Regulated expression of renal and intestinal UT-B urea transporter in response to varying urea load

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Inoue, Hideki, Shelley D. Kozlowski, Janet D. Klein, James L. Bailey, Jeff M. Sands, and Serena M. Bagnasco. Regulated expression of renal and intestinal UT-B urea transporter in response to varying urea load. Am J Physiol Renal Physiol 289: F451–F458, 2005.—Production, recycling, and elimination of urea are important to maintain nitrogen balance. Adaptation to varying loads of urea due to different protein intake or in renal failure may involve changes in urea transport and may possibly affect urea transporters. In this study, we examined the expression of the UT-B urea transporter in rats fed a low-protein diet (LPD), a high-protein diet (HPD), and a 20% urea-supplemented diet. In the kidney, UT-B protein abundance increased in the outer medulla of both LPD-fed rats and 20% urea-fed rats, without changes in the inner medulla of either group compared with controls. In HPD-fed rats, UT-B protein abundance decreased significantly in both the outer and inner medulla. We identified expression of UT-B in the rat colon, as a 2.4-kb mRNA transcript and as an ~45-kDa protein, with apical localization in superficial colon epithelial cells. UT-B also is expressed in rat small intestine. In rat colon, UT-B protein abundance was mildly, but significantly, decreased in LPD-fed and 20% urea-fed rats. UT-B abundance also was examined in the colon of 7/8 nephrectomized, uremic rats and in HPD-fed rats and was not significantly different from that in control rats. These findings indicate that UT-B expression is regulated in response to different loads of urea, with a pattern that suggests involvement of tissue-specific regulatory mechanisms in kidney and colon.

Slc14A1 gene have a mild defect in urine concentration ability (29). Furthermore, recent studies have shown impaired ability to concentrate urine in transgenic mice lacking UT-B (38) or with combined deficiency of UT-A1 and UT-A3 (6), which becomes more severe when the UT-A1/A3 knockout mice are stressed by water restriction (6).

In recent years urea transporters also have been identified in other tissues, such as brain (4), testis (8), and intestine (16, 31, 32). The physiological role of urea transport in brain and testis is not known, but the ability of UT-A and UT-B knockout mice to reproduce is not impaired. In the intestine, the UT-B transporter has been identified in the colon of humans (16) and rats (present study; Ref. 33). An intestinal UT-A transporter isoform, UT-A6, has been characterized from human colon (6), and the presence of a UT-A transporter also has been detected in mouse intestine (32). Urea circulating in the blood can pass into the enteric lumen, where it is hydrolyzed into ammonia and carbon dioxide by the enzyme urease, produced by intestinal bacteria. Ammonia then can be incorporated into protein synthesized by the bacteria or be reabsorbed. Part of the absorbed nitrogen derived from urea hydrolysis can thus be "salvaged" and resynthesized to urea or remain available for other synthetic pathways, with a relatively little fraction eliminated with the feces under normal conditions (11, 17, 22, 37). Different protein intake can affect the production of urea and the generation of intestinal ammonia (13), and it seems likely that it also may influence the rate of urea transfer across the intestinal wall mediated by urea transporters. In this article, we describe the effect of different loads of urea on the expression of the urea transporter UT-B in the kidney and in the colon.

