Long-Term Regulation of Inner Medullary Collecting Duct Urea Transport in Rat

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Abstract. Facilitated urea transport is regulated acutely by arginine vasopressin (AVP) and hyperosmolality in rat terminal inner medullary collecting duct (IMCD). This study tested whether chronic diuresis or antidiuresis regulates facilitated urea transport. Basal and AVP-stimulated urea permeabilities ($P_{\text{urea}}$) were measured in perfused IMCD subsegments. Rats were made: (1) diuretic by giving them sugar water (with or without food) or furosemide; or (2) antidiuretic by water deprivation. They were then compared with untreated rats given food and water ad libitum. Terminal IMCD from untreated rats had a high basal $P_{\text{urea}}$ that was significantly increased by AVP. Diuresis significantly increased basal $P_{\text{urea}}$ in terminal IMCD in all five diuresis protocols. Water deprivation for 1 or 3 d had no effect on basal or AVP-stimulated $P_{\text{urea}}$ in the IMCD$_1$ subsegment of the terminal IMCD. In contrast, 3 d of water deprivation significantly increased both basal and AVP-stimulated $P_{\text{urea}}$ in the IMCD$_2$ subsegment; 1 d of water deprivation had no effect on basal or AVP-stimulated $P_{\text{urea}}$. Next, initial IMCD (IMCD$_1$) were studied. Initial IMCD from untreated rats had a low basal $P_{\text{urea}}$ that was not increased by AVP (10 nM). Water diuresis (with or without food) for 3 to 5 d had no effect on basal $P_{\text{urea}}$ but significantly increased AVP-stimulated $P_{\text{urea}}$. Furosemide diuresis and water diuresis for 1 or 7 d had no effect on either basal or AVP-stimulated $P_{\text{urea}}$ in initial IMCD. Water deprivation for 2 to 3 d, but not for 1 d, significantly increased basal $P_{\text{urea}}$ in initial IMCD, whereas water deprivation for 1 d increased AVP-stimulated $P_{\text{urea}}$. It is concluded that chronic changes in hydration cause heterogeneous changes in facilitated urea transport in rat IMCD subsegments. (J Am Soc Nephrol 9: 737-745, 1998)

The terminal inner medullary collecting duct (IMCD) has a facilitated urea transport process (1,2) that is stimulated acutely by arginine vasopressin (AVP) (2) and hypertonic NaCl (3). AVP also stimulates osmotic water permeability in the IMCD (2), which occurs through the AVP-regulated aquaporin-2 (AQP2) water channel (4). AVP regulates AQP2 acutely by regulating the shuttling of preformed AQP2 between intracellular vesicles and the apical plasma membrane (5–8), and long-term by varying the amount of AQP2 protein (5,9,10). Lankford et al. showed that terminal IMCD from rats that were deprived of water for 1 d had significantly higher values for basal (no AVP) osmotic water permeability than terminal IMCD from rats made water-diuretic by giving them sugar water for 1 d (11). However, Lankford et al. found no difference in basal urea permeability values in terminal IMCD from similarly treated rats (11). This result raises the question of whether AVP regulates the urea transporter long-term as it does for AQP2.

A cDNA (UT2) for an AVP-regulated urea transporter was cloned by You and colleagues from rabbit (12) and rat (13,14) inner medulla. Northern blots of rabbit or rat inner medullary mRNA, which are probed with a UT2 cDNA, reveal two transcripts: 4.0 (UT-A1) and 2.9 (UT-A2) kb (12–17). Shayakul and colleagues proposed that these two mRNA bands result from alternative splicing of a single gene, and they renamed the 4.0-kb mRNA “UT1” and reserved the name “UT2” for the 2.9-kb mRNA (14). The two mRNA differ at their 5’ end but share a common 3’ domain; the proteins encoded by these mRNA differ at their N-terminal but share a common C-terminal domain (14). Nielsen et al. prepared a polyclonal antibody to UT-A1 by immunizing rabbits with a polypeptide corresponding to this common C-terminal (18) and showed that the AVP-regulated urea transporter (VRUT) protein is present in the apical membrane and subapical vesicles in the rat IMCD.

