Published in final edited form as:

Pflugers Arch. 2012 December ; 464(6): 561–572. doi:10.1007/s00424-012-1157-0.

Molecular Mechanisms of Urea Transport in Health and Disease

Janet D. Klein, Mitsi A. Blount, and Jeff M. Sands

Renal Division, Department of Medicine, and Department of Physiology, Emory University School of Medicine, Atlanta, GA 30322

Abstract

In the late 1980s, urea permeability measurements produced values that could not be explained by paracellular transport or lipid phase diffusion. The existence of urea transport proteins were thus proposed and less than a decade later, the first urea transporter was cloned. The SLC14A family of urea transporters has two major subgroups, designated *SLC14A1* (or UT-B) and *SLC14A2* (or UT-A). UT-B and UT-A gene products are glycoproteins located in various extra-renal tissues however, a majority of the resulting isoforms are found in the kidney. The UT-B (*SLC14A1*) urea transporter was originally isolated from erythrocytes and two isoforms have been reported. In kidney, UT-B is located primarily in the descending vasa recta. The UT-A (*SLC14A2*) urea transporter yields 6 distinct isoforms, of which 3 are found chiefly in the kidney medulla. UT-A1 and UT-A3 are found in the inner medullary collecting duct (IMCD), while UT-A2 is located in the thin descending limb. These transporters are crucial to the kidney's ability to concentrate urine. The regulation of urea transporter activity in the IMCD involves acute modification through phosphorylation and subsequent movement to the plasma membrane. UT-A1 and UT-A3 accumulate in the plasma membrane in response to stimulation by vasopressin or hypertonicity. Long term regulation of the urea transporters in the IMCD involves altering protein abundance in response to changes in hydration status, low protein diets, or adrenal steroids. Urea transporters have been studied using animal models of disease including diabetes mellitus, lithium intoxication, hypertension, and nephrotoxic drug responses. Exciting new genetically engineered mouse models are being developed to study these transporters.

Urea ($\text{O}=\text{C}(-\text{NH}_2)_2$) is a small, highly polar molecule with low lipid solubility thus lowering the permeability of this molecule through artificial lipid bilayers (4×10^{-6} cm/s) that lack urea transport proteins [31]. A specific facilitated urea transport process was first proposed in 1987 in rabbit and rat terminal IMCDs [75]. These physiologic studies provided a functional characterization for a vasopressin-regulated urea transporter (reviewed in [43]) that subsequently led to the cloning of the first urea transporter in 1993 [96]. To date, two urea transporter genes have been cloned: the *SLC14A2* (UT-A) gene encodes 6 proteins and 9 cDNA isoforms; and the *SLC14A1* (UT-B) gene encodes 2 isoforms (Table 1, reviewed in [43]).

Traditional wisdom (and most textbooks) states that urea is freely permeable across cell membranes. Why, then, is a urea transport system necessary? Although urea's permeability across artificial lipid bilayers is quite low, it is not zero. With sufficient time, urea will diffuse across cell membranes and achieve equilibrium; however, in kidney, the transit time for tubular fluid through the collecting duct or for red blood cells through the vasa recta is too fast to allow urea concentrations to reach equilibrium solely by passive diffusion. In addition, studies showing that urea transporter proteins are expressed in several non-renal

organs suggest that urea was, mistakenly, considered to be freely permeable. Research has revealed significant information about the structure, function and regulation of these proteins (reviewed in [43]).

Over 75 years ago, Gamble and colleagues first described “an economy of water in renal function referable to urea” bolstering the importance of urea in the generation of a concentrated urine [32,27]. There is an emerging body of information on the roles that urea transporters play in the kidney under both normal and pathophysiological situations. The reduction in maximal urine concentrating capacity in several species, including humans, that occurs in periods of protein deprivation can be restored by urea infusion (reviewed in [43]). Genetically engineered mice lacking UT-A1/UT-A3 [26,28,38], UT-A2 [87], UT-B [92,93,50], or UT-A2 and UT-B [54] have decreased maximal urine concentrating ability. These reported phenotypes demonstrate that, although the mechanism by which the inner medulla concentrates urine remains controversial [24,72], urea and urea transporters must play a role in the inner medullary concentration of urine.

Urea Transporters

UT-A1 is expressed in the terminal IMCD and is detected in the apical plasma membrane (Table 1). UT-A3 is also expressed in the terminal IMCD; it is primarily detected in the basolateral plasma membrane but has also been detected in the apical plasma membrane in some studies. UT-A2 is expressed in thin descending limbs. UT-A4 mRNA is expressed in rat kidney medulla, although its protein has not been detected and UT-A4 mRNA has not been detected in mouse kidney. UT-A5 is in testis and UT-A6 is in colon. Neither UT-A5 nor UT-A6 are expressed in kidney. UT-B protein is expressed in descending vasa recta and red blood cells (Figure 1) (reviewed in [43]).

Vasopressin increases the phosphorylation and the apical plasma membrane accumulation of UT-A1 and of UT-A3 in freshly isolated suspensions of rat IMCDs [97,8]. UT-A1 is phosphorylated by vasopressin at serines 486 and 499 [9]. The vasopressin regulated phosphorylation site(s) on UT-A3 have not been determined, however, it is known that neither of the two conventional PKA consensus sites is involved [78]. Vasopressin stimulates urea transport and UT-A1 phosphorylation and apical plasma membrane accumulation through two cAMP-dependent pathways: PKA and Epac (exchange protein activated by cAMP) [91].

Although responsive to vasopressin, urea transporters are also responsive to changes in osmolality. During antidiuresis, inner medullary osmolality is high. Hyperosmolality increases urea permeability in rat terminal IMCDs, even in the absence of vasopressin, suggesting that it is an independent activator of urea transport (reviewed in [43]). Hypersomolality increases the phosphorylation and the plasma membrane accumulation of both UT-A1 and UT-A3, similar to activation by vasopressin [7,8,44,97].

Regulation of Urea Transporters in Health (Normal Physiology)

Vasopressin

UT-A1—As well as stimulating phosphorylation and membrane accumulation acutely, vasopressin exerts long-term regulation through changes in urea transporter protein abundance. These changes do not appear to be regulated by transcription (reviewed in [43]). Brattleboro rats are an animal model frequently used to investigate vasopressin effects. They have intact vasopressin receptors but lack vasopressin and have central diabetes insipidus. When Brattleboro rats are given vasopressin for 5 days UT-A1 protein abundance in the inner medulla is decreased [80,42]. When vasopressin is administered for 12 days instead of

the 5 days in the preceding studies, UT-A1 protein abundance is significantly increased [42]. The delayed increase in UT-A1 protein abundance is reflective of the timing for the increase in inner medullary urea content after vasopressin treatment of Brattleboro rats [34].

Consistent with this, when rats are water loaded for 3 days to downregulate vasopressin, their perfused terminal IMCDs have higher basal and higher vasopressin-stimulated urea permeabilities than terminal IMCDs from rats given water ad libitum [40]. Histochemistry confirms that UT-A1 expression is increased in 3 day water-loaded rats without a change in UT-A1's subcellular distribution [58].

Conversely, chronic down-regulation of vasopressin by water loading rats for 14 days (vs. 3 days) significantly decreases UT-A1 protein abundance [42]. Analysis of UT-A promoter I may explain the varied temporal response since it contains a tonicity enhancer element (TonE) but not a cAMP response element (CRE) (reviewed in [43]). Vasopressin administration may initially increase transcription of the Na-K-2Cl co-transporter, NKCC2/BSC1, in the thick ascending limb. This would result in increased NaCl reabsorption and increased inner medullary osmolality, which would then increase UT-A1 transcription through TonE.

