UT-B1 urea transporter is expressed along the urinary and gastrointestinal tracts of the mouse


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UT-B1 urea transporter is expressed along the urinary and gastrointestinal tracts of the mouse. Am J Physiol Regul Integr Comp Physiol 288: R1046–R1056, 2005. First published November 24, 2004; doi:10.1152/ajpregu.00286.2004.—Selective transporters account for rapid urea transport across plasma membranes of several cell types. UT-B1 urea transporter is widely distributed in rat and human tissues. Because mice exhibit high urea turnover and are the preferred species for gene engineering, we have delineated UT-B1 tissue expression in murine tissues. A cDNA was cloned from BALB/c mouse kidney, encoding a polypeptide that differed from C57BL/6 mouse UT-B1 by one residue (Val-8-Ala). UT-B1 mRNA was detected by RT-PCR in brain, kidney, bladder, testis, lung, spleen, and digestive tract (liver, stomach, jejunum, colon). Northern blotting revealed seven UT-B1 transcripts in mouse tissues. Immunoblots identified a nonglycosylated UT-B1 protein of 29 kDa in most tissues and of 36 and 32 kDa in testis and liver, respectively. UT-B1 protein of gastrointestinal tract did not undergo N-glycosylation. Immunohistochemistry and in situ hybridization localized UT-B1 in urinary tract urothelium (papillary surface, ureter, bladder, and urethra), prominently on plasma membranes and restricted to the basolateral area in umbrella cells. UT-B1 was found in endothelial cells of descending vasa recta in kidney medulla and in astrocyte processes in brain. Dehydration induced by water deprivation for 2 days caused a tissue-specific decrease in UT-B1 abundance in the urinary bladder and the ureter.

cDNA; immunohistochemistry; urothelium; water deprivation

IT IS NOW WELL ESTABLISHED that urea crosses cell plasma membranes through specific urea transporters, in addition to lipid bilayer passive diffusion. Urea transporters have been cloned mainly in humans and rats (2, 14, 22, 28, 29, 40, 45) and recently in mice (10, 43). They are allocated to two families (26). The UT-A family includes six isoforms, which are encoded by a single gene (Slc14a2). Four of them (UT-A1-UT-A4) were mainly assigned to kidney medulla, with a role in urea sequestration for urine concentration (25, 30, 36, 39). UT-A5 and UT-A6 are found, respectively, in the testis and the colon (9, 29). The UT-B family so far comprises only one transporter, encoded by a different gene (Slc14a1), whose disruption in mice resulted in a selective urea concentrating defect (43), and testicular hypertrophy (44). UT-B1 was originally cloned as hUT11, the red blood cell urea transporter carried by the Kidd blood group locus (21, 22). It has a wide tissue distribution in humans and rats (20, 23, 35, 38, 40) with, however, species and tissue differences in transcript and protein expressions. Indeed, UT-B1 transcripts were found in rat but not in human lung, liver, and spleen and in human but not rat skeletal muscle (20, 23, 35, 40). In rat, UT-B1 transcripts were repeatedly found in kidney, brain, and testis, whereas divergent findings have been reported pertaining to heart, lung, liver, small intestine, and colon (23, 35, 40). Their pathophysiological regulation has been predominantly examined in rat (see review in Ref. 24).

It has been stressed recently that the turnover of urea is much higher in mice than in rats, as indicated by much higher daily excretion of urea with respect to body weight or whole body urea pool (3). Accordingly, urea handling presumably requires adapted urea transport between body compartments. Recently, UT-A mRNA transcripts were reported in mouse kidney and several extrarenal tissues (testis, heart, brain, and colon) (10, 33). UT-B1 mRNA transcripts have been reported in mouse brain, kidney, spleen, urinary bladder but were not found in testis, small intestine, and colon (43). Regarding the protein, its tissue distribution in mice is poorly documented, and its regulation was restricted to the kidney (13).

