The urea transporter UT-A1 plays a predominant role in a urea-dependent urine-concentrating mechanism

Xiaoqiang Geng, Shun Zhang, Jinzhao He, Ang Ma, Yingjie Li, Min Li, Hong Zhou, Guangping Chen, and Baoxue Yang

From the State Key Laboratory of Natural and Biomimetic Drugs, Department of Pharmacology, School of Basic Medical Sciences, Peking University, Beijing, China, the Department of Physiology, Emory University School of Medicine, Atlanta, Georgia, USA, and the Key Laboratory of Molecular Cardiovascular Sciences, Ministry of Education, Beijing, China

Edited by Mike Shipston

Urea transporters are a family of urea-selective channel proteins expressed in multiple tissues that play an important role in the urine-concentrating mechanism of the mammalian kidney. Previous studies have shown that knockout of urea transporter (UT)-B, UT-A1/A3, or all UTs leads to urea-selective diuresis, indicating that urea transporters have important roles in urine concentration. Here, we sought to determine the role of UT-A1 in the urine-concentrating mechanism in a newly developed UT-A1–knockout mouse model. Phenotypically, daily urine output in UT-A1–knockout mice was nearly 3-fold that of WT mice and 82% of all-UT–knockout mice, and the UT-A1–knockout mice had significantly lower urine osmolality than WT mice. After 24-h water restriction, acute urea loading, or high-protein (40%) intake, UT-A1–knockout mice were unable to increase urine-concentrating ability. Compared with all-UT–knockout mice, the UT-A1–knockout mice exhibited similarly elevated daily urine output and decreased urine osmolality, indicating impaired urea-selective urine concentration. Our experimental findings reveal that UT-A1 has a predominant role in urea-dependent urine-concentrating mechanisms, suggesting that UT-A1 represents a promising diuretic target.

The physiological functions of UTs have been demonstrated via the phenotypic analysis of several UT-selective knockout (KO) mouse models (25–32). UT-A1 and UT-A3 are the major urea transporters contributing to the urine concentration confirmed by the UT-A1/A3-KO mice that exhibit a strong urine-concentrating defect. In basal conditions, UT-A1/A3-KO mice urine osmolality ($U_{\text{osm}}$) is reduced by 70%. Moreover, UT-A1/A3-KO mice were unable to raise their $U_{\text{osm}}$ after 24-h fluid restriction (25). These results highlighted the main role of UT-A1 and UT-A3 in transporting urea from the collecting duct to the deep inner medullary tissue. In 2015, Klein et al. (33) generated transgenic mice merely expressing UT-A1 but not UT-A3. They found that transgenic expression of UT-A1 alone in mice lacking UT-A1/A3 was sufficient to restore urine-concentrating ability, indicating the prior role of UT-A1 in the urea delivery of inner medulla and in the urine-concentrating mechanism. However, because UT-A1 is mainly expressed in the apical plasma membrane and UT-A3 is expressed in the basolateral plasma membrane of the principle cells at the end of IMCD (13, 34), it is necessary to determine the relative contribution rate of UT-A1 and UT-A3 to urea-dependent urine-concentrating mechanism.

UT-A1–KO mice are a suitable model to determine the role of UT-A1 in intrarenal urea recycling and the urine-concentrating mechanism. Because both UT-A1 and UT-A3 share the same promoter as well as the sequences of the first nine exons in gene Slc14a2, it is difficult to delete UT-A1 without affecting UT-A3. However, there is a unique sequence at the 3′-coding region of UT-A3 that is located in the intron of UT-A1 (8, 35, 36). So, we designed an ingenious point synonymous mutation of the consensus splice site of UT-A1 mRNA to disrupt the gene transcription process without any influence on the expression of UT-A3.

In this study, we generated an innovative UT-A1–KO mouse model. The phenotypic analysis showed that UT-A1 deficiency led to a severe urea-selective urine-concentrating defect and produced 3-fold daily urine output compared with WT and 82% of all-UT–KO mice. Moreover, UT-A1–KO mice exhibited normal growth, unaffected kidney index, morphology, and electrolyte excretion. Extrarenal phenotype analysis indicated that UT-A1 deletion did not affect blood pressure and had no effect on glycol and lipid metabolism. The experimental results suggest that UT-A1, but not other UTs, plays a predominant role in urea-dependent urine concentration.

This article contains supporting information.

*For correspondence: Baoxue Yang, baoxue@bjmu.edu.cn.
Urea transporter UT-A1–null mice

Figure 1. CRISPR/Cas9 system for UT-A1 KO and identification of UT-A1–KO mice. A, organization and restriction map of the mouse Slc14a2 gene and the alternatively spliced transcriptions of UT-A3 and UT-A1. Rectangles indicate exon segments that constitute coding sequences. Red letters represent the synonymous mutant bases of exon 9 by using CRISPR/Cas9 system. B, Western blotting analysis of kidney using UT-A2 (UT-A1 C-terminal) or UT-A3 (UT-A1 N-terminal) antibody. C, relative protein levels of UT-A1, UT-A2, and UT-A3 in kidney. The data are normalized by the intensity of GAPDH. The data are presented as the means ± S.D. (n = 3). The data are analyzed by Student’s t test. **p < 0.001 versus +/- . Delta UT-A1, UT-A2, and UT-A3 immunofluorescence of kidney inner and outer medulla stained by UT-A3 or UT-A2 antibody. -/-, UT-A1-KO; +/- , WT.