UT-A2 and UT-A3—The mRNA abundances of UT-A2, UT-A2b, UT-A3 and UT-A3b are reduced in the inner medulla of water loaded rats and rise in rats and mice that are water restricted or treated with dDAVP or vasopressin (reviewed in [43]). Histochemistry shows decreased UT-A2 and UT-A3 expression in 3 day water-loaded (vasopressin depleted) rats [58]. Similarly, UT-A2 and UT-A3 protein abundances are decreased in rats that have been treated with furosemide. Conversely, UT-A2 and UT-A3 are increased in the medulla of water deprived or dDAVP treated rats (reviewed in [43]). The regulation of UT-A2 could involve transcriptional mechanisms stimulated by vasopressin since UT-A2 is under the control of UT-A promoter II, which contains a CRE element [64]. UT-A3 is under the control of UT-A promoter I, which contains a tonicity-responsive enhancer (TonE) and could be mediated by tonicity-responsive transcription [64].

UT-B—Histochemistry shows increased UT-B1 expression in 3 day water-loaded rats without a change in UT-B1's subcellular distribution [58]. Another study reports that UT-B1 protein abundance is substantially down-regulated by long-term dDAVP infusion [85]. The effect of vasopressin or dDAVP on UT-B1 mRNA also varies with time and kidney region [70]. UT-B2 abundance is unaltered by vasopressin [82].

Adrenal steroids

Adrenalectomy produces a urine concentrating defect, although the mechanism is unclear [39,77,37,15]. Glucocorticoids such as dexamethasone increase the fractional excretion of urea in rats [51]. Two studies have examined the effect of glucocorticoids on UT-A1 protein abundance and obtained opposite results. In one study, rats were adrenalectomized and compared to sham-operated rats, each receiving a stress dose of dexamethasone or vehicle [65]. Compared to sham-operated rats, adrenalectomy increased urea permeability in terminal IMCDs and UT-A1 protein abundance in the inner medullary tip [65]. Dexamethasone treatment of the adrenalectomized rats decreased urea permeability in terminal IMCDs and UT-A1 protein abundance in the inner medullary tip [65]. The second study employed a different model where all rats were adrenalectomized and given aldosterone replacement alone or in addition to dexamethasone [56,15]. Rats lacking glucocorticoids had lower UT-A1 and UT-A3 protein abundances than animals given dexamethasone [56,15]. The reason for the different findings regarding UT-A1 between these studies is unclear, but may be due to the different treatment protocols.

Administering dexamethasone (at stress levels) to normal rats decreases UT-A1 and UT-A3 mRNA abundances in the inner medullary tip but does not change UT-A2 mRNA abundance [68]. Dexamethasone also decreases UT-A promoter I activity [68], suggesting that the decrease in UT-A1 and UT-A3 mRNA abundance is transcriptionally regulated. Consistent with transcriptional down-regulation, dexamethasone treatment for 14 days resulted in reduced UT-A1 and UT-A3 with an associated increase in urea excretion [56].

Mineralocorticoids (aldosterone) also decrease UT-A1 protein abundance in the inner medulla of adrenalectomized rats [33]. This decrease can be blocked by the mineralocorticoid-receptor antagonist, spironolactone [33]. The decrease resulting from dexamethasone is not blocked by spironolactone indicating that each adrenal steroid hormone works through its own receptor [33]. After feeding rats with a high salt diet to cause aldosterone-induced volume expansion, both UT-A1 and UT-A3 protein abundances are decreased (Figure 2) [90].

Angiotensin II

Infusing angiotensin II into the renal artery increases urine osmolality [22]. Animal studies involving a transgenic deletion of angiotensin converting enzyme (ACE) or angiotensinogen, as well as pharmacological inhibition of the angiotensin II pathway with an ACE inhibitor, resulted in impaired urine concentrating ability (reviewed in [43]). Even though mice that lack ACE have a normal appearing medulla, they have a pronounced urine concentrating defect [21] and UT-A1 protein abundance is decreased to 25% of the level seen in wild-type mice [49]. Administering angiotensin II to these mice for 2 weeks does not correct either the urine concentrating defect or the reduction in UT-A1 protein abundance [49]. The increase in urine osmolality in response to angiotensin and the increase in UT-A1 phosphorylation by PKC in response to hypertonicity [46] suggested that there might be a synergy between angiotensin and PKC in the urea transporter response. Surprisingly, when UT-A1 was studied in PKC α knockout mice there was no difference in the response to angiotensin when compared to littermate control animals [81].

Glucagon

Although the fractional excretion of urea is increased by glucagon [1,2,52], glucagon's effect on urea transport in the IMCD have varied between studies. One study showed that glucagon decreases urea permeability in perfused rat terminal IMCDs and UT-A1 protein abundance in rat IMCD suspensions [94]. Glucagon's effect was mediated by stimulating a PKC signaling pathway [94]. However in other studies, glucagon did not alter basal or vasopressin-stimulated urea permeability in perfused rat IMCDs nor cAMP production in either the initial or terminal IMCD [61,36].

Ageing

Normal ageing results in a decrease in urine concentrating ability (reviewed in [73]). UT-A1, UT-A3 and UT-B protein abundances are reduced in kidneys of aged rats [69,18,86]. Treatment of aging rats with a supra-physiologic dose of dDAVP results in increased urine osmolality. UT-A1, UT-A2 and UT-B protein abundances were also increased by a supra-physiologic dose of dDAVP, but not to the levels observed in younger rats [17].

Regulation of Urea Transporters in Disease (Pathophysiology)

Diabetes mellitus

Type 1—Rats treated with streptozotocin develop type 1 diabetes mellitus and show a characteristic osmotic diuresis in addition to increases in urea excretion, corticosterone production and plasma vasopressin levels [63,13,84]. The timeline of changes in transport

proteins in the diabetic inner medulla reflects different regulatory pathways during the different phases of disease development (Figure 3). UT-A1 protein abundance decreases at 3–5 days after inducing diabetes mellitus but not in adrenalectomized, diabetic rats [48,41]. The decrease in UT-A1 protein abundance is reversed by administering dexamethasone to adrenalectomized rats with diabetes mellitus, suggesting that glucocorticoids mediate the early decrease in UT-A1 protein [48].

Following the initial decrease in UT-A1 at 3–5 days of diabetes mellitus, UT-A1 mRNA and protein are increased at 10–21 days of diabetes mellitus in normal rats with increases in different parts of the inner medulla occurring at different times [4,41]. UT-A1 protein abundance does not increase in diabetic Brattleboro rats, indicating that vasopressin is necessary for this increase [42]. Although repletion of vasopressin does not increase UT-A1 phosphorylation in Brattleboro rats, it does in diabetic Brattleboro rats. This indicates that vasopressin is necessary for the increase in UT-A1 protein abundance and phosphorylation that occurs at 10–21 days after diabetes mellitus is induced [42].

The increased UT-A1 protein abundance in the diabetic rat kidney inner medulla is accompanied by a shift in the glycoprotein forms expressed in inner medullary tip versus base (Figure 3) [41,44,10]. The base of the inner medulla, which normally expresses only the 97 kDa glycoprotein form of UT-A1, shows both the 97 kDa and 117 kDa forms in the diabetic rat inner medulla [41,44,10]. This more mature glycosylated 117 kDa form, which contains n-acetylglucosamine is associated with lipid rafts more than the mannose rich 97 kDa glycoprotein form that is found in non-lipid rafts [14]. The inner medulla of diabetic rats has increased 117 kDa UT-A1 levels with high amounts of N-acetylglucosamine and sialic acid, largely associated with lipid rafts [14]. UT-A3 also exists as two glycoprotein forms of 65 and 45 kDa; the 65 kDa form is more stable and is enriched in lipid rafts [79].