Two studies have examined whether AVP regulates the urea transporter long-term by measuring the change in UT2/UT1 mRNA abundance in inner medullary mRNA obtained from hydrated or dehydrated rats (13,16). These two studies used different protocols for varying hydration: one study fed rats sugar water for 3 d to induce water diuresis or deprived rats of...
water for 3 d (13), whereas the second study added two different amounts of water to the rat chow for 5 wk to induce moderate or large changes in water intake, or administered deamino-8-oAVP for 5 wk to induce antidiuresis (16). Both studies found that the abundance of the 2.9-kb UT-A2 mRNA band, but not the 4.0-kb UT-A1 mRNA band, decreased after hydration and increased after dehydration, suggesting that VRUT protein and AVP-regulated urea transport are regulated long-term by AVP (13,16). Therefore, the purpose of this and the companion paper by Terris et al. (19) is to determine whether long-term changes (days) in hydration affects urea transport in perfused IMCD subsegments and/or VRUT protein in the inner medulla.

**Materials and Methods**

Pathogen-free male Sprague Dawley rats (National Cancer Institute, Frederick, MD) were kept in filter-top cages with autoclaved bedding throughout the study. The rats were fed a standard, 18% protein diet (NIH-31, Ziegler Brothers, Gardner, PA). Urine was collected immediately before animal sacrifice, and osmolality was measured using a vapor pressure osmometer (model 5500; Wescor, Logan, UT). Hematocrit was measured by collecting blood in heparinized microhematocrit capillary tubes (Fisher Scientific, Pittsburgh, PA) and spun in an Adams Autocrit centrifuge (model CT-2905; Clay Adams, Parsippany, NJ). Rats were weighed before and after altering their hydration status, using the following protocols:

- **Untreated (control):** Rats were given food and water *ad libitum* for 3 to 5 d.
- **Water Diuresis:** Rats were fed 10% glucose in water without additional food for 1, 3 to 5, or 7 d to induce a water diuresis. We combined the results from rats treated for 3, 4, or 5 d because there were no differences in the data obtained from these rats.
- **Water Diuresis + Food:** We showed previously that feeding rats a low protein diet for 2 wk increases urea permeability in the initial IMCD (20,21). To ensure that the results of the water diuresis protocol were due to water diuresis rather than to feeding the rats only glucose, additional animals were fed 10% glucose in water and given free access to the standard rat food for 3 to 5 d. We combined the results from rats treated for 3, 4, or 5 d because there were no differences in the data obtained from these rats.
- **Furosemide:** To compare the response to water diuresis to a second form of diuresis, a furosemide diuresis was induced by inserting a sustained-release furosemide pellet (pellet no. D-141, Innovative Research of America, Toledo, OH) subcutaneously into the backs of rats to administer 10 mg per day of furosemide for 3 to 5 d. During this period, rats received food and water *ad libitum*. We combined the results from rats treated for 3, 4, or 5 d because there were no differences in the data obtained from these rats.
- **Water Deprivation:** Rats were given free access to the standard diet but given no water for 1 or 2 to 3 d. We combined the results from rats treated for 2 or 3 d because there were no differences in the data obtained from these rats.

**Tissue Preparation for Tubule Microperfusion**

Twenty minutes before each experiment, furosemide (5 mg, intraperitoneally) was administered to reduce medullary osmolality and prevent osmotic shock to the inner medulla when it was removed from the animal and placed into dissecting solution (described below) (1). Initial (IMCD₁) or terminal (IMCD₂ or IMCD₃) IMCD were dissected as described previously (1,2,22) in a dissecting solution gassed with 95% O₂/5% CO₂ and containing (in mM): 118 NaCl, 25 NaHCO₃, 2 CaCl₂, 2.5 K₂HPO₄, 1.2 MgSO₄, 5.5 glucose, and 4 creatinine. The tubules were perfused using standard techniques in a 37°C bath, which was exchanged continuously and bubbled with 95% O₂/5% CO₂ gas (1,2,22).

