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REVIEW

The emerging physiological roles of the *SLC14A* family of urea transporters

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In mammals, urea is the main nitrogenous breakdown product of protein catabolism and is produced in the liver. In certain tissues, the movement of urea across cell membranes is specifically mediated by a group of proteins known as the *SLC14A* family of facilitative urea transporters. These proteins are derived from two distinct genes, UT-A (*SLC14A2*) and UT-B (*SLC14A1*). Facilitative urea transporters play an important role in two major physiological processes – urinary concentration and urea nitrogen salvaging. Although UT-A and UT-B transporters both have a similar basic structure and mediate the transport of urea in a facilitative manner, there are a number of significant differences between them. UT-A transporters are mainly found in the kidney, are highly specific for urea, have relatively lower transport rates and are highly regulated at both gene expression and cellular localization levels. In contrast, UT-B transporters are more widespread in their tissue location, transport both urea and water, have a relatively high transport rate, are inhibited by mercurial compounds and currently appear to be less acutely regulated. This review details the fundamental research that has so far been performed to investigate the function and physiological significance of these two types of urea transporters.

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Abbreviations
AQP, aquaporin; IMCD, inner medullary collecting duct; KO, knockout; MDM2, murine double minute; MeACPTU, 1-(3-azido-4-chlorophenyl)-3 methyl-2-thiourea; pCMBS, p-chloromercuribenzenesulphonate; PKA, protein kinase A; SGLT1, sodium-dependent glucose transporter; tDL, thin descending limbs; TMD, transmembrane spanning domain; UNS, urea nitrogen salvaging; UT, urea transporter

Overview

Urea is the main breakdown product of protein catabolism in mammals. It is produced in the liver via the ornithine-urea cycle and has classically been viewed simply as a toxic nitrogenous waste product, emitted from the body in both urine and faeces. Urea (H_2NCONH_2) is a water soluble molecule and was originally thought to simply pass slowly across cell membranes via passive diffusion. However, the cloning of the specific proteins responsible for the enhanced *trans*-epithelial urea transport that is present in certain tissues has revolutionized our understanding of the physiological importance of urea.

Urea transporters (UTs) allow the rapid movement of urea molecules across cell membranes. Although this can occur in either direction through the transporters, net urea movement can only occur down a concentration gradient. This equilibrative movement of urea is also independent of ions such as sodium and chloride (You *et al.*, 1993). These proteins are generally referred to as 'facilitative urea transporters', and are distinct from the undefined class of proteins responsible for the 'active' uptake of urea against concentration gradients.

Urea transporters are derived from two separate genes: *SLC14A1* (UT-B) and *SLC14A2* (UT-A). These UT-A and UT-B genes are on the same chromosome (18q12.1-q21.1), suggesting gene duplication from a common ancestor (Olives *et al.*, 1996). The Kidd (Jk) blood group, previously known to be located on this chromosome, does in fact represent the human UT-B urea transporter (Olives *et al.*, 1995).The human UT-A gene consists of 26 exons covering 476 kb, with

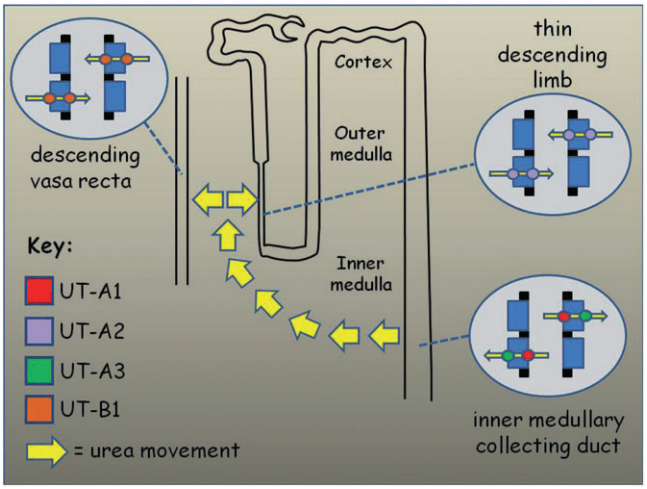

Figure 1

Schematic model of the kidney nephron showing role of *Slc14a* transporters in the renal urinary concentrating mechanism. Blood urea is freely filtered at the glomerulus and ~40% is constitutively reabsorbed in the proximal tubule. The remaining urea can be reabsorbed across the epithelial cells of the inner medullary collecting duct, mainly via apical UT-A1 transporters and basolateral UT-A3 transporters that are both regulated by vasopressin. This has the effect of increasing urea concentration in the kidney medulla and hence preventing the excessive water loss that would otherwise occur due to the osmotic effect of the urinary urea. In order to maintain this high medullary concentration, it is necessary for the urea to be recycled and the concentration gradient not to be dissipated. This is achieved by the facilitated movement of urea across the apical and basolateral of both the thin descending limbs (via UT-A2 transporters) and the descending vasa recta blood vessels (via UT-B1 transporters).

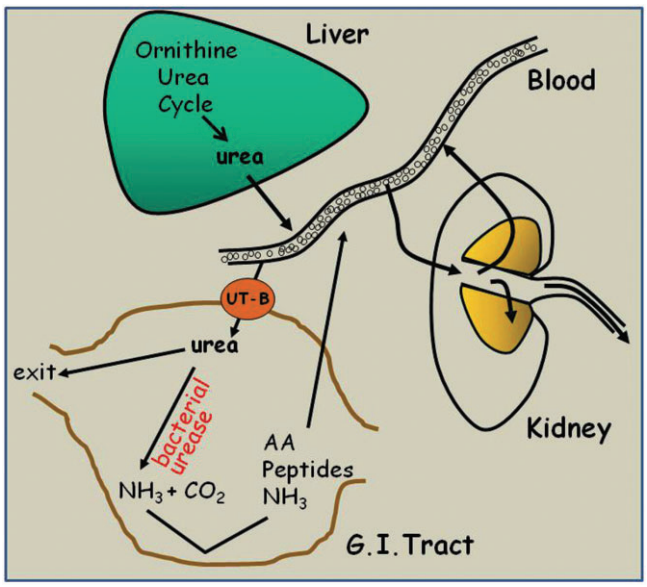

Figure 2

Model of the urea nitrogen salvaging process. Urea is produced in the liver via the ornithine-urea cycle and passed into the blood. Urea can then pass (a) into the kidney, where it is freely filtered and either reabsorbed or excreted, or (b) via UT-B urea transporters into specific regions of the gastrointestinal tract that contain large bacterial populations (e.g. rumen, caecum, colon). Within these regions urea is broken down by the bacterial enzyme urease into ammonia and carbon dioxide. The ammonia can either be reabsorbed directly back into the blood or be utilized as a nitrogen source by the bacteria to produce amino acids and peptides, which themselves can then be reabsorbed. The return of the nitrogen to the mammalian host in these different forms represents the 'salvaging' of the original urea nitrogen.