There is also a change in the vasopressin sensitivity of the diabetic rat inner medulla. In the normal rat inner medulla, only urea transport in the tip is subject to stimulation by vasopressin, whereas in the diabetic kidney, urea permeability in both the tip and the base is increased by vasopressin [67]. This may be a consequence of increased UT-A1 membrane association in the diabetic inner medulla relative to the control, non-diabetic inner medulla (Figure 4).

In normal animals, angiotensin stimulates urea permeability synergistically with vasopressin. In the diabetic animal when angiotensin receptors are blocked with candesartan, there is an increase in both UT-A1 and UT-A3 protein, apparently to reduce the loss of solute during uncontrolled diabetes [10]. This suggests that angiotensin may have different modes of action in the chronic disease state compared with its acute effect. In other studies, diabetic rats treated with the nitric oxide synthase inhibitor L-NAME displayed a decrease in both UT-A1 and UT-A3 total protein expression [16]. Despite decreased expression, the diabetic-induced glycosylation of UT-A1 remained increased. Further studies addressing the function of the urea transporter glycosylation states and lipid rafts in diabetes will likely yield interesting findings.

UT-A2 has been shown to be decreased in the streptozotocin-induced diabetic rat [4]. The abundance of mRNA for UT-A2 is unaltered by diabetes, so the decrease occurs post-translationally and may reflect a general increase in protein degradation in the catabolic diabetic state.

Type 2—The knowledge of the responses of urea transporters in type 2 diabetes is less advanced compared with our information about these transporters in type 1 diabetes. The most commonly used rat model of type 2 diabetes is the obese Zucker rat model, which has

a genetic mutation of the leptin receptor gene [88]. UT-A1 in obese Zucker rats is dramatically decreased compared to lean Zucker (non-diabetic) rats [6]. One of the differences between the type 1 and type 2 models is that the obese Zucker rats have pronounced hypertension [88], while the streptozotocin-induced type 1 diabetic rats have mild hypertension, if any. In normal Sprague-Dawley rats that were made hypertensive with angiotensin II, UT-A1 was decreased [47], so the decreased UT-A1 protein abundance in this type 2 diabetic model may be a response to the hypertension in the obese Zucker rats.

Lithium

Lithium is the most commonly used medication for the treatment of patients with manic-depressive (bipolar) disorder. Unfortunately, long-term treatment with lithium frequently causes nephrogenic diabetes insipidus (NDI) and an inability to concentrate urine (reviewed in [83]). A hallmark of lithium nephrotoxicity is a reduction in inner medullary interstitial osmolality. Contributing to this are lithium-induced changes in AQP2 [45,62] and reduced interstitial urea and NaCl concentrations [11]. Rats fed lithium also show a dramatic reduction in UT-A1 protein abundance in both inner medullary tip and base regions [45], as well as a reduction in UT-A3 protein abundance in the inner medullary tip [11]. This lithium-induced down-regulation of urea transporters severely compromises transepithelial urea transport, resulting in disruption of the osmotic gradient required for urine concentration. The abundance of UT-B in the inner medullary base is decreased in lithium treated rats [45].

Lithium enters the principal cells of the collecting duct through the epithelial sodium channel, ENaC, which has a higher permeability for lithium than for sodium. Treating lithium-fed rats with the ENaC blocker, amiloride, restored UT-A1 expression as well as urine concentrating ability [5]. Lithium is known to increase the tissue phosphodiesterase (PDE) concentration [19]. When lithium-treated rats were also provided with the PDE5-inhibitor sildenafil for 4 weeks, UT-A1 levels, as well as γ -ENaC levels, were normalized and polyuria was reduced [71].

Some of the symptoms of NDI can be reversed or eliminated by discontinuing lithium treatment. This can occur in as little as 3 weeks after discontinuing lithium. In many cases, however, discontinuing lithium treatment does not result in restored kidney function. In rodent studies, UT-A1 and UT-A3 protein levels returned to control values 14 days after stopping lithium (Figure 5); however, even with both urea transporters returning to basal values of expression, urine urea levels remained significantly lower than prior to lithium therapy [11]. This could be due to a lithium-induced defect in transporter function.

Kidney tissue from lithium treated animals has been subjected to proteomic analysis. Results showed that several proteins involved in signaling cascades that impact urea transporter function and/or trafficking have altered expression after 14 days of lithium treatment [66]. Immunohistochemistry showed that UT-A1 localization was unaffected, either by chronic lithium treatment or after recovery from lithium treatment, indicating that the trafficking machinery remains intact.

Acute treatment with lithium can also affect urea transport. Despite an increase in plasma vasopressin levels, lithium blocks cAMP production by inhibiting adenylyl cyclase. Lithium also increases cAMP-PDE activity, which further reduces cAMP concentrations [19]. The phosphorylation of UT-A1 by PKA at serine 486 and serine 499 is necessary for movement of the protein to the apical membrane where it must be located to transport urea [9]. The lack of cAMP in response to vasopressin during lithium treatment reduces the phosphorylation of the urea transporters and thus prevents trafficking of the transporter to the membrane. Without membrane insertion of these transporters, the osmotic gradient is

destroyed, ultimately leading to polyuria. Consistent with this analysis, a physiologic concentration of vasopressin stimulates urea transporter phosphorylation in IMCD suspensions from normal rats, but does not increase UT-A1 phosphorylation in IMCD suspensions from lithium-fed rats [45].

Hypertension

Dahl rats—Dahl salt-sensitive rats have increased urea transport in their terminal IMCDs and increased protein abundances of UT-A1 and UT-A3 in the inner medulla [25]. These rats also have increased levels of 11 β -hydroxysteroid dehydrogenase type II, the enzyme that inactivates corticosterone [25]. Inactivating glucocorticoids would lessen the repression of UT-A promoter I activity, thereby increasing UT-A1 and UT-A3 transcription, and mRNA and protein abundances [68].

Angiotensin and norepinephrine—In a different rodent model of hypertension, rats were treated with a very high dose of angiotensin II. Blood pressures ranged from 130 mmHg in control animals to 200 mmHg within three days of treatment [47]. The treated animals had a significant decrease in inner medullary urea transporter protein. Glucocorticoids and mineralocorticoids can reduce UT-A1 levels, so angiotensin II-induced increases in aldosterone levels could have been responsible for the decreased UT-A1 abundance. Two pieces of evidence argue against this. First, when the mineralocorticoid receptor blocker spironolactone was given in addition to the angiotensin II, the decrease in UT-A1 was indistinguishable from the response to angiotensin II alone. Second, when blood pressure was increased by treatment with norepinephrine instead of angiotensin II, UT-A1 abundance was again reduced. These studies show that UT-A1 is decreased with high blood pressure [47].

Renal disease models

Nephrotic syndrome—Administering adriamycin, an anti-cancer chemotherapeutic agent, to rats for 3 weeks results in the development of proteinuria and decreased UT-A1 protein abundance in the inner medulla, compared to vehicle-treated rats [30]. Recently, however, we showed that UT-A1 protein abundance was reduced in non-pair-fed nephrotic animals, but not their pair-fed counterparts, suggesting a nutritional bias [12]. In these adriamycin-induced nephrotic syndrome animals: 1) NKCC2 was dramatically reduced; 2) NCC and AQP2 were modestly reduced; and 3) active gamma-ENaC was increased. We concluded that these transporter changes could be a response to increased sodium reabsorption in the cortical collecting ducts along with the disruption of the osmotic gradient along the medullary collecting duct [12].

