Novel bUT-B2 urea transporter isoform is constitutively activated

P. Tickle, A. Thistlethwaite, C. P. Smith, and G. S. Stewart

1Faculty of Life Sciences, The University of Manchester, Manchester, UK; and 2School of Biology and Environmental Science, University College Dublin, Dublin, Ireland

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Tickle P, Thistlethwaite A, Smith CP, Stewart GS. Novel bUT-B2 urea transporter isoform is constitutively activated. Am J Physiol Regul Integr Comp Physiol 297: R323–R329, 2009. First published May 27, 2009; doi:10.1152/ajpregu.00199.2009.—Our previous studies have detailed a novel facilitative UT-B urea transporter isoform, bUT-B2. Despite the existence of mouse and human orthologs, the functional characteristics of UT-B2 remain undefined. In this report, we produced a stable MDCK cell line that expressed bUT-B2 protein and investigated the transepithelial urea flux across cultured cell monolayers. We observed a large basal urea flux that was significantly reduced by known inhibitors of facilitative urea transporters; 1,3-dimethylurea (P < 0.001, n = 17), thionicotinamide (P < 0.05, n = 11), and phloretin (P < 0.05, n = 9). Pre-exposure for 1 h to the antidiuretic hormone vasopressin had no effect on bUT-B2-mediated urea transport (NS, n = 3). Acute vasopressin exposure for up to 30 min also failed to elicit any transient response (NS, n = 9).

Further investigation confirmed that bUT-B2 function was not affected by alteration of intracellular cAMP (NS, n = 4), intracellular calcium (NS, n = 3), or protein kinase activity (NS, n = 4). Finally, immunoblot data suggested a possible role for glycosylation in regulating bUT-B2 function. In conclusion, this study showed that bUT-B2-mediated transepithelial urea transport was constitutively activated and unaffected by known regulators of renal UT-A urea transporters.

UT-B2 isoform; protein expression; rumen

MAMMALIAN FACILITATIVE UREA transporters allow the passage of urea across cell membranes, down a concentration gradient, in a phloretin-sensitive manner (21). They were first cloned from rabbit kidney medulla (32) and are derived from the UT-A (Slc14a2) and UT-B (Slc14a1) genes (21). In recent years the vital role of renal urea transporters in the urinary concentrating mechanism has become well established (3, 30, 31). In addition, UT-A and UT-B urea transporters have also been identified in the gastrointestinal tract of numerous species, including mice (14, 23), rats (10), humans (9, 21), cattle (16, 25), and sheep (15, 17, 20).

We have previously suggested that gastrointestinal urea transporters play a vital role in regulation of the urea nitrogen salvaging (UNS) process (26). During UNS urea from mammalian blood enters the gastrointestinal lumen and is broken down by bacterial urease into carbon dioxide and ammonia. The nitrogen from the ammonia can then be utilized by the bacterial population to synthesize the amino acids and peptides required for their growth (12). Importantly, these bacterially derived products and ammonia can also be reabsorbed by the mammalian host and used to maintain nitrogen balance (12). The mutually beneficial process of UNS is therefore vital to the symbiotic relationship between mammals and their gastrointestinal bacteria, especially in ruminants such as cattle and sheep.

In previous studies, we have shown that facilitative urea transporters mediate urea movement across bovine ruminal tissue (25) and that dietary regulation of ruminal urea transporter expression and epithelial localization occurs (28). These findings have illustrated the potential importance of facilitative urea transporters in the regulation of ruminate nitrogen balance. We have previously isolated a novel UT-B splice variant from bovine rumen, bUT-B2 (25), whose expression level is significantly altered by changes in dietary intake (28). No functional investigation of this bUT-B2 isoform has previously been performed, except for oocyte expression studies to confirm that it transported urea and was blocked by inhibitors of facilitative urea transporters (25). In this current study, we have therefore investigated the functional regulation of the bUT-B2 protein when stably expressed in a MDCK cell line.

MATERIALS AND METHODS

Antiserum. To study the expression of bUT-B2 protein, we used a anti-bUT-B2 polyclonal antibody, BUTB-PAN (28). This antibody was raised against an immunizing peptide that corresponded to amino acids of 421–439 in the COOH-terminal end of bUT-B2 (H2N-EEENRFYLOQSKRTVQGPL-COOH). To study the expression of rUT-A1, we used the previously characterized COOH-terminal mouse UT-A1 antibody ML194 (24).