In the present study, we isolated a cDNA encoding mouse UT-B1 and identified the different transcripts and protein isoforms in various mouse tissues. We disclosed that UT-B1 is present in the urothelium of all urinary tract parts and in several segments of the gastrointestinal tract. Finally, we found that 48-h thirsting decreased UT-B1 expression in the urinary bladder and the ureter and did not alter that in the kidney.

MATERIALS AND METHODS

PCR amplification of mouse UT-B1 cDNA. Specific mouse sense primer (SP-1, 5'-GCCTGTATGGCACACCTGATGGCTGTG-3') and anti-sense primer (AS-1, 5'-CCAGGTACAAGGTGGCAGGT-GAAC-3'), which correspond to mouse residues 319–327 (AC-MAHLMAV) and 328–335 (VHLPACTW), respectively, were deduced from BLAST search between human urea transporter hUT-B1 (accession no. Y19039) and GenBank mouse expressed sequence tag entries using Gapped BLAST and PSI-BLAST programs (1). Mouse kidney Marathon-Ready cDNA premade from whole kidneys pooled from BALB/c males (Clontech Laboratories, Palo Alto, CA) was used as template to amplify the 5’ part of mUT-B1 (where m is mouse) cDNA by hemi-nested PCR amplification between two adaptor primers (API and AP2) and AS-1 primer according to the manufacturer’s instructions. Similarly, the 3’ part of mUT-B1 cDNA was amplified between SP-1 primer and two adaptors primers API and AP2. Amplification products were subcloned and sequenced on both strands using an ABI Prism 310 genetic analyzer (Applied Biosystems, Foster City, CA). Sequence entries using Gapped BLAST and PSI-BLAST programs (1).

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City, CA). In a second step, full-length mUT-B1 cDNA was PCR amplified from mouse kidney Marathon-Ready cDNA between SP-2 (5’-ATAGGCCATGGAAGATAGTCCCAC-3’) and AS-2 (5’-GGCCGTTCTTACAGGGGGGC-3’), deduced from the 3’ and 5’ parts of the mUT-B1 sequence and subcloned into the EcoRV-digested pT7TS vector before sequencing control. All primers were synthesized by Genset (Paris, France). Expand high-fidelity PCR system (Roche Applied Sciences, Meylan, France) was used for DNA amplification.

Animals and tissues. The experimental protocols were approved by the Animal Care Committee of Paris 5 University. Adult male C57BL/6 mice (Charles River, L’Arbresle, France) were used. The animals were killed by cervical dislocation or an overdose of pentobarbital sodium. Organs (lung, brain, spleen, heart, gastrocnemius muscle, seminal vesicles, testis, urinary bladder, kidney, liver, stomach, jejunum, and colon) were immediately harvested and placed in ice-cold PBS. Cerebrum, cerebellum, and brain stem were collected from the encephalon. The cortex, the outer and inner stripe of outer medulla, and the inner medulla were dissected from the kidney slices.

A fragment of right ventricle was sampled from the heart. Data were collected on a minimum of three mice.

Dehydration protocol. Male mice were randomly placed in groups of four or five mice per cage, with free access to food and water. Dehydration was achieved by withdrawing the drinking water bottle for 48 h. Because thirsted mice did not void urine, urine osmolality was not measured. Hence, dehydration was ascertained by body weight loss and increased plasma osmolality.

RNA isolation. Tissues fragments were homogenized in ice-cold RNA NOW reagent (Biogentex, Seabrook, TX), and total RNA was extracted according to the manufacturer’s protocol. For quantitative Northern blot analysis of UT-B1 mRNA in urinary bladder, total RNA was isolated using Versagene RNA isolation. Tissues fragments were homogenized in ice-cold PBS. Cerebrum, cerebellum, and brain stem were collected from the encephalon. The cortex, the outer and inner stripe of outer medulla, and the inner medulla were dissected from the kidney slices. A fragment of right ventricle was sampled from the heart. Data were collected on a minimum of three mice.