Cisplatin—Administering cisplatin, an anti-cancer chemotherapeutic agent, to rats for 5 days results in the development of acute kidney injury accompanied by an increase in urine volume and a decrease in urine osmolality [20]. However, UT-A1 and UT-A2 protein abundances are unchanged in the inner and outer medulla, respectively [20].

Cyclosporine—Treating patients with the immunosuppressant anti-rejection drug cyclosporine frequently results in nephrotoxic effects. Treating rats with cyclosporine for 4 weeks reduced urine concentrating ability [59]. Cyclosporine reduced UT-A2, UT-A3, and UT-B protein abundances but did not change UT-A1 protein abundance [59]. While plasma vasopressin levels were not altered by cyclosporine, vasopressin infusion did restore urine concentrating ability to cyclosporine treated animals [59]. Cyclosporine treatment increased the number of TUNEL-positive (apoptotic) cells and the number of apoptotic cells correlated with UT-A3 expression [59].

Cyclosporine is an inhibitor of protein phosphatase 2b, also known as calcineurin. Tacrolimus (also known as FK506) is another immunosuppressant drug administered to transplant patients and is also an inhibitor of calcineurin. Recently, we examined the effects of tacrolimus on UT-A1 phosphorylation and activation by membrane insertion [35]. In this study, UT-A1 was identified as a substrate for dephosphorylation by calcineurin. It was also determined that UT-A1 could be dephosphorylated by multiple phosphatases but the PKA-phosphorylated serine 486, the phosphorylation site necessary for apical membrane trafficking, is dephosphorylated by calcineurin (Figure 6). Interestingly, the response of phosphorylated UT-A1 appears to differ between acute and chronic tacrolimus treatment with acute treatment activating urea transport by promoting membrane accumulation of UT-A1, as opposed to the response to chronic treatment that tends to lower the ability of phosphorylated UT-A1 to associate with the membrane and may be a contributing factor to the observed nephrotoxicity of these drugs [35].

Ureteral obstruction—In rats with either bilateral or unilateral ureteral obstruction, the abundances of UT-A1, UT-A3 and UT-B proteins are reduced in the medulla [55]. All three urea transporter abundances remained reduced 2 weeks after release of bilateral ureteral obstruction [55].

Sepsis—Urinary concentration defects frequently occur in conjunction with sepsis. Recently Kuper et al studied endotoxemia induced in rats and reported reduced levels of UT-A1 and AQP2 along with other transport proteins. They correlated the decrease with reduced TonEBP/NFAT5 transcriptional activity in conjunction with increased nitric oxide production [53].

Chloroquine—Chronic administration of chloroquine, a widely used anti-malaria drug, results in polyuria. In a recent study, we found that chloroquine resulted in a down-regulation of AQP2 and NKCC2; however, UT-A1 and UT-A3 abundances were unaffected in chloroquine-treated rats. Chloroquine dampened cAMP production in the inner medulla and resulted in a marked reduction of UT-A1 and AQP2 in the apical plasma membrane in response to vasopressin in chloroquine-treated rats. We concluded that the chloroquine-induced polyuria likely occurs as a result of lowered cAMP production thus altering UT-A1 trafficking [89].

Genetic ablation of urea transporters

UT-B1 knock-out mice

Humans lacking UT-B1, the Kidd antigen, are unable to concentrate their urine above 800 mOsm/kg H₂O, even following overnight water deprivation and exogenous vasopressin administration [74]. An analogous mouse model has been developed and used to study the physiologic role of UT-B1, the UT-B1 knock-out mouse. UT-B1 knock-out mice have impaired urine concentrating ability, achieving a maximal urine osmolality of 2,400 mOsm/kg H₂O, compared to 3,400 in a wild-type mouse [92]. This supports the hypothesis that urea transport in red blood cells is important for efficient countercurrent exchange [60]. In normal mice, both red blood cells and perfused rat descending vasa recta exhibit phloretin-inhibitable urea transport and express UT-B1 protein, suggesting that urea transport in red blood cells and descending vasa recta occurs via UT-B1 (reviewed in [43]). In the UT-B1 null mice, the functional impairment is seen in early life, but in later life, there is also a renal medullary atrophy that is not apparent at young ages, suggesting that the severe polyuria and hydronephrosis contributes to the later structural impairment [98].

In UT-B1 knock-out mice, UT-A2 protein abundance is increased while UT-A1 and UT-A3 abundances are unchanged [50]. This finding suggests that UT-A2 is upregulated to

compensate for the loss of UT-B1, since both UT-A2 and UT-B1 are involved in urea recycling, may have explained the mild phenotype observed in UT-B1 knock-out mice and in humans lacking UT-B1/Kidd antigen [50]. However, this hypothesis was not supported by the findings in the UT-B1/UT-A2 knock-out mouse (see below) [54].

UT-A2 and UT-B1/UT-A2 knock-out mice—UT-A2 knock-out mice have an impaired ability to concentrate urine, likely resulting from impairment of urea recycling [87]. Since UT-B1 and UT-A2 are present in adjacent areas within the kidney, and because knockout of UT-B1 resulted in increased UT-A2 in the UT-B1 knockout mice, Lei et al. made a double knockout of both UT-B1 and UT-A2 to see if the relationship between urea handling by these two transporters could be elucidated. Surprisingly, the deletion of UT-A2 appeared to partially correct the concentrating defect in mice with only UT-B knocked out [54]. The authors concluded from these studies that UT-A2 may function to move urea into stores in response to changes from diuresis to antidiuresis in an acute response instead of playing a role in maintaining urea concentration during the normal steady state [54].

UT-A1/UT-A3 knock-out mice—Mice with genetic knock-out of both UT-A1 and UT-A3 have reduced urine concentrating ability and inner medullary interstitial urea content, and lack vasopressin-stimulated or phloretin-inhibitable urea transport in their IMCD [26,28]. Since a human correlate with genetic lack of UT-A1 or UT-A3 has not yet been identified, we will refer those interested in these models to these excellent reviews of the subject [23,29,57].

Future directions for research

Since knock-out mice lacking any of the urea transporters are polyruic, urea transporter inhibitors are potential targets for the development of a novel class of diuretics. Recently, an inhibitor of UT-B1 that is metabolically stable and works at nanomolar potency was reported [3,95]. Further development of this intriguing compound and the development of inhibitors of other urea transporters would be quite exciting. An inhibitor the IMCD urea transporters, UT-A1 and UT-A3, is particularly attractive as a drug target since these are located in the last portion of the nephron and hence should have minimal downstream effects on electrolyte excretion, in contrast to conventional diuretics that act in more proximal portions of the nephron.

Acknowledgments

This review was supported by NIH grants R01-DK41707, R01-DK89828, R21-DK91147 to JMS and JDK, and NIH grants K01-DK82733 and R03-DK91501 as well as Norman S. Coplon Extramural Grant, Satellite Healthcare to MAB.