MDCK-bUT-B2 cells. MDCK cells stably transfected with pFRT/lacZeo (termed “MDCK-FLZ” cells) were cultured as previously described (19). The bUT-B2 cDNA obtained from RT-PCR (25) was subcloned into pCDNA5/FRT (Invitrogen, Carlsbad, CA) and cotransfected with pOG44 into the MDCK-FLZ cells using an Amaxa nucleofector (Amamaxa, Cologne, Germany), according to the manufacturer’s instructions. Cells were selected with 300 μg/ml hygromycin after 24 h, and individual clones were isolated following 2 wk. Three bUT-B2 clone cell lines were isolated (GS1, GS2, and GS3), and protein expression was assessed using semiquantitative immunoblotting. Of the three original clones, one clone (GS3) was used for the majority of transepithelial flux experiments in the study and was termed MDCK-bUT-B2. In addition, the previously investigated MDCK-rUT-A1 and the basic MDCK-FLZ cell lines were also used for this functional study as positive and negative controls, respectively. Epithelial monolayers were cultured on semipermeable polyester supports (0.4 μm pore; Transwell, Corning, UK). Monolayers were fed fresh culture media on day 2 following seeding, then daily until use. The resistance of each monolayer was measured daily using an epithelial volt-ohmmeter resistance meter (World Precision Instruments). After becoming confluent, membranes developed a transmembrane resistance, and only membranes that showed sequential increases in resistance were used for flux experiments.

Transepithelial flux experiments. Urea flux experiments were performed as described by Frohlich and colleagues (7) at 37 ± 0.2°C in an apical-to-basolateral direction using 14C-labeled urea (0.8 μCi/ apical well) as the tracer. Transwell inserts containing epithelial monolayers were transferred from well to well at 3-min intervals, and the basolateral solution was collected for radioactivity measurements.
Baseline levels of flux were recorded from 4–6 wells containing Hanks’ balanced salt solution (HBSS) (GIBCO, Paisley, UK) and 5 mM urea, after which wells also contained test compounds (e.g., phloretin). For preincubation experiments, test compounds were added to the basolateral transwell chamber, and cells were incubated for 30 or 60 min at 37°C prior to the beginning of the experimental flux measurements. All test compounds were of certified grade and were made up either in sterile dH2O or anhydrous DMSO. Stocks were AVP, 100 μM; cyclopiazonic acid (CPA), 2.5 mM; BAPTA-AM, 100 mM; thionicotinamide, 100 mM; dimethylurea, 1 M; phloretin, 100 mM; PMA, 10 nM; 8-bromo-cAMP, 10 nM; and H89, 10 mM. Stocks were then diluted in HBSS before experimental use.

Immunoblotting. MDCK cells were washed twice in PBS and harvested using 0.05% trypsin-EDTA (GIBCO, UK), while bovine rumen papillae protein was obtained as previously described (25). Cell protein was homogenized with an automated homogenizer and specifically prepared buffer (300 mM mannitol, 12 mM HEPEs, pH 7.6). Homogenates were spun at 1,000 g at 4°C for 5 min, and the pelleted cellular debris was removed. Samples were then spun at 17,000 g for 20 min, and the pellet of plasma membrane-enriched protein was retained. (Note that for serial centrifugation experiments only, the resultant supernatant was subjected to one further spin at 100,000 g for 30 min—with the 100,000 g pellet produced enriched for intracellular membranes.) The total protein concentrations were determined using a Bio-Rad protein assay reagent kit (Bio-Rad, Hercules, CA). Deglycosylation experiments were undertaken by preincubating protein for 1 h at 37°C with and without the presence of PNGaseF enzyme (New England Biolabs, Ipswitch, MA). Five times reducing Laemmli sample buffer (5% SDS, 25% glycerol, 0.32 M Tris, pH 6.8, bromophenol blue, 5% beta-mercaptoethanol) was added to protein samples in a ratio of 1:4, and samples were then heated at 60°C for 15 min. Twenty micrograms of protein were run on a 12% SDS-polyacrylamide gel, and protein was transferred electrophoretically to a nitrocellulose membrane (Protran, Gelman Sciences, Northampton, UK). Membranes were blocked with 5% nonfat milk in washing buffer (15 mM Tris-HCl, 150 mM NaCl, pH 8.0, 0.01% Tween 20) and probed overnight with either BUTF-PAN (1:10,000), BUTB-PAN (1:10,000) preincubated for 2 h in 1 μg/ml specific immunizing peptide, or ML194 (1:1,000) antibody. After 3 × 10 min washes in washing buffer, 1 h incubation in HRP-linked anti-chicken (Aves Labs, Tigard, OR) or anti-rabbit secondary (Dako, Glostrup, Denmark) antibody, respectively was performed. Further 3 × 10 min washes in washing buffer were followed by signal detection using a chemiluminescence kit (EZ-ECL, Geneflow, Staffordshire, UK) and ECL film (GE Healthcare, Staffordshire, UK). Images of developed film were captured with an Image Reader LAS-1000 package.