similarly large genes being present in mouse and rat (Smith and Fenton, 2006). In contrast, the human UT-B gene is smaller, consisting of 11 exons spread over 30 kb (Lucien *et al.*, 1998). Again, the UT-B gene is of a similar size in both mouse and rat (Yang and Bankir, 2005). [Note: a third urea transporter gene, UT-C, has so far only been identified in two species of fish (Mistry *et al.*, 2005).]

The most studied physiological role for urea transporters is in the urinary concentration mechanism. Renal UT-A transporters within the kidney nephron help facilitate the reabsorption and recycling of urinary urea, hence increasing medullary urea concentration – see Figure 1. This prevents the problematic osmotic effect that urinary urea would otherwise have and hence prevents excess water loss in the urine (Fenton *et al.*, 2004). During reasonable levels of dietary nitrogen intake, urea transporters are therefore vital in the production of concentrated urine and hence in maintaining body fluid balance. Detailed discussion of the precise renal function of urea transporters will not be presented here, as this topic has been the subject of an excellent recent review (see Fenton, 2009).

A second important physiological role for urea transporters is now emerging in respect to its role in the process of urea nitrogen salvaging (UNS) in the mammalian intestinal tract. This process supplies intestinal bacteria with a source of nitrogen that they utilize for their growth and is hence vital in maintaining the symbiotic relationship between mammals and their bacterial populations, particularly in ruminant species (for detailed review – see Stewart and Smith, 2005). The crucial first step in UNS is the movement of urea from the blood into the intestinal tract, via UT-B urea transporters in the epithelial layers – see Figure 2. UT-B proteins have now been identified in various intestinal tissues and species, such as bovine rumen (Stewart *et al.*, 2005), rat caecum (Collins *et al.*, 2011) and, most interestingly, human colon (Inoue *et al.*, 2004; Collins *et al.*, 2010). Urea transporters may therefore indirectly play a significant role in both nutritional balance and intestinal health.

Isoforms

Both the *Slc14a*1 and *Slc14a2* genes produce multiple isoforms, via the process of alternative splicing (for review of genomic organization – see Smith and Fenton, 2006). There are six known *Slc14a2* (UT-A) transporters and two *Slc14a1* (UT-B) transporters. Figure 3 shows a schematic representation of these eight urea transporter proteins. Some of these isoforms have yet to be fully characterized in more than one

species at present. For example, cDNA sequences for UT-B2 have been reported in human caudate nucleus (GenBank Acc. No. AK091064) and mouse thymus (GenBank Acc. No. AK153891), but the proteins have yet to be investigated. Evidence is also emerging of the existence of further novel isoforms, particularly for UT-B transporters. For example, a cDNA clone from human thalamus appears to encode a novel 281-amino acid UT-B protein (GenBank Acc. No. AK127452) that has a truncated N-terminus compared with the UT-B1 transporter. Because the current nomenclature for *Slc14a* urea transporters was not originally utilized, the previous aliases used in the literature are listed in Table 1. This table also details the small variations in amino acid length between species that occurs for certain transporters and includes a basic guide to tissue distribution (for further details see distribution section below).

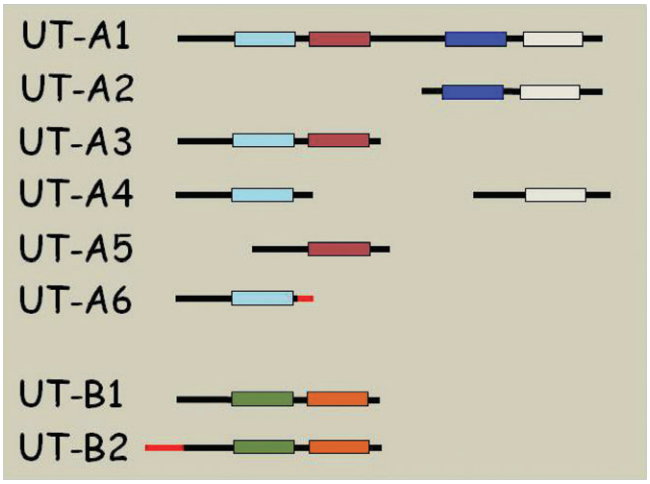

Figure 3

Schematic representation of the different isoforms of UT-A and UT-B urea transporters. The different boxes represent regions of hydrophobic amino acids. The black lines show coding sequences which are common, while the red lines show coding sequences that are unique to that particular isoform (i.e. derived from novel exons) (adapted from Smith, 2009).

Biochemistry and genetics

The proposed basic structure for both UT-A and UT-B urea transporters consists of 10 transmembrane spanning domains (TMDs), a large extracellular loop containing an N-glycosylation site, plus intracellular amino and carboxy terminals (Olives *et al.*, 1994) – see Figure 4. The main exception to this 10 TMDs structure is UT-A1, which is proposed to have 20 TMDs, and is basically UT-A2 and UT-A3 combined by a 73-amino acid central linking loop. This central loop region of UT-A1 contains serine 486, which is responsible for protein kinase A (PKA) activation of this isoform (Mistry *et al.*, 2010). A recent paper also reports that the N-terminal 81-amino acids in rat UT-A1 are required for transport activity (Huang *et al.*, 2010a), although further investigation is still required to fully understand its precise role. Interestingly, bacterial homologues of kidney urea transporters have recently been used to show that urea transporters function in the plasma membrane as multimers, for example, as dimers in *Actinobacillus pleuropnemoniae* (Raunser *et al.*, 2009) or as trimers in *Desulfovibrio vulgaris* (Levin *et al.*, 2009).

