High urea and creatinine concentrations and urea transporter B in mammalian urinary tract tissues

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Spector DA, Yang Q, Wade JB. High urea and creatinine concentrations and urea transporter B in mammalian urinary tract tissues. Am J Physiol Renal Physiol 292: F467–F474, 2007. First published July 18, 2006; doi:10.1152/ajprenal.00181.2006.—Although the mammalian urinary tract is generally held to be solely a transit and storage vehicle for urine made by the kidney, in vivo data suggest reabsorption of urea and other urine constituents across urinary tract epithelia. To determine whether urinary tract tissue concentrations are increased as a result of such reabsorption, we measured urea nitrogen and creatinine concentrations and determined whether urea transporter B (UT-B) was present in bladder, ureter, and other tissues from dogs and rats. Mean urea nitrogen and creatinine concentrations in dogs and rats were three- to sevenfold higher in urinary tract tissues than in serum and were comparable to those in renal cortex. In water-restricted or water-loaded rats, urea nitrogen concentrations in bladder tissues fell inversely with the state of hydration, were proportional to serum urea nitrogen concentration in every animal. Immunoblots of rat and dog urinary tract tissues demonstrated the presence of UT-B in homogenates of bladder and ureter, and immunocytochemical analysis localized UT-B to epithelial cell membranes. These findings are consistent with the notion that urea and creatinine are continuously reabsorbed from the urine across the urothelium, urea in part via UT-B, and that urine is thus altered in its passage through the urinary tract. Urea reabsorption across urinary tract epithelia may be important during conditions requiring nitrogen conservation and may contribute to pathophysiological states characterized by high blood urea nitrogen, such as prerenal azotemia and obstructive uropathy.

The lower urinary tract in fish, amphibians, and reptiles participates in water and solute homeostasis as a consequence of regulated water and solute transport across their respective urinary tract epithelia. In contrast, the mammalian urinary tract has long been thought not to participate in water and solute homeostasis but, instead, to function solely as a transit and storage site for urine produced by the kidney (5). However, a number of in vivo studies have suggested that many urinary tract epithelia, including water, urea, and various ions, can pass through the epithelial (ureothelial) lining in a regulated manner (6, 11, 16–18, 25). Levinsky and Berliner (11) reported loss of water, potassium, osmolality, and up to 20% of urea from “artificial urine” or urine slowly perfusing dog ureter and bladder. Similar data were obtained by Walser et al. (25), who noted net loss of potassium, creatinine, and 7% of urea from urine perfusing ureters over a 3-min period in moderately dehydrated rats. Furthermore, although Parsons and co-workers (16) demonstrated a net urea transport of only 1.2% across stretched rabbit urothelia, they noted a 21% urea loss from urine over 45 min in an in vivo preparation of rabbit bladder.

The sites and mechanisms underlying and regulating urothelial transport of urea and other substances have only recently begun to be identified. Vectorial transport might occur across the apical cell membrane of the so-called “umbrella” epithelial cells lining the urinary tract lumens or the tight junction between these cells. Although simple diffusion and/or urothelial “leaks” might account for some tranurothelial transport, epithelial cell transporters and channels have recently been described in urothelia. Several workers have demonstrated the existence of the aldosterone-responsive, amiloride-sensitive epithelial sodium channel (ENaC) in the urothelia of rats and rabbits and localized ENaC to epithelial cell cytoplasm and membranes (reviewed in Refs. 13 and 20). Furthermore, Spector and co-workers recently reported the presence of aquaporins (AQP) 1–3 (21) and urea transporter (UT)-B in rat urothelia (22). UT-B was localized (by immunocytochemistry) to epithelial cell cytoplasm and to all epithelial cell membranes, except the apical luminal membrane (22). UT-B thus seems ideally situated to facilitate movement of urea from the “umbrella cells” lining the urinary tract lumen through the deeper layers of epithelial cells and then (down the steep gradient) to the underlying capillary network. If this hypothesis is true, urea (and, presumably, creatinine) traversing the apical membrane and in transit through the epithelial cell layers would presumably result in higher concentrations in urinary tract tissue than in other body tissues. Here we describe, for the first time, tissue concentrations of urea nitrogen and creatinine in the lower urinary tract of rats and dogs, the effect of hydration status on urea nitrogen concentrations in these same tissues, and the presence and localization of the facultative UT-B in dog urinary tract tissues.