References

1. Ahloulay M, Bouby N, Machet F, Kubrusly M, Coutaud C, Bankir L. Effects of glucagon on glomerular filtration rate and urea and water excretion. *Am J Physiol Renal, Fluid Electrolyte Physiol.* 1992; 263:F24–F36.
2. Ahloulay M, Déchaux M, Laborde K, Bankir L. Influence of glucagon on GFR and on urea and electrolyte excretion: Direct and indirect effects. *Am J Physiol Renal, Fluid Electrolyte Physiol.* 1995; 269:F225–F235.
3. Anderson MO, Zhang J, Liu Y, Yao C, Phuan PW, Verkman AS. Nanomolar Potency and Metabolically Stable Inhibitors of Kidney Urea Transporter UT-B. *J Med Chem.* 2012; 55(12): 5942–5950.10.1021/jm300491y [PubMed: 22694147]

4. Bardoux P, Ahloulay M, Le Maout S, Bankir L, Trinh-Trang-Tan MM. Aquaporin-2 and urea transporter-A1 are up-regulated in rats with type I diabetes mellitus. *Diabetologia*. 2001; 44 (5): 637–645. [PubMed: 11380083]
5. Bedford JJ, Leader JP, Jing R, Walker LJ, Klein JD, Sands JM, Walker RJ. Amiloride restores renal medullary osmolytes in lithium-induced nephrogenic diabetes insipidus. *Am J Physiol Renal Physiol*. 2008; 294 (4):F812–F820. [PubMed: 18216143]
6. Bickel CA, Knepper MA, Verbalis JG, Ecelbarger CA. Dysregulation of renal salt and water transport proteins in diabetic Zucker rats. *Kidney Int*. 2002; 61 (6):2099–2110. [PubMed: 12028450]
7. Blessing NW, Blount MA, Sands JM, Martin CF, Klein JD. Urea transporters UT-A1 and UT-A3 accumulate in the plasma membrane in response to increased hypertonicity. *Am J Physiol Renal Physiol*. 2008; 295 (5):F1336–F1341. [PubMed: 18715940]
8. Blount MA, Klein JD, Martin CF, Tchapyjnikov D, Sands JM. Forskolin stimulates phosphorylation and membrane accumulation of UT-A3. *Am J Physiol Renal Physiol*. 2007; 293 (4):F1308–F1313. [PubMed: 17686955]
9. Blount MA, Mistry AC, Fröhlich O, Price SR, Chen G, Sands JM, Klein JD. Phosphorylation of UT-A1 urea transporter at serines 486 and 499 is important for vasopressin-regulated activity and membrane accumulation. *Am J Physiol Renal Physiol*. 2008; 295 (1):F295–F299. [PubMed: 18495802]
10. Blount MA, Sands JM, Kent KJ, Smith TD, Price SR, Klein JD. Candesartan augments compensatory changes in medullary transport proteins in the diabetic rat kidney. *Am J Physiol Renal Physiol*. 2008; 285 (6):F1448–F1452. [PubMed: 18417538]
11. Blount MA, Sim JH, Zhou R, Martin CF, Lu W, Sands JM, Klein JD. The expression of transporters involved in urine concentration recover differently after ceasing lithium treatment. *Am J Physiol Renal Physiol*. 2010; 298:F601–F608. [PubMed: 20032119]
12. Bou Matar RN, Malik B, Wang XH, Martin CF, Eaton DC, Sands JM, Klein JD. Protein abundance of urea transporters and aquaporin 2 change differently in nephrotic pair-fed versus non-pair-fed rats. *Am J Physiol Renal Physiol*. 2012; 302:F1545–F1553. [PubMed: 22461302]
13. Brooks DD, Nutting DF, Crofton JT, Share L. Vasopressin in rats with genetic and streptozotocin-induced diabetes. *Diabetes*. 1989; 38:54–57. [PubMed: 2909412]
14. Chen G, Howe AG, Xu G, Froehlich O, Klein JD, Sands JM. Mature N-linked glycans facilitate UT-A1 urea transporter lipid raft compartmentalization. *FASEB J*. 2011; 25:4531–4539. [PubMed: 21965602]
15. Chen YC, Cadnapaphornchai MA, Summer SN, Falk S, Li C, Wang W, Schrier RW. Molecular Mechanisms of Impaired Urinary Concentrating Ability in Glucocorticoid-Deficient Rats. *Journal of the American Society of Nephrology*. 2005; 16 (10):2864–2871.
16. Cipriani P, Kim SL, Klein JD, Sim JH, von Bergen TN, Blount MA. The role of nitric oxide in the dysregulation of the urine concentration mechanism in diabetes mellitus. *Front Physiol*. 2012; 3:176.10.3389/fphys.2012.00176 [PubMed: 22685437]
17. Combet S, Geffroy N, Berthouaud V, Dick B, Teillet L, Verbavatz J-M, Corman B, Trinh-Trang-Tan M-M. Correction of age-related polyuria by dDAVP: molecular analysis of aquaporins and urea transporters. *Am J Physiol Renal Physiol*. 2003; 284 (1):F199–F208. [PubMed: 12388383]
18. Combet S, Teillet L, Geelen G, Pitrat B, Gobin R, Nielsen S, Trinh-Trang-Tan MM, Corman B, Verbavatz JM. Food restriction prevents age-related polyuria by vasopressin-dependent recruitment of aquaporin-2. *American Journal of Physiology: Renal Physiology*. 2001; 281 (6):F1123–F1131.
19. Dousa TP. Interaction of lithium with vasopressin-sensitive cyclic AMP system of human renal medulla. *Endocrinology*. 1974; 95:1359–1366. [PubMed: 4372038]
20. Ecelbarger CA, Sands JM, Doran JJ, Cacini W, Kishore BK. Expression of salt and urea transporters in rat kidney during cisplatin-induced polyuria. *Kidney Int*. 2001; 60 (6):2274–2282. [PubMed: 11737600]
21. Esther CR Jr, Marrero MB, Howard TE, Machaud A, Corvol P, Capecchi MR, Bernstein KE. The critical role of tissue angiotensin-converting enzyme as revealed by gene targeting in mice. *J Clin Invest*. 1997; 99:2375–2385.