Reverse transcription and PCR. Total RNA (0.1 μg) was used for the first PCR (Titan One tube RT-PCR system, Roche Diagnostics, Mannheim, Germany) [50°C for 30 min (1 cycle); 94°C for 2 min (1 cycle); 94°C for 30 s, 60°C for 30 s, 72°C for 1 min (30 cycles); 72°C for 7 min (1 cycle)] between primers SP-1 (positions -396 to -413, exon 6) and AS-2 (positions -1197 to -1218, exon 11). A nested PCR was then carried out with one-tenth of the first reaction between primer SP-3 (positions -524 to -545, exon 7) and AS-4 (positions -973 to -993, exon 9), using Expand high-fidelity PCR system (Roche Diagnostics). For primer designation, position +1 refers to the first nucleotide of the initiation codon the mUT-B1 gene (GenBank accession no. AJ420967).

Northern blot analysis. UT-B1 mRNA was revealed by the PCR amplified full-length mUT-B1 cDNA probe (1,170 bp). The probe was labeled with [α-32P]dCTP (Dupont-NEN) using a random primed labeling kit (Roche Applied Sciences). Total RNA (5–15 μg) was denatured and resolved in a 1% formaldehyde-agarose gel. Preservation of RNAs was controlled by visualization, under UV light, of the 28S and 18S ribosomal RNA bands. RNAs were transferred overnight by capillary blotting to nitrocellulose membranes (Hybond C-Extra, Amersham, Les Ulis, France). Membranes were baked in a vacuum oven (2 h, 80°C) and hybridized at high stringency with radiolabeled probe. Membranes were exposed to autoradiographic films after a final wash in buffer containing 0.1× SSPE, 0.1% SDS at 55°C.

In situ hybridization. In situ hybridization was performed as previously described (15). Deparaffinized 5-μm-thick sections were digested for 15 min at 37°C with 15 μg/ml protease K (Sigma, St. Louis, MO) in a buffer containing 20 mM Tris-HCl, pH 7.5, and 2.5 mM CaCl2. The UT-B1 cDNA probe was labeled with [35S]dCTP using Megaprime DNA labeling system (Amersham) to 5.108 cpm/μg DNA. Hybridization with UT-B1 probe (0.2 μg/ml) was performed overnight at 45°C in 40% formamide, 4× SSC, 10% dextran, 1× Denhardt, 0.8 mg/ml yeast tRNAs, and 0.5 mg/ml denatured salmon sperm DNA. Sections were washed twice at 45°C for 15 min in 40% formamide and 4× SSC, once at 60°C for 30 min in 4× SSC, and three times at room temperature for 20 min in 2× SSC.
dehydration, sections were dipped into NTBII autoradiographic emulsion (Kodak, Rochester, NY) and exposed at 4°C for 12–28 days. Slides were finally immersed in D19 developer (Kodak), fixed in A44 Kodak, and counterstained with hematoxylin and eosin. For negative controls, sections were treated by RNase A (Sigma) at 50 μg/ml at 37°C for 30 min before the hybridization or were incubated with the nonrelevant 35S-labeled cDNA probe to human renin (5).

**Antibodies.** UT-B was detected by an affinity-purified rabbit polyclonal antibody against the carboxy terminus (residues 365–384) of rat UT-B1. This antibody has been shown to cross-react with murine UT-B1 (38).

