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# UT-B1 Mediates Transepithelial Urea Flux in the Rat Gastrointestinal Tract

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**Abstract** The process of urea nitrogen salvaging plays a vital role in the symbiotic relationship between mammals and their intestinal bacteria. The first step in this process requires the movement of urea from the mammalian bloodstream into the gastrointestinal tract lumen via specialized proteins known as facilitative urea transporters. In this study, we examined both transepithelial urea fluxes and urea transporter protein abundance along the length of the rat gastrointestinal tract. Urea flux experiments that used rat gastrointestinal tissues showed significantly higher transepithelial urea transport was present in caecum and proximal colon ($P < 0.01$, $n = 8$, analysis of variance [ANOVA]). This large urea flux was significantly inhibited by 1,3,dimethylurea ($P < 0.001$, $n = 8$, ANOVA) and thiourea ($P < 0.05$, $n = 6$, unpaired $t$-test), both known blockers of facilitative urea transporters. Immunoblotting analysis failed to detect any UT-A protein within rat gastrointestinal tissue protein samples. In contrast, a 30-kDa UT-B1 protein was strongly detected in both caecum and proximal colon samples at significantly higher levels compared to the rest of the gastrointestinal tract ($P < 0.01$, $n = 4$, ANOVA). We therefore concluded that UT-B1 mediates the transepithelial movement of urea that occurs in specific distal regions of the rat gastrointestinal tract.

**Keywords** UT-A · UT-B · Transepithelial urea transport · Dimethylurea · Intestine · Colon

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Urea is the main breakdown product of protein catabolism in mammals and was originally viewed as a simple waste product that was excreted in the urine (Stewart and Smith 2005). However, in recent years, it has become recognized that urea plays a vital role in a number of physiological processes, including osmoregulation and nutrition (Smith 2008). The movement of urea across plasma membranes can be regulated through a group of proteins known as facilitative urea transporters. In mammals, these transporters are derived from either the UT-A (Slc14a2) or UT-B (Slc14a1) genes (Smith 2008).

It is now believed that urea transporters localized within the mammalian gastrointestinal tract play an important nutritional role in the process of urea nitrogen salvaging (UNS) (Stewart and Smith 2005). Urea produced in the liver enters the bloodstream and then passes, via urea transporters, into the gastrointestinal tract. It is subsequently metabolized by intestinal bacteria into ammonia and carbon dioxide by urease enzymes (Stewart and Smith 2005). The ammonia is used as a nitrogen source for bacterial growth, and in turn, bacterial amino acids and peptides can be digested and reabsorbed by the mammalian host (Stewart and Smith 2005). This symbiotic relationship has long been known to be crucial to the nutritional balance of ruminant animals such as cattle and sheep. More recently, evidence is starting to emerge that the UNS process is also important in the nutrition of monogastric

mammals, such as rats and humans—particularly in the distal regions of the gastrointestinal tract (e.g., colon).

Urea transporters, particularly UT-Bs, have been localized in the gastrointestinal tract of various mammalian species, including cattle (Stewart et al. 2005), sheep (Ludden et al. 2009), mice (Lucien et al. 2005), rats (Timmer et al. 2001), and humans (Inoue et al. 2004). Importantly, there is also evidence that the expression of urea transporters is regulated by dietary factors (Inoue et al. 2005; Simmons et al. 2009), indicating a specific nutritional role for these proteins. However, although urea transporter mediated urea flux has been shown in certain tissues—for example, bovine rumen (Stewart et al. 2005)—comprehensive studies investigating all the different segments of the gastrointestinal tract of particular mammalian species have not been performed. In this study, we have characterized urea transporter mediated transepithelial urea flux throughout the rat gastrointestinal tract. In addition, we have compared this to the protein abundance of UT-A and UT-B urea transporters in these gastrointestinal tissues. Our findings strongly suggest that UT-B1 mediates transepithelial urea transport in specific distal regions of the rat gastrointestinal tract.

## Materials and Methods

### Rat Tissue Preparations

Adult male Wistar rats (250–300 g) were purchased from the biomedical facility at University College Dublin. Animals were housed under controlled environmental conditions with a 12:12 h light/dark cycle with free access to water and standard laboratory chow. Maintenance and welfare of animals, along with approved methods of killing them, were in accordance with the University College Dublin Animal Research Ethics Committee. After the animals were humanely killed, a midline laparotomy was performed and the relevant gastrointestinal segments (stomach, duodenum, ileum, caecum, or colon) were removed. Intestinal segments were opened longitudinally along the antimesenteric border, cleaned of enteric matter, and carefully stripped of underlying muscle layers by blunt dissection. Rat stomachs were opened along the greater curvature, and the outer muscle was dissected from the mucosa using a blistering technique (Hopkins et al. 2002).