UT-A1 is an effective diuretic target, and the UT-A1 inhibitors might be developed as potential diuretics without electrolyte disturbance.

Results

Generation of UT-A1–KO mice by CRISPR/Cas9 system

UT-A1–KO mice were generated by CRISPR/Cas9 system based on the specific structure of UT-A gene Slc14a2 (8) as described under “Experimental procedures” and shown in Fig. 1A. Western blotting analysis showed that UT-A1 and UT-A3 were expressed in the renal inner medulla, and UT-A2 was expressed in the renal outer medulla in WT mice. UT-A1 was specifically deleted in the IMCD, UT-A2 and UT-A3 expressions were not affected in UT-A1–KO mice (Fig. 1B and C). Immunofluorescence confirmed that UT-A1 was completely deleted, UT-A3 expressed in basolateral side of IMCD and UT-A2 expressed in outer medulla were not influenced (Fig. 1D).

These results confirmed that UT-A1 was successfully knocked out. Genotype analysis of offspring from breeding of UT-A1-KO heterozygous mice indicated a nearly 1:2:1 fitting to the Mendelian distribution (46 WT, 92 heterozygous, and 41 UT-A1-KO).

Urine-concentrating ability in UT-A1–KO mice

An analysis of growth by mouse body weight (age, 1–8 weeks) showed no difference between WT and UT-A1–KO mice (data not shown). Kidney index was not obviously different between UT-A1–KO mice, all-UT–KO mice, and WT mice (Fig. 2A). Daily urine output was shown in Fig. 2B. The UT-A1–KO mice exhibited severely polyuria, excreting over three times the urine compared with litter-matched WT mice, which was ~82% of the all-UT–KO mice (Fig. 2B and Table S1). The average urine osmolality of UT-A1–KO mice was markedly lower than that of WT mice and was similar to all-UT–KO mice (Fig. 2C and Table S1). Urine-concentrating ability was measured in response to 24-h water deprivation. Urine osmolality increased substantially in the WT mice but only slightly in the UT-A1–KO mice, in line with all-UT–KO mice (Fig. 2C). There was no significant difference in the gross morphology of the kidneys of the two genotypes.

Histological examination showed that there was dilatation of collecting ducts in the IM in UT-A1–KO mice on account of polyuria (Fig. 2D). The composition of the aqueous component of the IM was shown in Fig. 2E. Similar to all-UT–KO mice, the IM urea concentration in UT-A1–KO mice was significantly lower than that in WT mice. There was no obvious difference in sodium, potassium, and chloride concentrations between UT-A1–KO mice, all-UT–KO mice, and WT mice (Fig. 2E). Moreover, because of the pivotal role of UT-A1 in the urine concentrating mechanism in kidney, we adopted the transcutaneous measurement of glomerular filtration rate (GFR) technology and found that the GFR of UT-A1–KO mice (200.1 ± 88.1 μl/min) was similar to that of WT mice (230.2 ± 77.5 μl/min) (data not shown), suggesting that UT-A1 deletion does not influence glomerular filtration rate and hemodynamics.

Osmolality and urea concentration were measured in urine and plasma under basal condition. Urinary urea concentration was significantly low in UT-A1–KO mice, in keeping with all-UT–KO mice. However, plasma urea concentration was similar in UT-A1-KO, all-UT–KO and WT mice. There was no significant difference in plasma sodium, potassium, and chloride concentrations between UT-A1–KO mice, all-UT–KO mice, and WT mice (Fig. 2E). Moreover, lipid metabolism was unaffected in the absence of UT-A1 (Table S1).

Urea excretion ability

To evaluate the contribution of UT-A1 in the kidney’s capacity to dispose urea loads (~1/10 of daily urea excretion) as previously described (30). In the first 2 h after administration of urea load, WT mice significantly increased their urea concentration and urinary osmolality with a modest rise in urinary flow rate, whereas UT-A1–KO mice showed little rise in urinary osmolality and urinary urea concentration with rapidly increased urine flow rate (Fig. 3, A–C). The time
course of urea excretion was similar in both UT-A1–KO mice and WT mice (Fig. 3D). After urea excretion returned to basal level (6–8 h after the load), urinary osmolality and urea concentration in WT mice remained elevated. However, in UT-A1–KO mice, urinary osmolality was only modestly elevated, and urine output declined slightly, indicating that mice could not accumulate urea in the medulla after an acute urea load without UT-A1. Administration of exogenous urea significantly improved the ability of the kidney to concentrate other urinary solutes in WT mice, but this was not observed in UT-A1–KO mice (Fig. 3E). Because of the large urine output in the primary stage, the excretion of non-urea solutes was increased slightly in both WT and UT-A1–KO mice responding to the urea load. The excretion of non-urea solutes was similar in these two genotypes (Fig. 3F).

