Urea may regulate urea transporter protein abundance during osmotic diuresis

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THE RENAL MEDULLA IS THE LOCATION in which water excretion is controlled through the production of concentrated or dilute urine. Several solute transport proteins play a major role in the urinary concentrating mechanism, including urea transporters and the Na\(^{+}\)-K\(^{+}\)-2Cl\(^{-}\) cotransporter (NKCC2/BSC1). Among the urea transporters, UT-A1 is expressed in the inner medullary collecting duct and is important for vasopressin-regulated urea reabsorption (reviewed in Ref. 21). UT-A2 and UT-B are expressed in the thin descending limb and descending vasa recta, respectively, and are important for intrarenal urea recycling (reviewed in Ref. 21). NKCC2/BSC1 is expressed in the thick ascending limb of the loop of Henle and is responsible for the active reabsorption of NaCl that drives the single effect to concentrate urine (reviewed in Ref. 22).

Several studies show that UT-A1, as well as NKCC2/BSC1 and aquaporin-2 (AQP2), protein abundances increase after 5 days of uncontrolled diabetes mellitus due to streptozotocin (2, 10, 11, 19, 27). We showed that UT-A1 protein abundance does not change in streptozotocin-treated Brattleboro rats, indicating that vasopressin is necessary for the increase in UT-A1 protein abundance because Brattleboro rats lack vasopressin (11). When we administered vasopressin to Brattleboro rats and then induced diabetes mellitus, UT-A1 abundance increased. Interestingly, UT-A1 abundance was increased more in vasopressin-infused diabetic Brattleboro rats than in Brattleboro rats receiving vasopressin alone. These findings imply that vasopressin is necessary but cannot be the signal to increase UT-A1 abundance because the two groups of Brattleboro rats received the same amount of vasopressin (11).

Urea is the major urinary solute in mammals, comprising 40–50% of total urinary solute in rats on a normal diet. Rats with streptozotocin-induced diabetes mellitus do have an increase in absolute urea excretion, but the relative amount (percentage) of urea in total urinary solute is actually decreased (to ~20%) due to the marked increase in glucose excretion (10). The ongoing osmotic diuresis due to nonreabsorbable glucose causes water to be retained in the tubule lumen, which dilutes the urine urea concentration. Several urea-specific signaling pathways have been identified in cultured mIMCD3 cells and the renal medulla (reviewed in Refs. 3 and 28), suggesting the possibility that changes in the percentage or concentration of urea in the urine, tubular fluid, or medullary interstitium could be a factor that regulates UT-A1 protein abundance. Consistent with this possibility, urea has been shown to inhibit Na\(^{+}\)-K\(^{+}\)-2Cl\(^{-}\) cotransport in medullary thick ascending limb cells (9). In the present study, we tested the hypothesis that an increase in any urinary solute other than urea would increase UT-A1 abundance, similar to diabetes mellitus-induced glucose diuresis, whereas an increase in total urinary solute due to urea itself would not result in an increase in UT-A1. If this were true, then it would suggest that a reduction in urine or interstitial urea results in an increase in UT-A1 protein abundance in an attempt to restore inner medullary interstitial urea and preserve urine-concentrating ability.

METHODS

Animal preparation. All animal protocols were approved by the Emory University Institutional Animal Care and Use Committee. Male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA), weighing 125–200 g, received free access to a standard powdered rat chow (Testdiet 5001, Purina) containing 23% protein and 50% carbohydrate. All rats were anesthetized with ketamine/xylazine (10/1 mg/kg) and an ileal mesenteric venous (IV) catheter was placed. Rats were allowed at least 24 h to recover from the surgery before the experiments were started. Diabetic Brattleboro rats and normal rats received 1% streptozotocin (Sigma) in drinking water. Normal rats were maintained on 1% normal saline in drinking water. The rats were killed by overdose of ketamine/ xylazine. Glucose for the intraperitoneal injection was made freshly at 100 mg/kg to 300 mg/kg in saline. The solutions were prepared and reconstituted before each experiment. The urine was collected for 3 h, and the blood was collected from the inferior vena cava, and the plasma was obtained by centrifugation at 3000 g for 15 min. Glucose was determined using a glucose oxidase method.

