

Regulation of Urea Transporter Proteins in Kidney and Liver

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Abstract

Due to urea's role in producing concentrated urine, its transport is critically important to the conservation of body water. Within the renal inner medulla, urea is transported by both facilitated and active urea transport mechanisms. The vasopressin-regulated, facilitated urea transporter (UT-A1) in the terminal inner medullary collecting duct (IMCD) permits high rates of transepithelial urea transport and results in delivery of large quantities of urea into the deepest portions of the inner medulla where it is needed to maintain a high interstitial osmolality for maximal urine concentration. Four cDNA isoforms of the UT-A urea transporter family have been cloned. In addition, there are three secondary active, sodium-dependent, urea transport mechanisms in IMCD subsegments: (1) active urea secretion in the apical membrane of the terminal IMCD from untreated rats; (2) active urea absorption in the apical membrane of the initial IMCD from low-protein fed or hypercalcemic rats; and (3) active urea absorption in the basolateral membrane of the initial IMCD from furosemide-treated rats.

This review will focus on integrative studies of the rapid and long-term regulation of urea transporters in rats with reduced urine concentrating ability. These studies led to the surprising result that the basal-facilitated urea permeability in the terminal IMCD and UT-A1 protein abundance are increased during *in vivo* conditions associated with an impaired urine concentrating ability. In contrast, there are two response patterns of active urea transporters: (1) hypercalcemia, a low-protein diet, and furosemide result in induction of active urea absorption in the initial IMCD, albeit by different mechanisms, and inhibition of active urea secretion in the terminal IMCD; while (2) water diuresis results in up-regulation of active urea secretion in the terminal IMCD without any active urea absorption in the initial IMCD. The first pattern contributes to the urine concentrating defect by increasing urea delivery to the base of the inner medulla, thus decreasing urea delivery distally to the inner medullary tip. The second response pattern will directly decrease urea content in the deep inner medulla.

UT-A urea transporters are also expressed outside the kidney. Recent studies show that the liver has phloretin-inhibitable urea transport and that it occurs via a 49 kDa UT-A protein. When rats are made uremic, the abundance of this 49 kDa UT-A protein increases in the liver *in vivo*. This up-regulation of the 49 kDa UT-A protein may allow hepatocytes to increase ureagenesis to reduce the accumulation of ammonium and/or bicarbonate in uremia. **Key Words:** Urea, urea transporter, vasopressin, urine concentrating mechanism, collecting duct.

Introduction

UREA PLAYS A UNIQUE AND IMPORTANT ROLE in the urinary concentrating mechanism. Its importance to generating a concentrated urine has been appreciated since 1934 when Gamble and colleagues (1) described "an economy of water in renal function referable to urea." Maximal urine concentrating ability is decreased in protein-deprived or malnourished humans or animals, and is restored by urea infusion (1–8). Thus, any

solution to the question of how the inner medulla concentrates urine needs to take into account some effect derived from urea. The passive mechanism for urinary concentration (9, 10) requires that the inner medullary interstitial urea concentration exceed the urea concentration in the lumen of the thin ascending limb. If an inadequate amount of urea is delivered to the deep inner medulla, then the chemical gradients necessary for passive NaCl absorption are not established and urine concentrating ability is reduced. The major mechanism for delivering urea to the inner medullary interstitium is urea absorption across the terminal inner medullary collecting duct (IMCD) (11). We reported evidence for facilitated (or carrier-mediated) urea transport across the terminal IMCD in 1987 (12). Subsequently, four cDNA isoforms of the urea

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transporter (UT-A) family have been cloned (Table) (13–19), and polyclonal antibodies against urea transporter proteins are now available (14, 20, 21).