To evaluate the mechanisms by which mice of different genotypes chronically adapt their renal function to different levels of urea excretion, WT and UT-A1–KO mice were fed for 1 week with a diet containing low, normal, or high protein content (10, 20, or 40% casein, as the only source of protein), respectively. Protein intake had a significant influence on urine output, which increased in the two groups with the amount of diet protein. Under different protein content circumstances, the urine flow rate was higher in UT-A1–KO mice than in WT mice (Fig. 4A). Urinary osmolality and urea concentration were increased obviously with the amount of diet protein in WT mice but not changed in UT-A1–KO mice (Fig. 4, B and C), suggesting that the long-term accumulation of urea in the IM was disrupted to a great extent without UT-A1. With each protein content diet, the osmolar excretion and urea excretion were similar in the two groups (Fig. 4, D and E). Non-urea solute excretion elevated along with the higher protein intake, and there was no significant difference between two groups with the same protein content (Fig. 4F).

Blood pressure observed in UT-A1–KO mice

Because the lower blood pressure observed in UT-B-KO and all-UT–KO mice compared with WT mice (30, 37), we measured blood pressure of WT and UT-A1–KO mice using a computerized tail-cuff system with a photoelectric sensor (37). The results showed that diastolic blood pressure (95.3 ± 6.2 mm Hg in UT-A1–KO versus 92.2 ± 7.0 mm Hg in WT), systolic blood
Urea transporter UT-A1–null mice

Figure 3. Renal handling of acute urea loading. 300 μmol of urea were injected intraperitoneally just after the first 2-h urine collection (time 0). A, urinary osmolality (U_{osm}). B, urine output. C, urinary urea concentration (U_{urea}). D, urea excretion (exc.). E, non-urea solute concentration (U_{non-urea solutes}). F, excretion of non-urea solutes. The data are presented as means ± S.D. (n = 6). The data are analyzed by one-way ANOVA and post hoc Bonferroni test. *, P < 0.05 versus +/+ or −/−, UT-A1-KO; +/−, WT.

Discussion

UT-A1 and UT-A3 are expressed in the inner medullary collecting duct apical plasma membrane and basolateral plasma membrane, respectively (13, 34). Deletion of UT-A1/A3 results in severe urine concentration deficiency and urea-selective diuresis, indicating the significant role of UT-A1 and UT-A3 in the urine-concentrating mechanism. Further study identified that UT-A1 transgenic restoration successfully rescued the urine-concentrating defect in UT-A1/A3-KO mice (33). These studies suggest the predominant role of UT-A1 in urine concentrating. The specific UT-A1-KO mouse is a good model to confirm the importance of UT-A1 versus UT-A3 in urea-dependent urine-concentrating mechanism.

Generating the UT-A1-KO mouse is a challenging task because the tricky gene structure of SLC14A2 encodes the UT-A1 to UT-A6 proteins simultaneously. It was impossible to generate the UT-A1–KO mice simply by conventional frameshift mutation because the UT-A1 gene structure covers six UT-A members. Based on a small piece of DNA sequence shared for the UT-A3 coding region and UT-A1 splicing site, respectively, we designed an elegant strategy by a point synonymous mutation of consensus splice site nucleotides of UT-A1 to inhibit the splicing process, thereby destroying the integrity of the UT-A1 mRNA transcript and knocking out the UT-A1 protein specifically. However, the protein expression of other UT-As would not be affected by such synonymous mutation. The mouse model with specific UT-A1 deletion was successfully generated and validated by means of functional analysis.

Experimental results showed that UT-A1–KO mice exhibited a grossly normal phenotype, except high urine output and low urine-concentrating ability. The inner medullary urea concentration in UT-A1–KO mice was significantly lower than that in WT mice, and no obvious difference was found in sodium, potassium, or chloride concentrations in blood between the two genotypes. By comparing the data from UT-A1-KO mice and all-UT-KO mice, it is clear that UT-A1 itself sufficiently mediates the urea reabsorption of IM, UT-A3, or other UTs in kidney but could not restore the impaired urine-concentrating mechanism, suggesting UT-A1 as an effective diuretic target.

Unlike the all-UT–KO and UT-B-KO mice, UT-A1 deletion had no influence in blood pressure. No change was found in either plasma creatinine or GFR in UT-A1–KO mice, demonstrating that UT-A1 deletion did not influence normal glomerular filtration rate. Importantly, plasma Na^+, K^+, Cl^−, and Ca^{2+} were similar between UT-A1–KO, all-UT–KO, and WT mice, indicating that deletion of UT-A1 increased urine output without affecting electrolyte metabolism.

There appeared no morphological change in the cortex and outer medulla of UT-A1–KO mice, except for the collecting duct dilatation caused by large urine output, similar to all-UT–KO mice. UT-A1–KO mice showed much greater urine output than WT mice and slightly lower urine output than all-UT–KO mice. The reason is that less urea was reabsorbed at the end of IMCD (by UT-A1), and the existence of UT-B and UT-A3 partially contributes to maintain inner medullar urea concentration. Compared with WT mice, neither an acute nor a chronic urea load increased urinary urea concentration and osmolality, indicating that UT-A1–KO mice could not accumulate urea in the IM. These results were highly similar to all-UT–KO mice, suggesting the predominant role of UT-A1 in urea reabsorption and concentration in kidney.