Statistical analysis. All data values are shown as mean averages ± SE, with N representing the number of experiments and n representing the number of transwells tested. One-way ANOVA was used, with groups deemed statistically significant if P < 0.05 using Tukey post hoc test (Instat, GraphPad Software, San Diego, CA).

RESULTS

Initial immunoblot analysis was performed using bovine blood and rumen protein samples and the anti-bUT-B antibody BUTF-PAN (see Fig. 1A). Strong signals of 30–50 kDa in bovine blood and 30–55 kDa in bovine rumen were all completely ablated by preincubation with the immunizing peptide, suggesting BUTF-PAN specifically detected bUT-B proteins. Further immunoblots using protein from MDCK-FLZ and bUT-B2 cell line clones, GS1 and GS2 (see Fig. 1B) were then analyzed. Using BUTF-PAN, signals of 32 and 36–55 kDa were detected in GS1 and GS2 bUT-B2 clone protein but not in the protein sample from MDCK-FLZ cells. In addition, these bUT-B2 signals were not obtained when preimmune serum was used. Next, immunoblot analysis was performed using deglycosylated protein from bovine blood (containing bUT-B1 only) and the bUT-B2 clone GS3 (seeFig. 1C). For bUT-B1, a clear 40- to 50-kDa signal was deglycosylated to bands at 30
and 32 kDa after 1 h incubation with PNGaseF enzyme. In contrast, MDCK-bUT-B2 GS3 protein displayed the large 36–55 kDa smear previously observed in GS1 and GS2 protein, which was deglycosylated to 32- and 36-kDa signals. Taken altogether, these data illustrated that all bUT-B2 clones successfully expressed glycosylated bUT-B protein that was specifically detected by the BUTB-PAN antibody.

Next, we investigated bUT-B2-mediated urea transport using bUT-B2 clone cells mounted on transwell supports. Transepithelial 14C-labeled urea flux experiments revealed that under basal conditions, bUT-B2 monolayers displayed a high level of urea transport, which was reversibly inhibited by 50 mM 1,3-dimethylurea (DMU) (see Fig. 2A). Importantly, no such DMU-sensitive urea transport was present in the original untransfected MDCK-FLZ cells. Compared with MDCK-FLZ controls, significantly greater DMU-sensitive transepithelial urea flux was present in all three bUT-B2 clones—GS1 (P < 0.001, n = 3), GS2 (P < 0.01, n = 3) and GS3 (P < 0.001, n = 3) (see Fig. 2B). Furthermore, GS3 flux was greater than GS2 flux (P < 0.01), and immunoblot analysis showed that this occurred along with an apparent increased glycosylation of bUT-B2 protein in the GS3 cells (see Fig. 2C). Because it produced the largest DMU-sensitive urea flux, the GS3 clone was used for all remaining experiments and, henceforth, was termed MDCK-bUT-B2. As predicted from previous studies (25), bUT-B2-mediated urea flux was inhibited by a range of known urea transporter inhibitors (see Fig. 2D). The largest inhibition was observed with 50 mM DMU (P < 0.001, n = 17). In addition, both 2 mM thionicotinamide (P < 0.05, n = 11) and 1 mM phloretin (P < 0.05, n = 9) also significantly reduced bUT-B2-mediated urea transport.

Investigation of the % inhibition obtained with a range of DMU concentrations (1 to 200 mM) revealed that bUT-B2 was less sensitive than rUT-A1 to 10 mM DMU (P < 0.001, n = 3) (see Fig. 3A). These data also illustrated that maximal inhibition of bUT-B2 was obtained with 50 mM DMU. Similar experiments detailing the % inhibition obtained with different phloretin concentrations (0.1 to 1 mM) showed that bUT-B2 was less sensitive to 0.5 and 1 mM phloretin than rUT-A1 (P < 0.01, n = 3) (see Fig. 3B). In agreement with data shown in Fig. 2D, the maximal inhibition of bUT-B2-mediated urea flux obtained with 1 mM phloretin was considerably less than that obtained with 50 mM DMU.