UT-A Urea Transporter Proteins

UT-A1 protein is expressed in the apical membrane of the rat terminal IMCD; urea transport is stimulated by vasopressin when UT-A1 is heterologously expressed in *Xenopus* oocytes (13, 18, 20, 22). UT-A2 is expressed in thin, descending limbs of short-looped nephrons (20). Urea transport is not stimulated by cAMP analogs when UT-A2 is heterologously expressed in either *Xenopus* oocytes or human embryonic kidney cells (13, 15, 16, 18, 19, 22, 23). UT-A1 and UT-A2 share identical 3' cDNA and C-terminal amino acid sequences, but differ at their 5' (N-terminal) ends (13). Thus, UT-A2 is basically the C-terminal half of UT-A1 (Fig. 1). UT-A3 has the same 5' cDNA and N-terminal amino acid sequence as UT-A1 but has a unique 3' (C-terminal) end and is basically the N-terminal half of UT-A1 (19). Although UT-A4 has the same 5' and 3' cDNA, and N- and C-terminal amino acid sequences as UT-A1, it is smaller than UT-A1 and basically consists of the N-terminal quarter of UT-A1 spliced into the C-terminal quarter of UT-A1 (19). Both UT-A3 and UT-A4 mRNAs are expressed in kidney medulla (although their exact tubular location is unknown), and neither is expressed in cortex (19). Both UT-A3 and UT-A4 are stimulated by cAMP analogs when heterologously expressed in human embryonic kidney cells, suggesting that both are regulated by vasopressin (19).

Antibodies have been made to the C-terminus (20, 21) and intracellular loop region (14) of UT-A1. Based upon the cDNA sequence of the four

UT-A isoforms, the C-terminus antibody should detect UT-A1, UT-A2, and UT-A4, while the loop region antibody should detect only UT-A1 (14, 19, 24). Western blots of inner medullary tip proteins generally show bands at 97 and 117 kDa using either antibody (14, 20, 21). Preliminary studies suggest that both the 97 and 117 kDa proteins are glycosylated versions of UT-A1 (25). 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 (20, 26), consistent with the pattern of facilitated urea permeabilities measured in perfused rat collecting duct segments (11, 12, 27, 28).

Functional studies show that phloretin-inhibitable urea transport is present in both the apical and basolateral membranes of the rat terminal IMCD, with the apical membrane being the rate-limiting barrier for vasopressin-stimulated urea transport (29). UT-A1 immunostaining is observed in the apical membrane and intracellular cytoplasmic vesicles of the rat terminal IMCD, but no immunostaining is seen in the basolateral membrane (20). Recently, Wade and colleagues (30) identified a 67 kDa UT-A protein in the basolateral membrane of the rat terminal IMCD, suggesting that it may be the basolateral membrane urea transporter.

Rapid Regulation of UT-A1

Vasopressin. Adding vasopressin to the bath of a perfused rat terminal IMCD results in vasopressin binding to V_2 -receptors, stimulating adenylyl cyclase, generating cAMP, stimulating protein kinase A (PKA), and ultimately increasing facilitated urea permeability (12, 31–34). One possible mechanism for this increase is regulated trafficking of UT-A1, as suggested by comparison

TABLE
UT-A Facilitated Urea Transporter Family

Isoform	Synonym	mRNA (kb)	MW (kDa)	AVP Stim	Location	References
UT-A1	UT1	4	97; 117	Yes	IMCD	(13, 14, 18, 20)
UT-A2	UT2	2.9	55	No	tDL	(15–17, 20)
UT-A3		2.1	4	Yes	Medulla*	(19)
UT-A4		2.5	60	Yes	Medulla*	(19)

Isoform names are based upon the systematic nomenclature for urea transporters proposed in reference 60.

mRNA size is based upon northern analysis.

Molecular mass (MW) is based upon western analysis.

AVP Stim = stimulation by arginine vasopressin. AVP Stim is based upon an increase in urea flux by vasopressin in heterologous expression systems (*Xenopus* oocytes, human embryonic kidney cells).

IMCD = inner medullary collecting duct.

tDL = thin descending limb.

Medulla* — exact tubular location unknown.