With the addition of the new UT-A1-KO model, there are seven models of UT KO mice, including UT-B-KO (27), UT-A1/A3-KO (25), UT-A2-KO (29), UT-A2/B-KO (28), UT-A1/A3-KO with UT-A1 rescue (33), all-UT-KO (30), and UT-A1-KO. All these mice were alive at adult age. The degree of urine-concentrating deficiency in these mice was compared by urine output, urine osmolality, and urine urea concentration (Fig. 5, A–C). The relative urine output was shown as follows: all-UT-KO mice > UT-A1/A3-KO mice ≥ UT-A1–KO mice > UT-B-KO mice > UT-A2/B-KO mice > UT-A3-null mice (UT-A1/A3-KO with UT-A1 rescue) > UT-A2-KO mice ≥ WT mice. The relative urine osmolality was shown as follows: UT-A1/A3-KO mice ≤ all-UT-KO mice < UT-A1–KO mice < UT-B-KO mice < UT-A2/B-KO mice < UT-A2-KO mice < UT-A3-null mice < WT mice. The relative urine urea concentration was shown as follows: all-UT-KO mice < UT-A1–KO mice < UT-A1/A3-KO mice < UT-B-KO mice < UT-A3-null mice < UT-A2/B-KO mice < WT mice. The urine output volume of UT-A1–KO mice is 18% less than
that of all-UT-KO mice, which is attributable to the existence of UT-B and UT-A3. These data identified that UT-A1 is the most important UT in intrarenal urea recycling and urea-selective urine concentrating (Fig. 6).

This study confirmed that UT-A1 deletion did not affect GFR or the excretion principal solutes (Na\(^{+_1}\), K\(^{+_1}\), and Cl\(^{-2}\)). Previous findings indicate that UT inhibitors might be developed as novel diuretics without disturbing electrolyte balance and metabolism (30, 38–41). Actually, there have been certain UT inhibitors (e.g. PU-14, PU-48, PU1424, CB-20, and 8n) discovered and developed to inhibit all UTs (42–47). In this case, however, side effects can not be ignored because that UT-B-KO mouse was found to exhibit depression-like behavior (48) and to induce DNA damage and apoptosis in bladder (49), which attributed to elevated level of urea. Accordingly, non–UT-selective inhibitors may lead to elevated blood urea concentration and certain unpredictable side effects such as depression and bladder urothelial cell damage in addition to the diuresis bioactivity. By contrast, knockout of UT-A1 did not affect the blood urea level or other key physiological index excepting for the significant diuresis. The UT-A1–specific inhibitor possesses the most valuable potential to be developed into novel diuretic with the minimum side effects compared with non–UT-selective inhibitors. The UT-A1–KO mouse model we generated is an ideal animal model in which to assess the specificity of novel UT-A1 inhibitors.

**Experimental procedures**

**Generation of UT-A1–KO mice**

Based on the specific structure of UT-A gene Slc14a2 and using CRISPR/Cas9 system, we designed a point synonymous mutation of consensus splice site nucleotides of UT-A1 to inhibit the splicing process and destroy the integrity of the UT-A1 mRNA transcript and finally knocked out the UT-A1 protein (Fig. 1A). Offspring were genotyped by PCR followed by DNA sequencing analysis (30). Heterozygous founder mice containing the point synonymous mutation of consensus splice site nucleotides that disrupted Slc14a2 gene in the germline were bred to produce homozygous UT-A1–KO mice. All-UT–KO mice were generated as described previously (30). The protocols were approved by the Peking University Health Center Committee on Animal Research.

![Figure 4. Renal handling of long-term urea loading in mice fed diets containing 10, 20, or 40% protein. A, urine output. B, urinary osmolality (U\(_{\text{osm}}\)). C, urinary urea concentration (U\(_{\text{urea}}\)). D, urinary osmolar excretion (exc.). E, urea excretion. F, excretion of non-urea solutes. The data are presented as means ± S. D. (n = 6). The data are analyzed by one-way ANOVA and post hoc Bonferroni test. *, P < 0.05 versus +/+., −/−, UT-A1-KO; +/+., WT.](#)

![Figure 5. Urine-concentrating ability in different UT-KO mice. A, relative daily urine output. The data are normalized by the urine output of WT mice (+/+). B, relative urinary osmolality (U\(_{\text{osm}}\)). The data are normalized by the urine osmolality of WT mice in their individual research. C, relative urinary urea concentration (U\(_{\text{urea}}\)). The data are normalized by the urea concentration of WT mice in their individual research. The data are derived from Refs. 25, 27–30, and 33. The data for WT mice are mean values from Refs. 25, 27–30, and 33. 1/1, WT; 2/2, KO.](#)
Western blotting analysis