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Table 1. Urine and blood chemistries

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>DM</th>
<th>2.5% NaCl</th>
<th>4% NaCl</th>
<th>DM+10% Urea</th>
<th>4% NaCl+ 3% Urea</th>
<th>5% Urea</th>
<th>20% Urea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total urinary solute, mmol/day/100 g BW⁻¹</td>
<td>9.7±2.1</td>
<td>69±7.8</td>
<td>10.6±0.3</td>
<td>18.9±2</td>
<td>83.1±7.2</td>
<td>22.5±2.6</td>
<td>18.5±1.7</td>
<td>45±3.1</td>
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<tr>
<td>Uosm, mosmol/kgH₂O</td>
<td>1.97±0.25</td>
<td>85±42</td>
<td>1.68±174</td>
<td>1.52±222</td>
<td>917±65.4</td>
<td>1.314±162</td>
<td>1.747±214</td>
<td>1.211±85</td>
</tr>
<tr>
<td>Urea excretion, mmol/day/100 g BW⁻¹</td>
<td>4.5±0.8</td>
<td>14.3±2.1</td>
<td>3.6±0.1</td>
<td>5.0±0.6</td>
<td>39.3±3.8</td>
<td>10.7±1.7</td>
<td>11.4±0.8</td>
<td>37.3±3.1</td>
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<tr>
<td>Uosm, mosmol/kgH₂O</td>
<td>294±4.4</td>
<td>306±7</td>
<td>298±4.9</td>
<td>288±4.4</td>
<td>327.3±6.7</td>
<td>297.7±16</td>
<td>293.2±7.8</td>
<td>301.7±10.7</td>
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<tr>
<td>BUN, mg/dl</td>
<td>921±97</td>
<td>176±14</td>
<td>579±63</td>
<td>399±49</td>
<td>433±18.6</td>
<td>618±52</td>
<td>1.070±99</td>
<td>998.8±71</td>
</tr>
<tr>
<td>Blood glucose, mg/dl</td>
<td>ND</td>
<td>410±34</td>
<td>ND</td>
<td>ND</td>
<td>454±64</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>Urea in urinary solute, %</td>
<td>47±5</td>
<td>21±1</td>
<td>34.3±0.7</td>
<td>26.1±1.5</td>
<td>-48±1.6</td>
<td>47±3.4</td>
<td>62.3±1.3</td>
<td>83±4.5</td>
</tr>
<tr>
<td>Glucose in urinary solute, %</td>
<td>0</td>
<td>51±5</td>
<td>0</td>
<td>0</td>
<td>44±4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other solutes (Na, K, Cl, etc.), %</td>
<td>53±5</td>
<td>28.5±6</td>
<td>66±0.7</td>
<td>74±1.5</td>
<td>8.4±4.4</td>
<td>53±3.1</td>
<td>38.3±1.4</td>
<td>17.4±5</td>
</tr>
</tbody>
</table>

Values are means ± SD. n = 6 for experimental groups; n = 42 for controls for all groups combined. DM, diabetes mellitus; Uosm, urine osmolality; BUN, blood urea nitrogen; Psm, plasma osmolality; Csm, osmolar clearance; SFWR, solute-free water reabsorption; BW, body wt; ND, not done. *P < 0.05 for control. †P < 0.05 for 4% NaCl vs. 2.5% NaCl. ‡P < 0.05 for DM+urea vs. DM alone. §P < 0.05 for NaCl+urea vs. 4% NaCl alone. ‡P < 0.05 for 20% urea vs. 5% urea.

Fig. 1. Graphic representation of urine parameters comparing the different study groups. Units and statistical significance are provided in Table 1. Ctr, control; DM, diabetes mellitus; conc., concentration.
1.05% NaCl (standard diet). All rats received free access to water throughout the study. Osmotic diuresis due to glucose, NaCl, and/or urea was induced as follows.

To induce glucose diuresis, rats were made diabetic by injecting streptozotocin (60 mg/kg body wt prepared fresh in 0.1 M citrate buffer, pH 4.0, Sigma, St. Louis, MO) into a tail vein at 7 AM (10, 11, 13). Diabetes mellitus was confirmed by measuring a spot urine glucose at 24 and 48 h after streptozotocin injection (Ames N-Multistix SG, Miles, Elkhart, IN). Diabetic rats were fed the standard diet throughout the study. An additional group of rats was made diabetic, and 10% urea was added to the standard powdered diet to keep the percentage of urea in total urinary solute at the control level.