AVP is known to regulate the transport function of renal UT-A transporters (7, 18, 29). In this study, experiments revealed that preincubation for 1 h in 10−6 M AVP did not increase DMU-sensitive urea flux in MDCK-bUT-B2 monolayers (NS, n = 3) (see Fig. 4A). This was in contrast to the positive response obtained with MDCK-rUT-A1 (P < 0.001, n = 8), confirming that the lack of response in MDCK-bUT-B2 cells was not due to defective vasopressin signaling. The nonresponse of bUT-B2 was further confirmed by the unchanged signals observed in an immunoblot of MDCK-bUT-B2 plasma membrane-enriched protein samples from control and AVP-treated cells (see Fig. 4B). Again, this contrasted to a positive increase in plasma-membrane expression obtained for rUT-A1 in response to vasopressin, as expected from a previous report by Klein et al. (11). To investigate whether any transient response to vasopressin was occurring undetected, similar to that observed for mUT-A2 (18), MDCK-bUT-B2 cell monolayers were continuously monitored during 30 min of exposure to vasopressin (see Fig. 4C). Conclusively, bUT-B2 transport...
A: summary of the % inhibition obtained for bUT-B2-mediated and vasopressin-stimulated rUT-A1-mediated urea flux with 1 to 200 mM DMU (**p < 0.001 vs. rUT-A1 inhibition value, n = 3). B: summary of the % inhibition obtained for bUT-B2-mediated and vasopressin-stimulated rUT-A1-mediated urea flux with 0.1 to 1 mM phloretin (**p < 0.01 vs. rUT-A1 inhibition value, n = 3).

Fig. 3. A: summary of the % inhibition obtained for bUT-B2-mediated and vasopressin-stimulated rUT-A1-mediated urea flux with 1 to 200 mM DMU (**p < 0.001 vs. rUT-A1 inhibition value, n = 3). B: summary of the % inhibition obtained for bUT-B2-mediated and vasopressin-stimulated rUT-A1-mediated urea flux with 0.1 to 1 mM phloretin (**p < 0.01 vs. rUT-A1 inhibition value, n = 3).

Further investigation showed that bUT-B2-mediated transepithelial urea transport was not affected by altering intracellular calcium concentration; either by increasing it with 10 µM CPA (NS, n = 3) or by decreasing it with 30 µM BAPTA-AM (NS, n = 3) (data not shown). The effect of altering protein kinase activity was also studied. To stimulate PKC, MDCK-bUT-B2 monolayers underwent 30 min preincubation in 10 µM PMA, but this had no effect on the urea flux observed (NS, n = 4) (see Fig. 5). There was also no effect after preincubating for 30 min in a high 100-µM dose of H89 (NS, n = 3) (data not shown), which inhibits the activity of both PKA and PKC.

Finally, further immunoblot analysis of serially centrifuged MDCK-bUT-B2 and bovine ruminal protein was performed. These data revealed that the bUT-B2 signal in MDCK-bUT-B2 cells was predominantly within the plasma membrane-enriched 17,000 g protein sample (see Fig. 6A). This plasma membrane localization was also observed using bovine ruminal protein, in which the strong 36- to 55-kDa smear representing glycosylated bUT-B2 was again almost exclusively present in the plasma membrane-enriched sample. In contrast, only a 30-kDa signal that is known to represent an unglycosylated protein (28) was present within the intracellular membrane-enriched 100,000 g ruminal sample (see Fig. 6B).

DISCUSSION

In previous studies, we have shown that UT-B urea transporters are expressed in the bovine rumen and contribute towards transepithelial urea transport (25). More recently, we have demonstrated that bovine UT-B2 ruminal expression and localization are regulated by dietary intake (28), indicating an important role for this transporter in the urea nitrogen-salvaging process. To further investigate the functional regulation of bUT-B2, we have stably expressed this novel urea transporter isofrom in a MDCK cell line.

Initial immunoblot analysis confirmed the successful production of an anti-bUT-B antibody and MDCK cell lines that expressed bUT-B protein (see Fig. 1, A and B). Using the BUTB-PAN antibody, we showed that MDCK-bUT-B2 cells contained a 36- to 55-kDa signal that deglycosylated to 32- and 36-kDa proteins (see Fig. 1C). This 36-kDa signal is 4 to 6 kDa larger than the unglycosylated bUT-B1 signals obtained from bovine blood. Since bUT-B2 contains an additional 55 amino acids, this size difference is exactly as predicted and strongly indicates the 36-kDa band is bUT-B2. In contrast, the 32-kDa band is probably a form of bUT-B1 and suggests that bUT-B2 can easily be truncated to bUT-B1 within the cell by an as yet undetermined proteolytic processes.

