

Regulation of Renal Urea Transporters^a

JEFF M. SANDS

Renal Division, Department of Medicine, Emory University School of Medicine, Atlanta, Georgia.

Abstract. Urea is important for the conservation of body water due to its role in the production of concentrated urine in the renal inner medulla. Physiologic data demonstrate that urea is transported by facilitated and by active urea transporter proteins. The facilitated urea transporter (UT-A) in the terminal inner medullary collecting duct (IMCD) permits very high rates of transepithelial urea transport and results in the delivery of large amounts of urea into the deepest portions of the inner medulla where it is needed to maintain a high interstitial osmolality for concentrating the urine maximally. Four isoforms of the UT-A urea transporter family have been cloned to date. The facilitated urea transporter (UT-B) in erythrocytes permits these cells to lose urea rapidly as they traverse the ascending vasa recta, thereby preventing loss of urea from the medulla and decreasing urine-concentrating ability by decreasing

the efficiency of countercurrent exchange, as occurs in Jk null individuals (who lack Kidd antigen). In addition to these facilitated urea transporters, three sodium-dependent, secondary active urea transport mechanisms have been characterized functionally in IMCD subsegments: (1) active urea reabsorption in the apical membrane of initial IMCD from low-protein fed or hypercalcemic rats; (2) active urea reabsorption in the basolateral membrane of initial IMCD from furosemide-treated rats; and (3) active urea secretion in the apical membrane of terminal IMCD from untreated rats. This review focuses on the physiologic, biophysical, and molecular evidence for facilitated and active urea transporters, and integrative studies of their acute and long-term regulation in rats with reduced urine-concentrating ability.

Urea is important for the conservation of body water due to its role in the production of concentrated urine in the renal inner medulla. The passive mechanism hypothesis for urine concentration in the inner medulla, proposed by Kokko and Rector (1) and by Stephenson (2) in 1972, requires the delivery of large quantities of urea to the deepest portions of the inner medullary (papillary) interstitium. This is accomplished through the transport of urea via the vasopressin-regulated urea transporter (UT-A1) (Table 1) in terminal inner medullary collecting duct (IMCD) (3,4). In addition, the erythrocyte urea transporter (UT-B) (Table 1) minimizes the loss of urea from the inner medulla by increasing the efficiency of countercurrent exchange for urea in the vasa recta (5). Thus, the production of concentrated urine and the conservation of body water depend on urea transporters in both IMCD and erythrocytes.

Urea also serves a second function in the kidney: the excretion of nitrogenous waste. Because large quantities of urea need to be excreted daily, the kidney's ability to concentrate urea reduces the need to excrete water simply to remove nitrogenous waste. Urea within the collecting duct lumen can be balanced osmotically by a high interstitial urea concentration. If interstitial urea were unavailable to offset the osmotic effect of urea in the collecting duct lumen that was destined for

excretion, then the interstitial NaCl concentration would need to be much higher, necessitating an increase in NaCl reabsorption from thick ascending limbs with an accompanying increase in work (ATP consumption). This effect of urea led to Gamble and colleague's 1934 observation that there is "an economy of water in renal function referable to urea" (6).

The reader may wonder why collecting ducts or erythrocytes need urea transporters when most textbooks state that urea is freely permeable and equilibrates across cell membranes. Urea is a small molecule with a molecular weight of 60 Da that will diffuse across cell membranes and achieve equilibrium given enough time since it is not completely impermeable. However, urea is a highly polar molecule ($\text{O} = \text{C}(-\text{NH}_2)_2$) and has a low permeability through artificial lipid bilayers (4×10^{-6} cm/s) (7) that lack any transport proteins to facilitate its transfer. The transit time for tubule fluid through the collecting duct or for erythrocytes through the vasa recta is too rapid for urea to achieve equilibrium by simple lipid-phase diffusion or paracellular transport. Thus, both IMCD and erythrocytes require urea transporters to transport urea rapidly enough to produce concentrated urine. Additionally, regulated urea transport processes are needed to concentrate urea within the deepest portions of the inner medullary interstitium to maintain the urea gradient that is critical for the production of concentrated urine (1,2).

This review focuses on the physiologic, biophysical, and molecular evidence for facilitated and active urea transporters, and integrative studies of their acute and long-term regulation in rats with reduced urine concentrating ability. The reader is referred to other reviews of urea transport for areas not covered herein (8–11).

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Correspondence to Dr. Jeff M. Sands, Emory University School of Medicine, Renal Division, WMRB Room 338, 1639 Pierce Drive, NE, Atlanta, GA 30322. Phone: 404-727-2435; Fax: 404-727-3425; E-mail: jsands@emory.edu
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Table 1. Comparison of cloned facilitated urea transporters^a

Isoform ^b	Synonyms	Species	Tissue ^c	MW (kD) ^d	Reference
UT-A1	UT1	Rat	Kidney	96	(45)
UT-A2	UT2	Rabbit, rat, human	Kidney	43	(31, 38, 39, 41)
UT-A3		Rat	Kidney	45	(46)
UT-A4		Rat	Kidney	43	(46)
UT-B1	HUT11, Rach1, UT3	Human, rat	Bone marrow, kidney	42 to 43	(59, 61, 135)
UT-B2	UT11	Rat	Brain	45	(60)

^a MW, molecular weight.