To induce NaCl diuresis, rats were fed a diet containing a total of 4 or 2.5% NaCl by adding 3 or 1.5% NaCl, respectively, to the standard powdered diet (which contains 1.05% NaCl). An additional group of rats was fed 4% NaCl and 3% urea to keep the percentage of urea in total urinary solute at the control level. NaCl was chosen as the osmolyte in this study because introducing mannitol or similar osmolytes that are not absorbed by the gastrointestinal tract would result in osmotic diarrhea rather than osmotic diuresis. In addition, it is difficult to deliver sufficient amounts of these sugars parenterally to approximate the glucose levels that are produced in diabetes mellitus, whereas NaCl can readily be delivered and appropriate levels achieved.

To induce urea diuresis, rats were fed the standard diet to which 5 or 20% urea was added. Each osmotic diuresis group (n = 6 rats) was compared with its own control group (n = 4–6 rats), which was studied in parallel. Because our previous studies showed that UT-A1 protein abundance increases consistently from 10–20 days after streptozotocin-induced diabetes mellitus (10, 11), osmotic diuresis was induced for 15–20 days in all protocols, except that 4% NaCl diuresis was induced for both 5 and 15 days to distinguish whether changes seen at 15 days were temporally distinct as they are in diabetes mellitus. Two days before death, rats were put into metabolic cages and a 24-h urine collection was obtained to measure urine volume, osmolality (model 5500 vapor pressure osmometer, Wescor, Logan, UT), and urea concentration (Infinity BUN reagent, Sigma). After 15–20 days of osmotic diuresis, rats were killed and trunk blood was collected to measure urea nitrogen (BUN) and osmolality. Blood and urine glucose concentrations were measured only in the streptozotocin-treated rats (One Touch Profile Diabetes Tracking Kit, Lifescan, Milpitas, CA). The absence of glucosuria in the other groups was confirmed using urine dip-sticks. Osmolar clearance (\(C_{osm}\)) and solute free water reabsorption (SFWR) were calculated as follows: \(C_{osm} = \frac{U_{osm} \times P_{osm}}{U_{vol}}\); SFWR = \(C_{osm} - U_{vol}\), where \(U_{osm}\) is urine osmolality, \(P_{osm}\) is plasma osmolality, and \(U_{vol}\) is urine volume.

**Tissue urea concentration.** Kidneys were removed, and the medulla was dissected into outer medulla, inner medullary base, and inner medullary tip as previously described (10, 11). These tissue pieces from one kidney per rat were weighed and processed to determine interstitial urea concentration essentially as described by Schmidt-Nielsen and colleagues (23). Briefly, tissue (~10 mg/piece) was

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**Fig. 2.** Urea transporter (UT-A1) protein abundances in inner medullary (IM) base and tip of rats with DM and NaCl-induced diuresis. **Left:** Western blots of IM base proteins. **Middle:** Western blots of IM tip proteins. Both IM base and tip were probed with a specific antibody to UT-A and show the characteristic 117- and 97-kDa glycoprotein forms of UT-A1. CTR, control; d, days undergoing the feeding protocol. Within each gel, the left 4 lanes are control rat tissues, and the right 4 lanes are either DM or diuretic rat tissues. Each gel contains a representative group of samples from the experimental manipulation, and each lane is tissue from a separate animal. **Right:** densitometric analysis of the total groups (n = 6) with combined 117- and 97-kDa band densities averaged for each group. The y-axis is density in arbitrary units. Values are means ± SD, n = 6. *P < 0.05, diuretic vs. control.
were highest in rats with diabetes mellitus (glucose diuresis) and diabetes mellitus + urea and also increased in the 20% urea diuretic rats (Fig. 1). Urine osmolality was lowest in the two diabetic groups, but also reduced in the 4% NaCl + urea and the 20% urea diuretic rats. Urine urea excretion was highest in diabetes + urea and 20% urea diuretic rats but also increased in diabetic rats.

Urine urea concentration and the relative amount (percentage) of urea in total urinary solute were decreased in diabetes mellitus and NaCl diuresis and increased in urine diuresis. The percentage of urea in total urinary solute was maintained at the control level in diabetes + urea and in the 4% NaCl + urea diuretic rats, although the urine urea concentration was reduced in these groups compared with control. However, the urine urea concentrations in rats with diabetes + urea and 4% NaCl + urea diuresis were significantly increased compared with rats with diabetes and 4% NaCl diuresis without urea supplementation, respectively.