**Western blot analysis.** Tissue fragments were thoroughly homogenized (Ultra-Turrax) in ice-cold lysis buffer (250 mmol/l sucrose, 10 mmol/l triethanolamine, pH 7.6) containing protease inhibitors (Complete Mini EDTA-free protease inhibitor cocktail tablets; Roche Diagnostics). Protein concentrations were determined by the Bradford method (Bio-Rad, Hercules, CA). Samples were then solubilized in Laemmli buffer and heated at 65°C for 10 min before loading. Twenty micrograms of protein were separated by SDS-PAGE (10%) and transferred to polyvinylidene difluoride membranes. Equal protein loading was checked by coloration of the polyvinylidene difluoride membranes with Ponceau red. Blots were blocked for 45 min at room temperature with PBS containing 5% nonfat dry milk, followed by incubation with the primary antibody (0.25 μg/ml) for 2 h at room temperature. The membranes were then thoroughly washed and incubated for 60 min with a goat peroxidase-conjugated anti-rabbit IgG polyclonal antibody (0.2 μg/ml) (Promega, Madison, WI). Bands were visualized on Hyperfilm-ECL (Amersham) by chemiluminescence (ECL+; NEN, Boston, MA). Apparent molecular weights were determined using prestained protein marker, broad range (New England Biolabs, Beverly, MA).

**Protein N-deglycosylation.** Twenty micrograms of proteins were incubated for 1 h at 37°C in a buffer containing 0.5% SDS, 1% NP-40, 1% β-mercaptoethanol, 50 mM sodium phosphate, pH 7.5, and 5 units N-glycosidase F (Roche Applied Science). Control samples were run simultaneously in enzyme-free buffer in otherwise identical conditions.

**Immunohistochemistry.** Kidneys, ureters, urinary bladder, and urethra were fixed in formalin and embedded in paraffin. Deparaffinized 5-μm-thick sections were heated at 98°C for 30 min in target retrieval solution buffer (Dako, Trappes, France) for antigen retrieval. Endogenous peroxidase, avidin, and biotin activities were blocked with 0.3% H2O2, avidin, and biotin blockers (Dako), respectively. Anti-UT-B1 polyclonal antibody was then incubated at 1:100 dilution for 90 min at room temperature. Preimmune rabbit serum at the same dilution was used as negative control. Secondary biotinylated anti-rabbit IgG antibody (Amersham, Les Ulis, France) was used at 1:400 for 30 min. Streptavidin-peroxidase amplification was then performed with the Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA) according to the manufacturer’s instructions with diaminobenzidine as substrate (Dako). Sections were counterstained with hematoxylin.

Confocal laser microscopy was used to assess colocalization of UT-B1 and the endothelial marker CD34 on kidney sections. Combined immunofluorescence was performed as follows: after antigen retrieval as described above, the kidney sections were first incubated with the anti-UT-B1 polyclonal antibody revealed by biotinylated anti-rabbit IgGs and streptavidin-cyanin-2 (Amersham). The sections were then incubated with anti-human CD34 monoclonal antibody (Dako), which cross-reacts with mouse tissue, diluted at 1:25, and revealed by using cyanin-3-labeled anti-mouse IgG (Amersham). For

![Fig. 2. Reverse transcription-PCR detection of UT-B1 in murine tissues (lung, cerebrum, spleen, testis, urinary bladder, whole kidney, liver, colon, jejunum, and stomach). RNA was replaced by water in control amplification.](image-url)

![Fig. 3. Northern blot analysis of UT-B1 in mouse tissues. Fifteen micrograms of total RNA were separated on 1% agarose gels and transferred to nitrocellulose membranes. Membranes were hybridized with 32P-labeled full-length mUT-B1 cDNA. Left: presence of UT-B1 transcripts in urinary bladder and in kidney medulla zones [inner medulla and the inner stripe of outer medulla (ISOM)]. The cortex and the outer stripe (OSOM) of outer medulla are devoid of UT-B1 transcripts. Right: several transcripts are identified in testis, and the 3 parts of brain, with highest expression in the cerebellum. No transcript was found in digestive tract, spleen, gastrocnemius muscle, heart, and lung.](image-url)
negative controls, the primary antibodies were replaced by either preimmune rabbit serum or normal mouse IgG (Dako) at the concentrations of the primary antibodies. We observed sections using a Leica TCS SP confocal microscope (Leica Microsystems, Heidelberg, Germany).