### Ussing Chamber Experiments and Electrophysiology

For all tissues, the exposed epithelial layer and underlying lamina propria was mounted in an Ussing chamber (WPI, UK) with a circular window surface area of 0.63 $cm^2$. Tissues were bathed bilaterally with 5 ml of Krebs–Henseleit buffer (composition in mM: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $KH_2PO_4$ 1.2, $NaHCO_3$ 25, and D-glucose 11.1, pH 7.4) and were continuously gassed with 95% $O_2$–5% $CO_2$ at 37°C.

The potential difference (PD) across the tissue and the required short circuit current ($I_{sc}$) to maintain a zero PD were monitored using Ag/AgCl electrodes embedded in 3 M KCl agar attached to an automated voltage clamp (DVC 4000; WPI, UK). The PD and $I_{sc}$ were recorded with a MacLab analog–digital recorder (AD Instruments, UK). Baseline potential difference (mV) and short circuit current (μA $cm^{-2}$) values were recorded after an initial equilibration period of 30 min after the tissues were mounted. As an indicator of net active ion transport, $I_{sc}$ was recorded continuously for the duration of the experiment, while the PD was recorded every 30 s for 3 s by removing the voltage clamp via an automated timer (Pro-4, WPI, UK). The transepithelial electrical resistance (Ω $cm^2$) was calculated according to Ohm's law. Tissue capacity to respond to basolateral application of the cholinomimetic secretagogue, carbachol (10 μM), was used to confirm tissue viability at the end of experiments.

### Urea Transport Studies

Measurement of radiolabeled [$^{14}$C] urea in the absorptive (lumen to blood) and secretory (blood to lumen) directions was used to determine urea flux. Urea was added to give a total unlabeled concentration of 1 mM. [$^{14}$C] urea (0.1 μCi/ml) was added to either the apical or basolateral chamber, and in each case, an equivalent concentration of unlabeled substrate was present in the contralateral chamber. The tissues were equilibrated for 10 min, when 200-μl samples from both apical and basal chambers were taken. Samples (200 μl) were then taken every 30 min for up to 120 min. The urea transporter inhibitors, 1,3-dimethylurea (DMU; 50 mM, Sigma-Aldrich, Dublin, Ireland) or thiourea (100 mM, Sigma-Aldrich, Dublin, Ireland), were added at 40 min to both apical and basolateral chambers. Vehicle (dimethyl sulfoxide) was added to control chambers. Radioactivity was measured using a liquid scintillation analyzer (Packard Tricarb 2900 TR). Urea permeabilities were calculated from the disintegrations per minute by the apparent permeability coefficient (Papp) equation:

$$\mathrm{Papp}\left(\mathrm{cm\ s^{-1}}\right) = \frac{dQ}{dt} \times \frac{1}{(A.C_0)}$$

where Papp is the apparent permeability coefficient (cm $s^{-1}$), dQ/dt is the steady-state flux (mol $s^{-1}$), A is the surface area of membrane ($cm^2$), and $C_0$ is the initial concentration in donor chamber (mol $cm^{-3}$). Note: dQ/dt was caculated by measuring the slope of the graph plotted for accumulated radioactivity (in disintegrations per minute) against time.

### Antisera

To study the distribution of rat UT-A urea transporters, we used the previously characterized antibodies ML194 and MQ2 raised to the C terminals of mouse UT-A1 and mouse UT-A3, respectively (Stewart et al. 2004). These antibodies were the gift of Dr. Craig Smith (University of Manchester, UK). To study UT-B urea transporter distribution, we used the characterized UT-B antibody BUTB-PAN raised to the C terminal of bovine UT-B1 (Simmons et al. 2009). To study MCT1 monocarboxylate transporters, we used the commercially available antibody MCT12A (Autogen Bioclear, UK).