^b Isoform names are based on the systematic nomenclature for urea transporters proposed in reference 11.

^c Indicates tissue from which this isoform was originally cloned.

^d Predicted molecular mass based on amino acid sequence.

Facilitated Urea Transport

Although several early studies suggested that vasopressin could increase urea permeability across IMCD in mammals (12–15), direct evidence was not obtained until the late 1980s when three groups showed that vasopressin could increase passive urea permeability in isolated perfused rat IMCD (4,16,17). Rat terminal IMCD have a high basal urea permeability that is increased 400% after stimulation by vasopressin (3,4,18,19). This high urea permeability, especially after stimulation by vasopressin, was the first evidence that terminal IMCD contained a facilitated (or carrier-mediated) urea transporter (4). Even in the absence of vasopressin, rat terminal IMCD have a urea permeability that is 85 times greater than the calculated maximal urea permeability which could be achieved by simple lipid-phase diffusion and paracellular transport (4).

Physiologic Evidence for Facilitated Urea Transport

Urea transport by a facilitated or carrier-mediated transporter in the mammalian collecting duct was first proposed in 1987 (4). Before this proposal, two other mechanisms to increase urea delivery to the deepest portions of the inner medullary interstitium had been suggested: (1) urea reabsorption across the papillary surface epithelium (20); and (2) urea reabsorption by solvent drag through aqueous pores in IMCD (20–26). However, subsequent experimental studies showed that neither of these proposed mechanisms makes a significant contribution to urea delivery into the inner medulla.

The papillary surface epithelium has a low urea permeability, even with vasopressin present, precluding significant urea reabsorption across it (3). Solvent drag of urea, *i.e.*, urea and water sharing a common transport pathway and physically interacting in the pathway or pore, is determined by measuring the reflection coefficient for urea ($\sigma_u = 1 - P_u/P_w$). Early studies (13,16,27) did measure a reflection coefficient for urea of less than 1, suggesting the presence of solvent drag of urea. However, more recent studies remeasured the reflection coefficient for urea in rat terminal IMCD, including explicit measurement of the dissipation of the imposed urea gradient, and showed that the urea reflection coefficient is 1, *i.e.*, no solvent drag of urea (28,29). Other experimental findings also preclude solvent drag of urea: (1) in terminal IMCD, phloretin inhibits

urea transport, but not water transport, consistent with urea and water transport occurring by separate pathways (17,30); and (2) in *Xenopus* oocytes, heterologously expressed aquaporin-2 (AQP2), the vasopressin-regulated water channel, is impermeable to urea, whereas UT-A1, the vasopressin-regulated urea transporter, is impermeable to water (31,32). Thus, there are no experimental data to support either significant recycling of urea across the papillary surface epithelium or solvent drag of urea across terminal IMCD.

It should be noted that solvent drag is not required for perfused terminal IMCD to reabsorb water. A significant rate of fluid reabsorption occurs when rat terminal IMCD are perfused in the absence of an osmotic gradient but with a bath solution that is higher in NaCl and a perfusate solution that is higher in urea, due to rapid facilitated urea reabsorption, down its chemical gradient, generating an osmotic gradient for water reabsorption (29). Thus, rat terminal IMCD can perform osmotic work, even without solvent drag, by the combination of a high luminal urea concentration and a high rate of facilitated urea transport.

Inhibitor studies provided additional evidence for the concept of a facilitated urea transporter. As mentioned above, phloretin, an inhibitor of facilitated urea transport in erythrocytes (33,34), inhibits urea transport (but not osmotic water permeability) in terminal IMCD (17,30,35). In addition, urea analogs, such as thiourea, inhibit urea transport in terminal IMCD (30,35). Chou and colleagues used thiourea to test for saturation kinetics in perfused terminal IMCD and showed saturation of the facilitated urea transporter with a K_m of 20 mM thiourea (36). They also showed that the K_m for urea itself exceeded 800 mM urea; technical problems precluded their study of higher urea concentrations (36). Kishore and colleagues estimated a facilitated urea transporter turnover number of 0.3 to 1×10^{-5} , suggesting that the facilitated urea transporter is a channel rather than a carrier (37).

Molecular Cloning of the Kidney-Facilitated Urea Transporter: UT-A

The preceding physiologic studies established the existence of, and provided a functional definition for, a facilitated urea transporter in mammalian terminal IMCD. On the basis of this

definition, Hediger and colleagues expression-cloned UT-A2 (Table 1), a putative vasopressin-regulated urea transporter, from rabbit inner medulla (31). Subsequently, UT-A2 homologs from human and rat were cloned; they share 90% sequence identity with rabbit UT-A2 (38–41). Heterologous expression of UT-A2 cRNA in *Xenopus* oocytes results in a urea flux that can be inhibited by phloretin and urea analogs (31,38,39,42), consistent with the functional properties of the facilitated urea transporter in rat terminal IMCD (30). In contrast to rat terminal IMCD (43,44), cAMP analogs have no effect on urea flux in *Xenopus* oocytes (31,45) or human embryonic kidney cells (HEK-293 cells) that heterologously express UT-A2 (31,45,46), suggesting that UT-A2 is not a vasopressin-regulated urea transporter.