22. Faubert PF, Chou SY, Porush JG, Byrd R. Regulation of papillary plasma flow by angiotensin II. *Kidney Int.* 1987; 32:472–478. [PubMed: 3430949]
23. Fenton RA. Urea transporters and renal function: lessons from knockout mice. *Current Opinion in Nephrology and Hypertension.* 2008; 17:513–518. [PubMed: 18695393]
24. Fenton RA, Brond L, Nielsen S, Praetorius J. Cellular and subcellular distribution of the type II vasopressin receptor in kidney. *Am J Physiol Renal Physiol.* 2007; 293:F748–F760. [PubMed: 17553938]
25. Fenton RA, Chou C-L, Ageloff S, Brandt W, Stokes JB III, Knepper M. Increased collecting duct urea transporter expression in Dahl salt-sensitive rats. *Am J Physiol Renal Physiol.* 2003; 285 (1):F143–F151. [PubMed: 12684228]
26. Fenton RA, Chou C-L, Stewart GS, Smith CP, Knepper MA. Urinary concentrating defect in mice with selective deletion of phloretin-sensitive urea transporters in the renal collecting duct. *Proc Natl Acad Sci USA.* 2004; 101 (19):7469–7474. [PubMed: 15123796]
27. Fenton RA, Chou CL, Sowersby H, Smith CP, Knepper MA. Gamble's "economy of water" revisited: studies in urea transporter knockout mice. *Am J Physiol Renal Physiol.* 2006; 291 (1):F148–F154. [PubMed: 16478978]
28. Fenton RA, Flynn A, Shodeinde A, Smith CP, Schnermann J, Knepper MA. Renal phenotype of UT-A urea transporter knockout mice. *J Am Soc Nephrol.* 2005; 16 (6):1583–1592. [PubMed: 15829709]
29. Fenton RA, Knepper MA. Urea and renal function in the 21st century: insights from knockout mice. *J Am Soc Nephrol.* 2007; 18 (3):679–688. [PubMed: 17251384]
30. Fernández-Llama P, Andrews P, Nielsen S, Ecelbarger CA, Knepper MA. Impaired aquaporin and urea transporter expression in rats with adriamycin-induced nephrotic syndrome. *Kidney International.* 1998; 53 (5):1244–1253. [PubMed: 9573539]
31. Galluci E, Micelli S, Lippe C. Non-electrolyte permeability across thin lipid membranes. *Arch Int Physiol Biochim.* 1971; 79:881–887. [PubMed: 4112719]
32. Gamble JL, McKhann CF, Butler AM, Tuthill E. An economy of water in renal function referable to urea. *Am J Physiol.* 1934; 109:139–154.
33. Gertner R, Klein JD, Bailey JL, Kim D-U, Luo X, Bagnasco SM, Sands JM. Aldosterone decreases UT-A1 urea transporter expression via the mineralocorticoid receptor. *J Am Soc Nephrol.* 2004; 15 (3):558–565. [PubMed: 14978157]
34. Harrington AR, Valtin H. Impaired urinary concentration after vasopressin and its gradual correction in hypothalamic diabetes insipidus. *J Clin Invest.* 1968; 47:502–510. [PubMed: 5637139]
35. Ilori TO, Wang Y, Blount MA, Martin CF, Sands JM, Klein JD. Acute calcineurin inhibition with tacrolimus increases phosphorylated UT-A1. *Am J Physiol Renal Physiol.* 2012; 302:F998–F1004. [PubMed: 22205230]
36. Isozaki T, Gillin AG, Swanson CE, Sands JM. Protein restriction sequentially induces new urea transport processes in rat initial IMCDs. *Am J Physiol.* 1994; 266:F756–F761. [PubMed: 8203559]
37. Jackson BA, Braun-Werness JL, Kusano E, Dousa TP. Concentrating defect in the adrenalectomized rat. Abnormal vasopressin-sensitive cyclic adenosine monophosphate metabolism in the papillary collecting duct. *J Clin Invest.* 1983; 72:997–1004. [PubMed: 6309913]
38. Jacob VA, Harbaugh CM, Dietz JR, Fenton RA, Kim SM, Castrop H, Schnermann J, Knepper MA, Chou CL, Anderson SA. Magnetic resonance imaging of urea transporter knockout mice shows renal pelvic abnormalities. *Kidney Int.* 2008; 74 (9):1202–1208. [PubMed: 18854850]
39. Kamoi K, Tamura T, Tanaka K, Ishikashi M, Yamagi T. Hyponatremia and osmoregulation of thirst and vasopressin secretion in patients with adrenal insufficiency. *J Clin Endocrin Metabol.* 1993; 77:1584–1588.
40. Kato A, Sands JM. Evidence for sodium-dependent active urea secretion in the deepest subsegment of the rat inner medullary collecting duct. *J Clin Invest.* 1998; 101 (2):423–428. [PubMed: 9435315]

41. Kim D-U, Sands JM, Klein JD. Changes in renal medullary transport proteins during uncontrolled diabetes mellitus in rats. *Am J Physiol Renal Physiol.* 2003; 285 (2):F303–F309. [PubMed: 12697581]
42. Kim D-U, Sands JM, Klein JD. Role of vasopressin in diabetes mellitus-induced changes in medullary transport proteins involved in urine concentration in Brattleboro rats. *Am J Physiol Renal Physiol.* 2004; 286:F760–F766. [PubMed: 14644754]
43. Klein JD, Blount MA, Sands JM. Urea transport in the kidney. *Comprehensive Physiology.* 2011; 1 (2):699–729.
44. Klein JD, Fröhlich O, Blount MA, Martin CF, Smith TD, Sands JM. Vasopressin increases plasma membrane accumulation of urea transporter UT-A1 in rat inner medullary collecting ducts. *J Am Soc Nephrol.* 2006; 17:2680–2686. [PubMed: 16959825]
45. Klein JD, Gunn RB, Roberts BR, Sands JM. Down-regulation of urea transporters in the renal inner medulla of lithium-fed rats. *Kidney Int.* 2002; 61 (3):995–1002. [PubMed: 11849454]
46. Klein JD, Martin CF, Kent KJ, Sands JM. Protein kinase C alpha mediates hypertonicity-stimulated increase in urea transporter phosphorylation in the inner medullary collecting duct. *Am J Physiol Renal Physiol.* 2012:302. in-press.
47. Klein JD, Murrell BP, Tucker S, Kim Y-H, Sands JM. Urea transporter UT-A1 and aquaporin-2 proteins decrease in response to angiotensin II or norepinephrine-induced acute hypertension. *Am J Physiol Renal Physiol.* 2006; 291 (5):F952–F959. [PubMed: 16788141]
48. Klein JD, Price SR, Bailey JL, Jacobs JD, Sands JM. Glucocorticoids mediate a decrease in the AVP-regulated urea transporter in diabetic rat inner medulla. *Am J Physiol.* 1997; 273 (6):F949–F953. [PubMed: 9435684]
49. Klein JD, Quach DL, Cole JM, Disher K, Mongiu AK, Wang X, Bernstein KE, Sands JM. Impaired urine concentration and the absence of tissue ACE: the involvement of medullary transport proteins. *Am J Physiol Renal Physiol.* 2002; 283 (3):F517–F524. [PubMed: 12167603]
50. Klein JD, Sands JM, Qian L, Wang X, Yang B. Upregulation of urea transporter UT-A2 and water channels AQP2 and AQP3 in mice lacking urea transporter UT-B. *J Am Soc Nephrol.* 2004; 15 (5):1161–1167. [PubMed: 15100356]
51. Knepper MA, Danielson RA, Saidel GM, Johnston KH. Effects of dietary protein restriction and glucocorticoid administration on urea excretion in rats. *Kidney Int.* 1975; 8:303–315. [PubMed: 1195562]
52. Knepper MA, Gunter CV, Danielson RA. Effects of glucagon on renal function in protein-deprived rats. *Surg Forum.* 1976; 27:29–31. [PubMed: 1019891]
53. Kuper C, Fraek ML, Muller HH, Beck FX, Neuhofer W. Sepsis-induced urinary concentration defect is related to nitric oxide-dependent inactivation of TonEBP/NFAT5, which downregulates renal medullary solute transport proteins and aquaporin-2. *Crit Care Med.* 2012; 40(6):1887–1895. [pii]. 10.1097/CCM.0b013e31824e118600003246-201206000-00027 [PubMed: 22610191]
54. Lei T, Zhou L, Layton AT, Zhou H, Zhao X, Bankir L, Yang B. Role of thin descending limb urea transport in renal urea handling and the urine concentrating mechanism. *American Journal of Physiology - Renal Physiology.* 2011; 301 (6):F1251–F1259.
55. Li C, Klein JD, Wang W, Knepper MA, Nielsen S, Sands JM, Frokiaer J. Altered expression of urea transporters in response to ureteral obstruction. *Am J Physiol Renal Physiol.* 2004; 286 (6):F1154–F1162. [PubMed: 14982816]
56. Li C, Wang W, Summer SN, Falk S, Schrier RW. Downregulation of UT-A1/UT-A3 Is Associated with Urinary Concentrating Defect in Glucocorticoid-Excess State. *Journal of the American Society of Nephrology.* 2008; 19 (10):1975–1981.
57. Li X, Chen G, Yang B. Urea Transporter Physiology Studied in Knockout Mice. *Frontiers in Physiology.* 2012:3. [PubMed: 22279436]
58. Lim S-W, Han K-H, Jung J-Y, Kim W-Y, Yang C-W, Sands JM, Knepper MA, Madsen KM, Kim J. Ultrastructural localization of UT-A and UT-B in rat kidneys with different hydration status. *Am J Physiol Regul Integr Comp Physiol.* 2006; 290 (2):R479–R492. [PubMed: 16179486]
59. Lim S-W, Li C, Sun B-K, Kim W-Y, Han K-H, Oh Y-W, Lee J-U, Kador PF, Knepper MA, Sands JM, Kim J, Yang C-W. Long-term treatment with cyclosporine decreases aquaporins and urea