Table 1

The nomenclature of all the currently identified members of the *SLC14A* urea transporter family

Isoform	Aliases	Amino acid length	Tissue distribution
UT-A1	UT1 (Shayakul *et al.*, 1996) UrTI-A (Shayakul *et al.*, 2001)	920 (human) 929 (rat) 930 (mouse)	Kidney (collecting duct), Cochlea
UT-A2	UT2 (Smith *et al.*, 1995) UrT1-B (Shayakul *et al.*, 2001)	397 (human, mouse, rat, rabbit)	Kidney (thin descending limbs), heart, colon
UT-A3	UrT1-C (Shayakul *et al.*, 2001)	451 (human) 460 (rat) 461 (mouse)	Kidney (collecting duct)
UT-A4		466 (rat)	Kidney (unknown)
UT-A5		322 (mouse)	Testis
UT-A6		235 (human)	Colon
UT-B1	UT11 (Olives *et al.*, 1994) UT3 (Shayakul *et al.*, 1997) UrT2 (Shayakul *et al.*, 2001)	384 (cow, mouse, rat) 389 (human)	Red blood cells, kidney, colon, small intestine, caecum, heart, brain, liver, spleen, bone marrow, testis, liver, lung, rumen, salivary gland, cochlea, thymus, urinary tract
UT-B2		439 (cow)	Rumen
		440 (mouse)	Thymus
		445 (human)	Caudate nucleus

Alternative splicing is known to produce at least six different UT-A isoforms and two different UT-B isoforms, some of which have other aliases. The precise amino acid length of some of the transporters varies between species, while their tissue distribution is also variable.

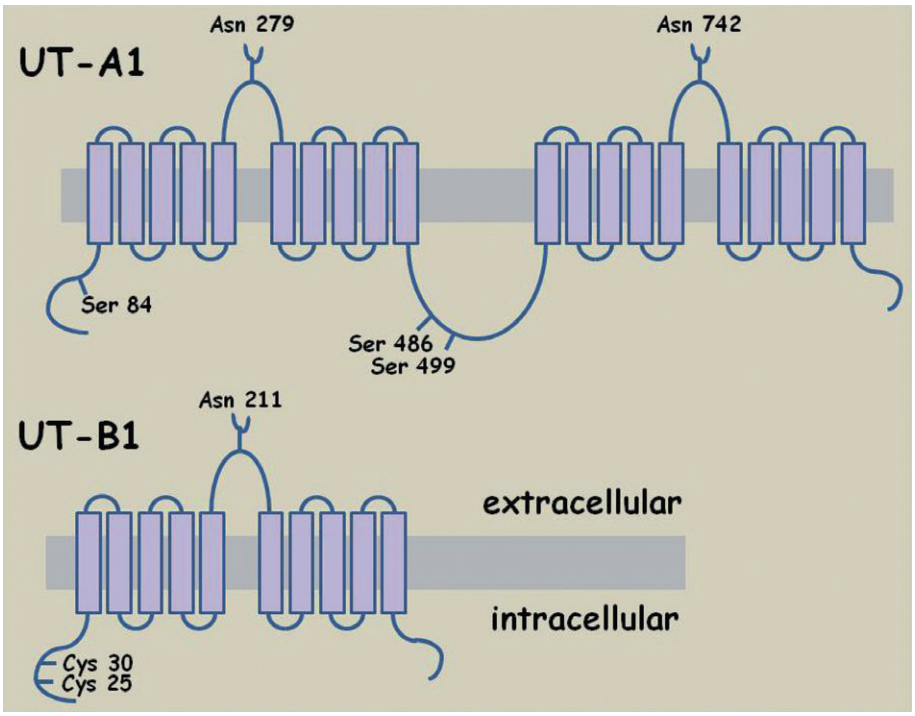

Figure 4

Schematic diagram representing topology of two *Slc14a* facilitative urea transporters: UT-A1 and UT-B1. Various important residues are highlighted in each transporter: the asparagine (Asn) residues known to be important in glycosylation; the serine residues (Ser) known to be involved in the phosphorylation events that regulate transporter function; the cysteine (Cys) residues important in targeting the protein to the plasma membrane.

As mentioned, urea transporters are N-linked glycosylated proteins that have a unique pattern of hydrophobicity, as shown originally for rabbit UT-A2 (You *et al.*, 1993). Rat UT-A1 has two glycosylated versions (97 & 117 kDa) that are both deglycosylated to an 88 kDa core 929-amino acid protein (Bradford *et al.*, 2001). We now know that glycosylation of UT-A1 at two sites (Asn 279 and Asn 742) is required in order for correct trafficking of the protein to the plasma membrane to occur in response to vasopressin (Chen *et al.*, 2006). The other main UT-A isoforms are also known to have N-linked glycosylations, such as the 55 kDa glycosylated rat UT-A2 protein (Wade *et al.*, 2000). The exact nature of the glycosylations can vary between species however. For example, rat UT-A3 has 44 & 67 kDa glycosylated forms that are deglycosylated down to 40 kDa (Terris *et al.*, 2001), while mouse glycosylated UT-A3 is a 45–65 kDa continuous smear that again shifts to a 40 kDa core protein (Stewart *et al.*, 2004).

The vital role of the human UT-B1 N-terminal in protein targeting to the plasma membrane has been clearly shown (Lucien *et al.*, 2002). Double mutation of the cysteine residues Cys 25 and Cys 30 prevented successful localization of hUT-B1 to the plasma membrane (Lucien *et al.*, 2002). UT-B urea transporters are also known to have N-linked glycosylations. Human UT-B1 gives a 46–60 kDa glycosylated protein that deglycosylates to 36 kDa in red blood cells (Olives *et al.*, 1995), while UT-B1 in human kidney gives a 41–54 kDa signal (Timmer *et al.*, 2001). This tissue-specific variation in the extent of glycosylation is characteristic of urea transporter proteins and occurs in other species, for example, rat glycosylated UT-B1 protein is 32 kDa in brain but 45–55 kDa in kidney (Trinh-Trang-Tan *et al.*, 2002). The glycosylation of human UT-B1 all occurs at the Asn 211 N-linked glycosylation site in the extracellular loop (Sidoux-Walter *et al.*, 2000). Interestingly, evidence from oocyte expression studies suggests that mutating this site surprisingly does not affect either membrane trafficking or function (Lucien *et al.*, 2002). However, it should be noted that UT-B glycosylation function may differ in a mammalian system, hence the precise role it plays has yet to be fully determined.