Epithelial monolayer transport experiments demonstrated that all three bUT-B2 clones possess a large, basally active transepithelial urea flux (see Fig. 2). This confirms that in all three clones, functional bUT-B2 transporters are expressed on both apical and basolateral membranes, as previously observed in bovine rumen tissue (25). This bUT-B2-mediated urea transport was significantly inhibited by a range of known urea inhibitors. To further investigate the functional regulation of bUT-B2, we have stably expressed this novel urea transporter isofrom in a MDCK cell line.

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Vasopressin is the key regulator of renal urea transport and has been shown to affect all major renal UT-A isoforms when they are expressed in MDCK cell lines. Acute exposure to vasopressin causes a sustained functional increase in both UT-A1 (7) and UT-A3 (27, 29), while having a more transient effect on UT-A2 (19). In contrast, for bUT-B2-mediated urea flux, vasopressin had no significant effect (see Fig. 4, A and B). In addition, there was no transient increase with acute vaso-
pressin exposure (see Fig. 4, C and D) or with 8-bromo-cAMP. Interestingly, basal bUT-B2-mediated urea transport was generally comparable in size to vasopressin-stimulated rUT-A1-mediated urea transport, suggesting that bUT-B2 is not significantly regulated because it is, at least to some extent, constitutively activated. This is the first stable UT-B cell line to be produced, and this lack of vasopressin response was as predicted, since there is no evidence in vivo that UT-B is sensitive to vasopressin (2). Although there are no reports of vasopressin receptor expression within bovine rumen tissue, this result does suggest that the previously reported regulation of urea entry into the rumen by vasopressin (8) is not due to direct effects on the bUT-B2 transporter.

Further investigation failed to determine the exact signaling pathway mechanisms involved in regulating bUT-B2-mediated urea transport. Altering intracellular levels of calcium, either through CPA or BAPTA-AM had no effect. The lack of effect of PMA (see Fig. 5) and a high concentration of H89 showed that bUT-B2 function was not altered by PKA or PKC. This is again in direct contrast with previous reports for UT-A isoforms, in which urea transporter function could be regulated by calcium (7), PKA (27), and PKC (29).

Preliminary data do suggest a significant role for glycosylation in regulating bUT-B2 function through control of plasma membrane localization. Initial observations noted that the GS3 bUT-B2 clone appeared glycosylated to a greater extent than GS2 (see Fig. 2C), a clone with a smaller DMU-sensitive flux (see Fig. 2B). Serial centrifugation analysis also showed that glycosylated bUT-B protein was predominantly expressed in

Fig. 4. A: summary of the effect of 1 h preincubation with 10^{-6} M AVP on MDCK-bUT-B2 and MDCK-rUT-A1-mediated urea flux. AVP had no effect on bUT-B2, while significantly stimulating rUT-A1 (**P < 0.01, ***P < 0.001 vs. basal flux; +++P < 0.01 vs. between control and AVP-treated basal fluxes, n = 3 to 8). B: immunoblots showing that the expression of bUT-B2 in plasma membrane-enriched protein samples was unaffected by chronic exposure to 10^{-6} M AVP (detected with BUTB-PAN antibody), while expression of rUT-A1 was increased as expected (detected by ML194 antibody). C: example of the acute (up to 30 min) effect of 10^{-6} M AVP on MDCK-bUT-B2-mediated and MDCK-rUT-A1-mediated urea flux. D: summary of the effects of acute AVP exposure, showing the lack of effect on MDCK-bUT-B2-mediated urea transport compared with the stimulation of MDCK-rUT-A1 mediated urea transport (***P < 0.001 increase vs. basal flux, n = 9).

Fig. 5. Summary of the lack of effect on MDCK-bUT-B2 urea flux of 30-min preincubation with 10 μM PMA (*P < 0.05 vs. basal flux, n = 4).