**Urea Permeability Measurement**

To determine urea permeability (Pₘₐₓ), 5 mM urea was added to the bath solution and 5 mM raffinose was added to the perfusate to create a 5 mM bath-to-lumen urea gradient without any osmotic gradient (1,2,22,23). The same Pₘₐₓ value is obtained regardless of whether a bath-to-lumen or lumen-to-bath urea gradient is imposed (1). Bath and perfusate solutions were otherwise identical to the dissecuting solution described above. First, basal Pₘₐₓ value was measured. Next, 10 nM AVP (Sigma, St. Louis, MO) was added to the bath, and after 30 min the response to AVP was measured (2,22). The urea concentration in perfusate, bath, and collected fluid was measured using a continuous-flow ultramicro-fluorometer, as described previously (1,2,22). Urea flux and urea permeability were calculated as described previously (1,2,22).

**Osmotic Water Permeability Measurement**

To determine osmotic water permeability (Pₒ), creatinine was used as a volume marker (2,23,24). Creatinine concentration in perfusate, bath, and collected fluid was measured using a continuous-flow ultramicro-colorimeter as described (2,24). Pₒ was measured by increasing bath osmolality to 490 mosmol/kg H₂O by adding NaCl (2,23,24). The perfusion rate and volume flux (Jᵥ) were calculated as described previously (2,24). The osmotic water permeability (Pₒ) was calculated using the equation of Al-Zahid *et al.* (25) as described (2,24). After three to four control collections, 100 pM AVP was added to the bath. After 30 min (23,24,26), a second set of three collections with a stable Pₒ value was obtained to assess the response to AVP.

**Statistical Analyses**

Data are presented as mean ± SEM (n), where n indicates the number of rats studied. Data from three to four collections were averaged to obtain a single value from each experimental phase in each tubule. To test for statistically significant differences, a two-way ANOVA was used followed by a multiple-comparison, protected *t* test (27).

**Results**

**In Vivo Parameters**

**Urine Osmolality.** Untreated rats had a urine osmolality of 910 ± 104 mosmol/kg H₂O (n = 8) (Figure 1). All five diuresis protocols (1, 3 to 5, and 7 d of water diuresis, water diuresis + food, and furosemide) significantly decreased urine osmolality compared with untreated rats. There was no significant difference in urine osmolality between rats made water-diuretic for 1, 3 to 5, or 7 d. Water deprivation for 1 or 2 to 3 d significantly increased urine osmolality compared with untreated rats; there was no significant difference between rats deprived of water for 1 or 2 to 3 d.

**Hematocrit and Body Weight.** Hematocrit levels were not significantly different between any group of rats except for rats deprived of water for 3 d, which had a significant increase in hematocrit (Table 1). All groups of rats lost weight except...
Long-Term Regulation of Urea Transport in Rat IMCD

for rats given sugar water for 1 d (in this group, weight was unchanged compared with untreated rats) and rats given sugar water and food ad libitum (rats in this group gained more weight than untreated rats) (Table 1).

Water Diuresis

Initial IMCD. Basal $P_{\text{urea}}$ in initial IMCD (IMCD₁) from rats made water-diuretic for 1, 3 to 5, or 7 d ($n = 6$) was low and similar to values obtained from untreated rats ($n = 5$) (Figure 2). AVP significantly increased $P_{\text{urea}}$ in initial IMCD from rats made water-diuretic for 3 to 5 d, but not from rats made water-diuretic for 1 or 7 d or from untreated rats.

Terminal IMCD. Basal $P_{\text{urea}}$ was significantly higher in terminal IMCD from rats made water-diuretic for 1 d ($n = 7$), 3 to 5 d ($n = 7$), or 7 d ($n = 5$) compared with untreated rats ($n = 5$) (Figure 3). AVP significantly increased $P_{\text{urea}}$ in terminal IMCD from untreated rats and rats made water-diuretic for 1 or 3 to 5 d (Figure 3, the three leftmost pairs of bars). AVP had no significant effect on $P_{\text{urea}}$ in terminal IMCD from rats made water-diuretic for 7 d (Figure 3, right pair of bars).

