Molecular and functional characterization of a urea transporter from the kidney of the Atlantic stingray

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IN GENERAL, MARINE ELASMOBRANCHS (sharks, skates, and rays) maintain body fluids hypertonic to the ambient environment. In the ocean, plasma osmolality is ~1,050 mosmol/kgH2O (for review, see Ref. 19), which is 2–10% higher than the osmolality of the seawater. This high plasma osmolality is achieved, in part, by the retention of urea (40); i.e., elasmobranchs are ureosmotic. Plasma urea concentrations average 350 mmol/l (range 209–453 mmol/l), and urea contributes 30–50% of the plasma osmolality (for review, see Ref. 19). Clearance of urea from the body is low, in large part because of reabsorption of 90–98% of the filtered load of urea by the kidneys (12, 15, 40).

Although most marine elasmobranchs are stenohaline (i.e., their range is restricted to marine habitats), a number of species are marginally euryhaline (i.e., they are also found in brackish environments, such as estuaries and river mouths) (7). Interestingly, a relatively small number of species (the euryhaline elasmobranchs) are able to exploit marine, estuarine, riverine, and even freshwater habitats. Most euryhaline elasmobranchs appear to migrate between habitats on a seasonal basis (35, 42). However, at least one species (Atlantic stingray) can reproduce and complete its life cycle in freshwater, whereas another (bull shark) has the potential to do so (22, 43).

Because the gills of elasmobranchs are freely permeable to water (5), in the ocean the small osmotic gradient between seawater and the extracellular fluid leads to an influx of free water into the fish. The kidneys maintain volume homeostasis by excreting urine hyposmolar to plasma (i.e., the osmotically driven uptake of water is balanced by the excretion of solute-free water).

A very high plasma osmolality and the free movement of water across the gills present a challenge to volume homeostasis for marginally euryhaline and euryhaline elasmobranchs when they move between environments of different salinities or are resident in low-salinity habitats. In response to this challenge, two strategies are employed: plasma osmolality is varied in the same direction as the change in external salinity by altering the plasma concentrations of urea and/or Na+ and Cl− (8, 10, 15, 16, 21, 30, 34, 41, 42, 48) and by altering renal excretory function inversely with changes in environmental salinity so that fluid efflux counteracts the osmotically driven water influx (15, 21, 28, 34, 48). However, for marginally euryhaline and euryhaline elasmobranchs, changes in internal osmolality are not directly proportional to changes in the external osmolality. Thus, when these fish exploit low-
salinity environments, there is an increase in the external-internal osmotic gradient (29, 30, 42). This osmotic gradient can be remarkable; for elasmobranchs in freshwater, it is in the range 600–650 mosmol/kg H₂O (29, 42). The increased gradient leads to increased osmotically driven water uptake (6, 8, 10, 15, 41). Fluid balance is returned toward the predilution level by a marked increase in renal excretory function, including increased glomerular filtration rate, urinary flow rate, free water clearance, and urea and electrolyte excretion (15, 16, 21, 28, 34, 48). The increase in urea excretion exceeds that of the other solutes, so that urea becomes the predominant urinary solute (21, 28, 48). The increase in urea excretion results from an increase in filtered load and a decrease in fractional reabsorption (21, 28, 34).

Interestingly, elasmobranchs remain ureosmotic in low-salinity environments. Urea remains a major osmolyte, contributing 30–40% to the extracellular fluid osmolality (8, 10, 15, 21, 34, 43, 48), and the kidneys remain the primary site for urea retention. The amount of urea reabsorbed by the renal tubules is markedly increased after exposure to a low-salinity environment; i.e., despite a lower fractional reabsorption of urea (decreasing from 90–98% to 66–84%), the increase in the filtered load of urea coupled with the high reabsorptive capacity of the renal tubules results in a large increase in the absolute amount of urea reabsorbed (data recalculated from Refs. 15, 28, and 34; see also Ref. 21). Thus the mechanisms that regulate renal urea reabsorption are important in the osmoregulatory and volume-regulatory processes of marine elasmobranchs.

