Urea and urine concentrating ability in mice lacking AQP1 and AQP3

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Submitted 13 January 2006; accepted in final form 24 February 2006

Zhao, Dan, Lise Bankir, Liman Qian, Dayu Yang, and Baoxue Yang. Urea and urine concentrating ability in mice lacking AQP1 and AQP3. Am J Physiol Renal Physiol 291: F429–F438, 2006. First published March 8, 2006; doi:10.1152/ajprenal.00011.2006.—Aquaporin-1 (AQP1) and aquaporin-3 (AQP3) water channels expressed in the kidney play a critical role in the urine concentrating mechanism. Mice with AQP1 or AQP3 deletion have a urinary concentrating defect. To better characterize this defect, we studied the influence of an acute urea load (300 μmol ip) in conscious AQP1-null, AQP3-null, and wild-type mice. Urine was collected and assayed every 2 h, from 2 h before (baseline) to 8 h after the urea load. Mice of all genotypes excreted the urea load in ~4 h with the same time course. Interestingly, despite their low baseline, the AQP3-null mice raised their urine osmolality and urea concentration progressively after the urea load to values almost equal to those in wild-type mice at 8 h. In contrast, urine volume fell in the last 4 h to about one-fourth of basal values. AQP1-null mice increased their urine flow rate much more than AQP3-null mice and showed no change in urine osmolality and urea concentration. The urea load strongly upregulated urea transporter UT-A3 expression in all three genotypes. These observations show that the lack of AQP3 does not interfere with the ability of the kidney to concentrate urea but impairs its ability to concentrate other solutes. This solute-selective response could result from the capacity of AQP3 to transport not only water but also urea. The results suggest a novel role for AQP3 in non-urea solute concentration in the urine.

transgenic mouse; vasopressin; urea transport; water transport; plasma urea

AT LEAST EIGHT FACILITATED WATER channel proteins, named aquaporins (AQPs), are expressed in the kidney and their localization has been studied in detail at the suborgan, cellular, and subcellular level (1, 24, 35, 36). Those that are expressed in plasma membranes of selected nephron segments and in vasa recta play a significant role in the urinary concentrating mechanism. AQP1 is expressed in the apical and basolateral plasma membranes in the proximal tubule and thin descending limb of Henle’s loop and in the endothelium of descending vasa recta throughout the medulla. AQP2 is expressed in principal cells of the entire collecting duct and undergoes vasopressin-regulated trafficking between subapical membrane vesicles and the apical plasma membrane, thus regulating water permeability of the luminal membrane in these cells. AQP3 and AQP4 are both expressed in the basolateral membrane of principal cells of the collecting duct and allow water exit from these cells. AQP4 is restricted to the inner medullary portion of the collecting duct. In contrast, AQP3 is expressed along the entire collecting duct in the cortex, outer, and inner medulla, with a maximum in the outer half of the inner medulla, and almost no expression at the tip of the papilla (9).

In addition, AQP3 is also expressed in the basilar cell layer of the ureter and bladder urothelium (32).

Deletion of AQP1 or AQP3 results in a severe urinary concentrating defect in basal conditions, but intriguingly, these two mouse models respond very differently to dehydration or dDAVP injection (21, 22). While AQP1-null mice are totally unresponsive to these stimuli, mice with AQP3 deletion, which exhibit a more severe concentrating defect in basal conditions, are able to increase markedly their urine osmolality to about one-third of that of wild-type mice (21).

Urea plays an important role in the urinary concentrating mechanism. It is the dominant urinary solute in carnivores and omnivores, and its concentration in urine is far higher than in the blood so that a large fraction of the concentrating effort of the kidney serves to concentrate urea. Several facilitated urea transporters (UTs) are highly expressed in the kidney (3, 6, 28, 29) and have been shown to play a significant role in the urinary concentrating mechanism, especially for countercurrent exchange of urea in the vasa recta and for delivery of concentrated urea at the tip of the papilla by the terminal collecting duct (6, 23). The deletion of UT-B or UT-A1/3 results in defects in urine concentrating ability (5, 12, 13, 37, 38).