Northern analysis of rat or rabbit inner medullary mRNA using a UT-A2 cDNA probe reveals two mRNA bands at 4.0 (UT-A1) and 2.9 (UT-A2) kb (31,38,40,41). You and colleagues initially published that both mRNA transcripts contained a single coding region for a single protein (31). Their next publication reported that these two mRNA bands were splice variants of a single gene with identical 3' ends but differing at their 5' ends so that splicing of the 4.0-kb mRNA produces an additional 1.6 kb of coding region (45). However, they did not clone the gene or provide any evidence that UT-A1 and UT-A2 originate from alternative splicing (45). Shayakul and colleagues reserved the name "UT2" (UT-A2, Table 1) for the 2.9-kb mRNA and renamed the 4.0-kb mRNA as "UT1" (UT-A1, Table 1) (45). Heterologous expression of UT-A1 in *Xenopus* oocytes results in a phloretin-inhibitable urea flux that is stimulated by vasopressin (45). The two UT-A transcripts localize to different regions of the medulla: the 4.0-kb UT-A1 mRNA is the predominant transcript in terminal IMCD, whereas the 2.9-kb UT-A2 mRNA is the predominant transcript in thin descending limbs (31,38,40,41,47).

Recently, two additional cDNA representing new UT-A isoforms have been cloned: UT-A3 and UT-A4 (46). Both UT-A3 and UT-A4 mRNA are present in rat medulla but not in cortex (46). Heterologous expression of UT-A3 or UT-A4 in HEK-293 cells results in phloretin-inhibitable urea transport, which is increased by cAMP, suggesting that both UT-A3 and UT-A4 may be regulated by vasopressin (46). Naruse and colleagues have cloned the rat UT-A gene and mapped its intron/exon boundaries (48). UT-A3 and UT-A4 are likely to arise from alternative splicing of this rat UT-A gene since the 5' end of rat UT-A1 is identical to the 5' end of UT-A3 and UT-A4; UT-A2 has a unique 5' end (45,46). In addition, the 3' end of rat UT-A1 is identical to the 3' end of UT-A2 and UT-A4; UT-A3 has a unique 3' end (45,46). The human UT-A gene is located on chromosome 18q12.1-q21.1 (39).

UT-A Urea Transporter Proteins

Polyclonal antibodies to rat UT-A1 have been made by immunizing rabbits with synthetic polypeptides to the C terminus (49,50) or the intracellular loop region of UT-A1 (11,51). Western blots of inner medullary tip proteins generally show bands at 97 and 117 kD using either anti-UT-A1 antibody (49–51); both the 97- and 117-kD proteins appear to represent

glycosylated versions of UT-A1 (52). UT-A1 protein is most abundant in the inner medullary tip, present in the inner medullary base, and not present in outer medulla or cortex (49,53), consistent with the pattern of urea permeabilities measured in perfused rat collecting duct segments (3,4,18,19). However, the amount of UT-A1 + UT-A2 + UT-A4 protein per millimeter tubule length does not vary between IMCD₁, IMCD₂, and IMCD₃ subsegments when it is measured by enzyme-linked immunosorbent assay of microdissected tubules (37).

Functional studies show that phloretin-inhibitable urea transport is present in both the apical and basolateral membranes of rat terminal IMCD, with the apical membrane being the rate-limiting barrier for vasopressin-stimulated urea transport (54). UT-A1 immunostaining is observed in the apical plasma membrane and intracellular cytoplasmic vesicles of terminal IMCD, but no immunostaining is seen in the basolateral plasma membrane (49). Recently, Wade and colleagues identified a 67-kD UT-A protein in the basolateral membrane of rat terminal IMCD, suggesting that this novel UT-A protein is the basolateral membrane urea transporter (55). Although UT-A1 has an immunolocalization pattern that is similar to AQP2 (56), the question of whether regulated trafficking of UT-A1 occurs is currently controversial (57,58). UT-A2 protein is expressed in terminal portions of short-looped thin descending limbs in the inner stripe of the rat outer medulla (49).

Molecular Cloning of Erythrocyte-Facilitated Urea Transporter: UT-B

Human UT-B1 (Table 1) was cloned by homology from UT-A2 and is the erythrocyte-facilitated urea transporter; they share 63% sequence identity (59). Heterologous expression of UT-B1 in *Xenopus* oocytes results in a urea flux that is inhibitable by phloretin, thiourea, and p-chloromercuriphenylsulfonate (42,59). The rat homologs (UT-B1, UT-B2, Table 1) of human UT-B1 have been cloned (60,61), and their mRNA are expressed in the inner stripe of outer medulla and in inner medulla (41,61). UT-B2 mRNA abundance correlates with urine osmolality in rat inner medulla (41). UT-B1 and/or UT-B2 protein is expressed in vasa recta endothelial cells (62), suggesting that UT-B1 and/or UT-B2 may be responsible for the phloretin-inhibitable urea transport measured in perfused rat descending vasa recta (63–65).