The countercurrent arrangement of the marine elasmobranch nephron (23) supports the role of passive mechanisms in renal urea reabsorption (3, 13). A phloretin-sensitive, facilitated urea transporter has been cloned from the kidney of the spiny dogfish shark, a marginally euryhaline elasmobranch (39). Because this urea transporter was highly expressed in the kidney, the authors proposed that it and possibly other homologous facilitated urea transporters contributed to tubular urea reabsorption. In a recent study, we observed that Atlantic stingrays held in harbor water reabsorbed 96% of the filtered load of urea, indicating that this species displays a high degree of renal urea conservation, even in an estuarine salinity (21). Unlike many dasyatid rays in this region, this species is commonly caught in oligohaline-to-freshwater habitats (17, 22, 29; W. Roumillat, personal communication). We therefore hypothesized that if phloretin-sensitive facilitated urea transporters played a role in tubular urea reabsorption in the elasmobranch kidney, urea transporters homologous to the spiny dogfish urea transporter would also be present in the kidneys of the Atlantic stingray, a phylogenetically distinct marine elasmobranch that, unlike the spiny dogfish, exploits habitats that cover a remarkable range of environmental salinities.

METHODS

Animals

The experiments were conducted with approval of the Medical University of South Carolina (MUSC) Institutional Animal Care and Use Committee.

Male Atlantic stingrays (Dasyatis sabina, Lesueur, 1824) were kindly supplied by the South Carolina Department of Natural Resources (Fort Johnson, Charleston, SC). The stingrays were caught in Charleston Harbor and neighboring estuaries by the South Carolina Department of Natural Resources during scheduled surveys of local fisheries. The stingrays were placed in transportation tanks until the survey vessel returned to Fort Johnson. The rays were then placed in a filtered, closed-circulating, 15,000-liter holding tank at the Department of Marine Biomedicine and Environmental Sciences, MUSC, located within the Marine Research Complex at Fort Johnson.

The salinity of the water in the holding tank was maintained at 28‰ (~850 mosmol/kg H₂O), water temperature was maintained between 20 and 24°C, and pH was monitored and, if necessary, adjusted to 8.0–8.3 with a seawater buffer (Marine Buffer, SeaChem, Stone Mountain, GA). The stingrays were fed a diet of shrimp during the holding period. The animals were allowed ≥1 wk to adapt to the holding conditions before euthanization by placement in buffered (pH 8.0–8.3) seawater containing aminobenzoic acid ethyl ester (MS-222, Sigma, St. Louis, MO). After euthanasia, tissues were quickly removed and snap-frozen in liquid nitrogen.

RT-PCR

Total RNA from whole kidney was isolated using TRIzol reagent (GIBCO BRL, Gaithersburg, MD). The RNA was reverse transcribed using Superscript II reverse transcriptase (GIBCO BRL) with an antisense primer (5′-AGC CAC CAG TAC CAG TCT CC-3′) corresponding to amino acids 133–139 of the Squalus acanthias urea transporter protein sequence (GenBank accession no. AAF66072; shUT) (39). Degenerate sense primers for PCR were derived to correspond to amino acids 60–66 [5′-GC(A/G)(A/C) CAG TTC TG(T/G) AAC-3′] and 81–86 [5′-CA(A/G) AAC CC(A/C) TGG TGG GC-3′] of the shUT protein sequence. cDNA was amplified in two separate reactions using Taq polymerase (GIBCO BRL) in a 50-μl reaction containing the following reagents (expressed as final concentrations): 5 μl of 10× Taq amplification buffer, 1.5 mM MgCl₂, 0.2 mM dNTP mix, 0.2 μM forward primer, and 0.2 μM reverse primer. Products were amplified according to the following parameters: initial denaturing for 5 min at 94°C followed by 32 cycles of 1 min at 94°C, 1 min at 55°C, and 2 min at 72°C. The final round was followed by extension for an additional 10 min at 72°C. Two PCR products of predicted length (147 and 237 bp) were gel purified and sequenced at the Biotechnology Resource Laboratory (MUSC). The sequence of the 237-bp product was used to design gene-specific primers for cloning using rapid amplification of cDNA ends (5′/3′-RACE).