METHODS

Animals. All animal protocols were reviewed and approved by the Emory University Institutional Animal Care and Use Committee. Male Sprague-Dawley rats weighing 125–200 g (Charles River Laboratories, Wilmington, MA) were used in these studies and assigned to groups of four to six animals per each experimental condition. All rats had unrestricted access to water throughout the study. Control rats had free access to standard powder rat chow (Testdiet 5001; Purina) containing 23% protein and 1.05% NaCl. To increase urea load and blood urea nitrogen (BUN), we fed rats for 12 days with a diet containing 20% urea, added to standard powder diet. To increase urea load, we fed rats for 12 days with a low-protein diet (LPD) containing 14% protein. To measure urine volume, we kept rats in metabolic cages for 24 h and collected urine for measurement of osmolality and urea. Urine osmolality was measured with a vapor pressure osmome-
eter (model 5500; Wescor, Logan, UT), and urea was measured in urine and in blood collected after death by using the Infinity BUN reagent (Sigma). In another group of rats, uremia was induced by 7/8 nephrectomy, as previously described (3), followed by 2 wk of feeding with a high (43%)-protein diet (HPD) (Teklad Premier Laboratory Diet, Madison, WI), with free access to water containing one-quarter saline. To test the effect of a high-protein diet, we matched rats fed HPD for 2 wk with control rats pair fed a standard diet. After death, kidneys were removed, dissected into cortex, outer medulla, and inner medulla, and processed for determination of protein and mRNA abundance. The intestine was opened, the fecal content was removed, and the internal surface of the ileum and colon was gently scraped with a blade to collect the mucosal layer. Fecal pellets were collected, weighed, homogenized into 7% trichloroacetic acid (Fisher), and centrifuged at 5,000 rpm for 10 min to precipitate protein. The supernatant was processed for determination of urea.

Western blot analysis. Proteins (10 μg/lane) were separated on 10% SDS-polyacrylamide gels and then transferred to a polyvinylidene difluoride membrane (Gelman Scientific, Ann Arbor, MI). Blots were blocked with 5% nonfat dry milk in Tris-buffered saline (TBS) at room temperature for 30 min and then incubated with an antibody to the carboxyl terminus of the rat UT-B (33) (this antibody reacts with rat, mouse, and human UT-B) at a dilution of 1:1,000 overnight at 4°C. After three washes in TBS with 0.5% Tween 20 (TBS/Tween), blots were incubated for 2 h with horseradish peroxidase-conjugated goat anti-rabbit IgG at a dilution of 1:5,000 (Amersham, Arlington Heights, IL) at room temperature. After two washes with TBS/Tween, the bound secondary antibody was visualized by chemiluminescence (ECL kit; Amersham).

Northern blot analysis. Total RNA was purified with TriPure isolation reagent (Roche, Indianapolis, IN). Northern hybridization was performed using a full-size marine UT-B cDNA probe (38), 86% identical with the human cDNA sequence, as previously described (27). To estimate mRNA abundance, we normalized the intensity of the UT-B signal to the intensity of glyceraldehyde-3-phosphate dehydrogenase or 18S RNA as internal control and compared the intensity in each experimental group and control.

Real-time PCR. Synthesis of cDNA was performed from 1 μg of total RNA with the SuperScript First-Strand Synthesis System Kit (Invitrogen, Carlsbad, CA). Real-time PCR was performed on 10 ng of RNA, using TaqMan Gene Expression primers and a probe for rat Slc14A1 (UT-B) and TaqMan Endogenous Control for rat β-actin (Applied Biosystems, Foster City, CA). PCR reactions were performed in triplicate, using TaqMan Universal Master Mix (Applied Biosystems) on an iCycler thermocycler (Bio-Rad, Hercules, CA). Negative controls, in which reverse transcription was not performed, were tested for each sample. Reaction efficiency based on standard curves for UT-B and β-actin ranged from 90 to 100%. Relative abundance of UT-B mRNA was estimated by normalizing for β-actin mRNA.

Immunolocalization. Ring segments of rat intestine were cut, fixed in 10% buffered formalin, and paraffin embedded. For immunohistochemistry, 5-μm sections of formalin-fixed, paraffin-embedded tissue were stained with the UT-B antibody at 1:2,000 dilution, followed by goat, horseradish peroxidase-conjugated anti-rabbit secondary antibody (Dako, Carpinteria, CA), as previously described (33). The primary antibody was omitted for negative control.

Statistics. Data are presented as means ± SD, and n indicates the number of rats per group. Differences between each experimental and control group were tested by unpaired, two-tailed Student’s t-test, with P < 0.05 indicative of significance.