### Immunoblotting

Rat kidney medulla or gastrointestinal tissue was homogenized with an automated homogenizer and a specifically prepared buffer (300 mM mannitol, 12 mM HEPES, pH 7.6). Homogenates were spun at 2500×*g* at room temperature for 5 min and the pelleted cellular debris removed. The remaining whole-cell homogenate was further spun at 17,000×*g* at room temperature for 30 min, producing a pellet of membrane-enriched protein. This pellet was resuspended in an appropriate volume of homogenization buffer. For loading onto Western blot test gels, 2 × reducing Laemmli buffer (5% sodium dodecyl sulfate, 25% glycerol, 0.32 M Tris, pH 6.8, bromophenol blue, and 5% $\beta$-mercaptoethanol) was added to protein samples in a 1:1 ratio and then heated at 65°C for 15 min. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis was performed on minigels of 12% polyacrylamide by loading ~10 µg of protein per lane. After transfer to nitrocellulose membranes, immunoblots were probed for 16 h at room temperature in the relevant primary antibody (ML194, MQ2, BUTB-PAN, or MCT12A). Immunoblots were then washed and probed with the relevant horseradish peroxidase–linked secondary antibody (either anti-chicken or anti-rabbit) for 1 h at room temperature. After further washing, detection of protein was performed with the EZ-ECL chemiluminescence detection kit (Geneflow, UK) and a LAS-4000 Image Reader (Fujifilm, USA). Densitometry analysis of the signals obtained was performed by ImageJ software (National Institutes of Health, USA).

### Statistical Analysis

Data are means ± standard error, with $n$ representing the number of samples. Unpaired $t$-tests and one-way analysis of variance (ANOVA) were used to test data, as appropriate. Groups were deemed statistically significant if $P < 0.05$, with ANOVA performed by the Dunnett post hoc test (Instat, GraphPad software).

## Results

We determined the functional transepithelial urea fluxes present in a range of rat gastrointestinal tissues in both apical-to-basolateral (i.e., absorption) and basolateral-to apical (i.e., secretion) directions (Fig. 1). For apical-to-basolateral experiments, there was a significantly larger $^{14}$C-radiolabeled urea flux in caecum/proximal colon tissue ($P < 0.01$, $n = 8$, ANOVA) (Fig. 1a). Importantly, this flux was significantly inhibited by 50 mM DMU ($P < 0.001$, $n = 8$, ANOVA), a known inhibitor of facilitative urea transporters. For basolateral-to-apical experiments, there was also a significantly larger $^{14}$C-radiolabeled urea flux in caecum/proximal colon tissue ($P < 0.001$, $n = 8$, ANOVA) that was inhibited by 50 mM DMU ($P < 0.001$, $n = 8$, ANOVA) (Fig. 1b).

To confirm that these large urea fluxes in caecum/proximal colon tissue were mediated by facilitative urea transporters, we investigated the effects of another known inhibitor: the urea analog thiourea (Fig. 2). Importantly,

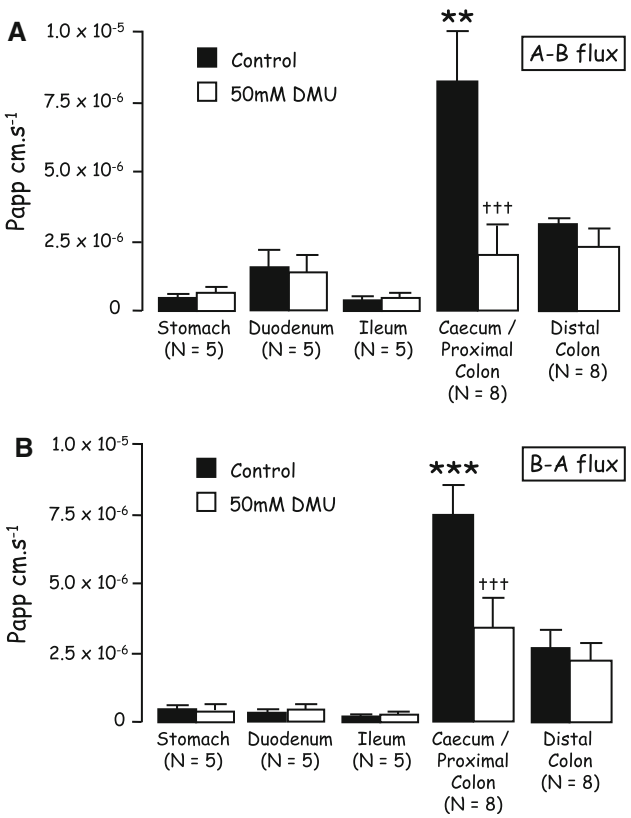


**Fig. 1** Transepithelial urea flux was significantly higher in rat caecum/proximal colon compared to other gastrointestinal tissues for both **a** apical-to-basolateral and **b** basolateral-to-apical directions. In both cases, the caecum/proximal colon urea flux was significantly inhibited by 50 mM DMU. Papp = apparent permeability coefficient. $^{**}P < 0.01$, $^{***}P < 0.001$ against other tissue fluxes, ANOVA; $^{\dagger\dagger\dagger}P < 0.001$ vs. control flux, ANOVA