Next, we measured basal and AVP-stimulated $P_{\text{urea}}$ in each of the terminal IMCD subsegments, IMCD₂ and IMCD₃, to determine whether the increase in basal $P_{\text{urea}}$ occurred throughout the terminal IMCD in rats made water-diuretic for 3 to 5 d. Basal $P_{\text{urea}}$ was significantly higher in both IMCD₂ and IMCD₃, from water-diuretic rats than from untreated rats ($n = 5$) (Figure 4). AVP significantly increased $P_{\text{urea}}$ in both IMCD₂ and IMCD₃, from untreated rats (Figure 4, solid lines). AVP had no significant effect on $P_{\text{urea}}$ in either IMCD₂ or IMCD₃, from water-diuretic rats (Figure 4, dashed lines). Because there was no significant difference in $P_{\text{urea}}$ between IMCD₂ and IMCD₃ in either untreated or water-diuretic rats, the results from all terminal IMCD subsegments were combined in subsequent diuretic protocols.

Table 1. Change in rat weight and hematocrit*

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Days Treated</th>
<th>Change in Body Weight (%)</th>
<th>Hematocrit (%)</th>
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<tbody>
<tr>
<td>Untreated</td>
<td>1</td>
<td>+2 ± 1 (n = 3)</td>
<td>42 ± 2 (n = 3)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>+6 ± 1 (n = 3)</td>
<td>41 ± 1 (n = 3)</td>
</tr>
<tr>
<td>Water diuresis (no food)</td>
<td>1</td>
<td>−1 ± 1 (n = 9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>−12 ± 1 (n = 6)</td>
<td>46 ± 1 (n = 3)</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>−21 ± 1 (n = 3)</td>
<td>46 ± 1 (n = 3)</td>
</tr>
<tr>
<td>Water diuresis with food</td>
<td>4</td>
<td>17 ± 5 (n = 3)</td>
<td>43 ± 1 (n = 3)</td>
</tr>
<tr>
<td>Furosemide diuresis</td>
<td>4 to 5</td>
<td>−10 ± 2 (n = 5)</td>
<td>46 (n = 1)</td>
</tr>
<tr>
<td>Water deprivation</td>
<td>1</td>
<td>−5 ± 1 (n = 6)</td>
<td>42 ± 2 (n = 3)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>−18 ± 1 (n = 3)</td>
<td>50 ± 1 (n = 3)</td>
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*Data are mean ± SEM. n = number of rats.

$^b$P < 0.05 versus untreated rats.
Osmotic Water Permeability in the Terminal IMCD.
Basal $P_f$ was similar in terminal IMCD from rats made water-diuretic for 3 to 4 d ($n = 5$) and from untreated rats ($n = 5$) (Figure 5). AVP significantly increased $P_f$ in terminal IMCD from untreated rats but not from water-diuretic rats.

Water Diuresis with Food

Initial IMCD. Basal $P_{\text{urea}}$ in initial IMCD from rats made water-diuretic for 3 to 5 d and given free access to food ($n = 5$) was low and similar to values obtained from untreated rats ($n = 5$) (Figure 6, left). AVP significantly increased $P_{\text{urea}}$ in initial IMCD from fed, water-diuretic rats but not from untreated rats.

Terminal IMCD. Basal $P_{\text{urea}}$ was significantly higher in terminal IMCD from fed, water-diuretic rats ($n = 7$) than from untreated rats ($n = 5$) (Figure 6, right). AVP significantly increased $P_{\text{urea}}$ in terminal IMCD from both untreated rats and fed, water-diuretic rats.

Furosemide

Initial IMCD. Basal $P_{\text{urea}}$ in initial IMCD from rats undergoing a furosemide-induced diuresis for 3 to 5 d and given free access to food ($n = 5$) was low and similar to values obtained from untreated rats ($n = 5$) (Figure 7, left). AVP had no significant effect on $P_{\text{urea}}$ in initial IMCD from either group.
Figure 7. In initial IMCD (left), AVP (10 nM) had no significant effect on urea permeability in untreated rats or in rats made diuretic with furosemide for 3 to 5 d. In terminal IMCD (right), basal urea permeability was higher in furosemide-treated rats than in untreated rats. AVP increased urea permeability in both groups of rats. *P < 0.05 versus basal; +P < 0.01 versus untreated. n = 5 to 6.

of rats. There was no significant difference in the response of $P_{\text{urea}}$ to AVP in the initial IMCD between untreated and furosemide-treated rats.