Interestingly, urea has been shown to play a role in the concentration of other solutes in the urine (7, 8, 14). Several studies in dogs, rats, and humans have shown that an infusion of urea (within certain limits) or a change from low to high protein intake induces an improvement in urine concentrating ability that does not concern only urea but also “non-urea solutes” (11, 16, 25, 30, 33).

To better characterize the concentrating defect of AQP1- and AQP3-null mice, we studied their response to an acute urea load using the same experimental protocol as that recently designed for the study of UT-B-null mice (5). In addition, we studied the expression of the main water channels and urea transporters involved in countercurrent exchanges in the renal medulla and their changes in expression after the urea load. The results show a very different pattern of changes in urinary concentrating ability for urea and non-urea solutes in these two genotypes, and reveal a novel, unexpected role for AQP3 in the kidney. They also reveal a dramatic upregulation of UT-A3, one of the facilitated urea transporters expressed in the terminal inner medullary collecting duct.

METHODS

Mice. Adult wild-type mice and transgenic mice lacking AQP1 or AQP3 protein were used in this study. The transgenic mice in a CD1

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genetic background were bred in our laboratory from parent mice obtained by targeted gene disruption, as previously reported (21, 22). All animal procedures were approved by the University of California San Francisco Committee on Animal Research.

**Acute urea load.** Six female mice of each of the three genotypes (body wt 20–25 g) were adapted to metabolic cages for 2 days before the experiments. As previously described (5), mice were fed a limited amount of pasty food per day consisting of 4 g synthetic dry powdered food (AIN 93G, Bioserv, Frenchtown, NJ) mixed with 1.3 ml water/g dry food. Restriction to 4 g dry food/day ensures a similar food intake for all mice and is sufficient to maintain stable body weight. Drinking water was provided ad libitum during the whole study.

During the experiment, urine was collected every 2 h after spontaneous voiding and/or bladder massage. The lower part of the metabolic cages was replaced by a tray covered with Parafilm. At 8:00 AM, each mouse had its bladder emptied by gentle abdominal massage and this urine was discarded. Thereafter, Parafilm sheets were inspected every 10 min and any urine found on the Parafilm was collected and placed in preweighed tubes containing 100 μl of paraffin oil (to prevent evaporation). Every 2 h, bladder urine was collected by abdominal massage and added to the urine from the same mouse previously collected on the Parafilm sheet during the same 2-h period. Urine collected from 8:00 to 10:00 AM corresponded to a basal period. The urea load was administered just after the 10:00 AM urine collection, and urine was further collected in 2-h periods for 8 h. The urea load was injected intraperitoneally and consisted of 300 μmol of 1 mol/l urea containing 300 μmol of urea, which is one-tenth of the daily urea excretion in mice (5).

Urine volume was measured gravimetrically. Urine osmolality was measured by freezing-point depression (microosmometer, Precision Laboratory). Urine urea concentration was measured by a colorimetric method using a commercial kit (Roche Diagnostics, Indianapolis, IN). Urine osmolality and urea excretions were calculated as well as the concentration and excretion of non-urea solutes (total osmoles minus urea).

**Blood sampling.** In separate groups of female mice of the three genotypes, treated as those used for urine collection, blood (~20 μl) was collected from small incisions on the tail in heparinized hematocrit capillary tubes before and at 10, 30, 60 min, 2, 4, and 8 h after the urea load. Two sets of four to six mice were used for these seven time points (3–4 measurements in each mouse). The tubes were centrifuged, and urea concentration in plasma was measured as in urine.

