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Topical Review

Molecular Mechanisms of Urea Transport

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Abstract. Physiologic data provided evidence for specific urea transporter proteins in red blood cells and kidney inner medulla. During the past decade, molecular approaches resulted in the cloning of several urea transporter cDNA isoforms derived from two gene families: UT-A and UT-B. Polyclonal antibodies were generated to the cloned urea transporter proteins, and their use in integrative animal studies resulted in several novel findings, including: (1) UT-B is the Kidd blood group antigen; (2) UT-B is also expressed in many non-renal tissues and endothelial cells; (3) vasopressin increases UT-A1 phosphorylation in rat inner medullary collecting duct; (4) the surprising finding that UT-A1 protein abundance and urea transport are increased in the inner medulla during conditions in which urine concentrating ability is reduced; and (5) UT-A protein abundance is increased in uremia in both liver and heart. This review will summarize the knowledge gained from studying molecular mechanisms of urea transport and from integrative studies into urea transporter protein regulation.

Key words: Urine concentrating mechanism — Vasopressin — Kidney — Glucocorticoids — Erythrocytes

Introduction

Urea is a highly polar molecule ((NH_2)-(C═O)-(NH_2)) with a molecular weight of 60 Da. Urea has a low lipid solubility through artificial lipid bilayers (4×10^{-6} cm/s; Galluci, Micelli & Lippe, 1971). Thus, in the absence of a transport protein, urea should have a low permeability across cell membranes. The high urea permeability across red blood cells and terminal inner medullary collecting ducts (IMCD) in the kidney was the initial evidence suggesting the presence of specific urea transporter proteins (Wieth et al., 1974; Sands, Nonoguchi & Knepper, 1987). Physiologic evidence establishing the concept of urea transporters was provided by transport studies of red blood cells and IMCDs performed in the 1970s and 1980s (reviewed in Sands, Timmer & Gunn, 1997; Sands, 1999a). In the last decade, several cDNA isoforms and two genes for urea transporters were cloned (Table 1). Through the use of cDNA probes and polyclonal antibodies to urea transporter proteins, urea transporters have been found in testis, liver, brain, and heart, and in some of these tissues, their abundance is altered in uremic rats (Effros et al., 1993; Hu et al., 2000; Duchesne et al., 2001). This review will focus on the knowledge gained through the use of molecular approaches to study the regulation of urea transporters in red blood cells and tissues.

Since most textbooks continue to state that urea is freely permeable across cell membranes, the reader may be surprised by the existence of urea transporters in red blood cells and kidney, and even more surprised by their expression in other organs. Urea's permeability across artificial lipid bilayers is very low but is not zero. Given sufficient time, urea will slowly diffuse across cell membranes and achieve equilibrium in the steady state. However, the transit time for red blood cells through the vasa recta or for tubule fluid through the collecting duct is too rapid to permit urea concentrations to reach equilibrium solely by passive diffusion. Furthermore, the expression of urea transporters in several non-renal tissues suggests that urea may have been mistakenly considered freely permeable due to a lack of knowledge regarding the widespread distribution of urea transporters.

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Table 1. Facilitated urea transporter genes and isoforms

Gene	Isoform	RNA (kb)	Protein (kDa)	AVP	Location	References
Slc14a1	UT-B1 (UT3)	3.8	43		DVR, RBC[1]	(Berger et al., 1998; Olivès et al., 1994; Timmer et al., 2001; Tsukaguchi et al., 1997)
	UT-B2 (UT11)	4.4	45		DVR, RBC[1]	(Couriaud et al., 1996; Promeneur et al., 1996; Timmer et al., 2001)
Slc14a2	UT-A1 (UT1)	4.0	97, 117	Yes	IMCD	(Bagnasco et al., 2001; Bradford et al., 2001; Shayakul et al., 1996)
	UT-A1b	3.5			Medulla*	(Bagnasco et al., 2000; Nakayama et al., 2001)
	UT-A2 (UT2)	2.9	55	No	tDL	(Nielsen et al., 1996; Olivès et al., 1996; Promeneur et al., 1996; Smith et al., 1995; You et al., 1993)
	UT-A2b	2.5			Medulla, heart, liver	(Bagnasco et al., 2000; Duchesne et al., 2001; Karakashian et al., 1999; Doran et al., 1999)
	UT-A3	2.1	44, 67	Yes*	IMCD	(Fenton et al., 2000; Karakashian et al., 1999; Shayakul et al., 2001; Terris et al., 2001)
	UT-A3b	3.7			Medulla	(Bagnasco et al., 2000)
	UT-A4	2.5	43	Yes	Medulla	(Karakashian et al., 1999)
	UT-A5[2]	1.4			Testis	(Fenton et al., 2000)

Isoform names are based upon the nomenclature proposed in (Sands et al., 1997) with original names in parenthesis. AVP: urea flux is stimulated by vasopressin in *Xenopus* oocytes or HEK-293 cells. Yes*: urea flux is stimulated in two studies but not in a third (Fenton et al., 2002c; Karakashian et al., 1999; Shayakul et al., 2001). IMCD: inner medullary collecting duct. tDL: thin descending limb. Medulla* (exact tubular location unknown). [1] Also expressed in several other tissues and endothelial cells. [2] Cloned from mouse only. DVR: descending vasa recta. RBC: red blood cells.

Role of Urea Transporters in the Urinary Concentrating Mechanism

The major physiologic role for urea transporters is in the urinary concentrating mechanism. Urea's importance to the production of a concentrated urine has been recognized since 1934 when Gamble and colleagues described "an economy of water in renal function referable to urea" (Gamble et al., 1934). Protein-deprived people and animals are unable to concentrate their urine normally, and a urea infusion reverses this defect (Gamble et al., 1934; Epstein et al., 1957; Hendrikx & Epstein, 1958; Crawford, Doyle & Probst, 1959; Levinsky & Berliner, 1959; Klahr & Alleyne, 1973; Pennell et al., 1975; Peil, Stolter & Schmidt-Nielsen, 1990). Thus, any hypothesis regarding the mechanism by which the kidney concentrates urine needs to include some effect derived from urea.

The passive mechanism hypothesis for urinary concentration was proposed 30 years ago by Kokko and Rector (1972) and by Stephenson (1972) (Fig. 1). This hypothesis requires that the concentration of urea in the inner medullary interstitium exceed that found in the lumen of the thin ascending limb. The urea concentration gradient permits the NaCl concentration in the interstitium to be lower than in the lumen of the thin ascending limb, thereby establishing a gradient for passive NaCl absorption in the absence of an osmotic gradient. If an inadequate amount of urea is transported into the deep inner medullary interstitium, then the chemical gradients necessary for passive NaCl absorption from the thin ascending limb cannot be established and urine concentrating ability is reduced. The primary mechanism for delivering urea to the deep inner medullary interstitium is urea absorption from the terminal IMCD, mediated by a facilitated urea transporter (Sands et al., 1987). Based upon functional measurements, both in red blood cells and in the isolated perfused IMCD, the properties of the putative facilitated urea transporters were established. Subsequently, several cDNA isoforms and two genes for urea transporters were cloned (Table 1).