MATERIALS AND METHODS

All research reported here adheres to the “Guiding Principles in the Care and Use of Animals” of the American Physiological Society and was approved by The Johns Hopkins University Animal Care and Use Committee.

Rat Experiments

Female Wistar rats (Harlan, Indianapolis, IN; ~215 g body wt) were maintained on an ad libitum intake of chow (Quality Lab Products) containing 14% protein. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Diets and resulting urine characteristics. Before experimental procedures, rats were placed in metabolic cages, maintained on ad libitum standard chow, and assigned to one of three treatment groups. 1) In the group allowed ad libitum water intake (ad lib, n = 18), average urine volume was 9.2 ml/day and average urine osmolality was 1,458 mosmol/kgH₂O. 2) In the rats subjected to 48 h of water restriction (water-restricted, n = 17) by complete withdrawal of drinking water, average urine volume was 4.0 ml/day and average urine osmolality was 3,263 mosmol/kgH₂O. 3) In the rats subjected to 48 h of water loading (water-loaded, n = 14) with 5% sucrose and water as the sole drinking fluid, average urine volume was 68.4 ml/day and average urine osmolality was 198 mosmol/kgH₂O. Urine was collected during the 24 h immediately preceding tissue collection.

Procedure for obtaining tissue samples. Rats were anesthetized with intraperitoneal thiobutabarbital (Inactin), and a midline abdominal incision was made. Blood samples were obtained for determination of serum urea and creatinine. Bladder, kidneys, and quadriceps muscle tissue (to act as a “control” tissue) were removed from each rat. In some rats, pieces of stomach, small and large intestine, and other tissues were obtained. For removal of residual urine from the luminal surface, bladders were opened longitudinally, immediately blotted on paper, rinsed with 0.1 ml of NaCl solution (150 mmol/l), and blotted three times. All tissues were weighed and then homogenized using 50 strokes of a hand-held homogenizer (VWR Micro tissue grinder). Then 200 µl of NaCl solution (150 mmol/l) were pipetted into the homogenizer, and the tissue was further homogenized with 50 strokes of the hand-held homogenizer. The homogenized samples were transferred to a microcentrifuge tube and spun at 14,000 rpm for 10 min, and the supernatant was used for analysis of urea nitrogen and creatinine concentrations.

Tissues were similarly obtained from an additional 12 rats (4 each of ad lib, water-loaded, and water-restricted rats) for subsequent desiccation for determinations of percent water content (see below).

Dog Experiments

Six healthy male beagle dogs and one male mixed-breed/beagle dog (18–35 pounds body wt) were maintained on ad libitum water and standard dog chow until the day of the experimental procedure.

Procedure for obtaining tissue samples. Dogs were anesthetized with gas anesthesia and monitored with ECG and pulse oximetry. An intravenous solution of lactated Ringer solution (infusion rate varied) produced a mild diuresis. After aspiration of bone marrow (for a different research protocol), a peripheral blood sample was obtained for analysis of urea nitrogen and creatinine, and the animals were euthanized with pentobarbital sodium. Immediately upon death, midline abdominal and skin incisions were made, and urine was obtained by needle puncture of the bladder. Bladder, ureter, renal cortex, and quadriceps muscle tissues were obtained within 2–3 min. Tissues were blotted, rinsed, and homogenized as described above for rat tissues for measurement of urea nitrogen and creatinine concentrations. A scissor incision was made to separate the mucosal surface from the muscle and serosal layer and, thus, divide additional pieces of dog bladder into two sections (mucosal and muscularis/serosal), which were separately processed and analyzed for urea nitrogen and creatinine. Additional pieces of all dog tissues were obtained for subsequent desiccation for determination of percent water content (see below).

Chemical Analysis

Urea nitrogen and creatinine concentrations in serum, urine, and supernatants of tissue samples were measured in duplicate using a blood urea nitrogen Analyzer 2 and a creatinine Analyzer 2, respectively (Beckman Instruments, Fullerton, CA).