**Fluorescence-based real-time RT-PCR.** Total RNA from the whole kidney was isolated by homogenization in TRIzol reagent (GIBCO BRL), and mRNA was extracted using an Oligotex mRNA minikit (Qiagen). cDNA was reverse-transcribed from mRNA with oligo(dT) (SuperScript II preamplification kit, BRL). Real-time PCR was carried out by using a LightCycler with a LightCycler FastStart DNA MasterPLUS SYBR Green I kit (Roche Diagnostics). Primers for UTs, AQP3, and β-actin were designed as previously reported (18). Primers for the V1a and V2 receptors were as follows: 5'- TCTATCGTC- CAGATGTTGGCAGTC-3' (sense) and 5'-GCTCTGGGACACAA- CCTGTAGGAG-3' (antisense) for the V1a receptor and 5'- TCTTCGCTCCTTCACGGCTG-3' (sense) and 5'-AATCCAGGTC- GCATAAGTGCG-3' (antisense) for the V2 receptor. Real-time PCR was carried out according to the manufacturer’s instructions, β-Actin was used as the reference gene, and pooled wild-type cDNA was used as the calibrator. PCR products were electrophoresed on a 2% agarose gel. Results are reported as calibrated ratios. All samples are normalized to the reference gene. Concentration ratios for each sample are then calibrated to calibrator samples so that quantification results are reported as a normalized ratio with the calibrator sample as the denominator: normalized ratio = ratio of sample (target/reference)/ratio of calibrator (target/reference).

**Western blot analysis.** Kidney tissue was homogenized in 250 mM sucrose containing 1 mM EDTA, 20 μg/ml PMSF, 1 μg/ml pepstatin A, and 1 μg/ml leupeptin (pH 7.4) with a glass Dounce homogenizer. Total protein was assayed using a Bio-Rad DC protein assay kit (Bio-Rad, Richmond, CA) and size-separated by SDS-PAGE using 10% polyacrylamide gels. Proteins were blotted to polyvinylidene difluoride membranes (Gelman Scientific, Ann Arbor, MI). Blots were incubated for 30 min at room temperature with blocking buffer [5% nonfat dry milk suspended in Tris-buffered saline (TBS; 20 mM Tris–HCl, 225 mM NaCl, pH 7.5)] and then with a 1:500 dilution of rabbit polyclonal serum raised against an NH2-terminal peptide (NH2- EEKDLRSSDEDIVKIEKPNER-COOH) or against a COOH-terminal peptide (NH2- QEKNRASMTKYQAYDVS-COOH) of mouse UT-A (generated by Abgent, San Diego, CA) or anti-UT-B (37). Blots were developed with an ECL plus kit (Amersham Biosciences). The National Institutes of Health Image program was used to quantify the intensity of the resulting bands. Parallel gels stained with Coomassie blue were done for confirmation of loading (data not shown).

**Immunofluorescence.** Kidney tissue samples were fixed with 4% paraformaldehyde in PBS for 4 h, infiltrated with 30% sucrose in PBS overnight, frozen in optimal cutting temperature compound with liquid nitrogen, and cut into 3-μm-thick sections using a cryostat. Immunostaining was done by standard procedures (21).

**Statistics.** All data are presented as means ± SE. Statistical significance of differences between each AQP-null genotype and wild-type mice was evaluated by Student’s t-test. Differences between post-urea periods and basal period in the same mice were evaluated by a paired t-test.

**RESULTS**

**Renal urea handling and plasma urea concentration.** During the basal period, AQP1- and AQP3-null mice concentrated their urine about three times less than wild-type mice. In previous studies, based on 24-h urine collection, the urine concentrating defect in AQP3-null mice was found to be more severe than that in AQP1-null mice (21), but this difference was not apparent in the present study when urine was collected for only 2 h in the morning. AQP1- and AQP3-null mice had similar urine outputs, osmolalities, and urea concentrations in the basal period (time 0 in Fig. 1, A–C). After administration of the hyperosmotic urea load, urea excretion increased abruptly during the first 2 h in all groups and decreased progressively thereafter, with a similar time course and intensity in mice of the three genotypes (Fig. 1D). However, this increase resulted from a very different pattern of changes in urine flow rate and urea concentration in the three groups. Wild-type mice increased their urea concentration and urine osmolality after the first post-load period and exhibited only a small and transient increase in urine flow rate, whereas AQP1-null mice increased their urine flow rate more intensely and showed no change in urine osmolality and urea concentration (Fig. 1, A–C). In AQP3-null mice, the increase in urine output was small and transient, with these mice being able to increase their urine urea concentration and osmolality to almost the same level as wild-type mice.