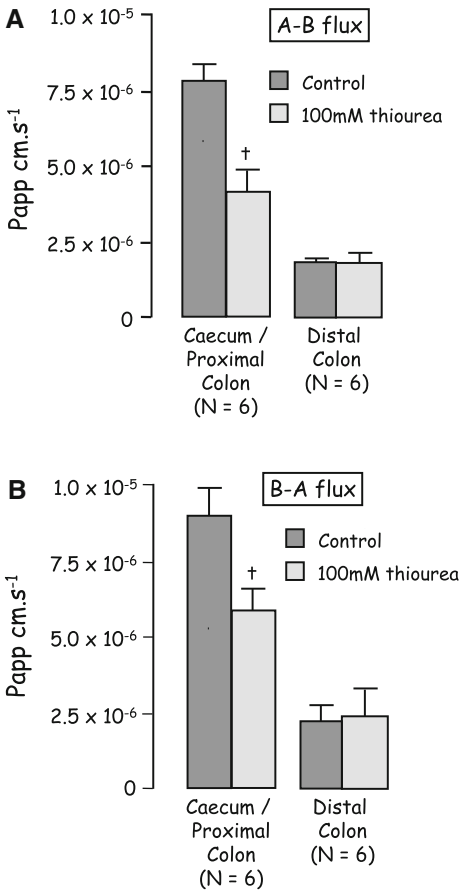


**Fig. 2** Transepithelial urea flux in rat caecum/proximal colon was significantly inhibited by 100 mM thiourea in both **a** apical-to-basolateral and **b** basolateral-to-apical directions. In contrast, thiourea had no effect on urea fluxes in distal colon. Papp = apparent permeability coefficient. $^{\dagger}P < 0.05$ vs. control flux, unpaired *t*-test

100 mM thiourea significantly inhibited both the apical-to-basolateral ($P < 0.05$, $n = 6$, unpaired *t*-test) (Fig. 2a) and the basolateral-to-apical ($P < 0.05$, $n = 6$, unpaired *t*-test) (Fig. 2b) urea fluxes in the caecum/proximal colon. In contrast, no such inhibitory effect was observed on the small basal urea fluxes present in the distal colon in both directions (NS, $n = 6$, unpaired *t*-test).

Investigation of the electrophysical properties of the caecum/proximal colon and the distal colon revealed some marked differences (Table 1). The distal colon tissue displayed a higher transepithelial resistance (120 ± 6 vs. 76 ± 8 Ω per cm$^2$, $P < 0.001$, $n = 6$, unpaired *t*-test), a higher voltage (8.1 ± 1.4 vs. 3.3 ± 0.7 mV, $P < 0.01$, $n = 6$, unpaired *t*-test) and, although not quite significantly different, a generally larger current (43 ± 8 vs. 25 ± 4 μA per cm$^2$, $P = 0.07$, $n = 6$, unpaired *t*-test). The change in current observed after the addition of 10 μM carbachol was not significantly different between the two regions (21 ± 7 vs. 33 ± 8 μA per cm$^2$, NS, $n = 6$, unpaired *t*-test) and confirmed tissue viability.

To investigate the distribution of urea transporters within the rat gastrointestinal tract, protein samples were probed with either UT-A or UT-B antibodies. Although the UT-A antibody ML194 successfully detected rat UT-A1 (97 and 117 kDa) and UT-A2 (30–55 kDa) proteins in rat kidney medulla, no UT-A signals were detected in protein samples from rat gastrointestinal tissues (Fig. 3a). Another UT-A antibody, MQ2, successfully detected UT-A3 (35–50 kDa) in rat kidney medulla, but again failed to detect any significant level of UT-A protein in the rat gastrointestinal protein samples (Fig. 3b).

In contrast, the UT-B antibody BUTB-PAN detected a strong 30-kDa signal in the protein samples from certain gastrointestinal tissues (Fig. 4a). This UT-B signal was predominantly found in the caecum and proximal colon, but it was also weakly detected in the distal colon. Further analysis revealed that the UT-B caecum signal was very similar to the 30-kDa signal found in rat red blood cells (Fig. 4b), which are known to contain the UT-B1 isoform (Trinh-Trang-Tan et al. 2002). However, the 30-kDa caecum signal was distinctly different from the UT-B2 isoform signals detected in an MDCK cell line expressing bovine UT-B2 (Fig. 4b). Preincubation of BUTB-PAN in its immunizing peptide (0.2 μg/μl) for 3–4 h before addition to the immunoblot completely abolished the 30-kDa UT-B1 signal in the proximal and distal colon samples. In contrast, preincubation in a similar amount of nonspecific peptide had no such effect (Fig. 4c).