Expression of Urea Nitrogen and Creatinine Concentrations

Urea nitrogen and creatinine concentrations in rat and dog tissue samples were initially expressed as milligrams per 100 g wet tissue weight. To express urea nitrogen and creatinine concentrations as milligrams per deciliter water and, thereby, better compare tissue urea nitrogen and creatinine concentrations in different tissues and in serum and urine and in concordance with past investigators (12, 24), we obtained tissue samples of bladder, renal cortex and inner medulla, and quadriceps muscle from an additional group of 12 rats (4 ad lib, 4 water-loaded, 4 water-restricted) in a manner identical to that described above. Wet tissue was weighed and then desiccated in centrifuge tubes at 40°C in a dry heating block for 7–10 days. Tissue weights were obtained daily until sample weights remained unchanged for 3 consecutive days, and desiccated tissue weight for each sample was derived from the mean of weights of the last 3 days. Tissue water (wt tissue wt – desiccated tissue wt) and percent tissue water ([tissue water wt ÷ wet tissue wt] × 100) were calculated for each sample. Average values for each tissue (e.g., bladder and muscle) were obtained for each rat hydration group, and urea nitrogen and creatinine concentrations for each tissue sample were recalculated as milligrams per deciliter tissue water. In dog experiments, duplicate tissue samples were obtained for four dogs, and similar desiccation procedures and analysis were performed to express urea nitrogen and creatinine concentrations as milligrams per deciliter tissue water in all dog samples.

Procedure for Immunoblotting

The procedure for immunoblotting was similar to that previously reported (22). For rat studies, animals were anesthetized with intraperitoneal thiobutabarbital, a midline abdominal incision was made, and ureters and bladders were rapidly removed, minced, and placed into an ice-cold isolation buffer solution composed of 250 mM sucrose and 10 mM triethanolamine, adjusted to pH 7.6 with 1 N HCl, and the protease inhibitors leupeptin (1 µg/ml) and phenylmethylsulfonyl fluoride (0.1 mg/ml). For dog studies, portions of dog bladder and ureter were taken within 2–3 min of euthanasia, and dog tissues were prepared as described above for rat tissues. All minced tissues were homogenized in ice-cold isolation solution using a Tissumizer homogenizer (Tekmar, Cincinnati, OH). Tissues were homogenized with five bursts of five strokes of a micro-saw-tooth generator. Homogenates were centrifuged at 4°C at 3,000 g for 10 min to separate incompletely homogenized tissue. Aliquots of the supernatant were obtained for measurement of total protein concentration using a bicinchoninic acid protein assay reagent kit (Pierce, Rockford, IL). Laemmli buffer (5×) was added to the remainder of the supernatant in a ratio of one part buffer to four parts homogenate, and samples were then heated to 60°C for 15 min to solubilize proteins, divided into aliquots, and stored at −80°C.

Antibodies

The affinity-purified polyclonal antibody to UT-B was raised in rabbits to an HPLC-purified synthetic peptide corresponding to the COOH-terminal 19 amino acids of human UT-B type 1 and has been extensively characterized (22). The UT-B antibody was a generous gift from Dr. J. M. Sands (Atlanta, GA).

Electrophoresis and Immunoblotting of Membranes

SDS-PAGE was carried out on minigels of 10% polyacrylamide. The proteins were electrophoretically transferred to nitrocellulose membranes. After the membranes were blocked with 5% nonfat dry milk in PBS, the primary antibody was applied overnight, usually at a 1:3,000 dilution of antibody in phosphate buffer solution containing 0.2% bovine serum albumin. The blots were exposed for 1 h to secondary antibody (donkey anti-rabbit IgG conjugated with horseradish peroxidase; Amersham Pharmacia Biotech). Blots were developed with enhanced chemiluminescence agents (Amersham Pharmacy Biotech) before exposure to X-ray film for visualization of sites of antigen-antibody reaction. Controls were carried out using antibody preabsorbed overnight with the immunizing peptide.
Table 1. Characteristics of rat groups