In the last 4 h, in wild-type mice, urine osmolality was higher and urine output lower than during the basal period (at 6 and 8 h in Fig. 1, A–C), which corresponds to the known ability of urea to improve the capacity of the kidney to concentrate urine. In wild-type mice, the concentration of both urea and non-urea solutes was influenced by this process. This improvement in urine concentration was absent in AQP1-null mice but strong in AQP3-null mice. The urine osmolality in AQP3-null mice was greatly enhanced and their urine output was markedly reduced in the last 4 h to values similar to those
seen in wild-type mice (Fig. 1, A and B). However, unlike wild-type mice, the concentration of non-urea solutes failed to increase, resulting in a progressive reduction of non-urea solute excretion from 78 ± 30 to 21 ± 10 μmol/2 h at 0 and 8 h, respectively (P < 0.02 by paired t-test) (Fig. 1, E and F). In the final urine collection (8 h), non-urea solutes represented only 11 ± 1% of total urinary solutes in AQP3-null mice vs. 21 ± 5% in wild-type mice (P = 0.05).

In AQP1-null mice, the excretion of non-urea solutes decreased from 39 ± 8 μmol/2 h during the basal period to 11 ± 7 μmol/2 h at 4 h but returned to basal values at 6 and 8 h (Fig. 1, E, F, and B). The transient fall occurred after the marked rise in urine flow rate and may thus be due to a temporary washout of solutes accumulated in the medulla.

Under basal conditions, plasma urea concentration was similar in AQP3-null mice to that in wild-type mice (9.2 ± 0.3 and 9.0 ± 0.6 mmol/l, respectively) but was lower in AQP1-null mice (5.9 ± 0.8 mmol/l, P < 0.02) (Fig. 2, time 0). After the urea load, plasma urea rose promptly, reached a peak at 30 min in all groups, and then returned to almost basal values after 4 h, in a parallel fashion in all mice genotypes. The magnitude of the rise from basal to peak level was also similar in the three groups (∼10 mmol/l), suggesting that urea was distributed in a roughly similar body fluid compartment in each genotype.

**Renal expression of urea transporters.** The effect of AQP1 and AQP3 deletion on UT expression was measured under basal conditions. In AQP1-null mice, Western blot analysis showed a reduction by ∼40–50% in protein abundance of the three UTs expressed in the nephron: UT-A1 and UT-A3 in the collecting duct and UT-A2 in thin limbs (Fig. 3, A and B). In contrast, the protein abundance of UT-B, the vasa recta/erythrocyte UT, was increased by 50%. In AQP3-null mice, the abundance of UT-A1, UT-A3, and UT-A2 proteins was also reduced (although not reaching statistical significance for UT-A1), but that of UT-B was unchanged. For the purpose of comparison in Fig. 3B, values in wild-type mice have been arbitrarily taken as unity, but it is interesting to note that UT-A3 protein was far more abundant than UT-A1 (Fig. 3C), as revealed by the anti-NH2-terminal UT-A1/3 antibody that binds to the same amino acid sequence in both UT-A1 and UT-A3 (34).
Under basal conditions, mRNA expression of the different UTs was altered in AQP-null mice in a similar fashion as the corresponding proteins (Fig. 4, time 0, open bars). All UT-A mRNA isoforms were reduced in AQP1- and AQP3-null mice, and UT-B mRNA abundance was enhanced only in AQP1-null mice. However, the magnitude of the differences was somewhat different. UT-A2 mRNA was reduced to a larger extent in AQP1- than in AQP3-null mice (to 110% and 75% of wild-type level, respectively). In AQP1-null mice, the reductions in UT-A2 mRNA and the elevation in UT-B mRNA were more intense than those in the corresponding proteins (compare Figs. 3 and 4).