UT-B Urea Transporter: *Slc14a1*

The red blood cell facilitated urea transporter, UT-B, was initially cloned from a human erythropoietic cell line (Olivès et al., 1994). Subsequently, it has been cloned from rat and mouse (Couriaud, Ripoche & Rousselet, 1996; Tsukaguchi et al., 1997; Yang et al., 2002). The human UT-B gene (*Slc14a1*) arises from a single locus located on chromosome 18q12, which is close to, but distinct from, the gene for UT-A, *Slc14a2* (Olivès et al., 1995, 1996; Lucien et al., 1998). The mouse urea transporter genes (*Scl14a1* (UT-B)

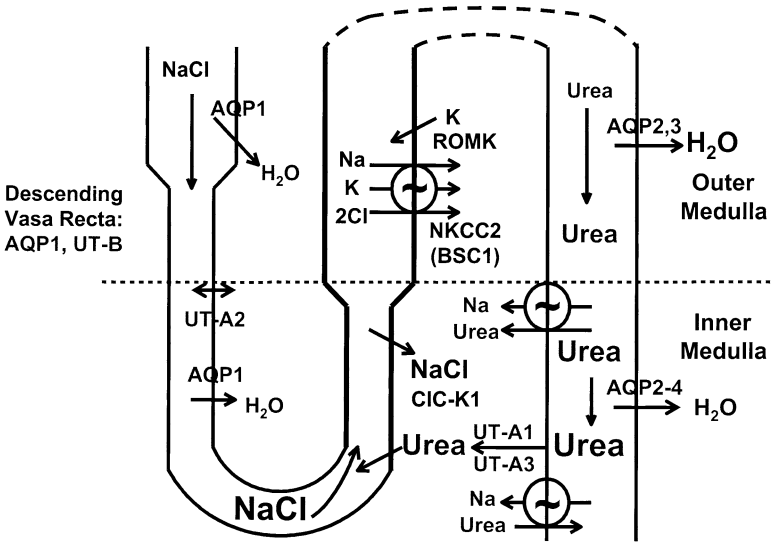

Fig. 1. Diagram showing the location of the major transport proteins involved in the urine concentrating mechanism in the outer and inner medulla. *UT*, urea transporter; *AQP*, aquaporin; *NKCC/BSC*, Na-K-2Cl cotransporter; *ROMK*, renal outer medullary *K* channel, C1C-K1, chloride channel.

and *Scl14a2* (UT-A)) also occur in tandem on chromosome 18 (Fenton et al., 1999). The Kidd (or Jk) antigen, one of the minor blood group antigens, is also located in the same region of human chromosome 18 as are the two urea transporter genes (Lucien et al., 1998). In people, UT-B protein is the Kidd antigen and several mutations of the UT-B/Kidd antigen (*Scl14a1*) gene have been reported (Olivès et al., 1995, 1996; Lucien et al., 1998; Sidoux-Walter et al., 2000; Lucien et al., 2002).

The human UT-B gene is approximately 30 kb in length, includes 11 exons, and its coding region begins in exon 4 and extends through exon 11 (Lucien et al., 1998). Human reticulocytes express 4.4 and 2.0 kb mRNA transcripts (due to alternative polyadenylation signals) but only a single 45 kDa protein (Lucien et al., 1998). Both the N- and C-termini of human UT-B are located intracellularly (Lucien et al., 2002). UT-B has a consensus glycosylation site at asparagine 211, but mutation of this site does not affect urea transport in heterologous expression systems, nor does the JK*A/JK*B polymorphism (Lucien et al., 2002). The cysteines at residues 25 and 30, but neither one alone, are essential for targeting UT-B to the plasma membrane in *Xenopus* oocytes (Lucien et al., 2002). Human UT-B is inhibited by phloretin and pCMBS, but pCMBS-inhibition does not depend upon the cysteines at residue 151 or 236. Red blood cells from individuals that lack the Kidd antigen (Jk null or Jk(a-b-)) also lack phloretin-sensitive facilitated urea transport (Kimoto & Constantinou, 1990).

In rat, two cDNA sequences have been reported (UT-B1, UT-B2) that differ by only a few nucleotides at their 3′ end (Couriaud et al., 1996; Tsukaguchi et al., 1997). At present, it is uncertain whether UT-B1 and UT-B2 truly represent different rat UT-B isoforms, a polymorphism, or a sequencing artifact. Most investigators currently favor the hypothesis that rat UT-B1 and UT-B2 are not distinct isoforms since man has only a single isoform, but this has not been tested. UT-B1/UT-B2 mRNA is widely expressed in kidney and several other organs, including testis, brain, bone marrow, spleen, prostate, bladder, thymus, heart, skeletal muscle, lung, liver, colon, small intestine, and pancreas (Olivès et al., 1994, 1996; Couriaud et al., 1996; Promeneur et al., 1996; Tsukaguchi et al., 1997; Berger, Tsukaguchi & Hediger, 1998; Hu et al., 2000; Prichett et al., 2000; Timmer et al., 2001; Yang et al., 2002). However, northern analysis has not detected UT-B1/UT-B2 mRNA in ovary, placenta, salivary glands, monocytes, leukocytes, or B lymphocytes (Olivès et al., 1994; Promeneur et al., 1996; Tsukaguchi et al., 1997; Prichett et al., 2000).

Several studies have addressed the question of whether UT-B transports urea only, or water and urea, using two approaches: injecting UT-B1/UT-B2 cRNA into *Xenopus* oocytes and studying red blood cells from double-knockout mice that lack both UT-B and aquaporin 1 (AQP1). The oocyte studies yielded differing results: two studies report that UT-B can function as a water channel (Yang & Verkman, 1998; Yang et al., 2002) but a third study reports that UT-B functions specifically as a urea transporter (Sidoux-Walter et al., 1999). However, the study of red blood cells from UT-B/AQP1 double-knockout mice does show that UT-B can function as a water channel in red blood cells (Yang & Verkman, 2002). This study also concluded that the amount of water transported through UT-B under physiologic conditions is small (in comparison to AQP1) and is probably not physiologically significant (Yang & Verkman, 2002).