Immunoblot analysis with BUTB-PAN was repeated with protein samples from the gastrointestinal tracts from three further rats (Fig. 5a). In all four cases, the pattern of 30 kDa UT-B1 expression was the same: caecum > proximal colon >> distal colon. The densitometry results for

**Table 1** Baseline electrophysical parameters and responses to carbachol for rat caecum/proximal colon and distal colon[a]

| Site | $I_{sc}$ (μA/cm$^2$) | V (mV) | TEER (Ω/cm$^2$) | CCh response ($\Delta I_{SC}$) |
|---|---|---|---|---|
| Caecum/proximal colon ($n = 6$) | 25 ± 4 | 3.3 ± 0.7 | 76 ± 8 | 33 ± 8 |
| Distal colon ($n = 6$) | 43 ± 8 | 8.1 ± 1.4 | 120 ± 6 | 21 ± 7 |
| *P*-value (unpaired *t*-test) | 0.0719 | 0.0119 | 0.0013 | 0.2853 |

[a] Values are mean ± standard error; *n* number of rats, $I_{sc}$ short-circuit current, *V* voltage, *TEER* transepithelial electrical resistance, *CCh* carbachol

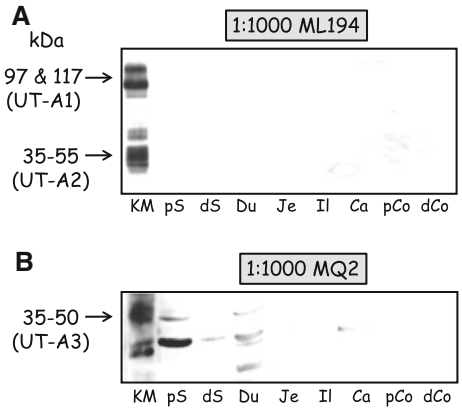


**Fig. 3** **a** Immunoblot of rat gastrointestinal tissue protein samples probed with a 1:1000 dilution of ML194 antibody. Although both UT-A1 (97 and 117 kDa) and UT-A2 (35–55 kDa) signals were detected in kidney medulla, as expected, no UT-A signals were obtained in any of the gastrointestinal tissues. **b** Immunoblot of protein samples probed with a 1:1000 dilution of MQ2 antibody also displayed no signals in any of the gastrointestinal tissues investigated, despite the detection of UT-A3 (30–50 kDa) in the kidney medulla. *KM* kidney medulla; *pS* proximal stomach; *dS* distal stomach; *Du* duodenum; *Je* jejunum; *Il* ileum; *Ca* caecum; *pCo* proximal colon; *dCo* distal colon

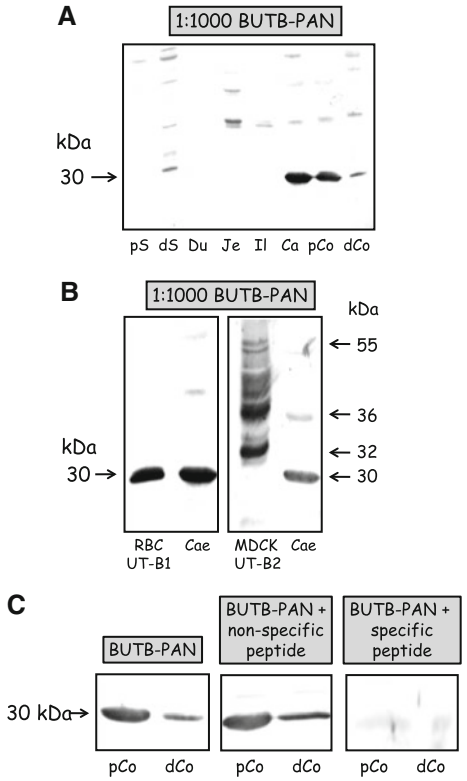


**Fig. 4** Immunoblot of rat gastrointestinal protein samples probed with a 1:1000 dilution of the UT-B antibody BUTB-PAN. **a** Strong 30-kDa UT-B signals were detected in protein samples of three gastrointestinal tissues—caecum, proximal colon, and distal colon. **b** The 30-kDa caecum signal was the same size as the UT-B1 signal detected in rat red blood cell protein, but different from the signals obtained in an MDCK cell line expressing the UT-B2 isoform. **c** The 30-kDa signal was abolished with prior incubation of BUTB-PAN antibody with its specific immunizing peptide (0.2 μg/μl), whereas no such effect was observed with incubation in a similar amount of a nonspecific peptide. *RBC* red blood cells; *pS* proximal stomach; *dS* distal stomach, *Du* duodenum, *Je* jejunum, *Il* ileum; *Ca* caecum; *pCo* proximal colon; *dCo* distal colon