Terminal IMCD. Basal $P_{\text{urea}}$ was significantly higher in terminal IMCD from furosemide-treated rats ($n = 6$) than from untreated rats ($n = 5$) (Figure 7, right). AVP significantly increased $P_{\text{urea}}$ in terminal IMCD from both groups of rats.

Water Deprivation for 1 Day

Initial IMCD. Basal $P_{\text{urea}}$ in initial IMCD was similar in untreated rats ($n = 5$) (Figure 8, solid line) and rats that were deprived of water for 1 d ($n = 5$) (Figure 8, dashed line). AVP significantly increased $P_{\text{urea}}$ in initial IMCD from rats deprived of water for 1 d but not in untreated rats.

Terminal IMCD. In the IMCD$_2$ portion of the terminal IMCD, basal $P_{\text{urea}}$ was similar in untreated rats ($n = 5$) (Figure 9, left, solid line) and rats that were deprived of water for 3 d ($n = 7$) (Figure 9, left, dotted/dashed line). AVP significantly increased $P_{\text{urea}}$ in IMCD$_2$ from both groups of rats. In contrast, in the IMCD$_3$ portion of the terminal IMCD, basal $P_{\text{urea}}$ was significantly higher in rats deprived of water for 3 d ($n = 7$) (Figure 9, right, dotted/dashed line) than in untreated rats ($n = 5$) (Figure 9, right, solid line). AVP significantly increased $P_{\text{urea}}$ in IMCD$_3$ from both groups of rats.

Discussion

The main result of this study is that basal and AVP-stimulated facilitated urea permeabilities vary in response to diuresis and antidiuresis in a heterogeneous manner among IMCD subsegments (Table 2). This result is surprising because these changes in urea transport do not correlate with the changes in UT2/UT1 mRNA abundance reported previously (13,16) and are more complex than expected.
Table 2. Summary of the effect of changes in in vivo hydration status on urea transporta

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Days Treated</th>
<th>Initial IMCD</th>
<th>Terminal IMCD</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Basal $P_{\text{urea}}$ b</td>
<td>Effect of AVP</td>
</tr>
<tr>
<td>Untreated</td>
<td>1</td>
<td>Low</td>
<td>No stimulation</td>
</tr>
<tr>
<td>Water diuresis (no food)</td>
<td>3 to 5</td>
<td>No change</td>
<td>No stimulation</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>No change</td>
<td>No stimulation</td>
</tr>
<tr>
<td>Water diuresis with food</td>
<td>3 to 5</td>
<td>No change</td>
<td>Stimulates</td>
</tr>
<tr>
<td>Furosemide diuresis</td>
<td>3 to 5</td>
<td>No change</td>
<td>No stimulation</td>
</tr>
<tr>
<td>Water deprivation</td>
<td>1</td>
<td>No change</td>
<td>Stimulates</td>
</tr>
<tr>
<td></td>
<td>2 to 3</td>
<td>Increased</td>
<td>Stimulates</td>
</tr>
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</table>

a IMCD, inner medullary collecting duct; AVP, arginine vasopressin (10 nM in bath).
b Basal $P_{\text{urea}}$ compared to value in untreated rats.

Diuresis
We previously fed rats sugar water without food to induce water diuresis and showed that it does not induce glucosuria or hyperglycemia (28). In the present study, we also studied rats given sugar water, but allowed food ad libitum to ensure that any changes in urea transport were due to water diuresis and not to lack of food. We performed this study because feeding rats a low-protein diet for 2 wk changes urea transport in initial IMCD (20,21). However, we found no difference in urea transport between rats given only sugar water and those allowed to eat food ad libitum (Figure 10), suggesting that the changes in urea transport in the present study are due to the water diuresis and not to the 3 to 5 d without food.

We found that every diuresis protocol (sugar water without food for 1, 3 to 5, or 7 d, sugar water with food, and furosemide treatment) increased basal urea permeability in terminal IMCD (Table 2). AVP significantly stimulated urea permeability in terminal IMCD in every protocol except feeding rats sugar water without food for 7 d. AVP-induced stimulation of urea permeability suggests that the increase is mediated by VRUT; the data in the companion paper by Terris et al. show that 117-kD VRUT protein is increased in the inner medulla under similar conditions (19).