For brain immunofluorescence studies, mice were anesthetized with ethyl carbamate (1.25 g/kg ip) and transcardially perfused with 4% paraformaldehyde in PBS (0.7 ml/min for 20 min). Brains were immersed in the fixative solution for 2 h at 4°C. Coronal sections (25 μm thick) were cut on a vibratome (Leica VT 1,000 S) and collected in PBS. PBS containing 0.1% Triton X-100 and 1% BSA (PBS-TBSA) was used for antibody dilution and washing. In the first step, free floating sections were rinsed three times in PBS for 5 min and incubated in PBS-TBSA for 30 min to block nonspecific binding sites. The sections were then incubated with UT-B1 antiserum or preimmune serum (dilution 1:100) overnight at 4°C. After three washes in PBS-TBSA, they were incubated for 2 h in the dark at room temperature with CY3-coupled sheep anti-rabbit IgGs (dilution 1:300) as secondary antibody. Glial fibrillary acid protein (GFAP) was used as a glial marker. It was revealed by an anti-mouse GFAP rabbit polyclonal antibody (dilution 1:200) and FITC-coupled goat anti-rabbit IgG (dilution 1:300) as secondary antibody. Sections were mounted in anti-quenching medium (Vectashield H-100; Vector Laboratories) and examined under laser-confocal microscope (Zeiss LSM 510).

Statistical analysis. Data are expressed as means ± SE. Groups were compared with Student’s t-test. Statistical significance was considered for P < 0.05.

RESULTS

Cloning of mouse UT-B1 transporter. Alignment of the deduced amino acid sequence with those of the other mouse and rat UT-B1 transporters revealed that the mUT-B1 from BALB/c encoded a polypeptide of 384 amino acids that differed by only a valine at position 8 instead of an alanine in mUT-B1 from C57BL/6 mice as previously reported (43) and exhibited 93% similarity with the rUT-B1 (Fig. 1). The extracellular N-glycosylation site (Asn-206) and the potential protein kinase C phosphorylation site (Ser-19) were conserved at the same positions.

Identification of tissues expressing mUT-B1 by RT-PCR. A single band of expected size (467 bp) was found in lung, cerebrum, spleen, testis, urinary bladder, kidney, liver, colon, jejunum, and stomach. No PCR products were found in heart, gastrocnemius skeletal muscle, seminal vesicles, or when ARN was omitted and replaced by water (Fig. 2).

Northern analysis of UT-B1 mRNA transcripts. Transcripts were detected in urinary bladder, kidney, testis, and brain in keeping with RT-PCR findings (Fig. 3 and Table 1). In the urinary bladder and kidney, three transcripts of 4.4, 2.8, and 1.7 kb were found. In kidney, these transcripts were not detected in the cortex and outer stripe of the outer medulla but were found in the inner stripe of the outer medulla and in the inner medulla. Among all tissues, testis had by far the highest abundance in UT-B1 mRNA, found as three transcripts (4.4, 2.8, and 1.7 kb).
2.1, and 1.4 kb). Within the central nervous system, two transcripts of 4.0 and 2.8 kb were easily detected, with the highest expression in cerebellum (Fig. 3). An additional transcript of 2.0 kb was visible in cerebellum under scrutiny. In seminal vesicles, liver, stomach, jejunum, colon, spleen, skeletal muscle, heart, and lung, no UT-B1 transcripts were detected even after prolonged exposure (Fig. 3).

**Characterization of UT-B1 proteins by immunoblotting.** UT-B1 proteins were revealed as several bands in lung, brain, spleen, skeletal muscle, testis, and kidney (Fig. 4 and Table 1). Along the gastrointestinal tract, distinct bands were visible in colon, jejunum, and stomach. In heart, bladder, and liver, multiple faint bands were found (Fig. 4). The fading of these bands after preadsorption of the antibody with the immunizing peptide confirmed their specificity (Fig. 5). It should be pointed out that, in the testis, among the three bands revealed, only the weakest one of 38 kDa was abated after preadsorption and was hence specific (Fig. 5B).