<table>
<thead>
<tr>
<th></th>
<th>Body wt, g</th>
<th>Food Intake, g/day</th>
<th>Water Intake, ml/day</th>
<th>Urine Volume, ml/day</th>
<th>Urine Osmolality, mosm/kg</th>
<th>SUN, mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ad lib</td>
<td>215.1±2.5</td>
<td>14.2±0.7</td>
<td>28.4±2.7</td>
<td>9.2±0.8</td>
<td>1.458±224</td>
<td>20.8±1.0</td>
</tr>
<tr>
<td>Water-loaded</td>
<td>218.7±3.1</td>
<td>13.7±0.6</td>
<td>99.4±9.7*</td>
<td>68.4±7.6*</td>
<td>198±39*</td>
<td>15.3±1.2*</td>
</tr>
<tr>
<td>Water-restricted</td>
<td>192.7±3.9†</td>
<td>9.5±0.5†</td>
<td>0*†</td>
<td>4.0±0.6†</td>
<td>3.264±521†</td>
<td>30.1±1.8†</td>
</tr>
</tbody>
</table>

Values are means ± SE of 4 animals for each hydration group. *P < 0.01 vs. ad lib. †P < 0.01 vs. water-loaded.

Procedures for Immunocytochemistry

Portions of dog bladders and ureters were fixed for immunolocalization by immersion of the tissues in 4% freshly prepared paraformaldehyde in PBS for 1 h. Tissues were then immersed in a cryoprotectant solution of 10% EDTA in 0.1 M Tris buffer, pH 7.4, for 1 h at 4°C and frozen on dry ice. Antibodies were immunolocalized on 8-μm frozen sections as previously described (22). Sections were incubated overnight at 4°C with primary antibodies diluted to 10 μg/ml. Secondary antibodies were species-specific goat anti-rabbit antibodies (Jackson Immunoresearch Labs, West Grove, PA) coupled to Alexa 488 and Alexa 568, respectively (Molecular Probes, Eugene, OR). Tissues thus treated were examined using standard immunofluorescent and confocal microscopy. Controls were carried out using antibody preabsorbed overnight with the immunizing peptide.

Statistics

Urea nitrogen and creatinine concentrations between groups of tissues and between hydration groups were compared by unpaired Student’s t-test. To correct for multiple comparisons, we used Bonferroni’s adjustment for reporting P values. Regression analysis was performed using JMP statistical software (version 5.1, SAS Institute, Cary, NC).

RESULTS

Characteristics of Ad Lib, Water-Restricted, and Water-Loaded Rats

As expected, 24-h urine volumes were greater and urine osmolality and serum urea nitrogen concentrations were significantly less in water-loaded than in ad lib rats (Table 1). Urine volumes at 24 h were less and urine osmolality and serum urea nitrogen concentrations were higher in water-restricted than in ad lib rats. Water content of rat muscle, bladder, and renal cortex and medulla tissues, expressed as percentage of wet tissue weight, for each hydration status group is shown in Table 2. Water content was slightly less in tissues of water-restricted than ad lib and water-loaded rats. Our values for percent water in rat renal tissues are similar to those of Valtin (24), who calculated values in rat papilla of 80% in rats allowed ad libitum access to water and 83% in dehydrated rats and in renal cortex of 75% in rats allowed ad libitum access to water and 76% in dehydrated rats.

Urea Nitrogen Concentrations in Rat Tissues

Urea nitrogen concentrations in serum and homogenates of quadriceps muscle, bladder, and renal cortex and in urine and inner medulla in individual ad lib rats are shown in Fig. 1. Urea nitrogen concentrations in muscle, bladder, and renal tissues are expressed as milligrams per deciliter tissue water. Urea nitrogen concentrations in quadriceps muscle tissue were similar (P = not significant) to those of corresponding blood samples, and both were significantly less (P < 0.001) than in renal and bladder tissues and urine. Concentrations of urea nitrogen in bladder tissue were higher than corresponding concentrations in serum and quadriceps muscle for every rat. Mean values were 20.8 ± 1.0 (SE) mg/dl in serum, 21.3 ± 1.0 mg/dl tissue water in quadriceps muscle, 65.2 ± 5.0 mg/dl tissue water in bladder, 75.2 ± 3.0 mg/dl tissue water in renal cortex (superficial and deep), 889.3 ± 67.6 mg/dl tissue water in renal outer and inner medulla, and 2431.1 ± 303.1 mg/dl in urine.