these UT-B1 signals showed that their abundance was significantly higher in the caecum ($P < 0.01$, $n = 4$, ANOVA) and the proximal colon ($P < 0.01$, $n = 4$, ANOVA) compared to all the other gastrointestinal tissues (Fig. 5b). Therefore, a strong correlation exists between UT-B1 protein abundance and urea transporter mediated transepithelial urea flux (Table 2), as indicated by the data from the DMU and thiourea flux experiments.

Finally, gastrointestinal samples were probed with an antibody that detected the monocarboxylate transporter MCT1, namely a 1:1000 dilution of MCT12A. The antibody successfully detected a strong signal of the expected 45-kDa size in protein samples (Fig. 6a), again predominantly in the caecum and colon but also more weakly in the stomach. Similar results were obtained from protein samples taken from two further rats (data not shown). Interestingly, MCT1 protein abundance was significantly higher in the caecum ($P < 0.01$, $n = 3$, ANOVA) and the distal colon ($P < 0.05$, $n = 3$, ANOVA) (Fig. 6b), rather than in the proximal colon as was the case with UT-B1.

## Discussion

In this study, we investigated the role of facilitative urea transporters along the length of the rat gastrointestinal tract. Initial functional studies showed that urea transport varied greatly between different rat gastrointestinal tissues. More precisely, transepithelial flux experiments revealed that large-scale urea transport was present in the caecum/proximal colon region, but not in any other tissue (Fig. 1). This finding agrees with a previous report that detailed a larger urea clearance was present in rat proximal colon compared to rat distal colon (Fihn and Jodal 2001). The location of this large urea flux also agrees with a role in UNS because this process mainly occurs in the caecum/proximal colon as a result of the limited availability of undigested material in the distal colon.

Our data strongly suggest that the transepithelial urea flux present in caecum/proximal colon was mediated by facilitative urea transporters. First, it is known that these proteins can transport urea in either direction and urea flux was present in both apical-to-basolateral (Fig. 1a) and basolateral-to-apical (Fig. 1b) directions. Second, the large urea flux in the caecum/proximal colon was inhibited by the urea analogs DMU (Fig. 1) and thiourea (Fig. 2), both of which are known blockers of facilitative urea transporters (Zhao

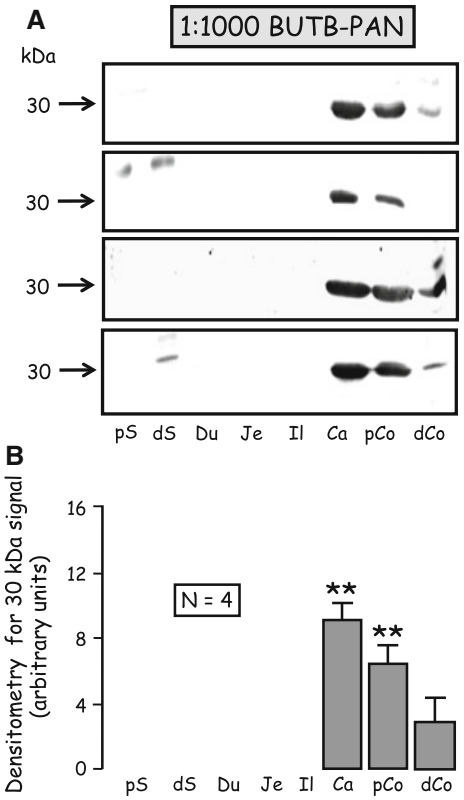


**Fig. 5** **a** Immunoblots of gastrointestinal protein samples from four rats probed with a 1:1000 dilution of BUTB-PAN. **b** Densitometry analysis of the 30-kDa UT-B1 signals obtained in the protein samples from the four rats showed that UT-B1 abundance was significantly higher in caecum and proximal colon. *pS* proximal stomach; *dS* distal stomach; *Du* duodenum; *Je* jejunum; *Il* ileum; *Ca* caecum; *pCo* proximal colon; *dCo* distal colon; $^{**}P < 0.01$ against UT-B1 abundance in other gastrointestinal tissues, ANOVA