Effect of diabetes mellitus and NaCl diuresis on UT-A1, UT-A2, UT-B, and NKCC2/BSC1 protein abundances. UT-A1 abundance in both portions of the inner medulla was significantly increased in diabetic rats (255% of control in inner medullary base, 171% of control in inner medullary tip), consistent with our previous results (10, 11), and in rats with 4% NaCl diuresis for 15 days (166% of control in inner medullary base, 162% of control in inner medullary tip) (Fig. 2). It was unchanged in 2.5% NaCl diuretic rats. Because UT-A1 abundance varies with time after streptozotocin injection (10), we tested whether there was a temporal change in UT-A1 after induction of 4% NaCl diuresis. UT-A1 abundance increased at 5 days of 4% NaCl diuresis in the inner medullary base, but not in the inner medullary tip, similar to the pattern of changes in UT-A1 at 5 days of diabetes (10).

In contrast, UT-A2 abundance in the outer medulla was significantly decreased in diabetic rats (66% of control) and in

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**RESULTS**

**Physiological parameters.** Table 1 shows the urine osmolality, volume, urea, glucose, and other solutes, and plasma urea, glucose, and osmolality. Total urinary solute and urine volume were highest in rats with diabetes mellitus (glucose diuresis)

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**Fig. 3.** UT-A2 protein abundances in outer medulla (OM) from rats with DM and NaCl-induced diuresis. Western blots of OM proteins in control (left 4 lanes) and diuretic (right 4 lanes) rats probed with anti-UT-A are shown. The 3- to 4-band glycoprotein “smear” centered at 50 kDa is characteristic of UT-A2 in the OM. Each gel contains a representative group of samples from the experimental manipulation, and each lane is tissue from a separate animal. Right: densitometric analysis of combined UT-A2 glycoprotein bands. The y-axis is density in arbitrary units. Values are means ± SD; n = 6. *P < 0.05, diuretic vs. control.
rats with 2.5% NaCl diuresis (72% of control) but was unchanged in 4% NaCl diuresis (Fig. 3) rats. UT-B abundance was unchanged in any portion of the medulla with either diabetes or 4% NaCl diuresis (Fig. 4). NKCC2/BSC1 abundance in the outer medulla (Fig. 5) was significantly increased in both diabetic rats (143% of control) and 4% NaCl diuretic (130% of control) but not in 2.5% NaCl diuretic rats.

Effect of urea feeding on diabetic and 4% NaCl diuretic rats. The data in Figs. 1 and 2 suggest the hypothesis that a decrease in urine urea below a certain level may be a signal that increases UT-A1 abundance in the inner medulla. To test this hypothesis, diabetic and 4% NaCl diuretic rats were fed supplemental urea to restore the percentage of urea in urine solute to control levels (Table 1, Fig. 1). Diabetic rats and 4% NaCl diuretic rats fed urea supplements did not show significant differences in UT-A1 (Fig. 6), UT-A2 (Fig. 7), or UT-B abundances (Fig. 8) compared with control rats, suggesting that restoring the percentage of urea in total urinary solute reverses the diabetes- or 4% NaCl diuresis-induced upregulation of UT-A1 abundance. NKCC2/BSC1 abundance (Fig. 9) was significantly increased in both rats with diabetes (124% of control) and 4% NaCl diuresis (121% of control). However, the percentage increases were somewhat smaller than the increases in rats with diabetes or 4% NaCl diuresis alone.