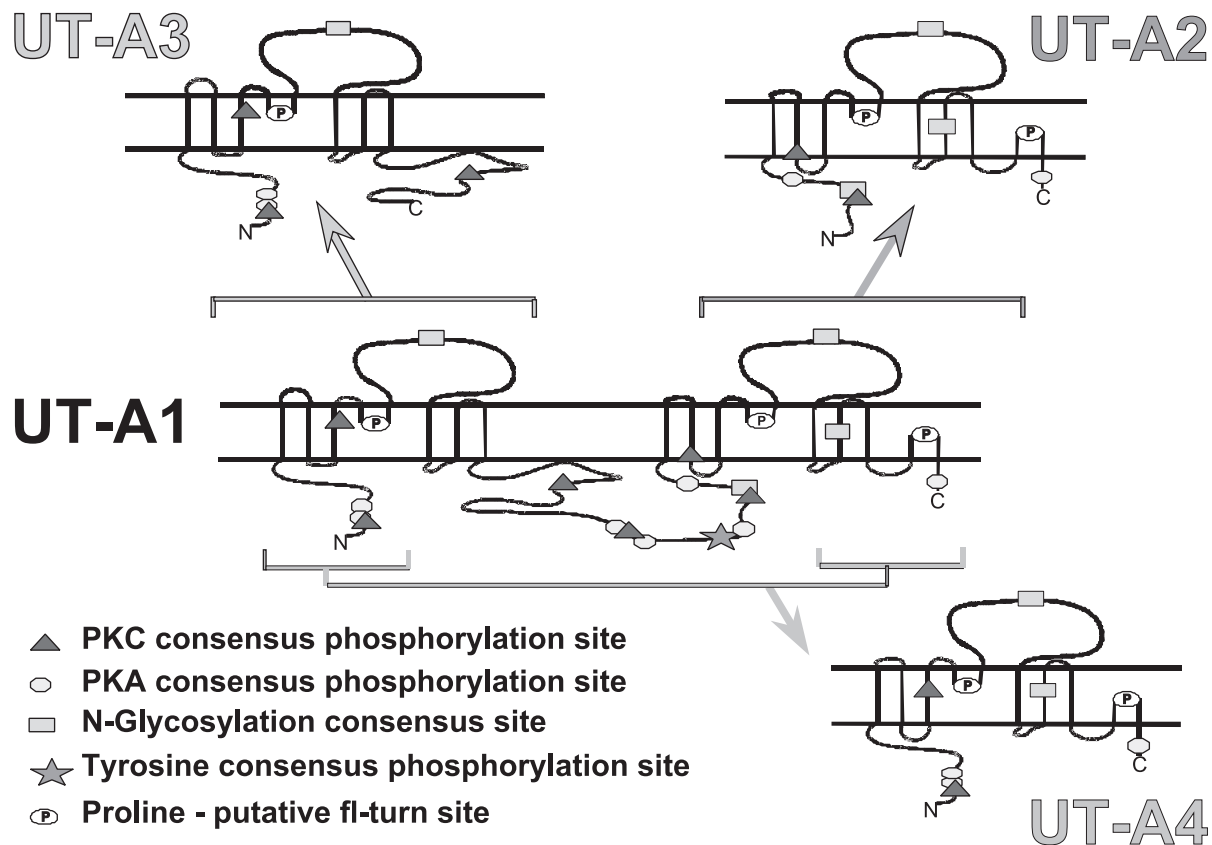


Fig. 1. Diagram illustrating a model of the predicted membrane topology for the four UT-A proteins and illustrating the structural relationships between them. Consensus sequences for phosphorylation or glycosylation are indicated. This figure is modified from reference 19.

to the mechanism by which vasopressin regulates aquaporin-2 (AQP2), the vasopressin-regulated water channel (AQP2 and UT-A1 have similar immunolocalization patterns and both are regulated rapidly by vasopressin [12, 20, 35]). However, Knepper and colleagues tested for regulated trafficking of UT-A1 in the rat terminal IMCD and showed that, in contrast to AQP2, trafficking of UT-A1 between the apical membrane and sub-apical vesicles does not occur (36). An alternative mechanism is that vasopressin alters the phosphorylation of UT-A1. The deduced amino acid sequence for UT-A1 contains several consensus sites for phosphorylation by PKA, as well as protein kinase C (PKC) or tyrosine kinase (19). However, no studies testing this potential regulatory mechanism have been published.