- transporters in rat kidney. *Am J Physiol Renal Physiol.* 2004; 287 (1):F139–F151. [PubMed: 14871880]
60. Macey RI. Transport of water and urea in red blood cells. *Am J Physiol.* 1984; 246:C195–C203. [PubMed: 6199982]
 61. Maeda Y, Terada Y, Nonoguchi H, Knepper MA. Hormone and autocrine regulation of cAMP production in rat IMCD subsegments. *Am J Physiol.* 1992; 263:F319–F327. [PubMed: 1354941]
 62. Marples D, Christensen S, Christensen EI, Ottosen PD, Nielsen S. Lithium-induced downregulation of Aquaporin-2 water channel expression in rat kidney medulla. *J Clin Invest.* 1995; 95:1838–1845. [PubMed: 7535800]
 63. Mitch WE, Bailey JL, Wang X, Jurkowitz C, Newby DN, Price SR. Evaluation of signals activating ubiquitin-proteasome proteolysis in a model of muscle wasting. *Am J Physiol Cell Physiol.* 1999; 276:C1132–C1138.
 64. Nakayama Y, Peng T, Sands JM, Bagnasco SM. The TonE/TonEBP pathway mediates tonicity-responsive regulation of UT-A urea transporter expression. *J Biol Chem.* 2000; 275 (49):38275–38280. [PubMed: 10995747]
 65. Naruse M, Klein JD, Ashkar ZM, Jacobs JD, Sands JM. Glucocorticoids downregulate the rat vasopressin-regulated urea transporter in rat terminal inner medullary collecting ducts. *J Am Soc Nephrol.* 1997; 8 (4):517–523. [PubMed: 10495779]
 66. Nielsen J, Hoffert JD, Knepper MA, Agre P, Nielsen S, Fenton RA. Proteomic analysis of lithium-induced nephrogenic diabetes insipidus: Mechanisms for aquaporin 2 down-regulation and cellular proliferation. *Proceedings of the National Academy of Sciences.* 2008; 105 (9):3634–3639.
 67. Pech V, Klein JD, Kozlowski SD, Wall SM, Sands JM. Vasopressin increases urea permeability in initial IMCDs from diabetic rats. *Am J Physiol Renal Physiol.* 2005; 289 (3):F531–F535. [PubMed: 15886274]
 68. Peng T, Sands JM, Bagnasco SM. Glucocorticoids inhibit transcription and expression of the rat UT-A urea transporter gene. *Am J Physiol Renal Physiol.* 2002; 282 (5):F853–F858. [PubMed: 11934695]
 69. Preisser L, Teillet L, Aliotti S, Gobin R, Berthouaud V, Chevalier J, Corman B, Verbavatz JM. Downregulation of aquaporin-2 and -3 in aging kidney is independent of V₂ vasopressin receptor. *Am J Physiol Renal Physiol.* 2000; 279 (1):F144–F152.
 70. Promeneur D, Bankir L, Hu MC, Trinh-Trang-Tan M-M. Renal tubular and vascular urea transporters: influence of antidiuretic hormone on messenger RNA expression in Brattleboro rats. *J Am Soc Nephrol.* 1998; 9 (8):1359–1366. [PubMed: 9697657]
 71. Sanches TR, Volpini RA, Massola Shimizu MH, Braganca AC, Oshiro-Monreal F, Seguro AC, Andrade L. Sildenafil reduces polyuria in rats with lithium-induced NDI. *Am J Physiol Renal Physiol.* 2012; 302(1):F216–225. [ajprenal.00439.2010 \[pii\]. 10.1152/ajprenal.00439.2010](https://doi.org/10.1152/ajprenal.00439.2010) [PubMed: 22031848]
 72. Sands JM. Critical role of urea in the urine-concentrating mechanism. *J Am Soc Nephrol.* 2007; 18 (3):670–671. [PubMed: 17251382]
 73. Sands JM. Urine concentrating and diluting ability during aging. *J Gerontol A Biol Med Sci.* 2012 in-press.
 74. Sands JM, Gargus JJ, Fröhlich O, Gunn RB, Kokko JP. Urinary concentrating ability in patients with Jk(a-b-) blood type who lack carrier-mediated urea transport. *J Am Soc Nephrol.* 1992; 2:1689–1696. [PubMed: 1498276]
 75. Sands JM, Nonoguchi H, Knepper MA. Vasopressin effects on urea and H₂O transport in inner medullary collecting duct subsegments. *Am J Physiol.* 1987; 253:F823–F832. [PubMed: 3688238]
 76. Sands JM, Timmer RT, Gunn RB. Urea transporters in kidney and erythrocytes. *Am J Physiol.* 1997; 273 (3):F321–F339. [PubMed: 9321905]
 77. Schwartz MJ, Kokko JP. Urinary concentrating defect of adrenal insufficiency. Permissive role of adrenal steroids on the hydroosmotic response across the rabbit cortical collecting duct. *J Clin Invest.* 1980; 66:234–242. [PubMed: 6156951]
 78. Smith CP, Potter EA, Fenton RA, Stewart GS. Characterization of a human colonic cDNA encoding a structurally novel urea transporter, UT-A6. *Am J Physiol Cell Physiol.* 2004; 287 (4):C1087–C1093. [PubMed: 15189812]