Single-amino acid mutations in human UT-A2 (Val/Ile 227 or Ala/Thr 357) have been associated with a decrease in blood pressure (Ranade *et al.*, 2001). These UT-A2 mutations have been linked with increased efficacy of the antihypertensive drug nifedipine (Hong *et al.*, 2007) and, more recently, shown to be associated with metabolic syndrome (Tsai *et al.*, 2010). Jk null individuals have mutations in UT-B1 and therefore have red blood cells that lack functional urea transporters. For example, the first recorded mutations were shown to cause the skipping of exon 6 or 7 during the transcription process, producing non-functional UT-B1 proteins that did not reach the plasma membrane (Lucien *et al.*, 1998). However, various Jk null polymorphisms occur in different populations around the world – see Table 2. Many of these mutations have functional consequences, such as the S291P mutation, whose failure to be expressed in red blood cells

Table 2

A list of the various mutations in *SLC14A1* (UT-B) urea transporters found in different populations around the world

Population	SNP/mutation	Consequence	Reference
Polynesian	Splice site mutation	Exon 6 missing	(Irshaid *et al.*, 2000)
Finnish	S291P	Prevents efficient trafficking to membrane	(Sidoux-Walter *et al.*, 2000)
English	Genomic deletion	Exons 4, 5 missing	(Irshaid *et al.*, 2002)
Swiss	T194Stop	Truncated exon 7	(Irshaid *et al.*, 2002)
Chinese	Splice site mutation	Exon 6 missing	(Meng *et al.*, 2005)
American			
Caucasians	G68Stop	Nonsense mutation	(Wester *et al.*, 2008)
Spanish	I262Stop	Nonsense mutation	
African	T319M	Missense mutation	

SNP, single-nucleotide polymorphism.

explains the Finnish Jk null phenotype (Sidoux-Walter *et al.*, 2000). However, some mutations have no functional effect. For example, the G838A mutation (Asp280 to Asn 280) is a common polymorphism (i.e. A or B Kidd allele) in human UT-B1, but does not affect transport function (Lucien *et al.*, 2002).

Pharmacology

Using a fluorescent based enzyme-linked immunosorbent assay, it was estimated that UT-A1 transporters in the rat renal inner medullary collecting duct (IMCD) had a turnover number of 100 000 urea molecules per second and that there were ~5 million copies of UT-A1 per IMCD cell (Kishore *et al.*, 1997). More recently, stopped flow fluorometry measurements of mouse UT-A transporters expressed in *Xenopus* oocyte plasma membranes showed transport rates of 46 000 and 59 000 urea molecules per second per protein for UT-A2 and UT-A3 respectively (MacIver *et al.*, 2008).

Two reports have independently estimated that there are 14 000 copies of UT-B1 per human red blood cell (Masouredis *et al.*, 1980; Mannuzzu *et al.*, 1993). It has therefore been calculated that human UT-B1 transporters have a turnover of up to 6 000 000 urea molecules per second (i.e. at least ~60-fold greater than UT-A transporters) (Mannuzzu *et al.*, 1993). Although not as impressive as the Aquaporin-1 (AQP1) water channel, which transports 3 billion water molecules per second (Gade and Robinson, 2006), one might argue that with this channel-like transport rate the correct terminology for these proteins is '*UT-B channels*'. Interestingly, the structure of a bacterial homologue urea transporter has recently been shown to consist of two oppositely orientated homologous halves, known as 'an inverted repeat motif' and operate by a channel-like mechanism (Levin *et al.*, 2009). Furthermore, this 'inverted repeat motif' is also found in channels that transport other neutral solutes, such as ammonia and water, for example, AQP1 itself (Murata *et al.*, 2000).

Urea transporters have a low affinity for urea, for example, rabbit UT-A2 Km > 200 mM (You *et al.*, 1993), and so are not saturated by the levels of between 2 and 10 mM of urea generally found in mammalian blood. Initial reports suggested that UT-A proteins are highly selective for urea and do not transport water (Hill *et al.*, 2005). Recent stopped flow fluorometry measurements of transporters expressed in *Xenopus* oocyte plasma membranes confirmed that mouse UT-A2 and UT-A3 did not transport water, ammonia or urea analogues, such as formamide, acetamide, methylurea and dimethylurea (MacIver *et al.*, 2008).

The observation that rat UT-B1, as well as facilitating the movement of urea, also transports water (1.4×10^{-14} $cm^3 \cdot s^{-1}$ per channel cf. 0.1×10^{-14} $cm^3 \cdot s^{-1}$ per channel for rat UT-A2) (Yang and Verkman, 1998) was initially controversial (Sidoux-Walter *et al.*, 1999). However, stopped flow light scattering measurements using mouse erythrocytes from knockout (KO) models confirmed UT-B1 has a water permeability (Pf) of 7.5×10^{-14} $cm^3 \cdot s^{-1}$ per channel (Yang and Verkman, 2002). This promiscuous nature of the UT-B channel has now been confirmed by the fact it also transports formamide, acetamide, methylurea, methylformamide, ammonium carbonate and acrylamide (Zhao *et al.*, 2007).

In humans, this relatively unselective UT-B mediated transport is shown by the fact that red blood cells from Jk null individuals (i.e. ones lacking UT-B1) have a decreased permeability to both urea and water (Meng *et al.*, 2005). Indeed, it has been reported that a known urea transporter inhibitor, the analogue thiourea, can actually go through human UT-B1 (Km = 40 mM), but not through human UT-A2 (Martial *et al.*, 1996).