Hyperosmolality. Increasing osmolality, either by adding NaCl or mannitol to both the perfusate and bath of a perfused rat terminal IMCD, increases facilitated urea permeability, independently of vasopressin (34, 37, 38). When osmolality is increased in the presence of vasopressin, there is an additive stimulatory effect on facilitated urea permeability (32, 34, 37, 38).

Both hyperosmolality- and vasopressin-stimulated facilitated urea permeability are inhibited by phloretin or thiourea (31, 37). However, increases in intracellular calcium (39) mediate hyperosmolality-stimulated facilitated urea permeability while increases in adenylyl cyclase mediate vasopressin-stimulated facilitated urea permeability (33). Thus, both hyperosmolality and vasopressin rapidly increase facilitated urea permeability, but they do so via different second messenger pathways.

Long-Term Regulation of UT-A1

Vasopressin. Two methods have been used to study whether vasopressin exerts a prolonged regulatory effect on IMCD-facilitated urea permeability and UT-A1 protein abundance: water loading or restriction to vary endogenous vasopressin levels; or administering exogenous vasopressin or dDAVP (desmopressin, a V_2 -selective vasopressin agonist) to Brattleboro rats (which have congenital central diabetes insipidus due to the lack of endogenous vasopressin). Increasing plasma vasopressin by either method decreases the abun-

dance of both the 97 and 117 kDa UT-A1 proteins in rat inner medulla (14). Consistent with this change in UT-A1 protein abundance, a perfused terminal IMCD from water loaded (3 days) rats has a higher basal and vasopressin-stimulated facilitated urea permeability than a terminal IMCD from rats given water *ad libitum* (40). Thus, these studies led to the surprising result that facilitated urea permeability and UT-A1 protein abundance are decreased when vasopressin levels are increased (i.e., down-regulation of UT-A1).

In response to either water loading or restriction, northern analysis shows no change in UT-A1 mRNA abundance (16, 18, 22), suggesting that the changes in facilitated urea permeability and UT-A1 protein abundance are not regulated by transcription. In contrast, UT-A2 mRNA abundance does fall in the inner medulla of water loaded (3 days) rats and rises in: (1) water-restricted (3 days) rats; (2) rats receiving dDAVP for 3 weeks; and (3) Brattleboro rats treated with vasopressin for 1 week (16, 18, 41). Recently, Wade and colleagues showed that administering dDAVP for 7 days to Brattleboro rats increases UT-A2 protein abundance in thin, descending limbs (42). Thus, regulation of UT-A2, but not UT-A1, could involve transcriptional mechanisms stimulated by vasopressin (43). No studies of the long-term regulation of UT-A3 or UT-A4 have been published.

Impaired urine concentrating ability. To address the question of whether UT-A1 is regulated both rapidly and long term by vasopressin, similar to the way that AQP2 is regulated by vasopressin, we tested the long-term regulation of UT-A1 in four animal models associated with a reduction in urine concentrating ability: water diuresis; low-protein diet; hypercalcemia; and furosemide diuresis (44). We predicted that a decrease in urine concentrating ability could result from: (1) a decrease in urea absorption and down-regulation of UT-A1 in the inner medullary tip; or (2) an increase in urea absorption and up-regulation of UT-A1 in the inner medullary base.