The human UT-B gene is located on the same locus (chromosome 18q12.1-q21.1) as the human UT-A gene and the human Kidd antigen blood group (a minor blood group) locus (39,66,67). Erythrocytes from individuals lacking the Kidd antigen (Jk(a-b-) or Jk null) lack facilitated urea transport and are unable to concentrate their urine above 800 mosmol/kg H₂O (68,69). The mechanism by which loss of the erythrocyte urea transporter UT-B1 could result in a mild urine-concentrating defect is as follows: (1) erythrocytes acquire urea as they traverse the descending vasa recta into the inner medulla; (2) the loss of facilitated urea transport prevents Jk null erythrocytes from losing urea rapidly as they traverse the ascending vasa recta (5); and (3) urea is trapped in the Jk null erythrocytes and lost from the medulla when these erythrocytes return to the

systemic circulation. Thus, the efficiency of countercurrent exchange is diminished, decreasing urine concentrating ability.

Active Urea Transport

Lassiter and colleagues showed that the urea concentration was greater in vasa recta than in adjacent collecting ducts in rats fed a low-protein diet or undergoing an osmotic diuresis, consistent with active transport of urea out of collecting ducts (70). Papillary micropuncture studies also supported active urea reabsorption from collecting ducts of low-protein fed rats (71,72). Active urea transport processes have been described in a variety of tissues, including: (1) a sodium-linked, active urea transport process in the renal tubule of the spiny dogfish *Squalus acanthias* (73); (2) phloretin-inhibitable, active urea secretion in rabbit proximal straight tubules (74); (3) sodium-independent and/or phloretin-inhibitable active urea transporters in the skins of *Bufo bufo*, *Rana esculenta*, *Bufo viridis*, and *Bufo marinus* (75–79); (4) active urea transport in tubules from dog (80,81) and frog (82,83); and (5) active urea transport in *Saccharomyces cerevisiae* (84).

We confirmed the existence of active urea transport by perfusing initial IMCD from rats fed a low (8%) protein diet for at least 3 wk and showing that a sodium-dependent, secondary active, urea reabsorptive transport mechanism was present that was not expressed in initial IMCD of rats fed a normal (18%) protein diet (85–87) (Figure 1). This “sodium-urea cotransporter” (Table 2) is: (1) inhibited by removing sodium from the lumen (but not from the bath), suggesting that it is located in the apical membrane; (2) inhibited when Na^+/K^+ -ATPase is inhibited with either ouabain or removing bath potassium; (3) not inhibited by phloretin; (4) not stimulated by vasopressin; and (5) encoded by a longer mRNA than UT-A1 based on initial expression cloning studies (85–88).

Recently, we have found two additional active urea transporters in rat IMCD subsegments (Figure 1). One is an active urea reabsorptive transport process that is expressed in initial IMCD from furosemide-treated rats (89). This active urea reabsorption differs from the one expressed in low-protein fed rats (Table 2) since it is: (1) inhibited by removing sodium from the bath (but not from the perfusate); (2) stimulated by vasopressin; and (3) inhibited by phloretin or ouabain (89). These results suggest a sodium-dependent, secondary active, urea reabsorptive transport mechanism in the basolateral membrane of initial IMCD (89).

The other newly described transport process is active urea secretion in the deepest portion of rat terminal IMCD, IMCD₃ (Figure 1) (90). Active urea secretion (Table 2) is: (1) inhibited by removing sodium from the tubule lumen (but not from the bath); (2) stimulated by vasopressin; and (3) inhibited by phloretin or ouabain (90). These results suggest a sodium-dependent, secondary active, urea secretory transport mechanism in the apical membrane of IMCD₃.

Regulation Of Urea Transporters

Vasopressin

Adding vasopressin to the bath of a perfused rat terminal IMCD results in vasopressin binding to V_2 receptors, stimu-

lating adenylyl cyclase, generating cAMP, stimulating protein kinase A, and ultimately increasing facilitated urea transport by increasing the number of functional urea transporters (V_{max}) without changing the transporter's affinity (K_m) for urea (4,30,43,44,91). The time course for vasopressin-induced stimulation of urea permeability, and the time course after vasopressin withdrawal, each consist of two distinct phases: an initial 10-min period during which facilitated urea permeability increases or decreases rapidly, followed by a second 10- to 60-min period during which facilitated urea permeability changes slowly (43,92,93).

Adding vasopressin to the lumen of a perfused rat terminal IMCD results in vasopressin binding to luminal V_2 receptors and an increase in facilitated urea transport (94). However, if vasopressin is added to the bath before it is added to the lumen, then luminal vasopressin decreases facilitated urea transport, suggesting that luminal vasopressin is a negative modulator of basolateral vasopressin (94).

Hyperosmolality

Increasing osmolality, either by adding NaCl or mannitol, increases facilitated urea permeability in perfused rat terminal IMCD independently of vasopressin (35,44,95). When osmolality is increased in the presence of vasopressin, they have additive stimulatory effects on facilitated urea permeability (35,36,44,95). Kinetic studies show that hyperosmolality, like vasopressin, increases the V_{max} rather than the K_m for facilitated urea transport, and inhibitor studies show that phloretin and thiourea inhibit hyperosmolality-stimulated facilitated urea permeability (35). However, increases in intracellular calcium (96) and protein kinase C (97) mediate hyperosmolality-stimulated facilitated urea permeability, whereas increases in adenylyl cyclase mediate vasopressin-stimulated facilitated urea permeability (43). Thus, both hyperosmolality and vasopressin increase facilitated urea permeability acutely by increasing V_{max} , but they do so via different second-messenger pathways.