Urea transport in perfused rat IMCD was originally shown to be inhibited by 250 μM phloretin and 200 mM of the urea analogues thiourea, methylurea and acetamide (Chou and Knepper, 1989). The $K_{1/2}$-value for thiourea inhibition was 27 mM, which was unaffected by vasopressin (see regulation section below) even though transport increased fourfold (Chou and Knepper, 1989). Rat IMCD urea transport was also inhibited by dimethylurea and phenylurea (Zhang and Verkman, 1990). Rat UT-A1, located in the IMCD, was later confirmed to indeed be inhibited by phloretin and urea analogues (Shayakul *et al.*, 1996), as well as by thionicotinamide (Frohlich *et al.*, 2004). Importantly, all other known UT-A isoforms are also inhibited by phloretin – including UT-A2

(You *et al.*, 1993), UT-A3 and UT-A4 (Karakashian *et al.*, 1999), UT-A5 (Fenton *et al.*, 2000) and UT-A6 (Smith *et al.*, 2004).

Human UT-B1 expressed in oocytes was initially confirmed to be inhibited by phloretin and thiourea (Olives *et al.*, 1994). In agreement with this, rat UT-B1 function in vasa recta was also inhibited by thiourea, methylurea, acetamide and phloretin (Pallone, 1994), with a $K_{1/2}$-value for thiourea of 19 mM, as well as dimethylurea (Yang and Verkman, 1998). More recently, mouse UT-B1 mediated transport in red blood cells has been shown to be inhibited by more than 60% by dimethylurea, acrylamide, thiourea and methylformamide (Zhao *et al.*, 2007). As expected, both bovine UT-B1 and UT-B2 isoforms were also inhibited by both phloretin and thionicotinamide (Stewart *et al.*, 2005). Interestingly, it has been suggested that UT-B proteins are more sensitive to phloretin inhibition than UT-A transporters – for example, IC_{50} phloretin: human UT-B1 = 75 μM versus human UT-A2 230 μM (Martial *et al.*, 1996). In addition, human red blood cell urea transport is also inhibited by mercurial compounds, such as p-chloromercuribenzenesulphonate (pCMBS) (Mannuzzu *et al.*, 1993), because human UT-B1 is pCMBS-sensitive (Olives *et al.*, 1994; Lucien *et al.*, 2002) with an IC_{50} of 150 μM (Martial *et al.*, 1996). In direct contrast, human UT-A2 is not inhibited by pCMBS (Olives *et al.*, 1996), while rat kidney UT-B1 is indeed pCMBS-sensitive (Pallone, 1994).

Finally, a human red blood lysis assay has been used to investigate the potential inhibitory effects of over 50 000 compounds on UT-B1 facilitated acetamide transport (Levin *et al.*, 2007). This research discovered 30 specific inhibitors that selectively inhibited UT-B but not UT-A transporters, while also having no effect on AQP1 (Levin *et al.*, 2007). These compounds were from the phenylsulphoxyoxazole, benzensulphonanilide, phthalazinamine and aminobenzimidazole classes (Levin *et al.*, 2007).

Some members of the aquaporin water channel group are also permeable to urea. For example, AQP7 is urea permeable and was originally localized in the testis (Ishibashi *et al.*, 1997). AQP7 has also been localized to the proximal tubule in rat kidney (Ishibashi *et al.*, 2000) and is hence located in a different nephron segment to the UT-A urea transporters. Although this implies that AQP7 could play a role in renal urea transport, a mouse AQP7 KO model does not actually display a urinary concentrating defect and shows the renal function of AQP7 to be the reabsorption of water and glycerol (Sohara *et al.*, 2005). AQP9 is another urea permeable aquaporin, which was originally located in human leukocytes (Ishibashi *et al.*, 1998). However, AQP9 KO mice have no change in their plasma urea levels and results showed AQP9 to be involved in glycerol metabolism in the liver (Rojek *et al.*, 2007). These results strongly suggest aquaporins do not play a significant physiological role in urea transport.

Urea has also been reported to pass through other co-transporters, such as the sodium-dependent glucose transporter (SGLT1) (Leung *et al.*, 2000). However, the actual urea transport rate through SGLT1 was $P_{urea} = 1.2 \times 10^{-7}$ cm·s^{-1} (Leung *et al.*, 2000), which is several orders of magnitude lower than P_{urea} values for urea transporters, for example, UT-A2 = 4.5×10^{-5} cm·s^{-1} (You *et al.*, 1993). It therefore again seems unlikely that these co-transporters have a significant physiological role in transporting urea.

Distribution

In the original research that cloned the first urea transporter, rabbit UT-A2 was discovered in the kidney, but also detected in the colon (You *et al.*, 1993). Human UT-A1 was detected in the kidney, as it was for other species such as rat (Nielsen *et al.*, 1996) and mouse (Fenton *et al.*, 2002b). Similarly, UT-A2 (Olives *et al.*, 1996), UT-A3 (Stewart *et al.*, 2004) and UT-A4 (Karakashian *et al.*, 1999) are also all found in the kidney of various species. Human UT-A3 protein has yet to be investigated, although a cDNA clone has been identified (Smith and Fenton, 2006) and an appropriately sized 2.4 kb transcript detected in human kidney medullary RNA (Bagnasco *et al.*, 2001). In contrast to these renal locations, mouse UT-A5 is found in the testis (Fenton *et al.*, 2000) and human UT-A6, a 235-amino acid protein including a novel 5a exon and unique 19-amino acid C-terminal, has been located in the colon (Smith *et al.*, 2004). Other tissues in which UT-A proteins have been found include heart (Duchesne *et al.*, 2001), cochlea (Kwun *et al.*, 2003), placenta (Damiano *et al.*, 2006), brain and liver (Fenton *et al.*, 2002b).