We found that in all four conditions, basal (no vasopressin) facilitated urea permeability was increased in the deepest portion of the IMCD (located in the inner medullary tip), the IMCD₃, and there was greater abundance of the 117 and 97 kDa UT-A1 proteins in the inner medullary tip (14, 21, 23, 40, 45–49). Surprisingly, therefore, basal facilitated urea permeability and UT-A1 protein abundance are increased during *in vivo* conditions associated with an impaired urine concentrating ability and a reduced plasma vasopressin level. This suggests that rather than the UT-A1 urea transporter being up-regulated to

generate urine concentrating ability, it may be up-regulated to prevent further reductions in urine concentrating ability and/or to reduce the loss of urea from the inner medulla during conditions with high urine flow rates. This could be a mechanism explaining the rapid increase in urine concentrating ability that occurs within 5–10 minutes after urea is infused into people, rats, or dogs who were malnourished or fed low-protein diets (1, 2, 4, 50): UT-A1 protein abundance is increased when urine concentrating ability is impaired and this response “prepares” the IMCD to restore inner medullary urea rapidly once urea (or protein) intake rises.

Role of glucocorticoids. Facilitated urea permeability was measured in perfused initial and terminal IMCD subsegments from rats undergoing adrenalectomy, adrenalectomy plus replacement with a physiologic dose of glucocorticoid (dexamethasone), or sham-operation. Compared to sham-operated rats, basal-facilitated urea permeability in the rat terminal IMCD was significantly increased in adrenalectomized rats and reduced in dexamethasone-treated rats (21). In contrast, there was no difference in basal- or vasopressin-stimulated facilitated urea permeability in the initial IMCD between the three groups of rats (21). Next, western analysis was performed on membrane or vesicle fraction proteins from the inner medullary tip or base, corresponding to the location of the terminal or initial IMCD, respectively. UT-A1 protein abundance was significantly increased in both membrane and vesicle fractions from the inner medullary tip of adrenalectomized rats (21). In contrast, there was no change in UT-A1 protein abundance in the inner medullary base (21). Northern analysis showed no change in UT-A1 mRNA abundance in either inner medullary region (21). Thus, glucocorticoids down-regulate the function and expression of UT-A1 in the rat terminal IMCD.

Diabetic rats. Next, we studied rats with uncontrolled diabetes mellitus, since this condition is associated with enhanced urea excretion and an increase in urinary corticosterone. Thus, diabetic rats provide a second *in vivo* model for testing the role of glucocorticoids in down-regulating UT-A1. Rats were made acutely diabetic by injecting streptozotocin (125 mg/kg body weight). UT-A1 protein abundance was significantly decreased in the inner medullary tip, but unchanged in the inner medullary base, of diabetic rats (47). AQP2 protein abundance was unchanged in the inner medulla, indicating that the decrease in UT-A1 was not a generalized, nonspecific response (47).

To determine whether the decrease in UT-A1 protein abundance was mediated by glucocorticoids, rats underwent adrenalectomy, adrenalectomy plus streptozotocin, and adrenalectomy plus streptozotocin plus replacement with a physiologic dose of glucocorticoid (51). Adrenalectomy blocked the decrease in UT-A1 protein due to diabetes, and glucocorticoid-replacement restored the diabetes-induced decrease (47). Thus, glucocorticoids are mediators of the reduction in UT-A1 protein in the inner medullary tip of rats with diabetes mellitus (47). This effect is independent of insulin, since insulin was missing from all three groups of rats (47).

Active Urea Transport

In rats given food and water *ad libitum*, we found no active urea transport in the IMCD₁ (initial IMCD) or IMCD₂ (52) (Fig. 2). However, we did find active urea secretion in the IMCD₃ (52). This active urea secretion is: (1) inhibited by removing sodium (and replacing it with N-methyl-D-glucamine) from the tubule lumen (but not from the bath), suggesting an apical membrane localization; (2) stimulated rapidly by vasopressin; and (3) inhibited by phloretin or ouabain (52). These functional properties suggest that this is a secretory "sodium-urea counter-transporter" in the apical membrane of the IMCD₃ (52).

