collecting duct (27).
The objectives of the present study were 1) to determine which of the three collecting duct water channels display increases in expression level in response to long-term increases in circulating AVP and 2) to determine whether the effect of AVP on water channel expression is dependent on increased interstitial tonicity.

METHODS

Animals. The animals used for these studies were pathogen-free male Sprague-Dawley rats (Taconic Farms, Germantown, NY) or male Brattleboro homozygous (di/di; Harlan-Sprague-Dawley, Indianapolis, IN) as indicated. Weights of control and experimental animals were carefully matched in each protocol, and all were equilibrated and maintained on an ad libitum intake of a diet containing a moderate level of sodium chloride (NHI 31-autoclavable rodent diet, 130 meq Na+/kg; Ziegler Bros., Gardner, PA). The rats were kept in filter-top microisolators with autoclaved feed and bedding, to maintain a pathogen-free state, or metabolic cages, as required.

Antibodies. Antibodies to aquaporin-2 (4), aquaporin-3 (5), and aquaporin 4 (33) were characterized previously. All polyclonal antibodies were raised to synthetic peptides (18–24 amino acids) corresponding to a region in the carboxyl termini of the respective water channels and were affinity purified using columns on which the immunizing peptides were immobilized (Immunopure Ag/Ab immobilization kit no. 2; Pierce, Rockford, IL). For this study, we prepared a new antibody against aquaporin-1 using the carboxyl terminal sequence for rat aquaporin-1 (2), HLN-CGGYVEYLDADDING-SRVEKMKPK-COOH. (Note the addition of a cysteine residue at the amino terminal end of the peptide to facilitate conjugation). The peptide was purified by high-performance liquid chromatography and conjugated to a carrier protein (keyhole limpet hemocyanin) via the added amino-terminal cysteine, and rabbits were immunized with the conjugate. Antiserum from a rabbit (LL266) with an enzyme-linked immunosorbent assay titer of greater than 1:32,000 was utilized for all experiments reported here. Labeling with the anti-aquaporin-1 antibody on immunoblots (see RESULTS) and immunocytochemistry (not shown) was identical to that seen in rat tissues with a previously characterized antibody raised against purified human aquaporin-1 (27).

Animal treatments. Four studies were performed to examine the long-term regulation of expression of aquaporins-1, -2, -3, and -4 in rat kidney. The first study, modified slightly from that of Lankford et al. (20), was designed to assess the effect of a 48-h water restriction on male, Sprague-Dawley rats (320–370 g). Initially, all 12 rats received sweetened water (600 mM sucrose) as the sole drinking fluid for a 48-h equilibration period. A moderate water diuresis developed, which was the control state for all animals. Following this equilibration period, six rats were thirsted for 48 h and six rats (hydrated controls) continued to receive the sweetened water for the same 48-h period.

The second study addressed the effects of a 5-day infusion of AVP (Sigma Chemical, St. Louis, MO) to Brattleboro homozygous (di/di) (150–160 g) rats. Under light methoxyflurane anesthesia, the rats were implanted with osmotic minipumps (model 2002, Alza, Palo Alto, CA). Six rats received AVP (2.52 pg/day) dissolved in 5% dextrose-0.05% acetic acid vehicle solution, and the remaining six rats received the vehicle only. This dose rate was chosen to yield plasma AVP levels approximately equivalent to the level

needed for maximal stimulation of osmotic water permeability in isolated perfused collecting ducts, i.e., 100 pM (32).

The third study was designed to evaluate the effects of a 5-day infusion of AVP in the setting of chronic furosemide treatment, which was utilized to decrease the corticomedullary interstitial osmotic gradient. All 12 Brattleboro rats (150–160 g) were implanted with osmotic minipumps (model 2M1.1; Alza) that delivered furosemide (Sigma Chemical) at a rate of 7.2 mg-100 g body wt-1-day-1 following a procedure originally reported by Kaisling and Stanton (17). The furosemide was dissolved in 0.9% sodium chloride and adjusted to pH 9–10 with 10 N sodium hydroxide. In addition to the furosemide, six rats were infused with 2.52 µg AVP/day by separate minipumps. Volume depletion was prevented by giving the rats a drinking solution containing 0.8% sodium chloride and 0.1% potassium chloride.

The fourth study examined the effects of a 5-day infusion of furosemide to Sprague-Dawley rats (140–160 g) in the setting of high levels of circulating AVP. All rats were infused with AVP (2.52 µg AVP/day by minipump) as described above. Six of these rats were also infused with furosemide (7.2 µg 100 g-1-day-1 by minipump) as described above. As with the previous study using furosemide, 0.8% sodium chloride and 0.1% potassium chloride was given as drinking fluid.