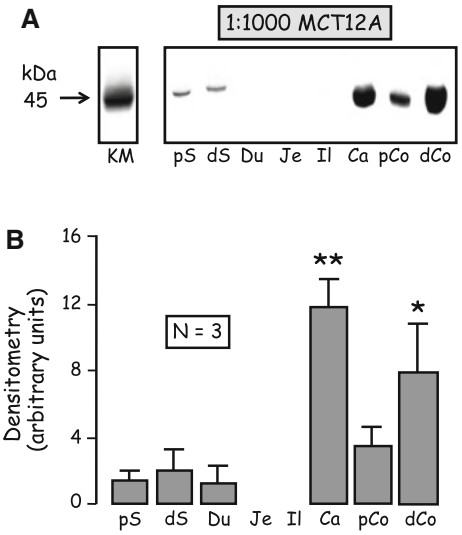


**Fig. 6** **a** Immunoblot analysis of gastrointestinal protein samples probed with a dilution of 1:1000 MCT12A antibody successfully detected a 45-kDa signal representing MCT1. **b** Densitometry analysis of 45-kDa immunoblot signals showed that MCT1 abundance was significantly higher in caecum and distal colon. *KM* kidney medulla; *pS* proximal stomach; *dS* distal stomach; *Du* duodenum; *Je* jejunum; *Il* ileum; *Ca* caecum; *pCo* proximal colon; *dCo* distal colon. $^{*}P < 0.05$, $^{**}P < 0.01$ against MCT1 abundance in other gastrointestinal tissues, ANOVA

et al. 2007). Furthermore, DMU was the more potent inhibitor, which was also previously reported for UT-B1-mediated urea transport in rat red blood cells (Zhao et al. 2007). Importantly, these urea analogs had no such inhibitory effects on the low basal levels of urea transport in the other segments of the gastrointestinal tract. However, because both UT-A and UT-B transporters have these functional characteristics, we investigated protein abundance levels to determine which group was responsible for the substantial transepithelial urea flux present in caecum/proximal colon tissue.

Although the UT-A antibodies strongly detected UT-A1, UT-A2, and UT-A3 in the kidney medulla positive control, no such signals were detected in the gastrointestinal tissues (Fig. 3). In contrast, the UT-B antibody BUTB-PAN detected a strong 30-kDa signal in protein samples from the caecum and proximal colon, while a weak signal was also present in distal colon (Fig. 4a). This 30-kDa signal was identical to that observed in the red blood cell lane (Fig. 4b), strongly suggesting that it represents UT-B1 urea transporters. It is also similar to the signal obtained for unglycosylated UT-B1 in rat kidney and brain in a previous study (Trinh-Trang-Tan et al. 2002). Further confirmation

**Table 2** Comparison of transporter-mediated urea flux and 30-kDa UT-B1 abundance in gastrointestinal tissue protein samples[a]

| Site | DMU-sensitive urea flux (A–B) | DMU-sensitive urea flux (B–A) | Thiourea-sensitive urea flux (A–B) | Thiourea-sensitive urea flux (B–A) | UT-B1 protein abundance |
|---|---|---|---|---|---|
| Stomach (proximal/distal) | 0.0 | 0.0 | – | – | 0.0 |
| Duodenum | 0.0 | 0.0 | – | – | 0.0 |
| Ileum | 0.0 | 0.0 | – | – | 0.0 |
| Caecum/proximal colon | 6.0 | 4.5 | 3.6 | 3.2 | 15.2 |
| Distal colon | 0.7 | 0.5 | 0.0 | 0.0 | 2.6 |

[a] *A–B* apical to basolateral (i.e., absorption), *B–A* basolateral-to apical (i.e., secretion)

that the 30-kDa signal does indeed represent a UT-B protein was obtained by the abolition of this signal in the presence of the original immunizing peptide, but not another nonspecific peptide (Fig. 4c). UT-B immunoblots were performed on samples from four rats in total (Fig. 5a), and densitometry analysis (Fig. 5b) confirmed that there was a strong correlation between 30 kDa UT-B1 protein abundance and transepithelial urea flux (Table 2).