Water Diuresis and Water Restriction

Vasopressin has a long-term regulatory effect on the collecting duct facilitated urea transporter UT-A1 (Table 3) (51,98), but this effect differs from vasopressin's effect on AQP2 (56). Making rats water-diuretic for at least 3 d (to decrease endogenous vasopressin) increases the basal (no vasopressin) facilitated urea permeability in rat terminal IMCD (98); 1 d of water diuresis increased basal urea permeability in one study (98) but not in a second (99). In rat initial IMCD, water diuresis for 1 to 7 d had no effect on basal facilitated urea permeability (98). Water-restricting rats for 1 d (to increase endogenous vasopressin) has no effect on basal facilitated urea permeability in rat terminal IMCD (98,99). However, at least 2 d of water restriction does increase basal facilitated urea permeability in initial IMCD (IMCD₁) and IMCD₃ (98). Increasing vasopressin, either by water restriction (to increase endogenous vasopressin) or by administering (exogenous) vasopressin to Brattleboro rats, decreases the abundance of both the 97- and 117-kD UT-A1 urea transporter proteins (51), consistent with the facilitated urea permeability measurements (98). Adminis-

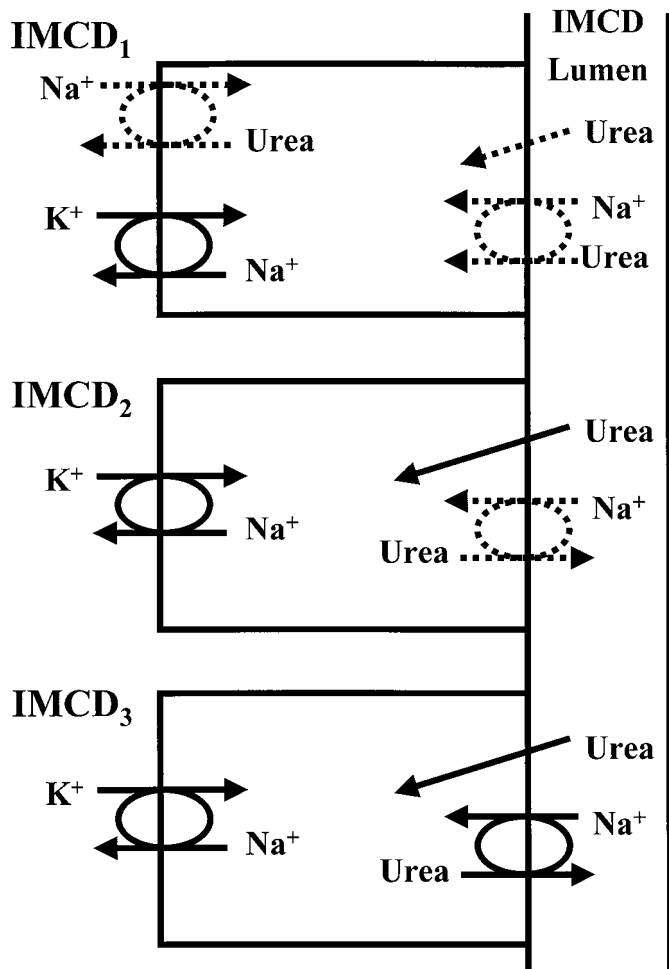


Figure 1. Urea transporters along the inner medullary collecting duct (IMCD). Cells are shown from the inner-outer medullary border (IMCD₁, top) to the papillary tip (IMCD₃, bottom) (136,137). The tubule lumen is shown on the right, and Na⁺/K⁺-ATPase is shown in the basolateral membrane on the left side of each cell. The arrows indicate facilitated urea transporters. The circles connecting two arrows indicate secondary active urea transporters. The solid symbols indicate urea transporters that are expressed in untreated rats. The dashed symbols indicate urea transporters that are not expressed in untreated rats but can be induced under various conditions. (Top) An IMCD₁ cell showing a facilitated urea transporter that is induced in low-protein fed and hypercalcemic rats (85,86,120) and two inducible, Na⁺-dependent, active urea transport processes: (1) a Na⁺-urea “cotransporter” in the apical membrane of low-protein fed (87) or hypercalcemic (102) rats; and (2) a Na⁺-urea “anti-porter” in the basolateral membrane of furosemide-treated rats (89). (Middle) An IMCD₂ cell showing a facilitated urea transporter (UT-A1) that is expressed in the apical membrane of untreated rats (4,49) and is upregulated in furosemide-treated, adrenalectomized, and hypercalcemic rats (50,98,120), and a Na⁺-dependent, active urea “anti-porter” that can be induced in the apical membrane of water-diuretic rats (102). (Bottom) An IMCD₃ cell showing a facilitated urea transporter (UT-A1) that is expressed in the apical membrane of untreated rats (4,49) and is upregulated in low-protein fed, furosemide-treated, adrenalectomized, and hypercalcemic rats (50,98,102,120), and a Na⁺-dependent, active urea “anti-porter” that is expressed in the apical membrane of untreated rats (4,49,90). This Na⁺-urea “anti-porter” is upregulated by water diuresis (90) and downregulated by a low-protein diet (102), furosemide (89), or hypercalcemia (102).

tering deamino-8-D-arginine vasopressin to Brattleboro rats also increases the abundance of the 55-kD UT-A2 protein in thin descending limbs (100).