In all studies, rats were maintained in metabolism cages to allow urine collections. Urine osmolality was measured in the 24-h urine collected preceding death of the rats using a vapor-pressure osmometer (model 5100C; Wescor, Logan, UT). Animals were killed by decapitation, and trunk blood was collected into heparinized beakers for determination of AVP levels and serum sodium concentration. The kidneys were rapidly removed into an ice-cold isolation buffer solution. This solution contained 250 mM sucrose plus 10 mM triethanolamine (Callbiochem, La Jolla, CA) and was adjusted to pH 7.6 with 1 N NaOH. It also contained protease inhibitors (1 µg/ml leupeptin (Bachem California, Torrance, CA) and 0.1 mg/ml phenylmethylsulfonyl fluoride (United States Biochemical, Toledo, OH)). The inner medulla, outer medulla, and cortex were separated using sharp, curved, iris scissors. AVP levels were determined in serum samples by radioimmunoassay (Incstar, Stillwater, MN).

Membrane preparation and immunoblotting. Membrane fractions from each of the three renal regions were prepared as previously described (5, 23, 33). Briefly, tissues were homogenized in ice-cold isolation solution (composition described above). Homogenates were centrifuged at 1,000 g for 10 min. Supernatants were saved, and the pellets were rehomogenized and centrifuged again at 1,000 g. For aquaporin-1, -2, and -3, supernatants from the two centrifugations were combined and centrifuged at 200,000 g. The resulting pellet contained plasma membranes and intracellular vesicles (5, 23). For aquaporin-4 determinations in the inner medulla, a sample of the 1,000 g supernatant was spun at 4,000 g, and this pellet was used for blots. Previous studies had established that virtually all of the aquaporin-4 in the inner medulla is present in this fraction (33). Inner and outer medullary pellets were resuspended in 100 µl of isolation solution, and cortical pellets were resuspended in 1,000 µl. Total protein concentration was determined with a Pierce bicinchoninic acid protein assay reagent kit. A quantity of 5× Laemmli buffer (7.5% sodium dodecyl sulfate, 30% glycerol, 50 mM tris(hydroxymethyl)aminomethane, pH 6.8, bromophenol blue) with 30 mg/ml dithiothreitol was added to the samples in a ratio of 1:4, which were then heated to 60°C for 15 min. Electrophoresis was performed on minigels of 12% polyacrylamide, and the proteins were transferred electrophotically to nitrocellulose membranes. After blocking with
Results

Effect of thirsting on aquaporin levels in kidney regions. In the first experiment, we compared water-loaded rats with rats that were thirsted for 48 h. Urine osmolality in the hydrated rats averaged 642 ± 161 compared with 1,903 ± 478 mosmol/kgH2O in the thirsted rats (P < 0.0001). As expected, the plasma AVP levels increased in response to thirsting (hydrated, 2.9 ± 1.8; thirsted, 16 ± 1.8 pg/ml; P < 0.001).

Figure 1 shows Western blotting results for aquaporin-2 in inner medulla, outer medulla, and cortex. In all three regions, two bands were observed as demonstrated previously (25), i.e., one sharp band at 29 kDa representing the nonglycosylated protein and one broad band at ~35 kDa representing the mature glycosylated protein. As we have demonstrated before (25), there was a striking upregulation of aquaporin-2 expression in inner medulla in response to thirsting (Fig. 1, top). In addition, there was also a marked increase in aquaporin-2 expression in outer medulla and cortex (Fig. 1, middle and bottom). Because the response was seen in the cortex where interstitial osmolality varies very little, we conclude that the increase in aquaporin-2 expression is not dependent on a large increase in local interstitial osmolality.

Figure 2 shows results in the same animals using the anti-aquaporin-1 antibody. Again, as with aquaporin-2, there are two bands, one at 28 kDa and a second broad band at ~35 kDa, as shown previously with another antibody (27). In contrast to aquaporin-2, there was no detectable increase in aquaporin-1 expression in any of the three regions with thirsting.

Figure 3 shows results in the same animals using the anti-aquaporin-3 antibody. As we have previously reported (5), thirsting increased the expression level of aquaporin-3 in the rat inner medulla (Fig. 3, top). Furthermore, thirsting also significantly increased the expression of aquaporin-3 in outer medulla and cortex (Fig. 3, middle and bottom), just as was seen for aquaporin-2. The fractional increase in aquaporin-3 expression appeared to be most pronounced in the outer medulla and cortex. Finally, aquaporin-4, which is detectable only in the inner medulla with this technique, was not changed in the thirsted rats (Fig. 4).