Concentrations of urea nitrogen in our rat renal cortex and medulla tissues were similar to those obtained by Valtin (24) in rats allowed ad libitum access to water [50.4 ± 15.1, 784 ± 73, and 1,633.8 ± 134.0 (SE) mg/dl tissue water in cortex (superficial), medulla, and papilla, respectively], despite differences in methodology and animal diet, size, and strain.

Table 2. Percent water in tissues of rats in different states of hydration

<table>
<thead>
<tr>
<th></th>
<th>Muscle</th>
<th>Bladder</th>
<th>Renal Cortex</th>
<th>Renal Inner Medulla</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ad lib</td>
<td>78.8±0.3</td>
<td>77.3±1.0</td>
<td>76.1±1.2</td>
<td>81.9±1.1</td>
</tr>
<tr>
<td>Water-loaded</td>
<td>77.3±0.6</td>
<td>78.9±0.9</td>
<td>77.2±0.8</td>
<td>82.9±1.5</td>
</tr>
<tr>
<td>Water-restricted</td>
<td>75.5±0.5*</td>
<td>75.9±0.7</td>
<td>75.8±0.9</td>
<td>81.0±1.6</td>
</tr>
</tbody>
</table>

Values are means ± SE of 4 animals for each hydration group. *P = 0.003 vs. ad lib.

Fig. 1. Urea nitrogen concentration in serum and urine (mg/dl) and in quadriceps muscle, bladder, and renal cortex and inner medulla (mg/dl tissue water) of individual rats allowed ad libitum access to water. Note different scales in A (serum, muscle, bladder, and renal cortex) and B (urine and renal inner medulla).
Urea Nitrogen Concentrations in Dog Tissues

Mean percent tissue water in dog tissues (n = 4 for all tissues) was 78.6 ± 0.5% (SE) in renal cortex [similar to values of 78.9–80.5% previously described by Levitin et al. (12)], 79.4 ± 1.2% in bladder, 66.5 ± 4.4% in ureter, and 75.6 ± 2.1% in quadriceps muscle. Urea nitrogen concentrations in dog serum, renal cortex, whole bladder, ureter, quadriceps muscle tissue, and urine are shown in Fig. 2. As in rats, urea nitrogen concentrations were expressed as milligrams per deciliter tissue water in bladder, ureter, muscle, and renal cortex tissues. Urea nitrogen concentrations in serum and quadriceps muscle were similar and, in every dog, were lower than values in corresponding bladder, ureter, and renal cortex tissues. Urea nitrogen concentrations in bladder walls that were split into epithelial (mucosal) and muscularis (serosal) halves (additional pieces of the same bladders in which whole bladder urea nitrogen was measured) are also shown. In each dog, urea nitrogen concentrations were higher in the epithelial than in the muscularis half of the split bladder wall. Mean urea nitrogen concentrations in dog tissues were 11.7 ± 1.5 (SE) mg/dl in serum, 17.2 ± 3.8 mg/dl tissue water in quadriceps muscle, 44.7 ± 7.9 mg/dl tissue water in bladder (whole), 77.3 ± 18.8 mg/dl tissue water in bladder (epithelial half), 39.5 ± 9.2 mg/dl tissue water in bladder (muscularis half), 38.6 ± 10.7 mg/dl tissue water in ureter, 47.6 ± 7.9 mg/dl tissue water in renal cortex, and 1,861.4 ± 315.0 mg/dl in urine. Urea nitrogen concentrations were significantly higher in bladder than in the epithelial and muscularis sections of the same bladder wall. Mean urea nitrogen concentration in renal cortex was in the range previously reported in dogs by Levitin and co-workers: 28.6 ± 3.3 mg/dl tissue water during water diuresis and 58.8 ± 12.9 mg/dl tissue water following dehydration.