Antibodies to the N- or C-terminus of UT-B (Xu et al., 1997; Hu et al., 2000; Timmer et al., 2001; Trinh-Trang-Tan et al., 2002) have been generated

that should detect both UT-B1 and UT-B2 proteins, if indeed there are two rat isoforms. Thus, in this review, I will refer to the rat protein(s) detected by UT-B antibodies simply as UT-B protein. By western blot, UT-B protein appears as a broad band between 45–65 kDa in human red blood cells and 37–51 kDa in rodent red blood cells (Timmer et al., 2001; Yang et al., 2002). In kidney inner or outer medulla, UT-B protein appears as a broad band between 41–54 kDa (Timmer et al., 2001; Trinh-Trang-Tan et al., 2002). In both red blood cells and kidney medulla, deglycosylation converts the broad band seen by western blot to a sharp, 32-kDa band (Timmer et al., 2001; Trinh-Trang-Tan et al., 2002). In kidney, but not in red blood cells, an additional 98-kDa band is detected whose molecular explanation is uncertain (Timmer et al., 2001). Both human and rodent kidney show UT-B immunostaining in the non-fenestrated endothelial cells that are characteristic of descending vasa recta, and phloretin-inhibitable urea transport is present in perfused rat descending vasa recta (Pallone, 1994; Pallone et al., 1994, 1995; Xu et al., 1997; Hu et al., 2000; Timmer et al., 2001; Trinh-Trang-Tan et al., 2002; Yang et al., 2002). UT-B protein is also present in many rodent tissues, including brain, testis, colon, heart, liver, lung, aorta, bladder, spinotrapezius muscle, and mesenteric artery, and in several cultured endothelial cell lines (Hu et al., 2000; Timmer et al., 2001; Trinh-Trang-Tan et al., 2002; Wagner et al., 2002). In endothelial cells grown in culture, UT-B promotes urea, thereby increasing intracellular urea and inhibiting L-arginine transport (Wagner et al., 2002). If a similar mechanism is present in endothelial cells in people with chronic kidney disease, then inhibition of arginine transport, a precursor of nitric oxide, could be contributing to the hypertension that is commonly present in these individuals (Wagner et al., 2002).

UT-B Protein during Development

UT-B protein is only weakly detected in rat kidney at fetal day 20 (Kim et al., 2002). UT-B immunostaining increases progressively after birth in the descending vasa recta, both in terms of the intensity of staining and the number of endothelial cells that stain for UT-B, until adult levels are achieved at age 21 days (Kim et al., 2002). Thus in rats, development of urine-concentrating ability coincides temporally with the increase in UT-B immunostaining in the descending vasa recta.

UT-B Protein and Urine-concentrating Ability

People lacking Kidd antigen are unable to concentrate their urine above 800 mOsm/kg H_2O, even following overnight water deprivation and exogenous vasopressin administration (Sands et al., 1992). A UT-B knockout mouse also has a reduced urine concentrating ability, achieving a maximal urine osmolality of 2400 mOsm/kg H_2O versus 3400 in a wild-type mouse (Yang et al., 2002). These findings support the hypothesis that UT-B-mediated urea transport in red blood cells and/or descending vasa recta is necessary to preserve the efficiency of countercurrent exchange (Macey, 1984).

Mathematical models of microcirculatory exchange between the descending and ascending vasa recta predict that UT-B is necessary to counterbalance the effect of AQP1 in the descending vasa recta, i.e., the efficiency of small solute trapping within the renal medulla will be decreased in the absence of UT-B, thereby decreasing the efficiency of countercurrent exchange and urine concentrating ability (Edwards & Pallone, 1997; 1998). Consistent with this hypothesis, the UT-B knockout mouse has impaired urea recycling and urine concentrating ability (Yang et al., 2002). Thus, the production of maximally concentrated urine requires UT-B protein expression in red blood cells and/or descending vasa recta (Edwards & Pallone, 1997, 1998; Macey & Yousef, 1988; Sands et al., 1992).

Long-term Regulation of UT-B in Kidney

In Brattleboro rats, which have central diabetes insipidus, administering vasopressin or dDAVP (Desmopressin, a V_2-selective vasopressin receptor agonist), for 6 hours reduces UT-B mRNA abundance in both the outer and inner medulla (Promeneur et al., 1998). However, administering vasopressin or dDAVP for 5 days increases UT-B mRNA abundance in the outer medulla (inner stripe portion) and the inner medullary base, but decreases it in the inner medullary tip (Promeneur et al., 1998). In normal rats, administering dDAVP for 7 days decreases UT-B protein abundance in the inner medulla, but furosemide also results in a more modest decrease in UT-B protein (Trinh-Trang-Tan et al., 2002). In either normal or Brattleboro rats, varying dietary protein between 10 and 40% has no effect on UT-B mRNA abundance in any portion of the kidney medulla (Hu, Bankir & Trinch-Trang-Tan, 1999). In uremic rats, UT-B mRNA and protein are reduced 5 weeks after 5/6 nephrectomy (Hu et al., 2000). Finally, in lithium-fed rats, UT-B protein abundance is markedly reduced in the inner medullary base (Klein et al., 2002a).

The UT-A Urea Transporter Family: *Slc14a2*

The UT-A family of urea transporters currently consists of 5 isoforms plus 3 variants that differ in the 3′ untranslated region. UT-A1 is the best studied and largest UT-A protein (Fig. 2). It is expressed in the

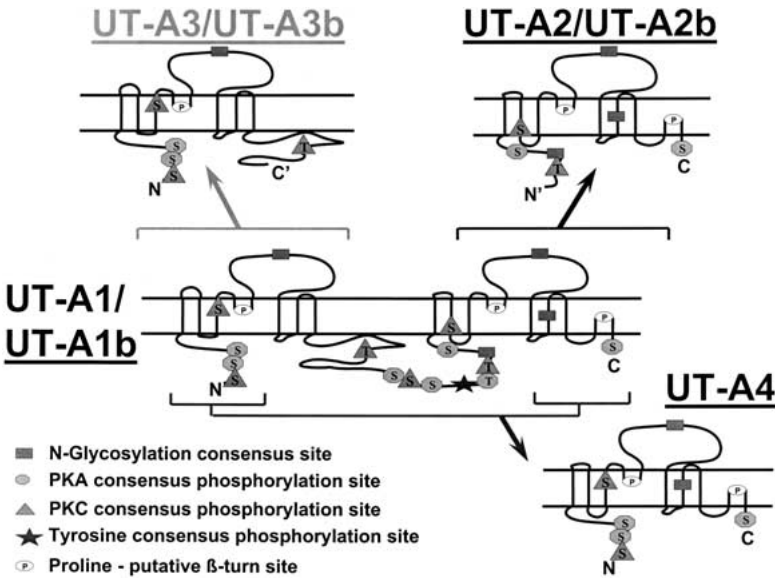

Fig. 2. Diagram showing the proposed structural relationship between the four kidney UT-A proteins. There is a high degree of homology between the four protein isoforms. There is no difference in the coding region of UT-A1, UT-A2, and UT-A3, and their respective b variants, so the latter are not shown separately in the diagram. UT-A1, UT-A3, and UT-A4 share common N-termini (N *vs.* N′). UT-A1, UT-A2, and UT-A4 share common C-termini (C *vs.* C′). Consensus sites for glycosylation and phosphorylation are indicated. *S*, serine; *T*, threonine.

apical membrane of the IMCD in humans (Bagnasco et al., 2001) and rodents (Nielsen et al., 1996; Kim et al., 2002). Human and rodent UT-A1 are stimulated by cyclic AMP (cAMP) when heterologously expressed in *Xenopus* oocytes (Promeneur et al., 1996; Shayakul, Steel & Hediger, 1996; Shayakul et al., 1997; Bagnasco et al., 2001; Fenton et al., 2002c).