Effect of urea diuresis. UT-A1 abundance was not changed in rats with 5 or 20% urea diuresis in either the inner medullary base or tip (Fig. 6), even though these rats had severe osmotic diuresis. Considering that the urine urea concentration is higher, but urine osmolality is lower, than those of control rats (Table 1, Fig. 1), this suggests that UT-A1 is responding to the urine urea concentration, rather than urine osmolality. In contrast, UT-A2 abundance in the outer medulla was significantly increased with both the 5% urea (163% of control) and 20% urea (155% of control) diuresis (Fig. 7). UT-B abundance in the outer medulla was also significantly increased in both rats with 5% urea (150% of control) and 20% urea (130% of control) diuresis but was unchanged in the inner medullary base (Fig. 8). NKCC2/BSC1 abundance showed a tendency to increase in both rats with 5 and 20% urea diuresis, but the increase was statistically significant only in 20% urea diuretic rats (Fig. 9).
Fig. 6. UT-A1 abundances in IM base and tip of normal and diuretic rats receiving urea supplement. Western blots of IM base proteins (left) and IM tip proteins (middle) in rats probed with anti-UT-A are shown. Within each gel, the left 4 lanes are control rat tissues, and the right 4 lanes are diuretic rat tissues. UT-A1 is identified with arrows. Each gel contains a representative group of samples from the experimental manipulation, and each lane is tissue from a separate animal. Right: densitometric analysis of the total groups (n = 6) with combined 117- and 97-kDa band densities averaged for each group. The y-axis is density in arbitrary units. Values are means \pm SD; n = 6. There were no significant differences in UT-A1 abundance between the urea-supplemented diuretic and control rats.

Fig. 7. UT-A2 abundances in OM of normal and diuretic rats receiving urea supplement. Western blots of OM proteins in control (left 4 lanes) and diuretic (right 4 lanes) rats probed with anti-UT-A are shown. Each gel contains a representative group of samples from the experimental manipulation, and each lane is tissue from a separate animal. Right: densitometric analysis of combined UT-A2 glycoprotein bands. The y-axis is density in arbitrary units. Values are means \pm SD; n = 6. *P < 0.05, experimental vs. control.
Tissue urea concentration. To determine whether the changes in urine urea reflected tissue urea, we determined tissue urea concentrations in 20% urea diuretic and 4% NaCl diuretic rats. Compared with control rats, tissue urea levels were significantly increased in all medullary regions in the 20% urea diuretic rats (Table 2). There was no difference in the tissue urea content of the 4% NaCl diuretic rats compared with control rats.

DISCUSSION

**UT-A1.** The major finding in this study is that UT-A1 protein abundance increases during osmotic diuresis whenever the relative amount (percentage) of urea in total urinary solute decreases. Regardless of whether glucose (diabetes mellitus) or NaCl induced the osmotic diuresis, thereby reducing the percentage of urea in the urine, UT-A1 abundance increased. When osmotic diuresis was induced by urea, the percentage of urea in the urine did not decrease and UT-A1 abundance did not increase.

To further test the role of urine urea, rats undergoing osmotic diuresis due to glucose or NaCl were given urea supplements to prevent the decrease in the percentage of urea in the urine. With the urea-supplemented diets, the percentage of urea in total urinary solute was adjusted so that is was similar to that in control rats. In both of the urea-supplemented groups, UT-A1 abundance did not increase, suggesting that the increase in UT-A1 is due, at least in part, to the decrease in the percentage of urea in the urine rather than to urine osmolality or the severity of the osmotic diuresis. In addition, it appears that the percentage of urine urea may need to decrease below a certain level because the smaller change in the percentage of urine urea induced by 2.5% NaCl diuresis did not increase UT-A1 abundance, whereas the larger change induced by 4% NaCl diuresis did increase it.

Despite the same percentage of urea in total urinary solute, the urea-supplemented rats still had a significant reduction in urine osmolality and urine urea concentration compared with control rats.

**Another finding in the present study is that UT-A1 increases in the inner medullary base before it increases in the tip.** In both diabetes mellitus (10) and 4% NaCl diuresis (present study), UT-A1 increases after 5 days in the inner medullary base but not in the tip. At 10–15 days, UT-A1 is increased in both the inner medullary base and tip in diabetes mellitus (10) and 4% NaCl diuresis (present study). Although we do not
have an explanation for this difference in response, it may be related to the difference in the cell types present in the collecting duct in the inner medullary base vs. tip: the base contains primarily principal cells, whereas the tip contains primarily inner medullary collecting duct cells (4, 5).

To further characterize the role of urea, we asked whether UT-A1 increases in response to a low urine urea concentration or osmolality. Usually, low urine urea concentrations and low urine osmolalities occur simultaneously, making it difficult to know which is responsible for an increase in UT-A1 abundance. However, these two conditions can be dissociated if urea is used to increase the urea level in total urinary solute above that found in control rats. Urine urea concentration increased significantly in 5% urea diuresis (the increase was not statistically significant in 20% urea diuresis), whereas urine osmolality decreased significantly in 20% urea diuresis (the decrease was not statistically significant in 5% urea diuresis). Urea diuresis did not change UT-A1 abundance despite the decrease in urine osmolality and the presence of osmotic diuresis, consistent with our previous finding (14), suggesting that UT-A1 increases in response to a reduced percentage of urea in urine and/or a reduced urine urea concentration, rather than to low urine osmolality.