Human UT-A1 was located in the renal IMCD (Bagnasco *et al.*, 2001). In rats, UT-A1 was present in the terminal portions of the IMCD (Nielsen *et al.*, 1996), while UT-A2 was found in the late part of descending thin limbs of short loops of Henle and the inner medullary part of descending thin limbs of long loops of Henle (Shayakul *et al.*, 1997). In mice, UT-A1 was again found in terminal IMCD, and UT-A2 located in short (type I) and long (type 3) thin descending limbs of the loops of Henle (Fenton *et al.*, 2002b). UT-A3 was also only located in the IMCD (Stewart *et al.*, 2004). Interestingly, mouse UT-A1 and UT-A3 co-localized with AQP2 in principal cells in IMCD (Fenton *et al.*, 2006). Mouse UT-A5 protein is localized to the peritubular myoid cells of the seminiferous tubules in the testis (Fenton *et al.*, 2000).

In IMCD cells, UT-A1 was mainly located at the apical membrane region (Nielsen *et al.*, 1996), while UT-A3 was mainly located at the basolateral membrane region (Shayakul *et al.*, 2001; Stewart *et al.*, 2004). However, it should be noted that UT-A1 is capable of going to the basolateral membrane (Frohlich *et al.*, 2004) and UT-A3 has been reported in one study at the apical membrane after vasopressin treatment (Blount *et al.*, 2007). UT-A2 was located on both apical and basolateral membranes in rats (Lim *et al.*, 2006) and mice (Fenton *et al.*, 2002b; Potter *et al.*, 2006). The subcellular location of UT-A4, UT-A5 and UT-A6 remain unclear at present.

In contrast to the mainly renal location of UT-A transporters, UT-B protein has been detected in numerous tissues. Initially, a triated urea analogue 1-(3-azido-4-chlorophenyl)-3methyl-2-thiourea ([^{3}H]MeACPTU) was used as a probe to photolabel human red blood cell urea transporters (Neau *et al.*, 1993). This technique detected a 40 kDa polypeptide in red blood cells, later called UT-B1, which was absent in Jk null red blood cells (i.e. showing that the Kidd antigen was a urea transporter) (Neau *et al.*, 1993). This UT-B1 transporter was initially detected in human bone marrow, erythrocytes and also the kidney, as 2.5 kb and 4.7 kb transcripts (Olives *et al.*, 1994). Rat UT-B1 was found in brain, spleen, kidney and testis, compared with rat UT-A2 which was only located in the kidney (Promeneur *et al.*, 1996). Rat UT-B1 was also found in urinary tract epithelia, thymus and lung (Tsukaguchi *et al.*,

1997). UT-B1 proteins were also detected in the heart (Meng *et al.*, 2009), bladder and gastrointestinal tract in mice (Lucien *et al.*, 2005), human colon (Inoue *et al.*, 2004), rat colon (Inoue *et al.*, 2005) and throughout sheep gastrointestinal tract, including the salivary glands (Ludden *et al.*, 2009). Bovine UT-B1 and UT-B2 isoforms are both present in the bovine rumen (Stewart *et al.*, 2005), while UT-B1 has also been reported in the cochlea of rats (Kwun *et al.*, 2003).

UT-B1 localized to non-fenestrated endothelial cells in descending vasa recta of human kidney (Xu *et al.*, 1997; Timmer *et al.*, 2001) and rat kidney (Pallone, 1994). In addition to its location in descending vasa recta, mouse UT-B1 has been reported in the renal proximal tubule and papillary surface epithelium (Jung *et al.*, 2003). UT-B1 was prevalent in the colonic epithelial cells in the human ascending colon (Collins *et al.*, 2010), while bovine UT-B protein was present in rumen epithelial layers (Stewart *et al.*, 2005). UT-B1 was also found in the Sertoli cells of the seminiferous tubules in the testis (Tsukaguchi *et al.*, 1997).

UT-B1 was located on both apical and basolateral membranes of descending vasa recta in both rats (Lim *et al.*, 2006) and mice (Jung *et al.*, 2003). UT-B1 was also located on both apical and basolateral membranes in rat testis Sertoli cells (Fenton *et al.*, 2002c). In agreement with these findings, bovine ruminal UT-B protein has been localized to both apical and basolateral membranes of epithelial cells (Stewart *et al.*, 2005; Simmons *et al.*, 2009). In addition, functional evidence for bovine UT-B2, when over-expressed in a Madin–Darby canine kidney (MDCK) cell line, also showed that this isoform is capable to going to both apical and basolateral membranes (Tickle *et al.*, 2009).

Regulation

IMCD plasma membranes limit the rate of *trans*-epithelial urea transport (Star, 1990) and it is known that the antidiuretic hormone vasopressin stimulates an increase in membrane urea permeability (Nielsen and Knepper, 1993). Vasopressin binds to V2 receptors on the basolateral membrane of IMCD cells, increasing cAMP levels and stimulating PKA – hence vasopressin stimulation of urea transport was prevented by PKA inhibition (Zhang and Verkman, 1990).

Vasopressin regulates rat UT-A1 function in the short term (Terris *et al.*, 1998), and its action is prevented by PKA inhibition (Frohlich *et al.*, 2006). Vasopressin activates PKA rapidly (within 5 to 10 min.) and stimulates phosphorylation of UT-A1 protein (Zhang *et al.*, 2002). This causes an increase in urea transport by increasing UT-A1 abundance at the apical plasma membrane in IMCD cells (Klein *et al.*, 2006a). This regulation of UT-A1 activity and membrane accumulation involves rapid phosphorylation of serine 486 (Klein *et al.*, 2010). Other UT-A1 amino acids reported to be phosphorylated in response to vasopressin include serine 84 (Hwang *et al.*, 2010) and serine 499 (Blount *et al.*, 2008) (see Figure 4).

Vasopressin also stimulates UT-A3 function (Stewart *et al.*, 2007), via a process again involving the stimulation of both phosphorylation and membrane accumulation (Blount *et al.*, 2007). Vasopressin firstly produces PKA-dependent stimulation of UT-A3 transporters in the basolateral membrane (Stewart *et al.*, 2009). Secondly, vasopressin also then stimulates casein kinase II-dependent trafficking of additional UT-A3 transporters to the basolateral membrane, via a process that is dependent on both protein kinase C and calmodulin (Stewart *et al.*, 2009).