UT-A2 was actually the first urea transporter that was cloned (You et al., 1993). It is expressed in thin descending limbs (Nielsen et al., 1996; Wade et al., 2000; Kim et al., 2002) and is not stimulated by cAMP analogs when heterologously expressed in either *Xenopus* oocytes or human embryonic kidney (HEK) 293 cells (You et al., 1993; Ashkar et al., 1995; Smith et al., 1995; Promeneur et al., 1996; Shayakul et al., 1996, 1997; Karakashian et al., 1999). UT-A2 is basically the C-terminal (3′) half of UT-A1; the two isoforms share identical C-terminal amino-acid and 3′ cDNA sequences but differ at their N-terminal (5′) ends (Shayakul et al., 1996; Bagnasco et al., 2001; Fenton et al., 2002b).

UT-A3 is expressed in the apical membrane of the IMCD (Terris, Knepper & Wade, 2001), similar to UT-A1 (Fig. 3). It is stimulated by cAMP analogs when heterologously expressed in HEK-293 cells or *Xenopus* oocytes in two studies (Karakashian et al., 1999; Fenton et al., 2002c) but not in a third (Shayakul et al., 2001). UT-A3 is basically the N-terminal (5′) half of UT-A1; the two isoforms share the same N-terminal amino-acid and 5′ cDNA sequence as UT-Al but differ at their C-terminal (3′) ends (Fenton et al., 2000; Karakashian et al., 1999; Shayakul et al., 2001).

UT-A4 has the same N- and C-terminal amino-acid (5′ and 3′ cDNA) sequences as UT-A1, but is smaller than UT-A1 and basically consists of the N-terminal (5′) quarter of UT-A1 spliced to the C-terminal (3′) quarter of UT-A1 (Karakashian et al., 1999). Although UT-A4's exact tubular location is unknown, UT-A4 mRNA is expressed in kidney medulla and is stimulated by cAMP analogs when heterologously expressed in HEK-293 cells (Karakashian et al., 1999).

UT-A5 is the only UT-A isoform that is not expressed in kidney (Fenton et al., 2000). UT-A5 is expressed in testis and is the shortest member of the UT-A family (Fenton et al., 2000). UT-A5's deduced amino-acid sequence begins at methionine 139 of mouse UT-A3, after which it it shares 100% homology and a common C-terminal amino-acid (3′ cDNA) end with UT-A3 (Fenton et al., 2000).

Three UT-A cDNA variants that do not differ in their coding regions, but have alternative 3′ untranslated regions, have been cloned and named UT-A1b, UT-A2b, and UT-A3b, respectively (Bagnasco et al., 2000). UT-A3b transcript is ~1.5 kb longer than the original cDNA while UT-A1b and UT-A2b transcripts are ~0.4 kb shorter than the original cDNAs (Bagnasco et al., 2000). All three variant UT-A mRNAs are expressed in rat inner medulla (Bagnasco et al., 2000).

Polyclonal antibodies have been made to 3 regions of UT-A1: the N-terminus (Wade et al., 2000); the C-terminus (Nielsen et al., 1996; Naruse et al., 1997); and the intracellular loop region (Terris et al., 1998). Consistent with the high degree of homology between the kidney UT-A cDNA isoforms, the C-terminus antibody detects UT-A1 (97 and 117 kDa), UT-A2 (55 kDa), and UT-A4 (43 kDa), the N-terminus antibody detects UT-A1, UT-A3 (44 and 67 kDa), and UT-A4, and the loop region antibody detects only UT-A1 (Naruse et al., 1997; Terris et al., 1998, 2001; Karakashian et al., 1999; Sands, 1999a). Western blot of inner medullary tip proteins probed

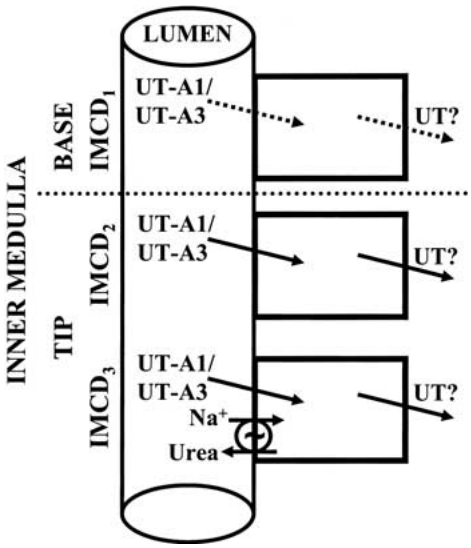

Fig. 3. Diagram showing the location of urea transporters in the inner medulla of normal rats (*solid arrows*). The names of the known urea transporters are indicated. The identity of the basolateral membrane urea transporter is unknown. *Arrows* indicate facilitated urea transporters. Circle connecting two arrows indicates secondary active urea transport. *Dotted arrows* indicate facilitated urea transport that is not present in normal rats but can be induced in rats fed a low- protein diet.

with any of the anti-UT-A1 antibodies shows bands at both 117 and 97 kDa; both bands represent glycosylated versions of a non-glycosylated 88 kDa UT-A1 protein (Bradford et al., 2001). UT-A1 also exists as a 206-kDa protein complex in native inner medullary membranes (Bradford et al., 2001). UT-A1 protein is most abundant in the inner medullary tip, only the 97 kDa protein is present in the inner medullary base, and no UT-A1 protein is present in outer medulla or cortex (Nielsen et al., 1996; Sands et al., 1998; Sands, 1999b).

Wade and colleagues succeeded in making a UT-A3-specific antibody, even though the C-terminus of UT-A3 differs from UT-A1 by only a single amino acid (Terris et al., 2001). Western blot of inner medullary tip proteins probed with the anti-UT-A3 antibody detects bands at both 67 and 44 kDa; both bands represent glycosylated versions of a non-glycosylated 40 kDa UT-A3 protein (Terris et al., 2001). UT-A3 protein is most abundant in the inner medullary tip, both glycoproteins are weakly detected in the inner medullary base and outer medulla, and no UT-A3 protein is detected in cortex (Terris et al., 2001).

Functional studies show that phloretin-inhibitable urea transport is present in both the apical and basolateral membranes of rat terminal IMCDs, with the apical membrane being the rate-limiting barrier for vasopressin-stimulated urea transport (Star, 1990). By immunohistochemistry, both UT-A1 and UT-A3 are expressed in the apical plasma membrane and intracellular cytoplasmic vesicles of terminal IMCDs, but not in the basolateral plasma membrane (Nielsen et al., 1996; Terris et al., 2001). Thus at present, none of the cloned UT-A or UT-B isoforms appear to mediate urea transport in the IMCD basolateral membrane.

Slc14a2: The UT-A Gene

The UT-A gene *Slc14a2* was initially cloned from rat (Nakayama et al., 2001); it was subsequently cloned from both human and mouse (Bagnasco et al., 2001; Fenton et al., 2002b). The rat UT-A gene is very long (approximately 300 kb) and contains 24 exons (Nakayama et al., 2001). UT-A1, UT-A3, and UT-A4 share a common transcription start site in exon 1 and translation start site in exon 4, with: UT-A1 being coded by exons 1–12 spliced to exons 14–23; UT-A3 being coded by exons 1–12; and UT-A4 being coded by exons 1–7 spliced to exons 18–23 (Nakayama et al., 2001). UT-A2 is unique with a transcription start site in exon 13, a translation start site in exon 16, and being encoded by exons 13–23 (Bagnasco et al., 2000). Thus, it is the only isoform that uses exon 13. Despite the difference in start sites, both UT-A1b and UT-A2b use exon 24 in the 3′ untranslated region.