Effect of Hydration Status on Urea Nitrogen Concentrations in Rat Tissues

Urea nitrogen concentrations in serum, muscle, and bladder in ad lib, water-loaded, and water-restricted rats are shown in Fig. 3. In bladder tissue, urea nitrogen concentrations were 65.2 ± 5.0, 46.1 ± 4.3, and 79.6 ± 8.4 mg/dl tissue water in ad lib, water-loaded, and water-restricted rats, respectively. In bladder tissue, urea nitrogen concentration was significantly less in water-loaded than in ad lib (P < 0.03) and water-restricted rats (P < 0.01) but not significantly (P = not significant) less in ad lib than in water-restricted rats. Mean urea nitrogen concentrations in serum and quadriceps muscle tissue water were similar for each of the three hydration groups. In serum and quadriceps muscle, urea nitrogen concentrations were higher in ad lib than in water-loaded animals and lower in ad lib than in water-restricted animals (P ≤ 0.01 for all comparisons). Mean urea nitrogen concentrations in renal cortex were similar to concentrations in bladder tissue in each hydration group. Table 3 shows mean concentrations of urea nitrogen in serum, urine, and tissue homogenates in groups of rats in different states of hydration and in dogs allowed ad libitum access to water.

Bladder urea nitrogen concentrations are plotted against logarithm of urine urea nitrogen concentrations for all rats in the three hydration groups in Fig. 4. In every animal, urine urea nitrogen concentration was greater than bladder urea nitrogen concentration. Bladder urea nitrogen concentration increased significantly as urine urea nitrogen increased.

Serum urea nitrogen concentrations are plotted against bladder urea nitrogen concentrations for each animal in the three hydration groups in Fig. 5. In every animal, bladder urea
nitrogen concentration was greater than serum urea nitrogen concentration. Serum urea nitrogen concentrations increased significantly as bladder urea nitrogen concentrations increased.

Creatinine Concentrations in Rat and Dog Tissues

Mean concentrations of creatinine in serum, urine, and tissue homogenates in groups of rats in different states of hydration and in dogs allowed ad libitum access to water are shown in Table 4.

In rats and dogs, creatinine concentrations were higher in quadriceps muscle (as expected, because creatinine is a product of metabolism in muscle tissues) than in serum for every animal. In rats and dogs, creatinine concentrations in bladder and ureter tissue homogenates were approximately twofold higher than in quadriceps muscle tissue, fourfold higher than in other tissues containing smooth muscle (colon and small intestine), and six- to sevenfold higher than in serum. In dog bladder tissues, creatinine concentrations were higher in the epithelial half (containing no muscle) than in the half containing smooth muscle. Light microscopy of both halves of the bladders confirmed separation of the epithelial tissue from the smooth muscle tissue.

Immunoblots of Bladder and Ureter Tissues

Immunoblot results for UT-B in homogenates of bladder and ureter tissues in rats and dogs are shown in Fig. 6. UT-B in tissue containing largely epithelial cells obtained by scraping the lining of the bladder lumen in both species is reflected as a broad ~40- to 54-kDa band (probably representing glycosylated UT-B), as previously reported in rats (12), and in whole bladder and ureter homogenates as a narrower band in rats and dogs.
dogs. Preabsorption of the UT-B antibody with the immunizing peptide ablated the UT-B signals (not shown). The differences in appearance between the bands of the epithelial scrapings and the whole organ homogenates reflect, in part, the much higher concentration of UT-B in epithelial cells than in whole organ homogenates (in the similarly loaded gel lanes), as well as heterogeneity and differences in tissue-specific expression of proteins, as has been noted previously for UT-A and UT-B (4).

Localization of UT-B in Dog Urothelia

Immunocytochemical studies of UT-B in dog bladder and ureter are shown in Fig. 7. For purposes of orientation, light-microscopic views of hematoxylin-and-eosin-stained bladder and ureter are shown. As we previously showed in rat urinary tract tissues, labeling for UT-B was strongly positive in epithelial cells lining the bladder and ureter and less intense in blood vessels subserving the epithelial cells. Preincubation of the UT-B antibody with the immunizing peptide ablated the epithelial labeling (not shown). Under higher-magnification confocal microscopy, antibody to UT-B (green stain) strongly labeled all epithelial membranes, except the apical membrane of the large epithelial (“umbrella”) cells lining the bladder and ureter, as we previously showed in rats (22). In dog bladder, as in rat bladder, UT-B also faintly stains a thin subepithelial layer of small cells that are not apparent in the rat or dog ureter. Some of these cells might be red blood cells and/or vascular endothelia (both of which contain UT-B), but the general pattern suggests a nonvascular origin. Antibody to AQP-3 (red...
stain) strongly labels the cell membranes of the basal (most intense) and intermediate layers of the epithelial cells in ureter and bladder.