79. Su H, Carter CB, Frohlich O, Cummings RD, Chen G. Glycoforms of UT-A3 urea transporter with poly-N-acetyllactosamine glycosylation have enhanced transport activity. *Am J Physiol Renal Physiol.* 2012; 303(2):F201–208.10.1152/ajprenal.00140.2012 [PubMed: 22535801]
80. Terris J, Ecelbarger CA, Sands JM, Knepper MA. Long-term regulation of collecting duct urea transporter proteins in rat. *J Am Soc Nephrol.* 1998; 9 (5):729–736. [PubMed: 9596069]
81. Thai TL, Blount MA, Klein JD, Sands JM. Lack of protein kinase C- α leads to impaired urine concentrating ability and decreased aquaporin-2 in angiotensin II-induced hypertension. *Am J Physiol Renal Physiol.* 2012; 303 (1):F37–F44. [PubMed: 22492943]
82. Tickle P, Thistlethwaite A, Smith CP, Stewart GS. Novel bUT-B2 urea transporter isoform is constitutively activated. *AJP - Regulatory, Integrative and Comparative Physiology.* 2009; 297 (2):R323–R329.
83. Timmer RT, Sands JM. Lithium intoxication. *J Am Soc Nephrol.* 1999; 10 (3):666–674. [PubMed: 10073618]
84. Trinder D, Phillips PA, Stephenson JM, Risvanis J, Aminian A, Adam W, Cooper M, Johnston CI. Vasopressin V₁ and V₂ receptors in diabetes mellitus. *Am J Physiol Endocrinol Metab.* 1994; 266:E217–E223.
85. Trinh-Trang-Tan M-M, Lasbennes F, Gane P, Roudier N, Ripoché P, Cartron J-P, Bailly P. UT-B1 proteins in rat: tissue distribution and regulation by antidiuretic hormone in kidney. *Am J Physiol Renal Physiol.* 2002; 283 (5):F912–F922. [PubMed: 12372766]
86. Trinh-Trang-Tan MM, Geelen G, Teillet L, Corman B. Urea transporter expression in aging kidney and brain during dehydration. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology.* 2003; 285 (6):R1355–R1365.
87. Uchida S, Sohara E, Rai T, Ikawa M, Okabe M, Sasaki S. Impaired urea accumulation in the inner medulla of mice lacking the urea transporter UT-A2. *Mol Cell Biol.* 2005; 25 (16):7357–7363. [PubMed: 16055743]
88. Van Zwieten PA, Kam KL, ijl AJ, Hendriks MGC, Beenen OHM, Pfaffendorf M. Hypertensive diabetic rats in pharmacological studies. *Pharmacological Research.* 1996; 33 (2):95–105. [PubMed: 8870024]
89. von Bergen TN, Blount MA. Chronic use of Chloroquine Disrupts the Urine Concentration Mechanism by Lowering cAMP Levels in the Inner Medulla. *Am J Physiol Renal Physiol.* 2012; 303(2):F201–208.10.1152/ajprenal.00547.2011
90. Wang X-Y, Beutler K, Nielsen J, Nielsen S, Knepper MA, Masilamani S. Decreased abundance of collecting duct urea transporters UT-A1 and UT-A3 with ECF volume expansion. *Am J Physiol Renal Physiol.* 2002; 282 (4):F577–F584. [PubMed: 11880317]
91. Wang Y, Klein JD, Blount MA, Martin CF, Kent KJ, Pech V, Wall SM, Sands JM. Epac regulation of the UT-A1 urea transporter in rat IMCDs. *J Am Soc Nephrol.* 2009; 20 (3):2018–2024. [PubMed: 19661162]
92. Yang B, Bankir L, Gillespie A, Epstein CJ, Verkman AS. Urea-selective concentrating defect in transgenic mice lacking urea transporter UT-B. *J Biol Chem.* 2002; 277:10633–10637. [PubMed: 11792714]
93. Yang B, Verkman AS. Analysis of double knockout mice lacking aquaporin-1 and urea transporter UT-B. *J Biol Chem.* 2002; 277 (39):36782–36786. [PubMed: 12133842]
94. Yano Y, Rodrigues AC Jr, deBraganca AC, Andrade LC, Magaldi AJ. PKC stimulated by glucagon decreases UT-A1 urea transporter expression in rat IMCD. *Pflugers Arch.* 2008; 456:1229–1237. [PubMed: 18449563]
95. Yao C, Anderson MO, Zhang J, Yang B, Phuan PW, Verkman AS. Triazolothienopyrimidine Inhibitors of Urea Transporter UT-B Reduce Urine Concentration. *J Am Soc Nephrol.* 2012; 23(7):1210–1220.10.1681/ASN.2011070751 [PubMed: 22491419]
96. You G, Smith CP, Kanai Y, Lee W-S, Stelzner M, Hediger MA. Cloning and characterization of the vasopressin-regulated urea transporter. *Nature.* 1993; 365:844–847. [PubMed: 8413669]
97. Zhang C, Sands JM, Klein JD. Vasopressin rapidly increases the phosphorylation of the UT-A1 urea transporter activity in rat IMCDs through PKA. *Am J Physiol Renal Physiol.* 2002; 282 (1):F85–F90. [PubMed: 11739116]

98. Zhou L, Meng Y, Lei T, Zhao D, Su J, Zhao X, Yang B. UT-B-deficient mice develop renal dysfunction and structural damage. *BMC Nephrol.* 2012; 13(6) 1471-2369-13-6 [pii]. 10.1186/1471-2369-13-6

\$watermark-text

\$watermark-text

\$watermark-text

Figure 1.

Urea transporters along the nephron. The cartoon and histology show the urea transporters (UT-A1/UT-A3, UT-A2, UT-B) along the nephron: UT-B found chiefly in the vasa recta; UT-A2 in the thin descending limb of the loop of Henle; UT-A1 (apical) and UT-A3 (basolateral) in the inner medullary collecting duct cells.

Figure 2.

Adrenal steroids and urea transporters. The diagram shows the known responses of urea transporters to adrenal steroids. Glucocorticoids, through transcriptional regulation, decrease both RNA and Protein abundance of UT-A1 and UT-A3. They do not decrease UT-A2 mRNA. Mineralocorticoids decrease both UT-A1 and UT-A3 protein levels through an undetermined mechanism. There is no data on the effect of mineralocorticoids on UT-A2 or UT-B.

UT-A1 protein in inner medullary (IM) tip of control (CTR) vs. diabetic (DM) rats.

Figure 3.

UT-A1 in the inner medullary tip of diabetic rats. A. Western blot analyses of IM tip lysates probed with anti-UT-A antibody. Samples were from 3 different control (CTR) rats (left) and 3 different diabetic (DM) rats (right). Both 117 and 97 kDa glycoprotein forms of UT-A1 (indicated by arrows) are increased over time after initiation of diabetes by STZ injection of Sprague-Dawley rats. B. The bar graph shows the summary densitometry of both bands in each bar with the black portion showing the contribution of the 117 kDa glycoprotein form and the white showing the 97 kDa contribution. *, $p < 0.05$ vs CTR total protein. N=6 rats/bar. Reproduced from [41].

UT-A1 in Control Rat IM

UT-A1 in Diabetic (STZ) Rat IM

Figure 4.

UT-A1 cellular location in the inner medullary collecting duct. Shown are 4 micron slices of perfusion fixed rat inner medulla from control (left) and STZ-diabetic (right) rats stained with antibody to UT-A1, visualized with peroxidase (brown positive stain). Nuclei were counterstained with hematoxylin. The apical localization of UT-A1 is more apparent in the diabetic rat IM than in the control animal.

Figure 5.

Lithium and Urea transporter abundance. Rats were fed a standard chow diet supplemented with lithium (40 mmol/kg) for 0 (control), 7 days (7d), 14 days (14d), or fed the diet for 14 days, then had lithium removed for 7 days (7d r) or 14 days (14d r). Top: schematic of serum lithium levels. Middle: Immunohistology of 4 micron slices of perfusion fixed rat inner medulla stained with antibody to UT-A1 and visualized with peroxidase (brown positive stain). Nuclei were counterstained with hematoxylin; micrographs were acquired at 40X magnification. Bottom: Western blots of IM tip probed for UT-A1 protein abundance (top) and tubulin as a loading control (Bottom). Each lane is tissue from a separate animal.

Figure 6.

Phosphorylation / dephosphorylation of UT-A1. Shown is a cartoon demonstrating phosphorylation of UT-A1 at serine 486 (a vasopressin sensitive, cAMP-mediated phosphorylation site) followed by movement to the apical membrane, dephosphorylation by calcineurin (CN), and release of cleaved phosphate by the calcineurin protein regenerating calcineurin for further action.

\$watermark-text

\$watermark-text

\$watermark-text

TABLE 1

Mammalian Facilitated Urea Transporter Gene Families.

Gene	Isoform	RNA (kb)	Protein (kDa)	Vasopressin Sensitive	Tissue Location
<i>Slc14a2</i>	UT-A1	4.0	97, 117	Yes	IMCD
	UT-A1b	3.5			medulla*
	UT-A2	2.9	55	No**	tDL, liver
	UT-A2b	2.5			medulla*, heart
	UT-A3	2.1	44, 67	Yes	IMCD
	UT-A3b	3.7			medulla*
	UT-A4§	2.5	43	Yes	medulla*
	UT-A5‡	1.4			testis
	UT-A6#	1.8			colon
<i>Slc14a1</i>	UT-B1	3.8	43		DVR, RBC‡
	UT-B2	3.7	43–54	No	Bovine rumen

Isoform names are based upon the urea transporter nomenclature proposed in [76]; AVP, urea flux is stimulated by vasopressin; IMCD, inner medullary collecting duct; tDL, thin descending limb;

* Medulla (exact tubular location unknown);

** No, no in rat but yes in mouse;

§, cloned from rat only;

‡, cloned from mouse only; DVR, descending vasa recta, RBC, red blood cells;

#, cloned from human only;

‡, also expressed in several other tissues and endothelial cells.

Citations to original publications can be found in [43].