Northern analysis shows no change in UT-A1 mRNA abundance during water diuresis or restriction (38,41,47), suggesting that the changes in facilitated urea transport and UT-A1 protein abundance are not regulated by transcription. However, UT-A2 mRNA abundance does decrease in the inner medulla of water-loaded (3 d) rats and increase in: (1) water-restricted (3 d) rats; (2) rats receiving deamino-8-D-arginine vasopressin, a V₂-selective vasopressin agonist for 3 wk; and (3) Brattleboro rats treated with vasopressin for 1 wk (38,41,101). The physiologic meaning of these changes in UT-A2 mRNA abundance is unclear.

In addition to upregulating facilitated urea transport in terminal IMCD, water diuresis upregulates the sodium-dependent, active urea secretory transporter (Table 4) (90,102). Active urea secretion is present in IMCD₃ from normal rats (90). It is upregulated 200% in IMCD₃ from water diuretic rats that are not given food and 500% in IMCD₃ from water-diuretic rats given food *ad libitum* (90). In addition, water diuresis induces active urea secretion IMCD₂ (Figure 1) (102). Water restriction does not alter active urea secretion in either terminal IMCD subsegment (90,102). Active urea secretion is not present in initial IMCD, regardless of the rat's hydration status (102). These results suggest that active urea secretion is physiologically important during water diuresis and not during water restriction.

Furosemide

Adding furosemide to the bath fluid of a perfused rat terminal IMCD inhibits vasopressin-stimulated facilitated urea permeability (103). However, treating rats for 3 to 7 d with furosemide significantly increases basal facilitated urea permeability in rat terminal IMCD (Table 3), but has no effect on basal facilitated urea permeability in initial IMCD (98). In addition, treating rats for 5 d increases the abundance of the 117-kD UT-A1 protein; the abundance of the 97-kD UT-A1 protein is unchanged (51). In contrast, treating rats with furosemide for 18 h or 8 d has no effect on UT-A1 mRNA abundance but decreases UT-A2 mRNA abundance (104). Thus, both water and furosemide diuresis increase basal facilitated urea permeability and the abundance of the 117-kD UT-A1 protein, but have no effect on UT-A1 mRNA abundance.

Unlike water diuresis, treating rats with furosemide for 3 to 5 d inhibits active urea secretion in IMCD₃ (Table 4) and does not induce it in IMCD₂ (90,102). Furosemide treatment (3 to 5 d) also induces a sodium-dependent, active urea reabsorptive transporter in the basolateral membrane of rat initial IMCD (Table 2) (89). Rats lose weight during the first 3 to 5 d of furosemide treatment, but come back into balance after 7 d (89), similar to the response of people treated with furosemide (105–107). Consistent with this time course, the induced active urea reabsorption is decreased after 7 d of furosemide (compared to the value measured after 3 to 5 d of furosemide) (89), suggesting that this transporter is involved in the initial re-

Table 2. Na⁺-dependent active urea transport mechanisms in IMCD subsegments^a

Characteristic	IMCD ₁ Na ⁺ -Urea “Co-transport”	IMCD ₁ Na ⁺ -Urea “Counter-transport”	IMCD ₂ Na ⁺ -Urea “Counter-transport”	IMCD ₃ Na ⁺ -Urea “Counter-transport”
Direction of net urea movement	Absorption	Absorption	Secretion	Secretion
Expression in normal rats	Not expressed	Not expressed	Not expressed	Expressed
Induced or upregulated by	Low-protein diet	Furosemide treatment	Water diuresis	Water diuresis
Downregulated by				Low-protein diet, furosemide- treatment, hypercalcemia
Na ⁺ -removal: perfusate	Inhibits	No effect	Inhibits	Inhibits
Na ⁺ -removal: bath	No effect	Inhibits	No effect	No effect
Cl ⁻ removal	Not tested	No effect	Not tested	No effect
Ouabain-sensitive	Yes	Yes	Yes	Yes
Vasopressin-sensitive	No	Yes	Yes	Yes
Phloretin-sensitive	No	Yes	Yes	Yes
Temperature-sensitive	Yes	Yes	Yes	Yes
Transport stimulated or inhibited by	Not tested	Bumetanide (stim.)	Triamterene (inhib.)	Triamterene (inhib.)
Localization ^b	Apical membrane	Basolateral membrane	Apical membrane	Apical membrane

^a Modified from reference 102. Data are from references 85–87, 89, 90, and 102. IMCD, inner medullary collecting duct; stim., stimulated; inhib., inhibited.

^b Localization is based on whether transport is inhibited by perfusate (apical) or bath (basolateral) Na⁺-removal.