**DISCUSSION**

Here we report, for the first time, concentrations of urea nitrogen and creatinine in mammalian urinary tract tissues. We found that rat and dog ureter and bladder contain tissue concentrations of urea nitrogen three- to fivefold higher than those in serum and other nonrenal tissues and comparable to concentrations of urea nitrogen in renal cortex. Furthermore, urea nitrogen concentrations in rat bladder tissue varied with the hydration status of the animal and correlated with urine and serum urea concentrations. Thus concentrations of urea nitrogen in bladder homogenates were lowest in water-loaded animals and highest in water-restricted animals. Similarly, concentrations of creatinine in rat and dog bladder were six to seven times those in serum. However, in contrast to urea, creatinine concentrations in rat bladder did not vary with state of hydration. This is likely in part due to background production of creatinine from bladder smooth muscle and, possibly, lack of a (known) creatinine transporter analogous to UT-B.

Since it has long been held that mammalian urinary tract function is solely for transit and storage (5) and since recent in vitro studies showed extremely low urothelial permeability to urea (as well as other substances) (3,14), these results are surprising. However, a number of factors, including long urine-epithelium contact time and increased hydrostatic pressures during peristalsis and bladder filling, might promote vectorial transport, and in vivo studies have shown that urea (and other substances) concentrations fell in urine or artificial urine perfusing renal pelvis, ureter, and bladder lumens (11, 19, 25). In those studies, urea must have been reabsorbed into surrounding urinary tract tissues, thereby raising ambient tissue concentrations. Such considerations, along with the finding of high levels of the organic osmolytes inositol, glycerophosphocholine, sorbitol, and betaine in epithelium and muscle of rabbit and rat urinary bladder (levels that rose in dehydrated animals), led Kwon and co-workers (10) to predict high levels of osmolality and urea in these tissues and to posit that the “compatible” organic osmolytes would help maintain cell volume in the setting of high osmolality without disturbing normal intracellular ion concentrations.

It seems likely that the source of urea and most of the creatinine in bladder and ureter tissues is luminal urine. In our rats in various states of hydration, urea concentration in bladder tissues was shown to correlate with urea concentration in urine, which were always much higher than urinary tract tissue concentrations. Furthermore, our data in dogs show higher urea and creatinine concentrations in the epithelium than in the muscularis half of split bladders, supporting the notion that the source of urea and creatinine in urinary tract tissues is urine bathing the apical membrane surface of the epithelial cells. If this is the case, it would seem probable that, within bladder tissue, urea and creatinine concentrations would be highest in epithelial cells, although we have no data to confirm this.

The exact site(s) and mechanism(s) whereby urea and creatinine might traverse the epithelial cell barrier(s) under physiological conditions are unknown. The permeability barriers to water and solute transport across the epithelial cell layer include the apical membranes of the large epithelial umbrella cells lining the lumen, the tight junctions between the epithelial cells, and the glycosaminoglycan layer, which lines the umbrella cell apical surface (8, 13). Despite these barriers, urea and other solutes could theoretically traverse the epithelial apical membrane or tight junction by simple diffusion down the steep urine-blood gradient or by “leak” across naturally occurring discontinuities or breaks in the permeability barrier(s) as a result, for example, of epithelial cell sloughing, and even by accompanying endocytosis of vesicles of apical membrane, which occurs during bladder contraction after voiding and during expansion and stretching of the bladder as urine accumulates (23). Finally, it is possible that apical membrane channels and transporters might, under certain circumstances [potentially including physiologically induced alteration of barrier(s) function], play a role in specific, regulated solute transport. This is apparently the case for sodium, which is reabsorbed via ENaC transporters located on the apical membrane of the umbrella cells (13). Although electrophysiological studies suggest the presence of other pathways for movement of sodium and potassium across the apical epithelial membrane (13), no specific channels or transporters other than ENaC have been identified at this site. In particular, the facilitative UT-B channel reported in rodents was found on all epithelial cell membranes, except the apical cell membrane (22). However, other urea-transporting channels, including, in particular, UT-A, may be present on the luminal cell apical membrane, since UT-A mRNA and protein have recently been identified (although not localized) in rat bladder tissues (4; unpublished data).