Next, we tested for active urea transport in IMCD subsegments from the four rat models associated with an impaired urine concentrating ability: low-protein diet; hypercalcemia; water diuresis; and furosemide diuresis. We found similar responses in rats fed an 8% protein diet for 3 weeks and in rats made hypercalcemic by feeding them a vitamin D analog, dihydrotachysterol, for 3 days. Active urea absorption was induced in the IMCD₁ from both low-protein fed and hypercalcemic rats. It was completely inhibited by: (1) removing sodium from the perfusate (and replacing it with N-methyl-D-glucamine) but not from the bath, suggesting an apical membrane localization; and (2) adding ouabain to the bath (45, 48, 53). These functional properties suggest that this is a reabsorptive "sodium-urea co-transporter" in the apical membrane of the IMCD₁. There was no active urea transport in the IMCD₂ from low-protein fed or hypercalcemic rats, and active urea secretion was completely inhibited in the IMCD₃ from low-protein fed or hypercalcemic rats (45, 48).

We also found active urea absorption in the IMCD₁ from furosemide-treated rats (54). In contrast to the active urea absorption in the IMCD₁ induced by low-protein feeding or hyper-

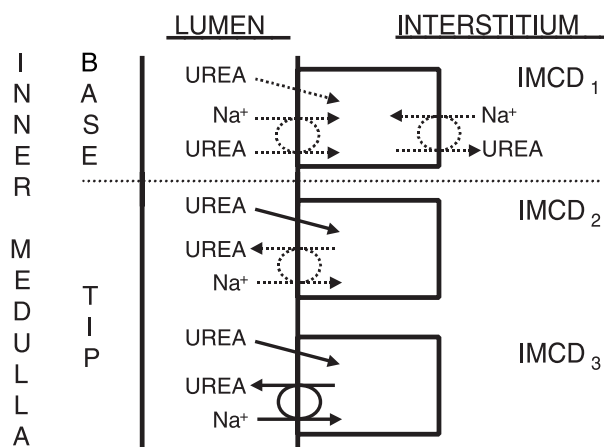


Fig. 2. Urea transporters expressed in rat inner medullary collecting duct subsegments. Collecting duct cells are illustrated starting at the inner-outer medullary border (IMCD₁, top) through the papillary tip (IMCD₃, bottom) (61, 62). The tubule lumen is shown on the left side of each cell. Arrows represent facilitated urea transporters. Circles connecting two arrows represent active urea transporters. Solid symbols represent urea transporters expressed in untreated rats. Dashed symbols represent urea transporters expressed only in rats with reduced urine concentrating ability. Top cell: an IMCD₁ cell showing a facilitated urea transporter that is induced in low-protein fed or hypercalcemic rats (45, 46, 49), and two inducible, Na⁺-dependent, active urea transport processes, one of which is a Na⁺-urea "co-transporter" in the apical membrane of low-protein fed (53) or hypercalcemic (48) rats, and the other, a Na⁺-urea "counter-transporter" in the basolateral membrane of furosemide diuretic rats (54). Middle cell: an IMCD₂ cell showing a facilitated urea transporter (UT-A1) that is expressed in the apical membrane of untreated rats (12, 20) and is up-regulated by hypercalcemia, furosemide, or adrenalectomy (21, 40, 49), and a Na⁺-dependent, active urea "counter-transporter" that can be induced in the apical membrane of water diuretic rats (48). Bottom cell: an IMCD₃ cell showing a facilitated urea transporter (UT-A1) that is expressed in the apical membrane of untreated rats (12, 20) and is up-regulated in low-protein, hypercalcemia, furosemide, or adrenalectomy (21, 40, 48, 49), and a Na⁺-dependent, active urea "counter-transporter" that is expressed in the apical membrane of untreated rats (12, 20, 52). This Na⁺-urea "counter-transporter" is up-regulated in water diuretic rats (52) and down-regulated in a low-protein fed, (48), hypercalcemic (48), or furosemide diuretic (54) rats.