The acute urea load induced very different changes in mRNA abundance of the four different UTs. A dramatic time-dependent 10-fold increase in UT-A3 mRNA was observed in all three genotypes. Contrasting with this intense response, the mRNA of UT-A1, the other collecting duct UT, did not change in wild-type mice and AQP1-null mice and increased by 30% in AQP3-null mice. The thin limb and vascular UTs either declined or did not change. In wild-type and AQP3-null mice, UT-A2 mRNA declined time dependently to less than one-fourth of basal but did not change in AQP1-null mice. UT-B mRNA did not change in wild-type and AQP3-null mice but fell modestly (by 20%) in AQP1-null mice.

To further characterize the increase in UT-A3 expression, the abundance of UT-A3 protein was evaluated by Western blotting before and 4 and 8 h after a urea load (Fig. 5). The protein abundance of UT-A3 was also increased in all three genotypes, although less intensely than the mRNA (2.8-fold in wild-type, 1.8-fold in AQP1-null mice, 2.0-fold in AQP3-null mice).

The increase in UT-A3 abundance could originate from a higher level of expression in the terminal inner medullary collecting duct or could also involve an extension of its expression toward an earlier portion of the medullary collecting duct. To evaluate this latter possibility, immunofluorescence studies were performed in wild-type mice before and 8 h after a hyperosmotic urea load. Because there is no UT-A3-specific antibody, we used two different antibodies, one raised against the NH2-terminal portion of UT-A proteins, which recognizes UT-A1 and UT-A3, and another raised against the COOH-terminal portion, which recognizes UT-A1 and UT-A2.

Under basal conditions (Fig. 6A and C), the first antibody strongly labeled the long thin papilla (Fig. 6A) and the second antibody weakly labeled the thin limbs in the outer medulla (Fig. 6C). Eight hours after the urea load, the labeling of the papilla with the NH2-terminal antibody was enhanced but did not extend to an earlier part of the collecting ducts, and the
labeling of the outer medulla with the COOH-terminal antibody was unchanged. At a higher magnification (Fig. 6, right), the NH2-terminal antibody revealed mainly intracytoplasmic vesicles and the intensity of this labeling was enhanced by the urea load.

Renal expression of AQPs and vasopressin receptors. The deletion of AQP1 or AQP3 induced some changes in the expression of other AQPs (Fig. 7, time 0, open bars). AQP1-null mice exhibited a 50% increase in AQP3 mRNA but no change in AQP2 or AQP4. AQP3-null mice showed a ~50% increase in AQP3 mRNA.
reduction in AQP2 mRNA and no change in AQP1 and AQP4. The acute urea load had little influence on AQP mRNA. It increased only modestly AQP2 in AQP3-null mice (at 4 and 8 h) and AQP3 in wild-type mice (only at 8 h). The urea load induced a 20–40% reduction in the abundance of V1a receptor mRNA in wild-type mice but did not change it significantly in AQP1- and AQP3-null mice (Fig. 8). The urea load induced an increase in V2 receptor mRNA in all three genotypes \( (P < 0.05 \text{ in AQP1-null mice and } P < 0.01 \text{ in the 2 other groups}) \), and the effect developed slowly (8 h after the load).

**DISCUSSION**

This study revealed marked differences in urea handling in mice with deletion of either AQP1 or AQP3, two models which have a concentrating defect under basal conditions. AQP1 deletion completely prevented any increase in urine osmolality after the administration of an exogenous urea load. In contrast, the same urea load induced a remarkable and prompt enhancement in urine osmolality in mice lacking AQP3. However, this improved ability to concentrate urine involved an imbalance in the proportion of urea and non-urea solutes in the urine. These results suggest a special role for AQP3 not only in water transport but also in urea transport in the collecting duct and lower urinary tract and shed new light on our understanding of the role of urea in the concentration of non-urea solutes. At the molecular level, this study shows a dramatic and selective adaptive response of the facilitated urea transporter UT-A3, independent of the operation of the urine concentrating mechanism.