The rat UT-A isoforms originate from a single gene (*Slc14a2*) which is an atypical gene since it has two promoter elements: promoter I, which is upstream of exon 1 and drives transcription of UT-A1, UT-A1b, UT-A3, UT-A3b, and UT-A4; and promoter II, which is located within intron 12 and drives transcription of UT-A2 and UT-A2b (Bagnasco et al., 2000; Nakayama et al., 2001). The initial 1.3 kb of UT-A promoter I does not contain a TATA box but has 3 CCAAT elements (Nakayama et al., 2000). However, expression of this 1.3 kb of DNA in a luciferase reporter gene construct and transfection into MDCK, mIMCD3, or LLC-PK1 cells results in promoter activity (Nakayama et al., 2000; Peng, Sands & Bagnasco, 2002). Promoter I's activity is increased by hyperosmolality, consistent with the presence of a tonicity enhancer (TonE) element (Nakayama et al., 2000). Promoter I activity is decreased by glucocorticoids, consistent with the decrease in the mRNA abundances of UT-A1, UT-A3, and UT-A3b in the inner medulla of rats given a stress dose of dexamethasone (Peng et al., 2002). Although promoter I contains a consensus glucocorticoid response element (GRE), this element does not mediate dexamethason's repressive effect (Peng et al., 2002).

As mentioned above, the transcription start site for UT-A2 is located in exon 13, almost 200 kb downstream from exon 1 (Bagnasco et al., 2000; Nakayama et al., 2001). This distance raised the possibility that there may be a second, internal promoter within intron 12, and transfection of a lucif-

erase reporter gene from this region of intron 12 into mIMCD3 cells does show evidence of promoter activity when the cells are stimulated with cAMP, but not under basal conditions (Bagnasco et al., 2000; Nakayama et al., 2001). Cloning and sequencing of 4.7 kb of intron 12 shows that a TATA box is present 40 bp upstream of the UT-A2 transcription start site in exon 13 and a cAMP response element (CRE) is present 300 bp upstream (Bagnasco et al., 2000; Nakayama et al., 2001).

The mouse and rat UT-A genes have a similar organization (Nakayama et al., 2001; Fenton et al., 2002b, 2002c). The mouse gene also is very long (over 300 kb), contains 24 exons, has two promoter elements, and promoter I contains a TonE element and its activity is increased by hypertonicity (Fenton et al., 2002b). Although neither the mouse nor rat promoter I contains a consensus CRE element, UT-A promoter I activity is increased by cAMP in mouse but not in rat (Nakayama et al., 2001; Fenton et al., 2002b). All mouse UT-A isoforms, including the testis isoform UT-A5, originate from a single mouse UT-A gene (Fenton et al., 2002b). However, the transcription start site for UT-A5 has not been determined and could be located downstream from the start site for mouse UT-A1 and UT-A3 (Fenton et al., 2002b).

The human UT-A gene is significantly shorter than rat or mouse (approximately 67.5 kb), contains 20 exons, and is located on chromosome 18 (Bagnasco et al., 2001). The difference in length between the human and rodent genes results from: (1) the 5′-untranslated region is almost entirely located in exon 1 in human, while in rodents, it spans the first three widely spaced exons; and (2) the 3′-untranslated region in human does not contain an exon analogous to rodent exon 24 (Bagnasco et al., 2001; Nakayama et al., 2001; Fenton et al., 2002b). Interestingly, single nucleotide polymorphisms in human UT-A2 are associated with variation in blood pressure in men, but not in women (Ranade et al., 2001).

Facilitated urea transporter cDNAs that are most homologous to UT-A2 have been cloned from frog, elasmobranch, gulf toadfish, Lake Migadi tilapia, eel, and pilot whale (Couriaud et al., 1999; Janech et al., 2002; Mistry et al., 2001; Smith & Wright, 1999; Walsh et al., 2000, 2001a; 2001b). A detailed discussion of these urea transporters is beyond the scope of this review and the reader is referred to the original citations for more information.

Rapid Regulation of UT-A

The primary method for investigating the rapid regulation of urea transport has been perfusion of rat IMCDs. This method provides physiologically relevant, functional data, although it cannot determine which urea transporter isoform is responsible for a specific functional effect since both UT-A1 and UT-A3 are expressed in terminal IMCDs (Fig. 3). Thus, the functional effects reviewed below may be due to transport mediated by UT-A1, UT-A3, or both. In the past few years, new findings have been published for 3 regulators of urea transport: vasopressin, angiotensin II, and hyperosmolality. The reader is referred to older reviews (Sands et al., 1997; Sands, 1999a, 2000), which discuss other factors that regulate urea transport.

Vasopressin

Adding arginine vasopressin (also known as antidiuretic hormone, ADH) to the bath of a perfused rat terminal IMCD results in binding to V_2-receptors, stimulation of adenylyl cyclase, generation of cAMP, and ultimately increase in facilitated urea permeability (Sands et al., 1987; Star et al., 1988; Sands & Schrader, 1991; Nielsen & Knepper, 1993). One mechanism for rapid regulation is vasopressin's effect of altering the phosphorylation of UT-A1 and/or UT-A3. The deduced amino-acid sequences for both UT-A1 and UT-A3 contain several consensus sites for phosphorylation (Fig. 2) by protein kinase A (PKA), as well as PKC and tyrosine kinase (Karakashian et al., 1999). Vasopressin does increase the phosphorylation of both the 117 and 97 kDa UT-A1 proteins within 2 minutes in rat IMCD suspensions, consistent with the time course (and dose response) for vasopressin-stimulated urea transport in perfused rat terminal IMCDs (Star et al., 1988; Wall et al., 1992; Nielsen & Knepper, 1993; Zhang, Sands & Klein, 2002). Both dDAVP and cAMP also increase UT-A1 phosphorylation, and PKA inhibitors block the phosphorylation of UT-A1 by vasopressin (Zhang et al., 2002). These findings strongly suggest that vasopressin rapidly increases urea transport in the rat terminal IMCD by increasing UT-A1 phosphorylation. At present, it is not known whether vasopressin also alters UT-A3 phosphorylation.

Another mechanism by which vasopressin could rapidly increase urea transport is regulation of trafficking of UT-A1 and/or UT-A3. However, regulated trafficking of UT-A1 by vasopressin does not occur in the rat IMCD (Inoue et al., 1999). At present, it is not known whether UT-A3 undergoes regulated trafficking in response to vasopressin.