Figure 5 presents quantitation of the changes in expression levels seen in response to thirsting, as determined by densitometry. Thirsting significantly increased the expression of aquaporin-2 and -3, but not that of aquaporin-1 or -4. Subsequent results will be given in the format shown in Fig. 5, rather than showing individual immunoblots.

Effect of AVP infusion on aquaporin levels in kidney regions. In the next experiment, we used Brattleboro rats, which manifest central diabetes insipidus. One group was infused with AVP for 5 days via osmotic

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Fig. 1. Immunoblots of aquaporin-2 in inner medulla, outer medulla, and cortex of hydrated and thirsted Sprague-Dawley rats. Quantities of 1, 3, and 10 μg of total protein were loaded onto the gel per lane for inner medulla, outer medulla, and cortex, respectively. Initially all 12 rats were given 600 mM sucrose as the sole drinking fluid for 48 h. Following this equilibration period, one-half of the animals were deprived of drinking water for the next 48 h while the remaining 6 continued to receive the sucrose water. In all 3 regions a mature band appears at 35 kDa and a nonglycosylated band at 29 kDa.

Fig. 2. Immunoblot of aquaporin-1 in inner medulla, outer medulla, and cortex of hydrated and thirsted Sprague-Dawley rats. Quantities of 3, 2, and 1 μg total protein were loaded onto the gel per lane for inner medulla, outer medulla, and cortex, respectively. Membrane samples were obtained from the same rats as those used for Fig. 1.

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5 g/dl nonfat dry milk for 30 min, the membranes were probed with affinity-purified antibodies to the respective aquaporins for 24 h at 4°C, washed, and exposed to secondary antibody (donkey anti-rabbit immunoglobulin G conjugated with horseradish peroxidase, Pierce no. 31458, diluted 1:5,000) for 1 h at room temperature. Sites of antibody-antigen reaction were visualized using luminol-based enhanced chemiluminescence (LumiGLO; Kirkegaard and Perry Laboratories, Gaithersburg, MD). The blots were quantitated by densitometry (Molecular Dynamics model PDS1-P90, ImageQuaNT v4.2 software).

For all experiments, control minigels were run prior to Western blotting and were Coomassie stained to confirm equality of loading in each lane. Several representative bands were quantified by densitometry to assure equality of loading.

Statistics. The data are reported as means ± SE. Statistical comparisons were made by use of unpaired Student's t-test.
Fig. 3. Immunoblot of aquaporin-3 in inner medulla, outer medulla, and cortex of hydrated and thirsted Sprague-Dawley rats. Quantities of 5.5, and 10 µg of total protein were loaded onto the gel per lane for inner medulla, outer medulla, and cortex, respectively. Membrane samples were obtained from the same rats as those used for Fig. 1.

Fig. 4. Immunoblot of aquaporin-4 in inner medulla of hydrated and thirsted Sprague-Dawley rats. A quantity of 10 µg of total protein was loaded onto the gel per lane. Membrane samples were obtained from the same rats as those used for Fig. 1.

We investigated the response to AVP in Brattleboro rats that were simultaneously infused with furosemide to prevent the maintenance of a normal interstitial osmotic gradient. All rats were infused with furosemide by minipump. Half of the furosemide-infused rats received AVP by minipump, and the other half received vehicle. Furosemide infusion prevented the large increase in urinary osmolality normally seen with AVP, although the difference was still significant (vehicle, 285 ± 19; AVP, 559 ± 20 mosmol/kg; P < 0.0001). AVP levels in the AVP-infused animals averaged 8.14 ± 1.9 pg/ml, and AVP was undetectable in controls.

Figure 7 shows the percent change in the levels of each of the four renal aquaporins in response to AVP infusion. As shown in Fig. 7A, furosemide infusion did not prevent the increase in aquaporin-2 expression caused by AVP infusion. Large increases in aquaporin-2 levels were seen in all three regions, as well as in inner medulla and outer medulla for aquaporin-3 (Fig. 7B). Furthermore, as was seen in the absence of furosemide infusion, AVP infusion did not alter the expression of either aquaporin-4 (Fig. 7C) or aquaporin-1 (Fig. 7D).