The basal urea permeability in terminal IMCD from rats fed sugar water without food for 7 d was extremely high. One possible explanation for the lack of AVP-induced stimulation of urea permeability in this condition is that the basal permeability is so high that AVP cannot increase it further. A second possibility is that 7 d (but not 1 to 5 d) of water diuresis reduces the ability of terminal IMCD to respond to AVP.

In contrast to the increase in basal urea permeability in the terminal IMCD, we found no effect of any diuresis protocol on basal urea permeability in the initial IMCD. In two of the five diuresis protocols, sugar water without food for 3 to 5 d and sugar water with food for 3 to 5 d, AVP caused a small but statistically significant increase in urea permeability. This increase is unlikely to be physiologically significant, because diuretic rats would be expected to have low plasma AVP levels. Thus, the meaning of this effect solely on AVP-stimulated urea permeability is unclear.

Two UT2 mRNA bands are present in rat medulla (13-16): a 4-kb UT-A1 cDNA and a 2.9-kb UT-A2 cDNA. Shayakul and colleagues showed that the 4.0-kb UT-A1 mRNA is expressed only in IMCD, whereas the 2.9-kb UT-A2 mRNA is expressed in thin descending limbs (29). Smith et al. used the same water diuresis protocol as that used in the present study, 3 d of feeding rats sugar water without food, and showed a decrease in the 4.0-kb UT-A1 mRNA band by Northern analysis and a decrease in the UT2/UT1 mRNA signal by in situ
hybridization in the inner medulla (13). However, Northern analysis of total kidney RNA showed no change in the 4.0-kb UT-A1 mRNA band during water diuresis, but did show an increase in the abundance of the 2.9-kb band (13). Promenour et al. used a different protocol for varying water intake (varying the water content of the rat chow or administering deamino-8-d-avp for 5 wk) and found a different result (16). They showed that the abundance of the 2.9-kb, but not the 4.0-kb, UT2/UT1 mRNA band varied with urine osmolality on Northern analysis of total kidney RNA (16). The differences in the changes in UT2/UT1 mRNA between these two studies (13,16) could be due to the marked differences in hydration protocols used. We do not have an explanation for the differences between our urea transport and VRUT protein (19) measurements and the UT2/UT1 mRNA abundance studies (13,16). However, these results are not necessarily contradictory, because UT2/UT1 mRNA levels may not correlate with urea transport or urea transporter protein levels if the predominant mode of regulation is at the translational or protein stability level.

The increase in urea permeability during diuresis is surprising because one would expect a decrease in urea permeability to decrease inner medullary urea concentration and reduce urine concentrating ability. However, the decrease in medullary urea accumulation during diuresis is believed to occur predominantly because there is a lower luminal urea concentration, and hence a smaller gradient driving urea exit from the IMCD. The smaller luminal urea concentration occurs because there is less water reabsorption in the more proximal portions of the collecting duct during diuresis. Because urine (and luminal) flow rates are higher during diuresis, a higher urea permeability may be required to maintain equilibration of urea concentration across the IMCD epithelium. The higher urea permeability would facilitate urea reabsorption or secretion, depending on the direction of the urea gradient. Several studies showed that inner medullary tissue urea content and concentration exceed urinary values during water diuresis in rats (30–33), suggesting that the increase in basal (no AVP) urea permeability observed in the present study occurs to increase urea secretion. This interpretation would be consistent with microcatheterization (34) and micropuncture (35,36) studies, which demonstrate urea secretion into the terminal IMCD during diuresis.

The decrease in urine concentrating ability and inner medullary urea concentration seen during diuresis could also be explained if more interstitial urea was returned to the body rather than being excreted by the kidneys. The former would occur if urea permeability and reabsorption increased, whereas the latter would occur if urea permeability decreased or urea secretion increased. If urea reabsorption increased during water diuresis accompanied by an increase in medullary blood flow, this could result in washout of urea from the medullary interstitium and an increase in the amount of interstitial urea being returned to the systemic circulation. In this case, it is possible that during the transition from diuresis to antidiuresis, interstitial urea content could be increased simply by an antidiuresis-induced reduction in medullary blood flow accompanied by a reduction in medullary urea washout. Thus, this could be a mechanism to enhance urea conservation during diuresis and aid in the transition from diuresis to antidiuresis.