RESULTS

Effect of low-protein, high-protein, and 20% urea feeding on renal UT-B abundance. No significant differences in weight were detected among rats fed LPD, HPD, or 20% urea compared with the control group. The studies on LPD and 20% urea-supplemented diet were repeated twice with similar results. Rats fed LPD showed significantly lower BUN values, similar urine volume, lower urea excretion, and lower urine osmolality compared with control rats (Fig. 1). Rats fed 20%...
urea showed significant increase in BUN values, urine volume, and urea excretion, but decreased urine osmolality, compared with controls (Fig. 1), consistent with osmotic diuresis. Urea accounted for 44% of urine osmolality in control rats and 43% in LPD rats, whereas in 20% urea-fed rats, urea accounted for 95% of urine osmolality.

In rats fed LPD, the abundance of the UT-B protein was significantly increased in the outer medulla (25%), compared with rats on a standard diet, but did not change in the inner medulla (Fig. 2). In 20% urea-fed rats, the abundance of the UT-B protein also was significantly increased in the outer medulla (60%) but was unchanged in the inner medulla (Fig. 3). As shown with real-time PCR, UT-B mRNA abundance in the outer medulla was significantly increased in 20% urea-fed rats but was without significant change in LPD-fed rats (Fig. 4). In the inner medulla, UT-B mRNA abundance was significantly higher in LPD-fed rats but without significant change in 20% urea-fed rats (Fig. 4).

In rats fed HPD, the abundance of the UT-B protein was significantly decreased in both the outer medulla and inner medulla (50% and 54%, respectively) compared with control animals (Fig. 5, A and B). As shown with real-time PCR, UT-B mRNA abundance in HPD-fed rats was significantly increased (2-fold) in the outer medulla but unchanged in the inner medulla (Fig. 5C).

Effect of urea load on colon UT-B expression. We recently characterized expression of the UT-B transporter in human colon epithelium (16), where it could mediate, at least in part, transfer of urea from the blood into the intestinal lumen. In the present study, we identified UT-B in scrapings of colon mucosa of rat, where it appears as a 2-kb mRNA transcript (Fig. 6A), similar in size to the predominant UT-B transcript in human colon, but with using RT-PCR with primers spanning the region common to UT-A6 and UT-A1 rat cDNA sequence, we could not detect evidence of a rat UT-A transcript homologous to the recently described human UT-A6 in rat colon (data not shown). A UT-B protein of ~45 kDa is detected by Western blot analysis in rat colon, similar in size to the one identified in human colon mucosa (Fig. 6B), and showing apical membrane localization in the superficial colon epithelial cells, as in the human colon epithelium (Fig. 6C). UT-B also is expressed in rat small intestine (Fig. 6, E and F).
We measured the abundance of the UT-B protein in the colon mucosa of LPD-fed and 20% urea-fed rats compared with controls (Fig. 7B). Because of low abundance and sample variability, we could not determine whether UT-B mRNA expression differs between LPD-fed rats or 20% urea-fed rats compared with rats fed standard chow.

We also tested the expression of UT-B in the colon of uremic rats compared with control sham-operated rats. The BUN values in sham-operated rats (17 ± 5 mg/dl), even after 2 wk of HPD, were comparable to those of rats fed a normal diet (18 ± 1 mg/dl). Uremic rats had the highest BUN (141 ± 56 mg/dl), much higher than that of 20% urea-fed rats (62 ± 5 mg/dl) (Fig. 8). Although very little urea was detectable in the feces because of breakdown by bacteria, fecal urea content differed among groups, with the highest values in uremic rats (0.58 ± 0.2 μmol/g) and in HPD-fed, sham-operated rats (0.43 ± 0.3 μmol/g) compared with control rats (0.15 ± 0.02 μmol/g). LPD-fed rats had the lowest value (0.09 ± 0.03 μmol/g) (Fig. 8). Relatively high fecal urea content also was detected in 20% urea-fed rats (0.34 ± 0.2 μmol/g), possibly reflecting a high amount of orally ingested urea, rather than urea transfer across the intestinal wall. There was no significant difference in the abundance of the UT-B protein in the colon of uremic rats compared with sham-operated controls (Fig. 9). We could not find significant changes in UT-B protein expression in the colon of HPD-fed rats compared with controls. It was difficult to evaluate UT-B mRNA abundance in colon samples. Thus we could not detect significant differences in UT-B mRNA expression among these different conditions and their respective controls.