<table>
<thead>
<tr>
<th>Tissue Urea Content, μmol/g dry tissue wt</th>
</tr>
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<tbody>
<tr>
<td>Control (n = 8)</td>
</tr>
<tr>
<td>IM tip</td>
</tr>
<tr>
<td>348±117</td>
</tr>
<tr>
<td>IM base</td>
</tr>
<tr>
<td>278±95</td>
</tr>
<tr>
<td>OM</td>
</tr>
<tr>
<td>106±44</td>
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</tbody>
</table>

Values are means ± SD. n, No. of rats; IM, inner medulla; OM, outer medulla. *P < 0.01 vs. control by ANOVA.

**Table 2. Tissue urea content**

**Fig. 9.** NKCC2/BSC1 abundance in OM of normal and diuretic rats receiving urea supplement. Western blots of OM proteins in control (left 4 lanes) and diuretic (right 4 lanes) rats probed with anti-NKCC2/BSC1 are shown. Experimental manipulations are identified to the far left of each gel pair. Within each gel, the left 4 lanes are control rat tissues, and the right 4 lanes are diuretic rat tissues. Each gel contains a representative group of samples from the experimental manipulation, and each lane is tissue from a separate animal. Right: densitometric analysis of the total groups (n = 6) with NKCC2/BSC1 band densities averaged for each group. The y-axis is density in arbitrary units. Values are means ± SD; n = 6. *P < 0.05, diuretic vs. control.
dances increase in both 5 and 20% urea diuresis, where the urine urea concentration increased, but was unchanged or decreased in NaCl diuresis and diabetes mellitus-induced glucose diuresis, conditions where the urine urea concentration is low. In addition, the renal medullary tissue urea concentration is high when the urine urea concentration is high. This suggests that UT-A2 and UT-B abundances increase when the medullary interstitial urea concentration is high, which would tend to increase intrarenal urea recycling during antidiuresis, thereby preventing the loss of urea from the medulla and maintaining medullary interstitial osmolality.

NKCC2/BSC1. NKCC2/BSC1 abundance is regulated by vasopressin-dependent and vasopressin-independent factors (reviewed in Refs. 8 and 16). Osmotic diuresis reduces sodium reabsorption in the proximal tubule, resulting in an increase in sodium delivery to the thick ascending limb. In the present study, NKCC2/BSC1 abundance increased in response to diabetes and 4% NaCl, both with and without urea, and to 20% urea. The increase in NKCC2/BSC1 is consistent with previous studies showing that NKCC2/BSC1 abundance increases when sodium delivery to the thick ascending limb increases (7, 12). NKCC2/BSC1 did not increase in response to the lesser degree of osmotic diuresis induced by 2.5% NaCl or 5% urea, suggesting that these regimens may not increase sodium delivery sufficiently to cause a measurable increase in NKCC2/BSC1.

**Summary and perspective.** During osmotic diuresis 1) UT-A1 abundance increases when the percentage of urea in total urinary solute and/or urine urea concentration is low, which will tend to promote the delivery of urea from the inner medullary collecting duct lumen to the inner medullary interstitium; and 2) UT-A2 and UT-B abundances increase when the urine concentration in the medullary interstitium is high, which will tend to enhance intrarenal urea recycling and prevent the escape of urea from the renal medulla. The changes in urea transporter abundances are relatively specific because NKCC2/BSC1 abundances increase during osmotic diuresis, regardless of urea’s contribution to urinary solute.

These changes also raise the possibility that urea transporters may be responding to, or sensing, urea. Is this feasible? Several urea-specific signaling pathways have been identified in cultured mIMCD3 cells and the renal medulla (reviewed in Refs. 3 and 28). Thus we speculate that cells in the renal medulla may be able to sense and respond to changes in urea, perhaps by activating urea-specific signaling pathways and regulating urea transporter abundance. Future studies will be needed to test this hypothesis.

**ACKNOWLEDGMENTS**


**GRANTS**

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