Fig. 6. A: immunoblot analysis of MDCK-bUT-B2 protein using BUTB-PAN antibody showed that bUT-B2 signals were predominantly found in the 17,000 g plasma membrane-enriched sample rather than 100,000 g intracellular membrane-enriched sample. B: expression of BUT-B signals in ruminal protein detected with BUTB-PAN was also predominantly within the 17,000 g plasma membrane-enriched sample, with only a 30-kDa unglycosylated signal present within intracellular membrane-enriched protein. (Key: 17 = 17,000 g pellet, containing plasma membrane-enriched protein; 100 = 100,000 g pellet, containing intracellular membrane-enriched protein.)
plasma membrane-enriched samples of both MDCK-bUT-B2 and bovine ruminal protein samples (see Fig. 6). A previous report described how glycosylation was vital to plasma membrane localization and, hence, function of rat UT-A1 (4). The authors of this study suggested that unglycosylated UT-A protein was less stable in the membrane and more likely to be broken down within the endoplasmic reticulum (4). Although no such effects have been reported for UT-B, this has so far only been investigated for human UT-B1 in an oocyte expression system (13) and not a mammalian cell line. Future research should, therefore, concentrate on the role of glycosylation in bUT-B2 cellular localization (i.e., mutational analysis of the predicted glycosylation site at Asn 206 within a stable cell line, etc.). It is certainly interesting to note that a recent study has described variation in the glycosylation state of UT-B expressed along the gastrointestinal tract of lambs (15), rather than in protein expression level per se. It, therefore, appears highly plausible that glycosylation plays a significant role in the functional regulation of gastrointestinal UT-B urea transporters in a number of species.

The lack of substantial short-term regulation of the constitutively activated bUT-B2 transporter indicates that protein expression level may be the key determinant in controlling the function of this ruminal urea transport mechanism. Extensive studies of the reported dietary regulation of expression level must now be undertaken to understand the long-term regulation of bUT-B2-mediated urea transport in the ruminal epithelium. In addition, further transepithelial flux experiments using bovine ruminal tissue (25) must also be performed to confirm the results obtained from this MDCK-bUT-B2 cell line. Using both native tissue and the MDCK expression system, researchers must now investigate the effects of known regulators of ruminal urea entry, such as ammonia, propionate, and carbon dioxide (1). The effect of butyrate should also be investigated, as prolonged exposure to increased concentrations of butyrate have previously been shown to increase ruminal urea entry (18). In addition, butyrate has been recently shown to alter expression levels of another membrane transporter, the peptide transporter PepT1, within the Caco-2 intestinal cell line (5). Since the levels of butyrate produced by ruminal bacterial fermentation are known to greatly vary with dietary intake, it would seem an ideal candidate by which UT-B2 expression, and hence ruminal urea entry, could be controlled to meet bacterial nitrogen demand. Finally, further work should also include production of a MDCK-bUT-B1 cell line to 1) confirm the lack of vasopressin sensitivity of the renal bUT-B1 isoform, and 2) compare both bUT-B isoforms to determine the functional significance of the additional 55 amino acids at the NH2 terminal of bUT-B2.

More generally, the significance of the bUT-B2 isoform being constitutively activated is now greater after the recent identification of this isoform in species and tissues other than the bovine rumen. For example, a 440 amino acid protein (GenBank accession no. BAE32238.1) representing mouse UT-B2 has been identified in thymus tissue, while a 445 amino acid protein (GenBank accession no. BAG52274.1) representing human UT-B2 has been located in caudate nucleus tissue. There is also emerging evidence for the existence of additional UT-B isoforms that also contain the 55-amino acid NH2-terminal sequence found in UT-B2—for example, within a pooled sample of human stomach, colon, and kidney (GenBank accession no. BC040128). The precise physiological roles for bUT-B2 and these other transporters are as yet unknown. However, investigation into their identity, localization, and functional significance will be an area for future focused research that should greatly expand our current understanding of gastrointestinal UT-B urea transporters.

**Perspectives and Significance**

This study is the first to report expression of a UT-B urea transporter in a stable cell line. The ruminal bUT-B2 transporter is constitutively activated in its basal state and is not significantly regulated by vasopressin, cAMP, calcium, or protein kinases. These findings provide novel information regarding the regulation of the UT-B2 urea transporter isoform. In addition, this study enhances our understanding of urea entry into the bovine gastrointestinal tract during UNS and as such has important implications for understanding bovine nutritional balance. Furthermore, our findings suggest more generally that gastrointestinal UT-B urea transporters are not regulated in a manner similar to that of renal UT-A urea transporters. Further work is now required to determine exactly how gastrointestinal UT-B function is controlled, particularly in reference to long-term regulation of protein expression level and the importance of glycosylation to cellular localization.

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