Table 3. Summary of facilitated urea transport and UT-A1 protein in IMCD subsegments

Condition	Initial IMCD	Terminal IMCD
Untreated	Low basal P _{urea} No effect of AVP on P _{urea}	High basal P _{urea} AVP stimulates P _{urea}
Water restriction		
1 day	No effect	No effect
≥2 days	↑ Basal and AVP-stim. P _{urea}	↑ Basal and AVP-stim. P _{urea} (IMCD ₃ only)
Water diuresis	No effect	↑ Basal and AVP-stim. P _{urea} ↑ UT-A1 protein
Furosemide	No effect	↑ Basal and AVP-stim. P _{urea} ↑ UT-A1 protein
Low-protein diet	Induces AVP-stim. P _{urea}	↑ Basal and AVP-stim. P _{urea} (IMCD ₃ only) ↑ UT-A1 protein
Hypercalcemia	Induces AVP-stim. P _{urea}	↑ Basal and AVP-stim. P _{urea}
Adrenalectomy	No change in UT-A1 protein	↑ UT-A1 protein
	No change	↑ Basal and AVP-stim. P _{urea}
	No change in UT-A1 protein	↑ UT-A1 protein
Diabetes mellitus		↓ UT-A1 protein

^a Data are from references 3, 4, 40, 50, 51, 85, 86, 98, 99, 102, 120, and 124. AVP, arginine vasopressin; stim., stimulated.

sponse and the subsequent adaptation to chronic furosemide administration.

Low-Protein Diet

Several studies show that maximal urinary concentrating ability is decreased in malnourished people and in humans and other mammals fed a low-protein diet (6,70,108–112). Inter-

estingly, infusing urea restores urine-concentrating ability in low-protein fed humans and other mammals within 5 to 10 min (108,113,114). A low-protein diet has profound effects on inner medullary function, including: (1) reduction in the fractional excretion of urea; (2) reduction in maximum urine-concentrating ability; and (3) reversal of the normal inner medullary urea concentration gradient so that the maximum

Table 4. Summary of active urea transport processes in IMCD subsegments^a

Subsegment	Untreated	Low-Protein Diet	Hypercalcemia	Furosemide	Water Diuresis
IMCD ₁	0	↑ Reabsorption	↑ Reabsorption	↑ Reabsorption	0
IMCD ₂	0	0	0	0	↑ Secretion
IMCD ₃	Secretion	↓ Secretion	↓ Secretion	↓ Secretion	↑ Secretion

^a Modified from reference 102. Data are from references 85–87, 89, 90, and 102.

inner medullary urea concentration is at the base of the inner medulla rather than at the inner medullary (papillary) tip (6,108–117).

A low-protein diet also has profound effects on urea transport in IMCD (Table 3). Feeding rats an 8% protein diet for 2 wk results in: (1) induction of vasopressin-stimulated facilitated urea permeability in initial IMCD (40,85,86); (2) increases in basal and vasopressin-stimulated facilitated urea permeability in IMCD₃ (102); and (3) increases in the 117-kD UT-A1 protein abundance in the inner medulla (51). In initial IMCD, the vasopressin-stimulated facilitated urea permeability that is induced by a low-protein diet is stimulated by hyperosmolality and inhibited by phloretin and thiourea (40,85). Thus, it has the same functional characteristics as the vasopressin-stimulated facilitated urea permeability that is normally expressed in terminal IMCD (40).

In contrast to rats fed a low-protein diet for 2 wk, rats fed a low-protein diet for 1 wk do not: (1) express vasopressin-stimulated facilitated urea permeability in initial IMCD (86); (2) have a reduction in the fractional excretion of urea (109); or (3) have a change in UT-A1 + UT-A2 + UT-A4 protein abundance in microdissected initial IMCD (37). Whether the mRNA abundance of any UT-A isoform changes in the inner medulla of low-protein fed rats is controversial (38,40,118).

Feeding rats a low-protein diet for 3 wk (Table 4): (1) induces sodium-dependent, active urea reabsorption in the apical membrane of initial IMCD (85–87); and (2) inhibits active urea secretion in IMCD₃ and does not induce it in IMCD₂ (85,102). Thus, both furosemide and a low-protein diet inhibit active urea secretion in IMCD₃ and induce active urea reabsorption in initial IMCD (Table 4). However, a low-protein diet induces an apical membrane active urea reabsorptive mechanism, whereas furosemide induces a basolateral membrane active urea reabsorptive mechanism.

Hypercalcemia

Hypercalcemia reduces urine-concentrating ability in humans and rats (119–121). Although varying perfusate calcium between 0 and 5 mM has no effect on facilitated urea permeability in terminal IMCD from normocalcemic rats (122), terminal IMCD from hypercalcemic rats have significantly increased values of both basal and vasopressin-stimulated facilitated urea permeability (120). The abundance of the 97- and 117-kD UT-A1 proteins are also increased in the inner medullary tip of hypercalcemic rats, consistent with the functional increase (120).

In addition, vasopressin significantly increases facilitated urea permeability in initial IMCD from hypercalcemic rats (120). Hypercalcemia also induces active urea reabsorption in the apical membrane of initial IMCD and inhibits active urea secretion in IMCD₃ (102). Thus, hypercalcemia and a low-protein diet induce similar changes in facilitated and active urea transporters in IMCD subsegments (Tables 3 and 4).