Angiotensin II

Angiotensin II has no effect on basal (no vasopressin) facilitated urea permeability, but increases vasopressin-stimulated urea permeability in rat terminal IMCDs and ^{32}P incorporation into both the 117 and 97 kDa UT-A1 proteins via a PKC-mediated effect (Kato et al., 2000). Mice that lack tissue angiotensin-

converting enzyme (ACE.2 mice), and hence lack angiotensin II, have a urine-concentrating defect despite a histologically normal medulla (Esther, Jr. et al., 1997). UT-A1 protein is decreased to 25% of the level in wild-type mice in the inner medulla of ACE.2 mice (Klein et al., 2002b). Neither the reduction in UT-A1 protein nor the urine-concentrating defect is corrected by administering angiotensin to ACE.2 mice for 2 weeks (Klein et al., 2002b). Thus, by augmenting the maximal urea permeability response to vasopressin, angiotensin II may play a physiologic role in the urinary concentrating mechanism.

Hyperosmolality

Increasing osmolality by adding mannitol or NaCl, but not urea, increases facilitated urea permeability in the perfused rat terminal IMCD, independently of vasopressin (Sands & Schrader, 1991; Gillin & Sands, 1992; Kudo et al., 1992). When osmolality is increased for 20 minutes in the presence of vasopressin, there is an additive effect to increase urea permeability (Chou et al., 1990; Sands & Schrader, 1991; Gillin & Sands, 1992; Kudo et al., 1992). Both hyperosmolality- and vasopressin-stimulated urea permeability are inhibited by the urea transport inhibitors phloretin and thiourea (Chou & Knepper, 1989; Gillin & Sands, 1992). However, increases in intracellular calcium and activation of PKC mediate hyperosmolality-stimulated urea permeability (Gillin et al., 1993; Kato et al., 2000), while increases in cAMP and activation of PKA mediate vasopressin-stimulated urea permeability (Star et al., 1988). Thus, hyperosmolality and vasopressin rapidly increase urea permeability through different second messenger pathways.

Long-term Regulation of UT-A in Kidney

Vasopressin

Surprisingly, the abundance of both the 117- and 97-kDa UT-A1 proteins in rat inner medulla and basal facilitated urea permeability in perfused terminal IMCDs are decreased when vasopressin is increased, regardless of whether this occurs by administering exogenous vasopressin or by water restriction (Kato et al., 1998; Terris et al, 1998). The decreases in UT-Al protein abundance and basal urea permeability probably do not result from a decrease in UT-A1 or UT-A1b mRNA abundance, since northern analysis shows no change in either mRNA abundance in response to either water loading or restriction in most studies (Smith et al., 1995; Promeneur et al., 1996, 1998; Shayakul et al., 1997; Bagnasco et al., 2000; Fenton et al., 2002b). However, one study does report that UT-A1 is decreased in water-restricted or vasopressin-treated Brattleboro rats (Shayakul et al., 2000).

Surprisingly, feeding rats a high (40%) or low (10%) protein diet for 1 week, compared to a control diet (20% protein), also has no effect on UT-A1 mRNA abundance in any portion of the kidney medulla (Ashkar et al., 1995; Hu et al., 1999). In contrast, UT-A1 mRNA is decreased in the inner medullary tip of low-protein fed Brattleboro rats (Hu et al., 1999), suggesting that there may be an interaction between vasopressin and dietary protein to affect UT-A1 mRNA abundance. Overall, transcriptional regulation does not appear to be the mechanism controlling changes in UT-A1 protein abundance in response to changes in hydration, vasopressin level, or dietary protein.

In contrast, UT-A2, UT-A2b, UT-A3, and UT-A3b mRNA abundances fall in the inner medulla of water-loaded rats and rise in rats and mice with increased vasopressin levels, regardless of whether this results from vasopressin administration or water restriction (Knox et al., 1980; Smith et al., 1995; Promeneur et al., 1996, 1998; Bagnasco et al., 2000; Shayakul et al., 2000; Fenton et al., 2002b). UT-A2 mRNA abundance is increased in the inner medullary base of rats fed a low (8%) protein diet for 1 week (Ashkar et al., 1995), but not in normal or Brattleboro rats fed a 10% protein diet (Hu et al., 1999). UT-A2 protein abundance is decreased by treating rats with urosemide (Leroy et al., 2000) and increased by administering dDAVP to Brattleboro rats (Wade et al., 2000). Thus, vasopressin may regulate UT-A2 by a transcriptional mechanism, consistent with the presence of a CRE element in promoter II and cAMP increasing promoter II's activity (Nakayama et al., 2000). UT-A2 expression can also be induced in mIMCD3 cells grown in hypertonic culture media in which osmolality is increased by adding equiosmolar NaCl and urea; mIMCD cells grown in isotonic culture media do not express any UT-A (Leroy et al., 2000; Nakayama et al., 2001).

Water deprivation also increases UT-A3 protein abundance (Terris et al., 2001). This effect could be transcriptionally mediated since promoter I contains a TonE element (Nakayama et al., 2000). The long-term regulation of UT-A4 or UT-A5 has not been studied to date. Thus, there may be multiple mechanisms by which vasopressin regulates the different UT-A protein and mRNA isoforms.

UT-A Proteins during Development

UT-A protein is not detected in the fetal kidney but appears in 1-day old rats, both in the IMCD (UT-A1) and the thin descending limb (UT-A2) (Kim et al., 2002). UT-A immunostaining increases progressively in both segments until adult levels are achieved at 21 days of age (Kim et al., 2002). Thus, the time course for the increase in UT-A1 and UT-A2 immuno-

staining coincides with the development of urine-concentrating ability in rats.

UT-A1 and Impaired Urine-Concentrating Ability

The long-term regulation of UT-A1 protein abundance has been studied in 6 animal models associated with reduced urine-concentrating ability: water diuresis; low-protein diet; hypercalcemia; furosemide diuresis; adrenalectomy; and lithium administration (Isozaki et al., 1993, 1994a; Ashkar et al., 1995; Klein et al., 1997, 2002a; Naruse et al., 1997; Kato et al., 1998; Sands et al., 1998; Terris et al., 1998; Kato & Sands, 1999). Surprisingly, in each of these animal models (except for lithium administration, which is discussed in more detail below), both UT-A1 protein abundance and basal facilitated urea permeability are increased in the deepest portion of the IMCD. The increase in urea permeability and UT-A1 protein abundance could be a mechanism for the rapid increase in urine concentrating ability that occurs within 5–10 minutes after urea is infused into malnourished or low-protein fed people and animals (Gamble et al., 1934; Levinsky & Berliner, 1959; Pennell et al., 1975; Wilson & Sonnenberg, 1982): 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.

Glucocorticoids

Adrenalectomy causes a urinary concentrating defect in man and animals (Schwartz & Kokko, 1980; Jackson et al., 1983; Kamoi et al., 1993). Administering dexamethasone to adrenalectomized rats decreases facilitated urea permeability in the rat terminal IMCD and UT-A1 protein abundance in the inner medulla (Naruse et al., 1997). Administering dexamethasone to normal rats decreases UT-A1, UT-A3, and UT-A3b mRNA abundances, but not UT-A2 mRNA abundance, in the inner medullary tip (Peng et al., 2002). As discussed above, this effect is likely to be transcriptionally regulated since dexamethasone decreases the activity of promoter I, which controls transcription of UT-A1 and UT-A3, but has no effect on promoter II, which controls transcription of UT-A2 (Peng et al., 2002).