Effect of long-term furosemide infusion on aquaporin levels in a setting of chronic AVP infusion. The last experiment was done to test whether furosemide infusion decreases the expression of any of the aquaporins. If any of the aquaporins are upregulated by interstitial hypertonicity, we would expect furosemide infusion to decrease the expression in the renal medulla. In these experiments, Sprague-Dawley rats received an infusion of AVP, and one-half of this group received in addition a 5-day infusion of furosemide. As expected, the urinary osmolality was markedly reduced by furosemide (AVP alone, 1,707 ± 118; AVP plus furosemide, 606 ± 47 mosmol/kg; P < 0.0001). AVP levels were not significantly different (AVP alone, 246 ± 20; AVP plus furosemide, 378 ± 51 pg/ml).

As illustrated in Fig. 8, instead of decreasing in response to furosemide, the expression level of all three of the collecting duct aquaporins (aquaporin-2, -3, and -4) increased in response to furosemide. We speculate that a portion of this increase could have been due to the general hypertrophic effect of furosemide on the collecting ducts and connecting tubules, as demonstrated by others (17), which would be expected to increase the levels of collecting duct-specific proteins relative to total protein levels. Consistent with this, the expression level of aquaporin-1, which is expressed upstream from the site of action of furosemide, was either decreased or was unchanged in the three regions.

DISCUSSION

The studies reported here have demonstrated that the expression levels of aquaporin-2 and aquaporin-3 increase in the kidney in response to elevated circulating AVP. In contrast, the levels of aquaporin-4 and aquaporin-1 were unaffected, indicating that the adaptation was selective for aquaporin-2 and -3. Because the upregulation of aquaporin-2 and aquaporin-3 expression occurred in the cortex as well as the medulla in
Fig. 5. Change in aquaporin-2 (A), aquaporin-3 (B), aquaporin-4 (C), and aquaporin-1 (D) water channel expression in inner medulla (IM), outer medulla (OM), and cortex (COR) of hydrated and thirsted Sprague-Dawley rats, as determined by densitometry of Western blots. Values are expressed as percent change (means ± SE) of thirsted animals relative to the mean for the hydrated controls. *Statistically significant (P < 0.05). Expression levels of aquaporin-4 in outer medulla and cortex were too low to be quantitated (NQ).

response to thirsting, we conclude that a large increase in interstitial tonicity is not a prerequisite for the response to AVP. Furthermore, the failure of furosemide infusion to prevent the increase in aquaporin-2 and -3 expression in response to AVP also supports the notion that the AVP-induced increase in expression levels of these two water channels does not require a large increase in local interstitial osmolality. Thus we conclude that the upregulatory action of AVP on aquaporin-2 and aquaporin-3 expression may be a direct

Fig. 6. Change in aquaporin-2 (A), aquaporin-3 (B), aquaporin-4 (C), and aquaporin-1 (D) water channel expression in inner medulla (IM), outer medulla (OM), and cortex (COR) in response to arginine vasopressin (AVP) infusion in Brattleboro rats, as determined by densitometry of Western blots. Values are expressed as percent change (means ± SE) relative to mean for vehicle-infused controls. *Statistically significant (P < 0.05). Expression levels of aquaporin-4 in outer medulla and cortex were too low to be quantitated (NQ).
Regulation of Renal Aquaporins in Rats

Effect of the hormone on collecting duct cells rather than an indirect effect of medullary hypertonicity. In the remainder of the discussion, we discuss these observations in greater detail in the context of the foregoing literature.

It has been recognized for many years that long-term restriction of fluid intake in humans results in an increase in the maximal urinary osmolality attainable in response to injections of vasopressin (7, 15). Based on studies in rats (4, 20), we have proposed that this long...
term enhancement of urinary concentrating ability is due in part to a "conditioned" increase in intrinsic water permeability of the renal collecting ducts. These studies demonstrated that fluid restriction for 24 h or more in normal rats (20) or long-term infusion of AVP in Brattleboro rats (4) increases the intrinsic water permeability of the renal collecting ducts even after the collecting ducts are removed from the short-term influence of AVP. In previous studies (4, 25), we have demonstrated that the increase in water permeability is associated with a marked increase in the abundance of aquaporin-2 in the collecting duct cells. In Brattleboro rats, the maximum stimulated water permeability of perfused inner medullary collecting duct segments was markedly enhanced after long-term AVP infusion in the rats, suggesting that these collecting ducts achieve a higher water permeability in vivo (4). Presumably, the larger number of water channels in the collecting duct cells allows more water channels to be translocated to the apical plasma membrane in response to an acute rise in intracellular CAMP, thus accounting for the higher water permeability. More recently, we have demonstrated that the expression level of aquaporin-3 is upregulated in the renal inner medulla in response to restriction of fluid intake in rats (5).