5′/3′-RACE

Initial cloning, 5′/3′-RACE was performed using reagents and the protocol provided in the Marathon cDNA amplification kit (Clontech, Palo Alto, CA). Briefly, poly(A)⁺ RNA was reverse transcribed, and second-strand cDNA was synthesized using the provided reagents. 5′-RACE was performed on Marathon adaptor-ligated cDNA using a gene-specific reverse primer (5′-CGA CCA ATA TGC CAT TGT ACC CAG...
PCR was performed using reagents described above, and amplification was carried out as follows: 1 min at 94°C followed by 30 cycles of 10 s at 94°C and 5 min at 68°C. Products were allowed to extend for 10 min at 72°C. A single PCR product of ~500 bp was amplified, gel purified, and inserted into pCRII-TOPO vector (Invitrogen, Carlsbad, CA). A second, gene-specific forward primer (5′-CTG GGA ACA GTG TTG GCA ACT TTG G-3′) was used to perform 3′-RACE. Amplification was carried out as follows: 1 min at 94°C followed by 30 cycles of 10 s at 94°C, 30 s at 60°C, and 4 min at 72°C. Products were allowed to extend for 10 min at 72°C. A PCR product of ~2 kb was identified, gel purified, and inserted into a pCRII-TOPO vector.

Competent Escherichia coli (One-shot, Invitrogen) were transformed with the pCRII-TOPO vector + insert and plated. Positive colonies were selected, and the plasmids were isolated. Plasmids containing the 5′- or 3′-RACE insert were sequenced as described above by primer walking. Overlapping 5′- and 3′-RACE products allowed the reconstruction of the almost full-length putative urea transporter cDNA.

Confatory cloning. SMART RACE (Clontech) technology was utilized to independently confirm the Marathon RACE product and to identify additional base pairs in the 5′-untranslated region (UTR). Poly(A)⁺ RNA was isolated from the kidney of a second stingray and reverse transcribed with Superscript II reverse transcriptase ( Gibco BRL) using the 5′-RACE cDNA synthesis primer and SMART II oligonucleotide. 5′-RACE was performed using a gene-specific reverse primer (5′-TAA CAC TGT GCC ATG CAA GGT TCA G-3′) complementary to nucleotides 1864–1888 within the 3′-UTR. PCR was performed using the reverse primer (0.8 μM final concentration) and reagents and protocols in the AdvanTage PCR cloning kit (Clontech). Amplification was carried out as follows: 1 min at 94°C followed by 35 cycles of 5 s at 94°C, 10 s at 65°C, and 4 min at 68°C. Products were allowed to extend for 10 min at 68°C. A PCR product of ~1.8 kb was gel purified and sequenced. A small aliquot of this product was reamplified under identical conditions. Reamplification was repeated until a suitable amount of DNA could be attained for in vitro transcription. All cDNA was gel purified and treated with phenol-chloroform-isomyl alcohol before precipitation with 7.5 M ammonium acetate (1.8 M final concentration) and ethanol. All cDNA was then dissolved in RNase-free water.