Regardless of the mechanism(s) and site(s) whereby urea traverses the epithelial permeability barrier, were urea to be present only in the intercellular spaces, the previously demonstrated presence of membrane AQP-3 (21) might cause these cells to dehydrate due to the consequent urea gradient. It seems likely that epithelial cell membrane UT-B allows urea to enter and exit epithelial cells, whether the luminal barrier is breached at the apical membrane or the tight junction; therefore, UT-B is probably important for maintenance of cell osmolarity and cell volume regulation. Furthermore, the presence of UT-B on all epithelial membranes apparently allows urea and creatinine to transit the entire epithelial cell layer (which thus acts functionally as a syncytium) to the underlying capillary complex subserving the epithelium. Here we have demonstrated that UT-B is not only present in rodent urothelia, but in dog urothelia as well, and that, as in rodents, it is localized strongly to the epithelial cell membranes and, to a lesser extent, in epithelial cell cytoplasm and small blood vessels in the stromal tissue adjacent to the epithelial layer. Since we have also found UT-B in human urothelia (unpublished observations), UT-B may be present in most mammalian species. From the subepithelial capillary plexus, reabsorbed urea must pass through the urinary tract venous drainage system to the general circulation. The concentrations of urea and creatinine in urinary tract venous effluent have never been measured but could theoretically be as high as the maximum concentrations found in the epithelial cells of urinary tract tissues (in rats, greater than whole bladder concentrations of 80 mg/dl for urea nitrogen). The persistent gradient of urea and creatinine between urinary tract tissues and serum, regardless of the animal state of hydration, suggests the possibility of a continuous reabsorption...
of both back into the systemic circulation, presumably with increased amounts of urea reabsorbed during dehydration when the epithelial tissue-blood gradient is highest. The magnitude of this urea flux is not known.

Our finding that urea and creatinine are present in high concentrations in urinary tract tissues adds to evidence suggesting that urine exiting the renal collecting ducts is modified as it transits the urinary tract. Schmidt-Nielsen (19) suggested a role for the renal pelvis in the modification of urine concentration and composition, and others have suggested the same for ureter (25) and bladder (11). The most obvious example of urinary tract modification of urine is in hibernating bears, which daily reabsorb their entire urine production, including urea, through their urinary tract (15). Although such reabsorption of urine is critical to survival in hibernating bears (for conservation of water, solutes, and nitrogen), the advantages of transurothelial vectorial transport of water, ions, and nitrogen-containing compounds in other mammalian species are not obvious and seem inimical to the apparent main function of the urinary tract, i.e., storage of urine until micturition. It is possible in the case of urea and creatinine that reclaimed nitrogenous wastes may play a role in nitrogen conservation under certain circumstances. Thus reabsorbed urea and creatinine might be metabolized by the gastrointestinal tract enterocytes and bacteria, and nitrogen may be subsequently utilized (as in hibernating bears) for protein synthesis in states such as malnutrition, growth in children, and low-protein diets (1, 2). Urothelial absorption of urea may also play a role in the well-known pathophysiological elevations of serum urea nitrogen in obstructive uropathy and in states of dehydration and volume depletion (“prerenal azotemia”). In these conditions, elevation of urea is out of proportion to the reduction in glomerular filtration rate (GFR) and the rise in serum creatinine. Although urea excretion in prerenal azotemia has been attributed to increased collecting duct reabsorption of urea and increased urea production (9), our data are consistent with a role for increased urothelial urea reabsorption as well.

Finally, our data suggest that estimates of GFR using urea and creatinine clearances might underestimate renal clearance values because of urothelial reabsorption of both substances. Errors in estimation of GFR would presumably be minimized by testing in the setting of high urine flow rates or by monitoring of urine contact with urothelia by bladder catheterization.

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REFERENCES

13. Lewis SA. Everything you wanted to know about the bladder epithelium but were afraid to ask. Am J Physiol Renal Physiol 278: F867–F874, 2000.