**DISCUSSION**

In this study, we have identified expression of the UT-B urea transporter in rat colon and small intestine and shown that the abundance of the urea transporter UT-B in the kidney and colon of rats varies with different loads of urea but does not seem to follow the same pattern. In the kidney, the abundance of UT-B protein increases in the outer medulla of both LPD-fed rats with a low urea load and 20% urea-fed rats with a high urea load. The increase in outer medullary UT-B abundance was smaller in LPD-fed rats than in 20% urea-fed rats. Compared with controls, urea-fed rats have higher concentrations of urea in blood and urine, urea-induced diuresis, and high urea in the medullary interstitium (18). In this condition, it is likely that the increment in UT-B abundance contributes to increased recycling of urea in the outer medulla, to prevent loss of urea by osmotic diuresis and to preserve medullary osmolality. Higher UT-A2 and UT-B protein abundance were recently described in urea-fed rats, even with lower urea load (5%), indicating activation of both tubular and vascular urea transporters in this setting (18). Compared with controls, urea-fed rats have higher concentrations of urea in blood and urine, and Peterson et al. (28) have shown previously that the medullary concentration of urea in rats fed a low-protein diet is significantly lower than in rats fed a standard diet. In this setting, the higher abundance of UT-B protein in the outer medulla of LPD-fed rats may promote increased recycling of urea, and preserve adequate levels of urea and osmolality in the medul-
lary interstitium, in the face of reduced availability of this solute in the body.

In rats fed a high-protein diet, UT-B expression fell in both the outer and inner medulla. In this case, reduced abundance of UT-B and decreased recycling of urea in the medulla would help to promote urea excretion. Urea production by the liver and urea excretion in the urine have been demonstrated to increase linearly with higher dietary proteins (20).

In the colon, we observed a downregulation of UT-B in LPD-fed rats and urea-fed rats but not in uremic rats and HPD-fed rats. The recent identification of an intestinal UT-A transporter in mouse and human colon suggests that more than one urea transporter may participate in the movement of urea across the enteric wall (6, 32). As mentioned in RESULTS, we could not demonstrate the presence of a UT-A transporter in the rat colon, and we are not aware of studies where this information is available. If UT-A were expressed in rat intestine, it would certainly contribute to the net movement of urea across the intestinal mucosa. We can only speculate on the possible effect that variation of UT-B may have on urea intestinal fluxes.

In LPD-fed rats, if urea could move from the blood into the intestinal tract through urea transporters, one would expect that lower urea in the blood of these animals would be associated...
with downregulation of intestinal urea transporters as a means to reduce loss of urea from the gut. Our data are consistent with this hypothesis, which also is supported by the lower content of urea in the feces of LPD-fed rats.

UT-B expression was downregulated in 20% urea-fed rats. In this condition, blood urea is higher than control, and increased abundance of the UT-B transporter to promote increased elimination of urea through the gut would be expected. However, the possibility that a relatively high content of ingested urea in the intestinal lumen of 20% urea-fed rats could lead to inhibition of UT-B expression in the colon mucosa cannot be excluded.

Uremic rats had the highest blood urea among all groups and the highest amount of urea in their feces, suggesting that an increased amount of urea was transferred from the blood to the gut in these animals. Although the intestinal UT-B protein appeared slightly higher in several uremic rats compared with control animals, the overall difference between uremic and control groups was not statistically significant. Thus the hypothesis that intestinal UT-B expression may be upregulated during uremia could not be verified by the results obtained under the conditions tested. The UT-B abundance is not increased in the colon of nonuremic rats fed HPD compared with normal controls, suggesting that increased urea load, per se, may not result in increased expression of intestinal UT-B. The possible regulation of intestinal UT-A expression and function relative to urea load and dietary protein content deserves further study.