In the present study, we have confirmed and extended the prior results. Restriction of fluid intake resulted in an increase in the abundance of aquaporin-2 and aquaporin-3 in all three regions of the kidney (Figs. 1, 3, and 5). The abundance of these two water channels also increased in response to AVP infusion in Brattleboro rats (Fig. 6). In contrast, the expression levels of neither aquaporin-1 nor -4 were affected by fluid restriction or AVP infusion. Thus, the effects of water restriction and increased AVP levels to increase water channel expression appear to be selective for aquaporin-2 and -3.

We investigated the stimulus for the increase in aquaporin-2 and -3 water channel expression. First, since the upregulatory response to thirsting was seen not only in the medulla but also in the cortex, where interstitial osmolality is maintained close to that of the general circulation (18), we conclude that the upregulatory response does not require a large increase in local interstitial osmolality. Furthermore, the increase in aquaporin-2 and -3 expression in response to AVP was not prevented by washout of the corticomedullary osmolality gradient by infusion of furosemide (Fig. 7). This observation adds further support to the view that the effect of AVP on aquaporin expression is not exclusively dependent on its effect to increase medullary interstitial osmolality. Based on these observations, we believe that the effect of water restriction and AVP infusion to increase aquaporin-2 and -3 expression in these studies is probably mediated predominantly by a direct effect of AVP on the collecting duct cells rather than through an alteration in medullary osmolality. However, it must be pointed out that these studies do not rule out the possibility that changes in interstitial osmolality could have an independent effect on water channel expression.

Although it is conceivable that regulation of aquaporin-2 and -3 levels can occur by a direct effect on translation or degradation of these proteins, it is most likely that the effects observed here are a result of changes in aquaporin-2 and -3 mRNA levels. In fact, thirsting has been reported to increase mRNA levels for aquaporin-2 (22, 37) in rats. Whether the thirsting-induced increases in aquaporin-2 and -3 mRNA levels are due to increased transcription rates or to increased mRNA stability has not been investigated. Cloning of the 5'-flanking region of the aquaporin-2 gene has revealed the presence of a CAMP-response element (CRE), which could play a role in the AVP-induced increase in aquaporin-2 expression (34). In contrast, no CRE has been identified in the upstream regulatory region of the aquaporin-3 gene (13). However, the aquaporin-3 gene does contain Sp1 and AP2 cis-regulatory elements, which have been associated with CAMP-mediated transcriptional regulation (1, 12, 28, 31).

One byproduct of these investigations is the observation that the degree of glycosylation of aquaporin-2 differs in the medulla and the cortex (Fig. 1). That is, a much greater fraction of the total amount of aquaporin-2 appears to be glycosylated in the inner medulla than in the cortex. In addition, as shown in Fig. 2, a large difference in glycosylation of aquaporin-1 is seen between cortex and medulla. However, with this water channel, the band corresponding to the glycosylated form is most abundant in the cortex. Neither the mechanistic basis nor the functional significance of these differences are ascertainable from the present studies.

An additional finding of this study was that furosemide infusion for 5 days caused a striking increase in abundance of all three of the collecting duct aquaporins (aquaporin-2, -3, and -4) (Fig. 8). This finding is probably attributable to the fact that chronic furosemide infusion causes marked hypertrophy of the renal collecting duct as well as other segments downstream from the site of furosemide action in the thick ascending limb (17). In contrast, chronic furosemide infusion did not increase the abundance of aquaporin-1, which is expressed in the proximal tubule and in the descending limb, i.e., at a site upstream from the thick ascending limb, which does not hypertrophy in response to chronic furosemide infusion.

Based on these studies, we suggest that the long-term regulation of aquaporin-2 and -3 expression in the renal collecting duct may play a role in the overall regulation in concentrating ability in the mammalian kidney. We have also observed that a long-term deficiency in aquaporin-2 expression as seen in the Brattleboro rat is associated with a marked downregulation of collecting duct water permeability (4). Consequently, clinical situations manifesting chronically low levels of AVP, such as compulsive water drinking and
central diabetes insipidus, can be expected to be accompanied by a “physiological nephrogenic diabetes insipidus” due to downregulation of water channel expression, which delays correction of the associated water balance disorder until the downregulation is corrected. Further studies will be required to definitively test this possibility.

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