Renal tissues (cortex, outer medulla, and inner medulla) were homogenized in tissue protein extraction reagent (Mei5, MF188-01, Beijing, China) containing a protease inhibitor mixture (catalog no. 11873580001, Roche). Total protein was assayed using BCA method (Pierce Biotechnology). The lysates were electrophoresed on polyacrylamide gels and electrotransferred to polyvinylidene difluoride membranes (Amersham Biosciences). After blocking, the membranes were incubated with antibodies against UT-A1, UT-A2, or UT-A3 (1:1000, provided by Dr. Guangping Chen, Emory University, Atlanta, GA, USA) (50, 51) and GAPDH (60004-1-lg, 1:5000 dilution; Proteintech, Rosemont, PA, USA). Goat anti-rabbit IgG or goat anti-mouse IgG (EASYBIO, Beijing, China) was added, and the blots were developed with an ECL plus kit (Biodragon, Beijing, China) and visualized with a chemiluminescence detection system (Syngene; GeneGnome XRQ, Cambridge, UK). Quantitation was performed by scanning and analyzing the intensity of the hybridization bands.

Immunofluorescence and hematoxylin and eosin staining

The kidney tissues were dissected out and fixed overnight in 4% paraformaldehyde. The specimens were transferred to a solution containing 20% sucrose in 0.1 M phosphate buffer, pH 7.4. Kidney was cut at 5 μm on a cryostat. The sections were blocked with 10% normal goat serum containing 1% (w/v) BSA, 0.1% Triton X-100, and 0.05% Tween 20 overnight at 4°C to avoid nonspecific staining. Then the sections were incubated with polyclonal UT-A1, UT-A2, or UT-A3 antibodies. The secondary antibodies (Cy3-goat anti-rabbit, 1:200; Jackson Immunoresearch Inc.) were added, incubated for 0.5 h, and then placed in a solution containing Hoechst (1:1000; Leagene, Beijing, China) for 1 min to stain the nuclei. The images were captured by laser confocal microscopy. Kidney tissues were obtained and fixed in 4% formaldehyde for paraffin embedding. Paraffin-embedded tissues were sectioned at 5 μm for hematoxylin and eosin staining. The images were captured by NanoZoomer-SQ (Hamamatsu Photonics, Hamamatsu City, Japan).

Urine-concentrating studies

Adult male WT and UT-A1–KO mice (8 mice/group; body weight, 22–25 g), were placed in metabolic cages adapted for mice (Harvard Apparatus, Holliston, MA, USA). After 2 days of adaptation to the cages, urine samples were collected for 24 h under paraffin oil (to prevent evaporation). In some experiments, urine samples were obtained from the same mice under basal conditions (unrestricted access to food and water) and after 24-h deprivation of food and water. 24-h urine output and water consumption were measured with metabolic cagels. Blood samples were collected in heparinized glass tubes by puncture of the periorbital venous sinus. Plasma was separated from blood cells by centrifugation. Urine osmolality was measured by freezing point osmometry (micro-osmometer; Precision Systems, Natick, MA, USA). Urine and plasma chemistries were measured by the Peking University Third Hospital Clinical Chemistry Laboratory (Beijing, China).

GFR measurement

For GFR measurement, the mice were anesthetized with isoflurane, and a miniaturized imager device built from two light-emitting diodes, a photodiode, and a battery (Mannheim Pharma and Diagnostics, MPD) was mounted via double-sided adhesive tape onto the shaved animal’s neck (52). For the duration of recording (~1.5 h), each animal was conscious and kept in a single cage. Before the intravenous injection of 150 mg/kg FITC-sinistrin (MPD), the skin’s background signal was recorded for 5 min. After removal of the imager device, the data were analyzed using MPD software. The GFR (μl/min) was calculated from the decrease in fluorescence intensity over time (i.e. plasma 1/2 of FITC-sinistrin) using a two-compartment model, the body weight of the mouse, and an empirical conversion factor (52).

Acute urea load

Adult male WT and UT-A1–KO mice (6 mice/group; body weight, 22–25 g) were adapted for 2 days before the urea load experiment to metabolic cages. Intraperitoneal injection was...
used to administer 300 μl of 1 m urea solution. Urine was collected in 2-h fractions for 2 h before and 10 h after the urea load and analyzed as described previously. Non-urea solute concentration was calculated by subtracting urea concentration from urine.

**Chronic alteration in protein intake**

Adult male WT and UT-A1–KO mice (6 mice/group; body weight, 22–25 g) were offered synthetic food with either low, normal, or high protein content (10, 20, or 40% casein, respectively, as the only source of protein). After 7 days on these diets, 24-h urine was collected and analyzed as described previously.

**Blood pressure measurement**

Blood pressure was measured using a computerized tail-cuff system (Hatteras Instruments, Cary, NC, USA) with a photoelectric sensor. Blood pressure was recorded daily at 9:00 a.m. and averaged over five consecutive recordings.

**Statistics**

Statistical analyses were performed using GraphPad Prism software (San Diego, CA, USA). All results are expressed as the means ± S.D. Each experiment was performed at least three times. The results of all replicates from each specimen were averaged. A two-tailed t test, one-way ANOVA analysis of variance followed by post hoc Bonferroni test, or a post hoc Bonferroni test was then performed to assess the differences between the groups. P values < 0.05 were considered statistically significant.

**Data availability**

All data in this study are contained within the article.