Immunoblotting after protein N-deglycosylation revealed an unglycosylated form with an apparent molecular mass of 29 kDa in most nonerythroid tissues (kidney, urinary bladder, cerebrum, and spleen) as in red blood cells, used as control (Fig. 6). This band was also present in gastrocnemius muscle, colon, and stomach and did not change after N-glycosidase F treatment. It should be stressed that in urinary bladder and liver, UT-B1 protein was barely visible due to multiple extents of glycosylation and could be easily observed after N-deglycosylation as 29- and 32-kDa bands, respectively. As concerns the jejunum, it was not possible to determine whether the 25-kDa protein (abated by antibody preadsorption, see Fig. 5) was a glycosylated form because, for unknown reasons, it was deleted after incubation in deglycosylation medium (not shown). Regarding testis, the specific band of ~38 kDa fell to 36 kDa (arrowhead) after enzyme treatment.

**Immunohistochemistry and in situ hybridization.** In murine kidney, UT-B1 protein and mRNA were detected in the microvessels of the inner and outer medulla (Fig. 7). The strongest signal was observed in the inner medulla where thin vascular structures, gathered in groups of two to four, were intensely labeled (Fig. 7, A and B). However, not all the microvessels were positive, as clearly shown by double immu-

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**Fig. 5.** Determination of the band specificity. Immunoblots shown in A and B were carried out simultaneously (20 μg of protein loaded). A: incubation with purified rUT-B1 antibody (0.25 μg/ml). B: incubation with rUT-B1 antibody (same concentration as in A) preabsorbed with excess immunizing peptide (10-to-1 molar ratio). Bands in A were abated in B, except for 2 intense nonspecific bands in testis. Apparent molecular weights are indicated on the right.

**Fig. 6.** Deglycosylation of UT-B1 in mouse tissues. Each lane was loaded with 20 μg of protein, except that of red blood cell membranes (RBCs), which were loaded with 2 μg of membrane protein. Top: N-glycosidase F (PNGase F) treatment revealed an intense 29-kDa band corresponding to the unglycosylated form of UT-B1 in RBCs, kidney, urinary bladder, cerebrum, and spleen. Bottom: PNGase F treatment did not affect the 29-kDa band found in gastrocnemius muscle, colon, and stomach, nor that of the 32-kDa band found in the liver (arrowhead), indicating that UT-B1 is expressed solely as nonglycosylated protein in these tissues. In testis, UT-B1 band was slightly lowered to 36 kDa (arrowhead) after enzyme treatment.
nofluorescence confocal microscopy (Fig. 7, E–H). In the inner stripe of the outer medulla, the signal was unevenly distributed, solely present in the vascular bundles (Fig. 7, I and J). Here again, the labeling was restricted to a part of the microvessels, the narrow vasa recta reminiscent of descending arterial vasa recta. In all of these layers, immunohistochemistry at high magnification and UT-B1-CD34 double-labeling immunofluorescence (Fig. 7, E–H and K) clearly showed that the signal was located in the membrane of endothelial cells. In the outer stripe of the outer medulla, only rare microvessels were labeled, and in the cortex no staining was found (data not shown). In situ hybridization revealed UT-B1 mRNA in microvessels in inner medulla and in vascular bundles in the outer medulla (Fig. 7, D and L). In negative control procedures, no signal was detected by either immunohistochemistry or in situ hybridization.

UT-B1 protein and mRNA were detected in the urothelium all along the urinary tract, including the papilla surface (Fig. 8, A–D), the ureter (Fig. 8, E–H), the bladder (Fig. 8, I–L), and the urethra (Fig. 8, M–P). The immunostaining was restricted to the basolateral membrane of the single-layer papillary surface epithelium in the kidney and to the uppermost cells of the multilayer epithelium of the urethra. In the ureter and the bladder, prominent UT-B1 immunostaining was visible at the periphery of the epithelial cells of intermediate and basal layers and was restricted to the basolateral membrane of umbrella cells. In negative control procedures, no signal was detected by either immunohistochemistry or in situ hybridization.