calcemia (45, 48, 53), this one is: (1) inhibited by removing sodium (and replacing it with N-methyl-D-glucamine) from the bath (but not from the perfusate), suggesting a basolateral membrane localization; (2) stimulated rapidly by vasopressin; and (3) inhibited by phloretin or ouabain (54). These functional properties suggest that this is a reabsorptive "sodium-urea counter-transporter" in the basolateral membrane of the IMCD₁. This active urea transport process is functionally similar to the sodium-urea counter-transporter in the IMCD₃ from rats given food

and water *ad libitum*, but transports urea in an opposite direction and is localized to the opposite cell membrane. At present, it is not possible to determine whether there are two sodium-urea counter-transporters, or a single transporter that can be sorted to different cell membranes depending upon the IMCD subsegment and the *in vivo* condition of the rat. There was no active urea transport in the IMCD₂ from furosemide-treated rats, and active urea secretion was completely inhibited in the IMCD₃ (54).

In contrast to the preceding three animal models, we found no active urea transport in the IMCD₁ from water diuretic rats (52). However, we found that water diuresis results in a significant increase in active urea secretion in the IMCD₃ and its induction in the IMCD₂, the middle portion of the IMCD, (48, 52). Thus, in contrast to the uniform pattern of long-term regulation of facilitated urea permeability, there are two response patterns of active urea transporters when urine concentrating ability is reduced: (1) hypercalcemia, a low-protein diet, and furosemide result in induction of active urea absorption in the IMCD₁, albeit by different mechanisms, and inhibition of active urea secretion in the IMCD₃; while (2) water diuresis results in up-regulation of active urea secretion in the IMCD₃ and its induction in the IMCD₂, without any active urea absorption in the IMCD₁ (45, 46, 48, 52–54).

These patterns of change in active urea transporters are consistent with our predictions about changes in urea transport and urine concentrating ability (*vide supra*). In the first response pattern (hypercalcemia, a low-protein diet, and furosemide), the induction of active urea absorption in the IMCD₁ contributes to the urine concentrating defect by increasing urea delivery to the base of the inner medulla, thereby decreasing urea delivery distally to the inner medullary tip; the accompanying inhibition of active urea secretion in the IMCD₃ may prevent an even greater reduction in urea content in the deep inner medulla. In the second response pattern (water diuresis), up-regulation of active urea secretion in the terminal IMCD subsegments (the IMCD₂ and the IMCD₃) will directly decrease urea content in the deep inner medulla.

Urea Transport in Liver

The only mammalian tissue which has phloretin-inhibitable urea transport, other than kidney and erythrocytes, is the liver (55–57). This suggests that liver cells may express a urea transporter protein in order to accelerate urea efflux following ureagenesis (55, 58). We tested

whether a cultured human liver cell line, HepG2 cells, transports urea by measuring thiourea influx. HepG2 cells had a high thiourea influx rate that was significantly inhibited by two urea transport inhibitors, phloretin and thionicotinamide (59). Western analysis of HepG2 cell lysate using our antibody to the C-terminus of UT-A1, which detects UT-A1, UT-A2, and UT-A4, revealed two protein bands, 49 and 36 kDa (59). We found the same proteins in cultured rat hepatocytes, freshly isolated rat hepatocytes, and in liver from rat, mouse, and chimpanzee (59). Deglycosylation of rat liver lysate had no effect on either band, and both bands were present when analyzed by native gel electrophoresis (59). Differential centrifugation of rat liver lysate showed that the 49 kDa UT-A protein is in the 200,000xg (membrane) fraction while the 36 kDa UT-A protein is in the cytoplasmic fraction (59).

Next, we made rats uremic by 5/6th nephrectomy and feeding them a high protein diet to determine whether the abundance of these UT-A proteins varies *in vivo*. The 49 kDa UT-A membrane protein was significantly increased in livers from uremic rats compared to pair-fed, control rats (59). Thus in liver, phloretin-inhibitable urea transport occurs via a 49 kDa UT-A protein, and uremia increases the abundance of this UT-A protein *in vivo*. This up-regulation of the 49 kDa UT-A protein may allow hepatocytes to increase ureagenesis to reduce the accumulation of ammonium and/or bicarbonate during uremia.

Acknowledgments

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