**References**

1. Fenton, R. A., Chou, C. L., Sovovsby, H., Smith, C. P., and Knepper, M. A. (2006) Gamble’s “economy of water” revisited: studies in urea transporter knockout mice. *Am. J. Physiol. Renal Physiol.* **291**, F148–F154 CrossRef Medline
2. Sands, J. M., Timmer, R. T., and Gunn, R. B. (1997) Urea transporters in kidney and erythrocytes. *Am. J. Physiol.* **273**, F321–F339 CrossRef Medline
3. Fenton, R. A., and Yang, B. (2014) Urea transporter knockout mice and their renal phenotypes. *Subcell. Biochem.* **73**, 157–152 CrossRef Medline
4. Yang, B., and Veerkman, A. S. (1998) Urea transporter UT3 functions as an efficient water channel direct evidence for a common water/urea pathway. *J. Biol. Chem.* **273**, 9369–9372 CrossRef Medline
5. You, G., Smith, C. P., Kanai, Y., Lee, W. S., Stelzner, M., and Hediger, M. A. (1993) Cloning and characterization of the vasopressin-regulated urea transporter. *Nature* **365**, 844–847 CrossRef Medline
6. Shayakul, C., and Hediger, M. A. (2004) The SLC14 gene family of urea transporters. *Pflugers Arch*: *Eur. J. Physiol.* **447**, 603–609 CrossRef Medline
7. Sands, J. M., and Blount, M. A. (2014) Genes and proteins of urea transporters. *Subcell. Biochem.* **73**, 45–63 CrossRef Medline
8. Fenton, R. A., Cottingham, C. A., Stewart, G. S., Howorth, A., Hewitt, J. A., and Smith, C. P. (2002) Structure and characterization of the mouse UT-A gene (Slc14a2). *Am. J. Physiol. Renal Physiol.* **282**, F630–F638 CrossRef Medline
9. Doran, J. J., Klein, J. D., Kim, Y. H., Smith, T. D., Kozlowski, S. D., Gunn, R. B., and Sands, J. M. (2006) Tissue distribution of UT-A and UT-B mRNA and protein in rat. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **290**, R1446–R1459 CrossRef Medline
10. Klein, J. D., Blount, M. A., Fröhlich, O., Denson, C. E., Tan, X., Sim, J. H., Martin, C. F., and Sands, J. M. (2010) Phosphorylation of UT-A1 on serine 486 correlates with membrane accumulation and urea transport activity in both rat IMCDs and cultured cells. *Am. J. Physiol. Renal Physiol.* **298**, F935–F940 CrossRef Medline
11. Nielsen, S., Terris, J., Smith, C. P., Hediger, M. A., Ecelbarger, C. A., and Knepper, M. A. (1996) Cellular and subcellular localization of the vasopressin-regulated urea transporter in rat kidney. *Proc. Natl. Acad. Sci. U.S.A.* **93**, 5495–5500 CrossRef Medline
12. Terris, J. M., Knepper, M. A., and Wade, J. B. (2001) UT-A3: localization and characterization of an additional urea transporter isoform in the IMCD. *Am. J. Physiol. Renal Physiol.* **280**, F325–F332 CrossRef Medline
13. Blount, M. A., Klein, J. D., Martin, C. F., Tchaplynikov, D., and Sands, J. M. (2007) Forskolin stimulates phosphorylation and membrane accumulation of UT-A3. *Am. J. Physiol. Renal Physiol.* **293**, F1308–F1313 CrossRef Medline
14. Stewart, G. S., Fenton, R. A., Wang, W., Kwon, T. H., White, S. J., Collins, V. M., Cooper, G., Nielsen, S., and Smith, C. P. (2004) The basolateral expression of mUT-A3 in the mouse kidney. *Am. J. Physiol. Renal Physiol.* **286**, F979–F987 CrossRef Medline
15. Kim, Y. H., Kim, D. U., Han, K. H., Jung, J. Y., Sands, J. M., Knepper, M. A., Madsen, K. M., and Kim, J. (2002) Expression of urea transporters in the developing rat kidney. *Am. J. Physiol. Renal Physiol.* **282**, F530–F540 CrossRef Medline
16. Kim, W. Y., Lee, H. W., Han, K. H., Nam, S. A., Choi, A., Kim, Y. K., and Kim, J. (2016) Descending thin limb of the intermediate loop expresses both aquaporin 1 and urea transporter A2 in the mouse kidney. *Histochem. Cell Biol.* **146**, 1–12 CrossRef Medline
17. Fenton, R. A., Stewart, G. S., Carpenter, B., Howorth, A., Potter, E. A., Cooper, G. J., and Smith, C. P. (2002) Characterization of mouse urea transporters UT-A1 and UT-A2. *Am. J. Physiol. Renal Physiol.* **283**, F817–F825 CrossRef Medline
18. Lucien, N., Bruneval, P., Lasbennes, F., Belair, M. F., Mandet, C., Cartron, J., Bailly, P., and Trinh-Trang-Tan, M. M. (2005) UT-B1 urea transporter is expressed along the urinary and gastrointestinal tracts of the mouse. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **288**, R1046–R1056 CrossRef Medline

**Author contributions**—X. G., S. Z., J. H., and Y. L. data curation; X. G. and J. H. software; X. G. formal analysis; X. G., S. Z., A. M., M. L., and G. C. investigation; X. G., A. M., Y. L., H. Z., G. C., and B. Y. funding acquisition; H. Z. validation; G. C. and B. Y. supervision; B. Y. conceptualization; B. Y. resources; B. Y. writing-review and editing.