Most previous studies concerning the mechanism by which urea improves the kidney’s ability to concentrate urine have been performed in larger mammals, usually under anesthesia and with constant intravenous infusion of isotonic saline. This protocol was difficult to adapt to mice because of their small size and low urine output. Moreover, any saline infusion would have perturbed the concentrating mechanism quantitatively (by bringing additional fluid) and qualitatively (by changing the proportion of urea and non-urea solutes in the urine). In the present study, the experiments were performed in conscious noninstrumented mice without prior treatment or saline infusion, and the urea load was given with as little fluid as possible. Thus they provide physiological results that apply to normal renal function in free-living mice.
The urea load was excreted with the same kinetics in wild-type mice and in mice with either AQP deletion. A similar kinetics was also observed previously in mice lacking the facilitated urea transporter UT-B (5). After 4 h, the urea excretion rate was back to or near pre-load values. The abrupt increase in plasma urea observed after the urea load peaked at 30 min and exhibited the same intensity in all three models. Plasma urea concentration then returned to the basal level with the same time course in all genotypes. Taken together, these results suggest that the ability of the kidney to excrete an additional load of urea is not influenced by the operation of the urine concentrating mechanism. It probably depends on changes in plasma urea level and on the resulting influence on urea filtration and secretion, a secretion that is now recognized to take place to a significant extent in mice (13, 37). Note that it may not be the same when urea excretion increases after protein ingestion because amino acids that result from protein breakdown are known to stimulate the secretion of hormones that have their own action on kidney function, and because the increase in plasma urea level is much less intense and abrupt (6).
In all three mice models, the transient increase in plasma urea and the resulting increase in plasma osmolality probably induced an increase in vasopressin secretion for the following reason. As previously underlined, the amount of urea administered acutely was relatively small compared with that excreted daily, but it was relatively large compared with the whole body urea pool (5, 36). Assuming this extra urea was mixed instantaneously with the body pool of urea, it should have increased plasma urea concentration about threefold and plasma osmolality by \( \sim 18 \text{ mosmol/l} \) (5). Even if urea is a less efficient osmole than sodium for stimulating vasopressin secretion (39), a significant rise in plasma vasopressin can be expected to occur in response to this osmotic load.

Thus after the urea load, the kidneys of all mice were probably subjected to a comparable increase in urea availability and in vasopressin-dependent increases in water and urea permeabilities in the collecting duct, mediated by AQP2 and UT-A1, respectively. Despite these similarities, marked differences were seen in the way the three mice models increased their urea excretion rate. Mice with AQP1 deletion did not exhibit any increase in urine urea concentration or osmolality, either during the excretion of the load itself (first 4 h) or after this excretion was completed (the last 4 h). In these mice, the increase in urea excretion was entirely accounted for by a marked increase in urine flow rate. After the urea load was almost fully excreted, the urine flow rate returned to basal values and there was no improvement in either urea or non-urea solute concentrations. The lack of facilitated water permeability in the proximal tubules, thin descending limb of Henle’s loops, vasa recta, and red blood cells thus totally prevented the kidney from making use of urea to improve urine concentration. Countercurrent exchange in the renal medulla and possibly a lower delivery of fluid to the loops of Henle by the pars recta of the proximal tubule are thus critical for this well-known urea-induced improvement in urine concentrating ability.

In contrast to AQP1-null mice, AQP3-null mice increased their urine osmolality and urea concentration remarkably. This effect lasted and was even amplified after the urea load was fully excreted to the point that these two parameters became equal to those seen in wild-type mice, although the initial values during the basal period were three times lower in AQP3-null mice than in wild-type mice. These mice did not even excrete the volume of fluid administered with the urea load (300 µl). Water excretion (= urine flow rate) rose above the basal level by only 100 µl during the first 2 h and then declined below the basal level for the remaining 6 h. In the last 2 h, urine flow rate was only 25% of the basal level (in the same urine samples, total osmolar excretion was reduced to 65% of the basal level, i.e., much less than water excretion). Thus urea induced a much greater improvement in urine concentrating ability and in water conservation in AQP3-null mice than in wild-type mice. Intriguingly, the lack of AQP3 resulted in a remarkable improvement in the capacity to make use of urea for concentrating urine.