In the brain, staining was observed at the border of the corpus callosum and striatum (Fig. 9, A and B). At this border, high magnification revealed staining of cellular processes surrounding blood microvessels (Fig. 9C). The colocalization of
UT-B1 with GFAP identifies these processes as astrocytic processes (Fig. 9D).

Effect of dehydration on UT-B1 abundance in urinary bladder and ureter. Thirsted mice lost 15% body weight after 48-h water deprivation (from 22.8 ± 0.8 to 19.3 ± 0.8 g, n = 12). Concurrently, the body weight of control mice was unchanged (22.6 ± 0.14 and 22.7 ± 16 g, n = 12). The plasma osmolality of thirsted mice was significantly higher than those of control mice (338 ± 2 vs. 321 ± 8 mosmol/kgH₂O, n = 6, P < 0.05). Thirsting significantly decreased UT-B1 protein abundance in urinary bladder and ureter, by 43 and 38%, respectively (Fig. 10, A and B). The expression of UT-B1 transcripts (2.8 and 4.4 kb) in the urinary bladder was obviously abated in dehydrated mice, whereas that of β-actin was unchanged (Fig. 10C). Dehydration did not alter UT-B1 protein abundance in kidney medulla, colon, testis and brain (Fig. 11).
DISCUSSION

Because mouse is now the preferred species for gene manipulations, the features of its normal physiology deserve thorough examination to subsequently detect abnormal phenotypes resulting from gene disruption. Moreover, urea turnover is particularly high in mouse compared with rat, as recently pointed out (3). The present study reports the cloning of murine UT-B1 urea transporter and identifies the UT-B1 transcripts and proteins in numerous mouse tissues. Hence, the presence of UT-B1 proteins was revealed along the urinary tract from kidney to urethra and along the gastrointestinal tract (stomach, jejunum, and colon). Finally, we found that, in thirsted mouse, UT-B1 expression was downregulated in the urinary bladder and in the ureter, in terms of both protein and mRNA abundance, whereas it was unchanged in kidney, testis, brain, and colon.

The present cloning of UT-B1 cDNA from BALB/c mouse validates the previously reported cloning from C57BL/6 mouse (43). The single difference at position 8 (valine instead of alanine) likely arises from strain polymorphism. The presence of UT-B1 mRNA was shown by RT-PCR in the lung, brain, spleen, testis, urinary bladder, kidney, liver, colon, jejunum, and stomach of the mouse. UT-B1 mRNA was not found here in mouse heart, skeletal muscle, and seminal vesicles by either RT-PCR or Northern blot. By contrast, a UT-B1 transcript was revealed in human and rat heart (35). Seven different transcripts were found here in mouse, compared with five in human (20, 22) and a single one in rat (23, 40) (Table 1). The sizes of UT-B1 transcripts revealed in this work and reported in previous studies in rat and human tissues are displayed in Table 1. The use of multiple polyadenylation sites likely explains the size differences between these multiple transcripts among mouse tissues and between mice, rats, and humans (18). A 29-kDa protein was found in most tissues. In jejunum, liver, and testis, the apparent molecular weights of UT-B1 are 25, 32, and 36 kDa, respectively (Table 1). The extents of glycosylation differ between the various tissues.

In the kidney, the location of UT-B1 mRNA and protein in mice is identical to that reported in human, rat, and mouse, i.e., endothelial cells of descending vasa recta in the medulla (13, 35, 38, 40, 42). Such a presence contributes to urine concentrating process, as demonstrated in UT-B1-null mice (3). However, dehydration achieved by 2-day water deprivation did not affect kidney UT-B1 expression in mouse (present study), such as in rat (37).