**Funding and additional information**—This work was supported by National Natural Science Foundation of China Grants 81620108209, 81974083, 81330074, and 81800388 (to A. M. and B. Y.); Beijing Natural Science Foundation Grant 71721113 (to B. Y.); and China Postdoctoral Science Foundation Grant 2018M630049 (to A. M.).

**Conflict of interest**—The authors declare that they have no conflicts of interest with the contents of this article.

**Abbreviations**—The abbreviations used are: UT, urea transporter; GFR, glomerular filtration rate; IM, inner medulla; IMCD, inner medullary collecting duct; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ANOVA, analysis of variance; KO, knockout.
Urea transporter UT-A1–null mice

19. Inoue, H., Jackson, S. D., Vikulina, T., Klein, J. D., Tomita, K., and Bagnasco, S. M. (2004) Identification and characterization of a Kidd antigen/UT-B urea transporter expressed in human colon. Am. J. Physiol. Cell Physiol. 287, C30–C35 CrossRef Medline

20. Kwun, Y. S., Yeo, S. W., Ahn, Y. H., Lim, S. W., Jung, Y. J., Kim, W. Y., Sands, J. M., and Kim, J. (2003) Immunohistochemical localization of urea transporters A and B in the rat cochlea. Hear Res. 183, 84–96 CrossRef Medline

21. Lucien, N., Sidoux-Walter, F., Roudier, N., Ripoche, P., Huet, M., Trinh-Trang-Tan, M. M., Cartron, J. P., and Bailly, P. (2002) Antigenic and functional properties of the human red blood cell urea transporter hUT-B1. J. Biol. Chem. 277, 34101–34108 CrossRef Medline

22. Prichett, W. P., Patton, A. J., Field, J. A., Brun, K. A., Emery, J. G., Tan, K. B., Riemann, D. J., McClung, H. A., Nadeau, D. P., Mooney, J. L., Suva, L. J., Gowen, M., and Nuttall, M. E. (2000) Identification and cloning of a human urea transporter HUT11, which is downregulated during adipogenesis of explant cultures of human bone. J. Cell. Biochem. 76, 639–650 CrossRef Medline

23. Berger, U. V., Tsukaguchi, H., and Hediger, M. A. (1998) Distribution of mRNA for the facilitated urea transporter UT3 in the rat nervous system. Anat. Embryol. (Berl.) 197, 405–414 CrossRef Medline

24. Geng, X., Lei, T., Zhou, H., Yao, W., Xin, W., and Yang, B. (2017) The knockdown of urea transporter-B improves the hematological properties of erythocyte. Clin. Hemorheol. Microcirc. 65, 249–257 CrossRef Medline

25. Fenton, R. A., Chou, C. L., Stewart, G. S., Smith, C. P., and Knepper, M. A. (2005) Renal phenotype of UT-A urea transporter knock-out mice. J. Am. Soc. Nephrol. 16, 1583–1592 CrossRef Medline

26. Yang, B., Bankir, L., Gillespie, A., Epstein, C. J., and Verkman, A. S. (2002) Role of thin descending limb urea transport in renal urea handling of erythrocyte. Proc. Natl. Acad. Sci. U.S.A. 99, 1518, 5775 CrossRef Medline

27. Lei, T., Zhou, L., Layton, A. T., Zhou, H., Zhao, X., Bankir, L., and Yang, B. (2011) Role of thin descending limb urea transport in renal urea handling and the urine concentrating mechanism. Am. J. Physiol. Renal Physiol. 301, F1251–F1259 CrossRef Medline

28. Uchida, S., Sohara, E., Rai, T., Ikawa, M., Okabe, M., and Sasaki, S. (2005) Impaired urea accumulation in the inner medulla of mice lacking the urea transporter UT-A2. Mol. Cell. Biol. 25, 7357–7363 CrossRef Medline

29. Jiang, T., Li, Y., Layton, A. T., Wang, W., Sun, Y., Li, M., Zhou, H., and Yang, B. (2017) Generation and phenotypic analysis of mice lacking all urea transporters. Kidney Int. 91, 338–351 CrossRef Medline

30. Bankir, L., Chen, K., and Yang, B. (2004) Lack of UT-B in vas a recta and red blood cells prevents urea-induced improvement of urinary concentrating ability. Am. J. Physiol. Renal Physiol. 286, F144–F151 CrossRef Medline

31. Klein, J. D., Sands, J. M., Qian, L., Wang, X., and Yang, B. (2004) Upregulation of urea transporter UT-A2 and water channels AQP2 and AQP3 in mice lacking urea transporter UT-B. J. Am. Soc. Nephrol. 15, 1161–1167 CrossRef Medline