Volume Expansion

People and animals become volume-expanded when given aldosterone and a high NaCl diet, but do not become volume-expanded when given aldosterone and a NaCl-free diet (Wang et al., 2002). Volume expansion reduces UT-A1 and UT-A3 protein abundances in the inner medulla, while UT-A2 protein abundance is unchanged (Wang et al., 2002). Following volume expansion, the time course for the decrease in UT-A1 protein parallels the decrease in serum urea concentration, while the decrease in UT-A3 is delayed (Wang et al., 2002). Pharmacologic inhibition of AT_1-receptors (given for 2 days) also decreases UT-A1 and UT-A3 protein abundances, suggesting that the suppression of the renin-angiotensin system that accompanies aldosterone-induced volume expansion may mediate the decrease in the abundance of UT-A1 and UT-A3 (Wang et al., 2002).

Lithium

Lithium is a widely used therapeutic agent for treatment of patients suffering from manic-depressive (bipolar) disorders. However, lithium can cause nephrogenic diabetes insipidus and an inability to concentrate urine (reviewed in Timmer & Sands, 1999). While the mechanisms by which lithium causes nephrogenic diabetes insipidus are not entirely understood, lithium-treated rats do have a marked reduction in inner medullary interstitial osmolality (Christensen et al., 1985) and AQP2 protein (Klein et al., 2002a; Okusa & Crystal, 1994; Marples et al., 1995).

Rats fed lithium for 10–25 days have a marked reduction in UT-A1 protein abundance in both the inner medullary tip and base (Klein et al., 2002a). In contrast to vasopressin's effect on IMCD suspensions from normal rats, vasopressin does not increase UT-A1 phosphorylation in IMCD suspensions from lithium-fed rats (Klein et al., 2002a). Thus, lithium administration differs from the other animal models associated with reduced urine-concentrating ability, which were discussed above, since it reduces UT-A1 protein abundance. At present, the reason for this difference is unknown.

UT-A in Rat Models of Human Diseases

Diabetes mellitus

In rats, uncontrolled diabetes mellitus (induced by streptozotocin) increases urea excretion and corticosterone production (Mitch et al., 1999). UT-A1 protein abundance is decreased in the inner medullary tip of rats at 3 days after streptozotocin injection, but does not decrease in adrenalectomized rats injected with streptozotocin (Klein et al., 1997). These findings suggest that the diabetes-induced increase in glucocorticoids is the mechanism for reducing UT-A1 protein abundance in rats with uncontrolled diabetes for 3 days (Klein et al., 1997).

After 21 days of uncontrolled diabetes, UT-A1 mRNA and protein are increased in the inner medulla (Bardoux et al., 2001). However, UT-A1 protein is decreased in 6-month old, obese Zucker rats, a

model of type II diabetes (Bickel et al., 2002). Thus, UT-A1 abundance may vary with the duration of diabetes and/or with the type of diabetes.

Renal Failure

Cisplatin administration to rats for 5 days results in acute renal failure, accompanied by an increase in urine volume and a decrease in urine osmolality, but no change in UT-A1, UT-A2, or UT-A4 protein abundances in the inner or outer medulla (Ecelbarger et al., 2001). Adriamycin administration to rats for 3 weeks results in proteinuria and decreased UT-A1 protein abundance in the inner medulla (Fernández-Llama et al., 1998). Inducing uremia by 5/6 nephrectomy in rats results in an increase in urine output and a decrease in urine osmolality at 5 weeks post-nephrectomy, accompanied by undetectable levels of UT-A1 mRNA and protein, and reduced levels of UT-A2 mRNA and protein (Hu et al., 2000).

Liver in Uremic Rats

The liver has phloretin-inhibitable urea transport, suggesting that liver expresses a urea transporter, possibly to accelerate urea efflux following ureagenesis (Effros et al., 1993; Hasegawa & Verkman, 1993; Walsh et al., 1994; Von Dahl & Haussinger, 1997). HepG2 cells, a cultured human hepatoblastoma cell line, has a high rate of urea influx that is inhibited by two urea transport inhibitors: thionicotinamide and phloretin (Klein et al., 1999). Western blot of HepG2 or rat liver proteins reveals two bands: a 49 kDa UT-A protein in the plasma membrane and a 36 kDa UT-A protein in the cytoplasm (Klein et al., 1999). Northern blot of rat liver reveals a 2.6 kb UT-A mRNA (Doran, Timmer & Sands, 1999), consistent with the size of either UT-A2b or UT-A4 (Table 1). DNA sequencing of this mRNA will be required to identify the liver isoform.

The abundance of the 49-kDa liver UT-A protein varies with uremia and/or acidosis in rats: it increases in livers from uremic rats but not in uremic rats given bicarbonate to correct their acidosis (Klein et al., 1999, 2002c). The abundance of this 49-kDa UT-A protein also increases in liver from non-uremic rats fed HCl to make them acidotic (Klein et al., 2002c). HCl-feeding also increases the abundance of the 117 kDa UT-A1 protein in kidney inner medulla (Klein et al., 2002c). Thus, acidosis, either directly or via a change in ammonium concentration, increases the abundance of the 49-kDa UT-A protein in liver and the 117-kDa UT-A1 protein in kidney (Klein et al., 2002c).

Testis in Uremic Rats

Phloretin-inhibitable urea transport is present in seminiferous tubules (Fenton et al., 2002a). Testis expresses UT-A5 (1.7 kb) and 3 other UT-A mRNA transcripts (approximately 4.0, 3.3, and 2.8 kb), some of which have not been detected in other rodent tissues (Doran et al., 1999; Karakashian et al., 1999; Fenton et al., 2000, 2002a). Seminiferous tubules also express UT-B protein and mRNA (Couriaud et al., 1996; Tsukaguchi et al., 1997; Hu et al., 2000; Timmer et al., 2001; Fenton et al., 2002a). UT-B mRNA abundance is not changed by uremia in testis (Hu et al., 2000).

Brain in Uremic Rats

Brain expresses both UT-A and UT-B mRNA and protein (Couriaud et al., 1996; Tsukaguchi et al., 1997; Berger et al., 1998; Doran et al., 1999; Klein et al., 1999; Hu et al., 2000; Timmer et al., 2001). UT-B mRNA is unchanged after 1 week of uremia, but is reduced to about 30% of control levels after 5 weeks (Hu et al., 2000).

Heart in Uremic Rats

Surprisingly, the heart expresses a urea transporter (Duchesne et al., 2001). Although the rationale is not as obvious as for red blood cells, kidney, or liver, cardiac hypertrophy is associated with an increase in polyamine synthesis, and since urea is a byproduct of the production of ornithine from arginine, it is possible that the heart needs a urea transporter to dispose of any urea that is produced intracellularly (Duchesne et al., 2001). Western blot shows that rat heart expresses 3 UT-A proteins: 56, 51, and 39 kDa, and that the abundance of the 56 kDa UT-A glycoprotein is increased by uremia (Duchesne et al., 2001). The abundance of the 56 kDa UT-A protein is also increased in hypertrophic hearts from non-uremic DOCA/salt hypertensive rats, and in short-term hypertension induced by a 3-day infusion of angiotensin II, before cardiac hypertrophy is apparent (Duchesne et al., 2001). Rat heart expresses only a single 2.7-kb UT-A mRNA transcript (Karakashian et al., 1999; Duchesne et al., 2001) and DNA sequencing shows that this is UT-A2b (Duchesne et al., 2001).