Another intriguing feature of AQP3-null mice is that they were able to remarkably concentrate urea in the urine, but not non-urea solutes. While urine osmolality and urea concentration increased markedly during the last 4 h in wild-type and AQP3-null mice, the concentration of non-urea solutes increased only in wild-type mice. Non-urea solutes became a significantly smaller fraction of total urinary solutes in the last 2 h in AQP3-null mice, suggesting that urea concentration occurred at the expense of that of other solutes, whereas in normal mice, urea and non-urea solute concentrations increased in parallel.

How could this unexpected phenotype of AQP3-null mice be explained? Because the improvement in urea concentration was greater in these mice than in wild-type mice, it may be assumed that the presence of AQP3 has some negative effect on urea concentration in the urine. In amphibian oocytes or in transfected cells, AQP3 has been shown to increase not only water permeability but also urea permeability (10, 17). It is conceivable that AQP3 in wild-type mice may contribute to some urea reabsorption along the collecting duct subsegments where it is expressed, thus reducing urea delivery to the tip of the papilla where vasopressin-sensitive UT-A1 is present. When the vasopressin level is increased and urine flow rate becomes relatively low, the contact time of tubular fluid with the apical membrane of the collecting duct may be long enough to allow fairly good equilibration of urea between the intracellular compartment of the principal cells and the lumen, where urea flows at a higher concentration than in the blood. Then, once in the cell, urea could diffuse in the extracellular fluid surrounding the collecting ducts via AQP3, and water could follow isosmotically because this AQP is permeable to both water and urea. This process can take place over a relatively long distance along the collecting duct because AQP3 is expressed in the cortex, the outer medulla, and the upper inner medulla (9). The consequence of this simultaneous exit of urea and water would be an increase in the concentration of all other (i.e., non-urea) solutes in the collecting duct lumen. This hypothesis appropriately explains the fact that, in mice lacking AQP3, the concentration of non-urea solutes was not improved by the urea load as it was in wild-type mice, whereas the concentration of urea itself was dramatically increased. It could also explain why urea concentration in cortex tissue and cortical interstitium is two to three times higher than in peripheral blood, an old finding that has remained unexplained (15, 26, 27).

Also intriguing is the fact that AQP3-null mice were able to concentrate urine after the urea load (present study) much better than they did after acute dDAVP administration or after water deprivation (21). The different duration of the experiments (8 h here vs. 1 h only for dDAVP and 36 h for water deprivation) might account, at least in part, for this difference. In addition, with increases in either an exogenous V2 receptor agonist or endogenous vasopressin, the proportion of the different solutes in the urine should not have been altered. In contrast, the urea load changed this proportion, making urea become a larger amount of the total osmoles. This extra urea may have been better concentrated in the urine because it was not reabsorbed in the collecting duct through AQP3 (as could have occurred in wild-type mice) and was thus delivered to the papilla through UT-A1, thus becoming available with a better efficiency for intrarenal urea recycling in vasa recta and thin limbs.

AQP3 is expressed not only in the collecting duct but also in the ureter and bladder (32). In vivo perfusion of the ureter and bladder in dogs showed that urea concentration in the perfused fluid (mimicking urine) fell significantly along these structures in an inverse flow-dependent way (greater flow with lower flow).
deserve to be underlined.