Finally, we measured the effect of 3 to 5 d of sugar water (without food) on a second AVP-regulated transport process in the terminal IMCD, osmotic water permeability. Diuresis had no significant effect on basal osmotic water permeability, but markedly blunted AVP-stimulated osmotic water permeability. The present result is consistent with the downregulation of AQP2 protein demonstrated by Nielsen et al. after 1 d of water diuresis in rats (5), but differs from Lankford et al.'s demonstration that basal osmotic water permeability is decreased after 1 d of water diuresis in terminal IMCD (11). This difference may be due to the longer duration of water diuresis used in the present study.

Antidiuresis

Water deprivation for 1 d is thought to primarily activate osmoregulatory mechanisms through increases in plasma osmolality and the osmotic release of AVP (37). Water deprivation for more than 1 d is thought to activate a more complicated set of physiologic responses, including both osmoregulatory and baroregulatory mechanisms (37), thus making it more difficult to infer the cause of any change. In the present studies, antidiuresis was induced by water deprivation both for 1 d and for 2 to 3 d. Even though the mechanistic interpretation of the longer period of water deprivation is more difficult, we studied both conditions, because some physiologic processes such as upregulation of aldose reductase activity (28) require 2 to 3 d of water deprivation to occur.

We found that 1 d of water deprivation had no effect on basal or AVP-stimulated urea permeability in the terminal IMCD, consistent with the data of Lankford et al. showing no effect of 1 d of water deprivation on basal urea permeability in the initial IMCD (11). In addition, in the initial IMCD (IMCD1), we found an increase in AVP-stimulated urea permeability after 1 d of water deprivation. This result is surprising because an increase in urea reabsorption across the initial IMCD would be expected to decrease urine concentrating ability by reducing urea delivery to the deeper portions of the inner medulla (38).

In contrast, 3 d of water deprivation significantly increased basal and AVP-stimulated urea permeabilities in the deepest portion of the IMCD, the IMCD3 subsegment, but not in the IMCD2 subsegment. This increase in urea permeability could aid in the maintenance of concentrated urine despite the progressive loss of body weight during 3 d of water deprivation, by promoting urea reabsorption into the papillary tip. We also found an increase in basal and AVP-stimulated urea permeabilities in the initial IMCD (IMCD1) after 2 to 3 d of water deprivation. Sonnenberg and Wilson proposed that water deprivation causes intra-inner medullary urea recycling by urea secretion in the IMCD1 and urea reabsorption in the IMCD3 (39). The patterns of change in urea permeabilities in the present study are consistent with this hypothesis.

Northern analysis of inner medullary or whole-kidney RNA shows an increase in the 2.9-kb, but no change in the 4.0-kb,
UT2/UT1 mRNA abundance in rats that were water-deprived for 3 d (13,16). The UT2/UT1 in situ hybridization signal also shows a large increase throughout the inner medulla (13). However, it is unlikely that the increase in urea transport in the present study is due to the increase in the 2.9-kb UT-A2 mRNA because it is not present in the IMCD3 by reverse transcription, even in IMCD3 from dehydrated rats (29).

**Summary and Perspective**

We found that chronic (days) changes in hydration in vivo resulted in heterogeneous and unexpected changes in urea transport (Table 2). Water or furosemide diuresis increased basal and AVP-stimulated urea permeability in the initial IMCD (IMCD1) and the deepest portion of the terminal IMCD (IMCD3). Quantitative evaluation of the physiologic impact of these changes in urea transport on the urine concentrating mechanism will require mathematical modeling in the future. Additional studies will also be necessary to explain the apparent differences between the change in urea transport found in this study and the change in UT2/UT1 mRNA reported previously (13,16). At present, investigators should exercise caution in drawing conclusions about changes in urea transport based solely on changes in UT2/UT1 mRNA abundance.

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**References**


20. Sands JM, Naruse M, Jacobs JD, Wilcox JD, Klein JD: Changes