The final set of immunoblots investigating the monocarboxylate transporter MCT1 (Fig. 6a) showed an abundance pattern similar to that previously reported in rat (i.e., caecum = colon > stomach > small intestine) (Kirat et al. 2009). MCT1 has been localized to the epithelial cells in rat colon (Iwanga et al. 2006) and as such may be involved in the transepithelial transport of the short chain fatty acids produced by UNS. Our finding that MCT1 abundance was significantly greater in distal colon (Fig. 6b) confirms that the differential colonic abundance pattern observed for UT-B1 is not due to variability in protein sample quality.

The results of this study show that UT-B rather than UT-A urea transporters are involved in the rat gastrointestinal UNS process. This confirms previous findings in other mammalian tissues in which large-scale UNS occurs—for example, in bovine rumen (Stewart et al. 2005). It is now known that UT-B transporters functionally differ from their UT-A counterparts in a number of key ways. Although UT-A is relatively impermeable to water (MacIver et al. 2008), UT-B proteins transport a significant amount of water (Yang and Verkman 2002). Estimations of the number of urea molecules transported per second per protein molecule also differ greatly, with UT-B ($\sim 5 \times 10^6$; Smith and Rousselet 2001) being $\sim$100-fold greater than UT-A ($\sim 5 \times 10^4$; MacIver et al. 2008). It has also been shown that while UT-A transporters can be acutely regulated by vasopressin via a second-messenger system (Stewart et al. 2009), UT-B proteins are unresponsive (Tickle et al. 2009). Hence, UT-A transporters are relatively selective, low-rate transporters that can be hormonally regulated—ideal for their role within the renal urinary concentration mechanism (Smith 2008). In contrast, UT-B proteins are relatively unselective, high-rate transporters, and it could be argued that a more accurate term for these proteins would be "UT-B channels." As such, UT-B proteins are well suited to enable rapid, large-scale movement of urea into the gastrointestinal tract.

The question still remains of how gastrointestinal UT-B transporters are regulated. Indirect evidence is now emerging from various mammalian species that it is likely to involve local changes in the intestinal pH affecting cellular UT-B localization. A recent study using sheep rumen determined that maximal urea transport was obtained in the presence of short chain fatty acids and a pH of 6.2 (cf. pH 7.4) (Abdoun et al. 2010). Because this transepithelial urea movement was sensitive to the urea transporter inhibitor phloretin, we may assume that it was mediated by UT-B proteins. Studies in bovine rumen have also shown that ruminal pH values of 6.2 (cf. pH 7.0) increased bUT-B2 abundance at the plasma membrane (Simmons et al. 2009). Our recent study in the human colon showed greater plasma membrane abundance of UT-B protein and functional urea transport occurred in ascending colon compared to descending colon (Collins et al. 2010). Because it has been reported that the pH values in these regions are $\sim$6.2 and $\sim$6.8, respectively (Nugent et al. 2001), we can see a strong pattern emerging across mammalian species where a pH of $\sim$6.2 enables maximal UT-B function. How do the findings of this study fit in with this general pattern? The functional heterogeneity of the rat colon is well established, and a recent report found the pH of the proximal colon to be $\sim$6.2, compared to a value of $\sim$7.3 for the distal colon (Talbot and Lytle 2010). We can therefore see that in the rat colon, as expected, a slightly acidic environment of pH $\sim$ 6.2 appears to lead to higher UT-B membrane abundance and hence greater UT-B-mediated transepithelial urea transport. Future studies must now concentrate on investigating this apparent mechanism of pH regulation of UT-B function.

Although the findings of this study strongly indicate that UT-B1 urea transporters play a functional role in UNS, the question remains as to the exact contribution of UNS to the overall nutritional balance of the rat. Although this has yet to be conclusively determined, some interesting observations can be inferred from studies in another rodent, the mouse. A previous report has shown that a similar 29-kDa unglycosylated UT-B protein exists in mouse colon (Lucien et al. 2005), although no comparison between proximal and distal colon was made. More interestingly, it has been noted that UT-B$^{-/-}$ knockout mice gained less weight than their wild-type littermates fed an identical diet (Yang and Bankir 2005). This intriguing observation suggests that removal of UT-B transporters reduces the capacity for UNS to occur, removing the beneficial effects of the symbiotic relationship with colonic bacteria and reducing the nutritional value of a given diet. This has important implications not only for our understanding of monogastric nutrition in general, but also to our future understanding of human health.

In conclusion, within the rat gastrointestinal tract, large transepithelial urea fluxes are present in caecum and proximal colon tissue. This regulated movement of urea occurs via UT-B1 facilitative urea transporters and is part of the UNS process that is thought to play an important role in mammalian nutrition.

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