The studies on the characterization of intestinal urea transporters have so far focused on the large intestine (6, 16, 32). Substantial secretion of urea has been shown to take place in the small intestine (11), and in this study we have shown that UT-B is expressed in ileum, where the role of urea transporters may be significant, and further investigation is required.

Fig. 7. A: UT-B protein abundance was significantly decreased in the colon of 20% urea-fed rats and in the colon of LPD-fed rats compared with control. A: UT-B protein abundance as detected using Western hybridization. Histogram shows results of densitometric analysis of UT-B protein expression (n = 4). *P < 0.05 vs. control. B: only a slight decrease in staining for UT-B is shown in the colon mucosa of LPD-fed and 20% urea-fed rats.

Fig. 8. BUN values and fecal content of urea in different groups of rats. HPD, sham-operated controls for the uremic group fed HPD; uremic, uremic rats. Values are means ± SD (n = 4–5).
The specific factors modulating renal and intestinal UT-B abundance in response to urea load remain to be clarified. The mechanisms regulating urea transporters have been mostly studied in the kidney, particularly for the UT-A transporter. Direct stimulation of the UT-A2 transporter expression by vasopressin (24, 35) and stimulation of UT-A1 and UT-A3 expression by hypertonicity (25) have been described. Expression of UT-A1 is inhibited by glucocorticoids (26, 27) and aldosterone (12). Vasopressin seems to inhibit the abundance of renal UT-B transporter (34). A direct effect of tonicity on UT-B has not been demonstrated, and the possible role of urea in the regulation of urea transporters expression has not been characterized. In a recent study, Kim et al. (18) suggested that a high interstitial concentration of urea may increase the abundance of UT-A2 and UT-B and proposed a stimulating effect of urea on the expression of these two transporters in the renal medulla. Although this possibility seems applicable to the renal findings in 20% urea-fed rats, associated with increased renal interstitial urea, LPD-fed rats have a lower concentration of urea in the blood and urine and a lower interstitial urea (28), which suggests that other factors must intervene to stimulate UT-B expression in this condition. The pattern of UT-B mRNA expression does not suggest a significant role of transcriptional regulation in the renal abundance of UT-B protein in the conditions tested.

The mechanisms controlling the abundance of intestinal UT-B and UT-A urea transporters are unknown. The regulated expression and activity of urea transporters in the gastrointestinal tract may influence the amount of nitrogen available for recycling by incorporation into protein synthesis, derived from hydrolysis of urea in the enteric lumen. This “nitrogen salvage” process may be valuable in protein-deficient conditions such as severe malnutrition or kwashiorkor, as indicated by analysis of urea kinetics in malnourished children, which showed a high utilization of nitrogen derived from urea salvage during recovery on both high- and low-protein diets (1). Decreased urea production and increased urea elimination through the gut are desirable when urea excretion by the kidney is impaired by renal failure. A beneficial role of protein restriction to reduce urea and other toxic waste products of protein metabolism during renal failure has been known for many years (14) and is widely used in the management of end-stage renal disease (19, 21, 23, 36). More recently, ways of exploiting increased elimination of urea and other uremic noxious metabolites through the intestine have been proposed, including oral administration of bacterial strains capable to hydrolyze urea, as an additional strategy to reduce the burden of uremic toxins during renal failure (5, 9, 15, 30). Whether and how these maneuvers affect urea enteric transporters remains to be elucidated. Nevertheless, identification of the factors regulating intestinal urea transport is important to understand how this process can be modulated in different pathophysiological states and may have potential therapeutic implications.

In summary, we have identified the UT-B transporter in rat small intestine and colon. We have shown that expression of the UT-B urea transporter is regulated in response to different loads of urea in the kidney and in the colon of the rat but that it does not seem to follow a similar pattern in the two organs. Our findings suggest the possible involvement of tissue-specific regulatory mechanisms, which remain to be determined.

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REFERENCES