Both UT-A1 (Shayakul *et al.*, 1996) and UT-A3 (Karakashian *et al.*, 1999; Stewart *et al.*, 2007) are stimulated by increased levels of cAMP, although the increase in human UT-A1 function is modest (Bagnasco *et al.*, 2001). PKA also stimulates the transport function of human UT-A6 (Smith *et al.*, 2004), but not UT-A2 (Fenton *et al.*, 2002b). In contrast, when mouse UT-A2 was expressed in a stable MDCK cell line it was acutely regulated by vasopressin, cAMP and calcium (Potter *et al.*, 2006). Increased tonicity in rat IMCD suspensions increased plasma membrane localization of UT-A1 and UT-A3 (Blessing *et al.*, 2008). Indeed, hypertonicity stimulates urea transport through a protein kinase C-mediated phosphorylation event (Wang *et al.*, 2010). Angiotensin II also increases vasopressin-stimulated urea transport in rat IMCD via a protein kinase C-dependent effect on UT-A1 (Kato *et al.*, 2000).

As well as insertion, regulation of urea transporter removal from the plasma membrane also occurs. For example, ubiquitination regulates the plasma membrane expression of the three main renal UT-A urea transporters: UT-A1, UT-A2 and UT-A3 (Stewart *et al.*, 2008). Ubiquitination and subsequent degradation of UT-A1 by the proteasome pathway involves MDM2 (murine double minute) E3 ubiquitin ligase (Chen *et al.*, 2008). UT-A1 is internalized by a dynamin-dependent mechanism, which is mediated by both caveolae and clathrin coated pit pathways (Huang *et al.*, 2010b). In contrast to all this, UT-B proteins have not been reported to be acutely regulated. For example, bovine bUT-B2 is constitutively activated when over-expressed in an MDCK cell line and is not stimulated by cAMP, calcium, vasopressin or protein kinases (Tickle *et al.*, 2009).

A number of factors have been shown to influence UT-A gene expression – including hydration state, dietary protein, glucocorticoids and mineralocorticoids. There are two promoters in the UT-A gene: one controlling UT-A1/UT-A3 expression (called UT-Aα or UT-A promoter I) and one controlling UT-A2 (called UT-Aβ or UT-A promoter II). In mice, the UT-Aα promoter is cAMP and tonicity sensitive, while the UT-Aβ promoter is only cAMP sensitive (Fenton *et al.*, 2002a), hence both UT-A2 and UT-A3 mRNA levels are increased in water-deprived mice (Fenton *et al.*, 2002a). Glucocorticoids have been reported to inhibit transcription and expression of UT-A1 and UT-A3 by decreasing UT-A promoter I activity in rats (Peng *et al.*, 2002), with similar findings for the UT-Aα promoter in mice (Fenton *et al.*, 2006). Interestingly, rat kidney UT-A1 has also been shown to be down-regulated by aldosterone via the mineralocorticoid receptor (Gertner *et al.*, 2004). In contrast, the down-regulation of UT-A1 by glucocorticoids in the same study was not via the mineralocorticoid receptor, but most likely through the glucocorticoid receptor (Gertner *et al.*, 2004). The implications of these findings are that both glucocorticoid agonists used for inflammatory conditions and mineralocorticoid antagonists used for diuresis are likely to alter *SLC14A2* transporter expression in the human kidney.

Glucocorticoids have been shown to have no effect on the UT-A promoter II (i.e. UT-Aβ) and so did not alter UT-A2 expression levels (Peng *et al.*, 2002). However, UT-A2 expression in the rat kidney can be influenced by other factors.

UT-A2 has a 4.0 kb RNA transcript that is regulated by dietary protein content, while a 2.9 kb transcript is responsive to hydration state (Smith *et al.*, 1995), thus meaning fluid and nitrogen balance can be regulated independently. It has also been reported that tonicity responsive regulation of rat UT-A1 and UT-A3 is mediated by the TonE/TonEBP pathway (Nakayama *et al.*, 2000).

Investigation of the regulation of UT-A protein level expression also confirms many of these findings. For example, osmolality and urea concentration regulate UT-A1 expression (Terris *et al.*, 1998), glucocorticoids down-regulate UT-A1 in rat terminal IMCD (Naruse *et al.*, 1997) and rat UT-A2 abundance is regulated by the antidiuretic hormone vasopressin (Wade *et al.*, 2000). Interestingly, one study has shown that water deprivation increased UT-A3 expression, but decreased UT-A1, in the rat IMCD (Lim *et al.*, 2006). The mechanisms for this differential effect on UT-A1 and UT-A3 are not yet clearly understood.

To date, little is known about the factors that influence expression of the UT-B gene. However, research at the protein level suggests a number of factors may be involved. Long-term vasopressin infusion has been shown to greatly decrease UT-B1 in rat kidney (Trinh-Trang-Tan *et al.*, 2002), while water derivation increased UT-B staining intensity of descending vasa recta (Lim *et al.*, 2006). Rat UT-B1 renal and intestinal protein expression is also regulated by low protein and urea diets (Inoue *et al.*, 2005). Furthermore, dietary intake has shown to alter abundance and localization of UT-B protein in the rumen of two species – cows (Simmons *et al.*, 2009) and sheep (Ludden *et al.*, 2009). Further research is now required to clarify how these dietary effects on UT-B proteins are actually regulated.

Pathology and clinical significance

Major dysfunction of UT-A protein has not been reported in humans. However, specific KO mouse models have been investigated and the lack of major dysfunction may well be due to the compensatory effects of up-regulating other urea transporter isoforms. For example, it has been reported that there are increased levels of UT-A2 in mice lacking UT-B (Klein *et al.*, 2004). The UT-A1/3 KO mice, lacking both the collecting duct urea transporters UT-A1 and UT-A3, have a severe concentrating defect when fed a normal 20% protein diet (Fenton *et al.*, 2004) – with increased fluid consumption, increased urine flow and decreased urine osmolality. More recently, these UT-A1/3 KO mice have also been shown to have increased blood pressure, plus increased chance of hydronephrosis and renal pelvic reflux compared with wild-type controls (Jacob *et al.*, 2008). UT-A2 KO mice have a much milder concentrating defect that is only detectable when on a low protein diet – suggesting UT-A2 mainly plays a role in maintaining inner medulla urea concentration when urea production is low (Uchida *et al.*, 2005). While these findings confirm that UT-A function is predominantly concerned with the renal urinary concentrating mechanism, the implications of UT-A mutations to the health of the human population is not yet fully understood.