32. Klein, J. D., Wang, Y., Mistry, A., LaRocque, L. M., Molina, P. A., Rogers, R. T., Blount, M. A., and Sands, J. M. (2016) Transgenic restoration of urea transporter A1 confers maximal urinary concentration in the absence of urea transporter A3. J. Am. Soc. Nephrol. 27, 1448–1455 CrossRef Medline

33. Nielsen, S., DiGiobien, S. R., Christensen, E. L., Knepper, M. A., and Harris, H. W. (1993) Cellular and subcellular immunolocalization of vasopressin-regulated water channel in rat kidney. Proc. Natl. Acad. Sci. U.S.A. 90, 11663–11667 CrossRef Medline

34. Bagnasco, S. M., Peng, T., Janech, M. G., Karkashian, A., and Sands, J. M. (2001) Cloning and characterization of the human urea transporter UT-A1 and mapping of the human Slc14a2 gene. Am. J. Physiol. Renal Physiol. 281, F400–F406 CrossRef Medline

35. Nakayama, Y., Naruse, M., Karakashian, A., Peng, T., Sands, J. M., and Bagnasco, S. M. (2001) Cloning of the rat Slc14a2 gene and genomic organization of the UT-A urea transporter. Biochim. Biophys. Acta 1518, 19–26 CrossRef Medline

36. Sun, Y., Lau, C. W., Jia, Y., Li, Y., Wang, W., Ran, J., Li, F., Huang, Y., Zhou, H., and Yang, B. (2016) Functional inhibition of urea transporter UT-B enhances endothelial-dependent vasodilatation and lowers blood pressure via L-arginine-endothelial nitric oxide synthase-nitric oxide pathway. Sci. Rep. 6, 18697 CrossRef Medline

37. Yang, B., and Bankir, L. (2005) Urea and urine concentrating ability: new insights from studies in mice. Am. J. Physiol. Renal Physiol. 288, F881–F896 CrossRef Medline

38. Verkman, A. S., Esteve-Font, C., Cil, O., Anderson, M. O., Li, F., Li, M., Lei, T., Ren, H., and Yang, B. (2014) Small-molecule inhibitors of urea transporters. Subcell. Biochem. 73, 165–177 CrossRef Medline

39. Li, M., Tou, W. L., Zhou, H., Li, F., Ren, H., Chen, C. Y., and Yang, B. (2014) Developing hypothetical inhibition mechanism of novel urea transporter B inhibitor. Sci. Rep. 4, 5775 CrossRef Medline

40. Ren, H., Wang, Y., Xing, Y., Ran, J., Liu, M., Lei, T., Zhou, H., Li, R., Sands, J. M., and Yang, B. (2014) Thienoquinolines exert diuresis by strongly inhibiting UT-A urea transporters. Am. J. Physiol. Renal Physiol. 307, F1363–F1372 CrossRef Medline

41. Li, F., Lei, T., Zhu, J., Wang, W., Sun, Y., Chen, J., Dong, Z., Zhou, H., and Yang, B. (2013) A novel small-molecule thienoquinolin urea transporter inhibitor acts as a potential diuretic. Kidney Int. 83, 1076–1086 CrossRef Medline

42. Li, M., Zhao, Y., Zhang, S., Xu, Y., Wang, S. Y., Li, B. W., Ran, J. H., Li, R. T., and Yang, B. X. (2020) A thiempipridine, CB-20, exerts diuretic activity by inhibiting urea transporters. Acta Pharmacol. Sin. 41, 65–72 CrossRef Medline

43. Zhang, Z. Y., Zhang, H., Liu, D., Lu, Y. Y., Wang, X., Li, P., Lou, Y. Q., Yang, B. X., Lou, Y. X., Lu, C., Zhang, Q., and Zhang, G. L. (2018) Pharmacokinetics, tissue distribution and excretion of a novel diuretic (P-48) in rats. Pharmaceutics 10, 124 CrossRef Medline

44. Zhao, Y., Li, M., Li, B., Zhang, S., Su, A., Xing, Y., Ge, Z., Li, R., and Yang, B. (2019) Discovery and optimization of thiempipridine derivatives as novel urea transporter inhibitors. Eur. J. Med. Chem. 172, 131–142 CrossRef Medline

45. Ran, J. H., Li, M., Tou, W. I., Lei, T. L., Zhou, H., Chen, C. Y., and Yang, B. X. (2016) Phenyolphthalazines as small-molecule inhibitors of urea transporter UT-B and their binding model. Acta Pharmacol. Sin. 37, 973–983 CrossRef Medline

46. Li, X., Ran, J., Zhou, H., Lei, T., Zhou, L., Han, J., and Yang, B. (2012) Mice lacking urea transporter UT-B display depression-like behavior. J. Mol. Neurosci. 46, 362–372 CrossRef Medline

47. Schreiber, A., Shulhevich, Y., Geraci, S., Hesser, J., Stespankou, D., Neudecker, S., Koenig, S., Heinrich, R., Hoecklin, F., Pilt, J., Friedemann, J., Schweda, F., Gretz, N., and Schock-Kusch, D. (2012) Transcutaneous measurement of renal function in conscious mice. Am. J. Physiol. Renal Physiol. 303, F783–F788 CrossRef Medline