not be identified in the present study, a few observations treating mechanism to an extent that overrides the more subtle water transport due to the lack of AQP1 in earlier nephron null mice. After the urea load, the abundance of AQP2 mRNA was doubled, that of UT-A1 was increased by 30%, and that of UT-A3 dramatically enhanced (by 10-fold). In contrast, UT-A2 (expressed exclusively in thin descending limbs), which was already reduced in basal conditions compared with wild-type mice, was further decreased after the urea load. Interestingly, the abundance of the vasopressin V2 receptor mRNA, which was normal in basal conditions, was doubled after the urea load. Altogether, the concomitant rise in vasopressin V2 receptors, aquaporins, and urea transporters in the collecting duct probably contributed jointly to the marked improvement in urine concentrating ability seen in AQP3-null mice after the urea load. Similar increases in V2 receptors, aquaporins, and urea transporters in the collecting duct did not allow AQP1-null mice to concentrate their urine after the urea load, as was the case in AQP3-null mice, because the additional defects in water transport due to the lack of AQP1 in earlier nephron segments and vasa recta (see above) compromise the concentrating mechanism to an extent that overrides the more subtle regulations occurring in the collecting duct.

Although the factors initiating these molecular changes cannot be identified in the present study, a few observations deserve to be underlined. 1) These changes are molecule specific (some channels/transporters increase whereas others decrease); thus they cannot simply result from physicochemical alterations in plasma or medullary interstitial tissue composition induced by urea infusion. 2) The dramatic and prompt increase in UT-A3 mRNA is remarkable because it occurred in all models regardless of whether they had a normal osmotic medullary gradient (wild-type). Thus urea itself, rather than osmotic pressure, is probably the stimulus inducing UT-A3 upregulation. Actually, UT-A3 is expressed exclusively in the terminal inner medullary collecting duct at the tip of the papilla, the site in the kidney where urea is accumulated to the highest concentration. We observed in wild-type mice that the marked increase in UT-A3 expression induced by the urea load was not due to an extension of its expression in a longer portion of the collecting duct but was restricted to the same subsegment where it is expressed in normal conditions. 3) UT-A1 mRNA, which is expressed in the same collecting duct subsegment as UT-A3, was increased only very modestly. Because UT-A1 and UT-A3 share the same 5'-UTR (GenBank accession nos. AF366052 and AF258602), they should share the same promoter (2, 31). Thus the different magnitude of the change in their mRNA abundance should involve a posttranscriptional regulation at the level of the alternative splicing or mRNA degradation. 4) Finally, it may be interesting to note that, in AQP3-null mice, the AQP and UT that are expressed selectively in the vasa recta and red blood cells, namely, AQP1 and UT-B, were expressed at a normal level in basal conditions, despite the very high diuresis, and did not exhibit any change in abundance after the urea load and its resulting consequences for blood and tissue composition.

In conclusion, this study confirmed with another approach, the critical role of AQP1-mediated water permeability in the ability of the kidney to concentrate urine. More importantly, it revealed a unique behavior in mice with AQP3 deletion. These mice, which have nephrogenic diabetes insipidus in normal conditions, can actually concentrate urine to a high level when given a urea load but at the expense of a reduction in the excretion of other solutes. These findings suggest a novel role for AQP3 in solute-selective urine concentration. The capacity of urea to enhance the concentration of non-urea solutes may rest on AQP3 and its capacity to transport both urea and water. Studies of isolated perfused collecting ducts from AQP3-null mice and wild-type mice will be necessary to measure urea and water permeability in the absence or presence of vasopressin and to evaluate the extent to which urea reabsorption through AQP3 could enhance the concentration of non-urea solutes in the luminal fluid.

ACKNOWLEDGMENTS

The authors thank Dr. A. S. Verkman for a critical reading of the manuscript.

Table 1. Summary of molecular data in AQP3 mice

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<th>V1aR</th>
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Basal condition: AQP3-null mice compared with WT mice

After the urea load: comparison with basal condition

AQP3, aquaporin-3; UT-A1, UT-A2, UT-A3, UT-B: urea transporters; V1aR and V2R, vasopressin receptors; WT, wild-type. Horizontal lines indicate no difference between AQP3-null and WT mice or no change after urea load in AQP3-null mice. n.a., No data are available. *Data are from Ref. 21.
REFERENCES