As previously mentioned, humans lacking red blood cell UT-B1 protein do exist (i.e. Jk null individuals). These patients displayed moderate maximal concentrating ability (~800 mOsm vs. ~1000–1100 mOsm in controls), that is, they have only a mild concentrating defect (Sands *et al.*, 1992). As expected, Jk null individuals have red blood cells that have a selective defect in urea transport (Olives *et al.*, 1995). Jk null individuals are rare within a population, for example, five out of 20 163 Thai individuals (i.e. 0.0002%) (Deelert *et al.*, 2010). However, after immunization anti-Jk3 forms and therefore it can be difficult to find donors suitable for these Jk null individuals (Deelert *et al.*, 2010).

UT-B KO mice have a 45-fold reduction in red blood cell urea permeability, 50% increase in urine output and 30% decrease in urine osmolality (i.e. mild, urea-selective concentrating defect (Yang *et al.*, 2002). Interestingly, the UT-B KO mice gain less weight than wild-type littermates, suggesting intestinal UT-B plays important role in weight gain (Yang and Bankir, 2005). Although it cannot be ruled out that this reduced weight gain was simply due to adverse effects on general health, the KO mice displayed no obvious signs of ill-health compared with the wild-type littermates. This proposed nutritional role for intestinal UT-B protein is linked to its involvement in the UNS process that helps maintain the symbiotic relationship between mammals and their intestinal bacteria (Stewart and Smith, 2005) – see Figure 2. Recent advances in our understanding of the importance of colonic bacteria populations to human health and nutrition strongly indicate that the UNS process may play a vital role in our well-being. Urea hydrolysis in the human gastrointestinal tract is regulated by diet (Fouillet *et al.*, 2008) and one may predict that this involves regulation of the UT-B1 proteins mediating *trans*-epithelial urea transport in the human ascending colon (Collins *et al.*, 2010) – in a manner similar to the dietary effects observed on ruminal UT-B transporters (Simmons *et al.*, 2009). Although the precise role of human colonic urea transporters still remain to be determined, it is intriguing to speculate that alteration of their normal function could alter bacterial populations and potentially contribute to disease states of the human colon.

Numerous factors have been shown to influence renal UT-A transporter expression. Excess of glucocorticoids produces a decrease in rat renal UT-A1 and UT-A3 abundance, and may explain impaired urinary concentrating capacity in human patients suffering from Cushing syndrome (Li *et al.*, 2008). Renal urea transporters are down-regulated by severe inflammation, such as occurs with sepsis-induced acute renal failure (Schmidt *et al.*, 2007), while UT-A1 is down-regulated in adriamycin-induced nephritic syndrome (Fernandez-Llama *et al.*, 1998). It has also been suggested that expressional changes in kidney UT-A protein may be responsible for reduced concentrating ability of mammalian kidney as ageing occurs (Combet *et al.*, 2003).

Rat UT-A1 in kidney medulla is down-regulated in angiotensin II-induced hypertension (Klein *et al.*, 2006b), while UT-A protein was increased in kidney of streptozotocin-induced diabetic rats compared with control rats (Bardoux *et al.*, 2001). Importantly, increases in UT-A1 (but not UT-B1) in renal medulla during diabetes mellitus may help limit fluid loss during this disease (Kim *et al.*, 2003). Lastly, there is increased UT-A1 and UT-A3 expression, and resulting function, in the IMCD of salt-sensitive rats (cf. salt-resistant rat) (Fenton *et al.*, 2003). There are also disease and

environmental-related changes in non-renal UT-A transporters. For example, pre-eclampsia appears to increase phloretin-sensitive urea transport and UT-A urea transporter abundance in the placenta (Damiano *et al.*, 2006), while UT-A protein abundance in the heart is increased during uraemia, hypertension and heart failure (Duchesne *et al.*, 2001).

There are a limited number of reports concerning changes in both UT-A and UT-B proteins. Decreases in UT-A1, UT-A3 and UT-B1 in rats with ureteral obstruction may explain reduction in the urinary concentrating ability in these animals (Li *et al.*, 2004). Chronic renal failure significantly decreases UT-A1, UT-A2 and UT-B1 in the rat kidney, and also decreased UT-B1 in rat brain (Hu *et al.*, 2000). A decrease in UT-A1 and UT-B1 abundance in the renal inner medulla has been reported in lithium-fed rats (Klein *et al.*, 2002). However, UT-A1 recovered to normal levels 14 days after cessation of lithium administration (Blount *et al.*, 2010). Treatment with the immunosuppressant drug cyclosporine reduces UT-A2, UT-A3 and UT-B1 levels in the kidney, explaining the impaired urine concentrating ability that is a main feature of cyclosporine-induced nephropathy (Lim *et al.*, 2004). The diuretic furosemide also moderately decreases UT-B1 abundance in rat kidney (Trinh-Trang-Tan *et al.*, 2002), which may have implications for its long-term use. Finally, there has also been a reported increase in UT-B1 abundance in the ageing rat brain (Trinh-Trang-Tan *et al.*, 2003). It is not yet known what might cause this change or whether it leads to dysregulation in cerebral function (Trinh-Trang-Tan *et al.*, 2003).

Conclusion

The family of *SLC14A* facilitative urea transporters play an important role in two major physiological processes, namely the urinary concentration mechanism and UNS. These facilitative transporters are found in specific locations within different tissues and are derived from two distinct genes: UT-A and UT-B. Generally, the UT-A and UT-B classes of urea transporters have a similar function, topology and basic structure. However, they do display marked differences in terms of substrate specificity, transport rates, inhibition, gene regulation, functional regulation and tissue localization. Although there are no major pathologies linked with urea transporter dysfunction, understanding these proteins has important clinical implications, especially within the context of renal disease.

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Conflicts of interest

There are no conflicts of interest to report regarding this article.

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