The present study also shows the presence of UT-B1 in papillary surface epithelium of mouse as in rat (31, 40) with a basolateral membrane location, confirming the observation of Jung et al. (13) but different from that of Spector et al. (31) who did not report such a polarization in rat. The three layers of urothelium lining the urinary tract comprise umbrella (uppermost), intermediate, and basal cells. In mouse as in rat (31),

Fig. 9. Immunohistochemical localization of UT-B1 in mouse brain by confocal microscopy. A and B: at low magnifications, staining is observed at the border of corpus callosum (CC) and striatum (S) but not in cortex (C). C: higher magnification reveals staining of foot processes (FP) in the vicinity of blood vessel (V) at the border of cortex and corpus callosum. D: UT-B1 staining (red) is localized in glial cells, as revealed by its colocalization with glial fibrillary acid protein staining (green). Bars = 100 μm in A, 50 μm in B, and 10 μm in C.
immunoreactive UT-B1 was found on the plasma membrane of intermediate and basal epithelial cells and was restricted to the basolateral area of umbrella cells of the ureter and the bladder. In the urethra, we found UT-B1 staining exclusively on the basolateral area of umbrella cells and no staining of intermediate and basal cells. Although UT-B1 function in these tissues is not yet understood, its protein expression in both bladder and ureter was intriguingly decreased after 2-day thirsting. In keeping with this finding, the mRNA expression of UT-B1 was reduced in the urinary bladder of dehydrated mice. As UT-B1 was unchanged in the other tissues, such a regulation concerns specifically the urothelium. Noteworthy, Spector et al. (31) reported that UT-B1 protein abundance was similar in the urinary bladder of rats after 2-day water deprivation and after 2-day water loading. The function and the mechanisms of UT-B1 regulation by dehydration in the urothelium deserve further investigations.

Within the central nervous system, UT-B1 mRNA is found in cerebrum, cerebellum, and brain stem of mouse, a location similar to that reported in rat (4, 23). UT-B1 protein was concentrated in a region inserted between the striatum and the corpus callosum in the vicinity of the rostral end of ventricles I or II. It is expressed in astrocyte processes directed to blood capillaries and endfoot processes surrounding these vessels.
Because one of the known functions of astrocytes is to regulate the content of extracellular space, UT-B1 presumably plays a role in urea homeostasis of the environment of central nervous system cells. The sizes of the three transcripts found in testes are different from those found in kidney, bladder, and brain (Table 1). They are translated into a protein of 36 kDa (vs. 29 kDa in most other tissues). In mouse testis, UT-B1 is presumably located on Sertoli cell membranes as shown in rat (8). Intriguingly, Yang et al. (43) did not find UT-B1 transcripts in testis of wild-type mice. Nevertheless, they reported decreased urea permeability of the blood-testis barrier and testis hypertrophy in male UT-B1 knockout mice (44) and no reduction in fertility (43).

The bulk of ureagenesis takes place in the liver, and the egress of urea from hepatocytes is seemingly ascribed to a phloretin-inhibited urea transport (7). In rat liver, urea transport egress of urea from hepatocytes is seemingly ascribed to a phloretin-inhibited urea transport (7). In rat liver, urea transport egress of urea from hepatocytes is seemingly ascribed to a phloretin-inhibited urea transport (7). In rat liver, urea transport egress of urea from hepatocytes is seemingly ascribed to a phloretin-inhibited urea transport (7). In rat liver, urea transport egress of urea from hepatocytes is seemingly ascribed to a phloretin-inhibited urea transport (7).

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In conclusion, the present study showed that, in mice, UT-B1 is encoded by multiple transcripts and translated into a 29-kDa protein in most tissues, except in testis and liver. It revealed the presence of UT-B1 along the urinary and gastrointestinal tracts and a downregulation by dehydration of UT-B1 in both urinary bladder and ureter.

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