Glucocorticoids

Adrenalectomy increases basal and vasopressin-stimulated facilitated urea permeability in rat terminal IMCD and the abundance of both the 97- and 117-kD UT-A1 proteins in the inner medullary tip (Table 3), compared with either sham-operated rats or adrenalectomized rats receiving a physiologic replacement dose of dexamethasone (50). This result suggests that glucocorticoids increase the fractional excretion of urea (123) by decreasing the amount of UT-A1 protein and facilitated urea reabsorption in rat terminal IMCD (50).

To examine the relevance of glucocorticoid-mediated regulation of facilitated urea transport to a common pathophysiologic condition, rats with uncontrolled diabetes mellitus induced by streptozotocin were studied, since these rats have increased corticosterone production and urea excretion. The 97-kD UT-A1 protein was significantly reduced in the inner medullary tip of diabetic rats compared with control rats (Table 3) (124). Although these results suggest that the decrease in UT-A1 protein in the inner medullary tip is mediated by the diabetes-induced increase in glucocorticoids (124), the experiment was repeated using three groups of adrenalectomized rats: (1) adrenalectomy alone; (2) adrenalectomy plus streptozotocin; and (3) adrenalectomy plus streptozotocin plus replacement with a physiologic dose of glucocorticoid (124). There was no significant difference in UT-A1 protein abundance between the adrenalectomy and the adrenalectomy plus streptozotocin rats. However, UT-A1 protein was significantly reduced in the inner medullary tip of glucocorticoid-treated adrenalectomized rats given streptozotocin compared to control adrenalectomized rats given streptozotocin but not given glucocorticoids (124). Thus, glucocorticoids regulate the abundance of the 97-kD UT-A1 protein independently of insulin in diabetic rats (124).

Other Agents

Oxytocin binds to V₂ receptors, increases cAMP production, and increases phloretin-inhibitable, facilitated urea permeability in rat terminal IMCD (125). Oxytocin-stimulated facilitated

urea permeability is unchanged by 2 d of water restriction in rat IMCD₂ (125).

Glucagon increases the fractional excretion of urea by 45% in rats (126,127), suggesting that glucagon has an effect on the tubular transport of urea. However, glucagon does not stimulate cAMP production in either initial or terminal IMCD (86,128), or change basal or vasopressin-stimulated facilitated urea permeability in terminal IMCD (86).

UT-A1, UT-A2, and UT-B1 urea transporter mRNA abundances are reduced 1 wk after 5/6 nephrectomy in the remnant kidney (129). At 5 wk after 5/6 nephrectomy, no UT-A1 or UT-B1 mRNA is detected by Northern analysis, and UT-A2 mRNA abundance is reduced by 95% (129).

Amphotericin-B inhibits vasopressin-stimulated, but not cAMP-stimulated, facilitated urea permeability in rat terminal IMCD (130). α_2 -Adrenergic agonists (clonidine, epinephrine) inhibit cAMP production and vasopressin-stimulated facilitated urea permeability in rat terminal IMCD (128,131). Atrial natriuretic factor (132) and insulin (133) have no effect on vasopressin-stimulated facilitated urea permeability in rat terminal IMCD. Finally, chlorpropamide stimulates basal facilitated urea permeability, but its effect on vasopressin-stimulated facilitated urea permeability has not been tested (134).

Summary of Studies of the Long-Term Regulation of Urea Transport

Basal facilitated urea permeability and UT-A1 protein abundance have been measured in the rat inner medulla in five conditions associated with a reduction in urine-concentrating ability: water diuresis, furosemide diuresis, low-protein diet, adrenalectomy, and hypercalcemia (Table 3). In all five conditions, basal facilitated urea permeability is increased in IMCD₃ and the abundance of the 117- and/or 97-kD UT-A1 urea transporter proteins are increased in the inner medullary tip. Thus, these studies lead to the surprising finding that basal facilitated urea permeability and UT-A1 protein abundance are increased during *in vivo* conditions associated with a reduced urine concentrating ability. These findings may be the mechanism that explains that rapid increase in urine concentrating ability after urea infusion into malnourished or low-protein fed people (6,108,113,117): UT-A1 is upregulated during reductions in concentrating ability to prepare the IMCD to rapidly restore inner medullary urea once urea (or protein) intake is restored.

In contrast to the single response pattern of facilitated urea transport, there are two response patterns of active urea transporters to reductions in urine concentrating ability (Table 4): (1) hypercalcemia, a low-protein diet, and furosemide result in induction of active urea reabsorption in initial IMCD, albeit by different mechanisms, and inhibition of active urea secretion in IMCD₃; whereas (2) water diuresis results in upregulation of active urea secretion in IMCD₃ and its induction in IMCD₂, without any active urea reabsorption in initial IMCD. In the first response pattern, the induction of active urea reabsorption in initial IMCD contributes to the urine concentrating defect by increasing urea delivery to the base of the inner medulla, thus decreasing urea delivery to the inner medullary tip; the accom-

panying inhibition of active urea secretion in IMCD₃ prevents an even further reduction in urea content in the deep inner medulla. In the second response pattern, the upregulation of active urea secretion in terminal IMCD will directly decrease urea content in the deep inner medulla.

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