Human heart expresses the three UT-A proteins expressed in rat (56, 51, and 39 kDa), but also expresses a 97 kDa UT-A protein (Duchesne et al., 2001). Terminally-failing (NYHA class IV) human hearts have a significant increase in the abundance of the 56- and 51-kDa UT-A proteins (Duchesne et al, 2001). Thus, UT-A proteins are expressed in both rat and human heart and their abundance increases in conditions that predispose to left ventricular hypertrophy, such as uremia or hypertension.

Active Urea Transport in Kidney

In addition to the facilitated urea transporters UT-A and UT-B, functional evidence exists for active urea

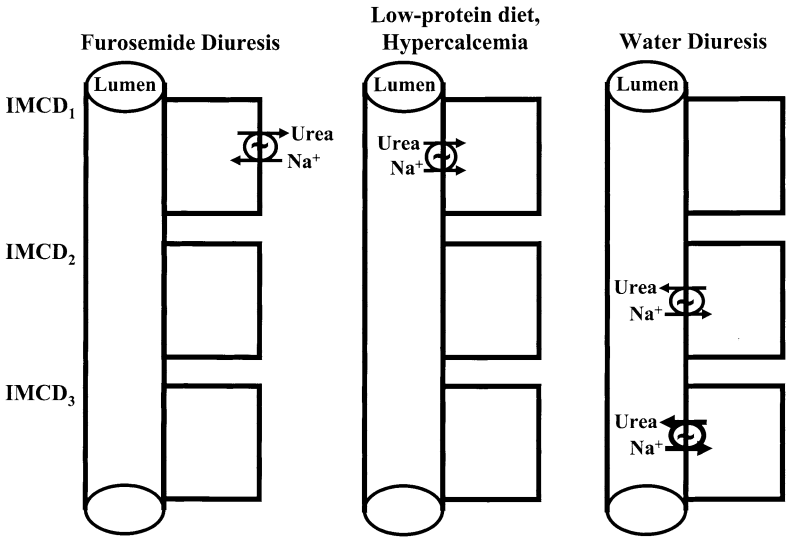

Fig. 4. Diagram showing location of secondary active urea transporters in four conditions associated with a urine concentrating defect.

transport mechanisms in the rat kidney collecting duct. Unfortunately, the active urea transporters have not been cloned to date, precluding an understanding of these transporters at the molecular level. The following sections will review the functional properties of the active urea transport processes in kidney and their long-term regulation.

Active Urea Absorption

Although active urea absorption does not occur in collecting ducts from ad libitum fed rats, it can be induced in the $IMCD_1$ from rats fed a low-protein diet or made hypercalcemic. Active urea absorption is completely inhibited by: (1) adding ouabain to the bath; or (2) removing sodium from the perfusate (and replacing it with N-methyl-D-glucamine) but not from the bath (Isozaki et al., 1993, 1994b; Kato & Sands, 1999). These functional properties suggest that this is an absorptive "sodium-urea cotransporter" located in the apical membrane of the $IMCD_1$ (Fig. 4). There is no active urea absorption in the terminal IMCD from low-protein fed or hypercalcemic rats (Isozaki et al., 1993; Kato & Sands, 1999).

Active urea absorption can also be induced in the $IMCD_1$ from furosemide-treated rats (Kato & Sands, 1998a). In contrast to the active urea absorption in the $IMCD_1$ induced by low-protein feeding or hypercalcemia (Isozaki et al., 1993, 1994b; Kato & Sands, 1999), this one is: (1) inhibited by phloretin or ouabain; (2) rapidly stimulated by vasopressin; and (3) inhibited by removing sodium (and replacing it with N-methyl-D-glucamine) from the bath but not from the perfusate (Kato & Sands, 1998a). These functional properties suggest that this is an absorptive "sodium-urea counter-transporter" located in the basolateral membrane of the $IMCD_1$. There is no active urea absorption in the terminal IMCD from furosemide-treated rats (Kato & Sands, 1998a) or in the IMCD from water diuretic rats (Kato & Sands, 1998b).

Active Urea Secretion

Although active urea secretion does not occur in the $IMCD_1$ (or initial IMCD) or $IMCD_2$ from ad libitum fed rats, active urea secretion is present (Fig. 3) in the $IMCD_3$ (Kato & Sands, 1998b). This active urea secretion is: [1] rapidly stimulated by vasopressin; [2] inhibited by phloretin or ouabain; and [3] inhibited by removing sodium (and replacing it with N-methyl-D-glucamine) from the tubule lumen but not from the bath (Kato & Sands, 1998b). These functional properties suggest that this is a secretory "sodium-urea counter-transporter" located in the apical membrane of the $IMCD_3$ (Kato & Sands, 1998b). A similar active urea transport mechanism is present in the basolateral membrane of the elasmobranch gill (Fines, Ballantyne & Wright, 2001).

In the rat $IMCD_3$, active urea secretion is completely inhibited by feeding rats a low (8%) protein diet, inducing hypercalcemia, and by treating rats with furosemide (Isozaki et al., 1993; Kato & Sands, 1998a, 1999). In contrast, water diuresis results in a significant increase in active urea secretion in the $IMCD_3$ and inducing it in the $IMCD_2$ (Kato & Sands, 1998b, 1999). Interestingly, active urea secretion in the apical membrane of the terminal IMCD is functionally similar to the active urea absorption in the basolateral membrane of the initial IMCD that is induced in furosemide-treated rats, except that it 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 different sodium-urea counter-transporters, or a sin-

gle transporter that can be sorted to different cell membranes, depending upon the IMCD subsegment and the in vivo condition of the rat.

Active Urea Transport and Urine-Concentrating Ability

Active urea transport changes in one of two patterns when urine-concentrating ability is reduced (Fig. 4): (1) water diuresis results in an increase in active urea secretion in the $IMCD_3$ and its induction in the $IMCD_2$, without any active urea absorption in the $IMCD_1$; and (2) a low-protein diet, hypercalcemia, and furosemide result in induction of active urea absorption in the $IMCD_1$, albeit by different mechanisms, and inhibition of active urea secretion in the $IMCD_3$ (Isozaki et al., 1993, 1994a, 1994b; Kato & Sands, 1998a, 1998b, 1999). In the first response pattern, the increase in active urea secretion in the terminal IMCD subsegments (the $IMCD_2$ and the $IMCD_3$) will directly decrease urea content in the deep inner medulla. In the second response pattern, the induction of active urea absorption in the initial IMCD contributes to the urine-concentrating defect by increasing urea delivery to the inner medullary base, thereby decreasing distal urea delivery to the inner medullary tip; the accompanying inhibition of active urea secretion in the $IMCD_3$ may prevent an even greater reduction in urea content in the deep inner medulla.

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