Northern Analysis

Poly(A)⁺ RNA (3 μg) from kidney, gill, liver, spiral valve, brain, testes, heart, and whole blood cells and total RNA (10 μg) from rectal gland were separated by electrophoresis, blotted, and cross-linked onto a positively charged nylon membrane (Hybond-N⁺, Amersham, Arlington Heights, IL) and then prehybridized in ULTRAhyb (Ambion, Austin, TX). To detect tissue-specific distribution of urea transporter mRNA, a hybridization probe corresponding to nucleotides 154–436 of the cloned urea transporter was constructed using the Marathon 5′-RACE product subcloned into a pCR4-TOPO vector (Invitrogen) and linearized by restriction digestion with AccI. An antisense [α-32P]UTP-labeled riboprobe was generated using the Maxiscript kit (Ambion). Hybridization was performed in ULTRAhyb overnight at 68°C (high stringency) or 63°C (low stringency). The membrane was washed twice for 5 min at room temperature in 2× saline-sodium citrate buffer + 1% SDS (low stringency) and then twice for 15 min at 65°C in 0.1× saline-sodium citrate buffer + 1% SDS (high stringency). The hybridized probe was visualized after incubation at ~80°C for 72 h (high stringency) or 1 wk (low stringency).

Multiple-Tissue RT-PCR

A third stingray was placed in buffered (pH 8.0–8.3) seawater containing amino-phenazic acid ethyl ester until swimming movements had ceased but sustained movement of the gill muscles was maintained (to allow adequate ventilation). The animal was placed dorsal side and head down on a slant board (~30°) so that the spiracles were completely under the water. The conus arteriosis was exposed by a ventral incision proximal to the heart. The conus arteriosis was cannulated, and the animal was perfused at low pressure with ice-cold elasmobranch Ringer solution. Tissues were quickly harvested, snap-frozen in liquid nitrogen, and stored at ~80°C until processed.

A fourth stingray was anesthetized as described above. Whole blood was collected using a 21-gauge needle attached to a heparinized syringe from the caudal haemal arch and centrifuged at 800 g for 1 min. Plasma and blood cells residing at the layer above the erythrocytes were removed. The erythrocytes were then resuspended in elasmobranch Ringer solution. This process was repeated twice to ensure complete removal of nonerythrocytes. Erythrocytes were then transferred to a 50-ml polyethylene tube, snap-frozen in liquid nitrogen, and stored at ~80°C until processed.

Total RNA was isolated from the tissues and erythrocytes using TRIzol reagent as described above. The RNA samples were pretreated with DNase I (Ambion), extracted with phenol-chloroform-isomyl alcohol, and precipitated with sodium acetate-isopropanol. Treated RNA (3 μg) from each tissue was reverse transcribed with an oligo(dT) primer as described above. PCR was conducted using stingray urea transporter-specific primers designed to regions that flank the nucleotide sequence of the riboprobe used for Northern analysis: 5′-ACA AAA TCC ATT CAT GGA GCA-3′ (forward) and 5′-TCT TCT CCG GGT ACG TCA CTT CGC ATA G-3′ (reverse). Degenerate primers to a conserved region of glyceraldehyde-3-phosphate dehydrogenase were used for positive controls: 5′-GAG TCT ACC GGT (AT) TFC TCC ACC ACC A-3′ (sense primer) and 5′-GAG TGA TCT TGC CCA CAC A CAG CCT TGG C-3′ (reverse primer). Amplification of cDNA was conducted using HotStarTaq master mix (Qiagen, Valencia, CA). Products were amplified according to the following parameters: initial denaturing for 14 min at 94°C followed by 30 or 35 cycles of 30 s at 94°C, 30 s at 56°C, and 3 min at 72°C. The final round was followed by extension for an additional 10 min at 72°C.

In Vitro Transcription

cRNA was transcribed directly from the 5′ SMART RACE product using the T7 promoter included in the universal primer. In vitro transcription was performed using the mMessage mMachine kit (Ambion). cRNA was precipitated with LiCl overnight and dissolved in 25 μl of RNase-free water. RNA quality was assessed by electrophoresis on a 2.2 M formaldehyde-1% agarose RNA gel, and concentration was determined by optical density at 260 nm.

Functional Characterization

Oocytes were surgically removed from gravid Xenopus laevis and prepared for [14C]urea uptake studies as described previously (20). Uptake was determined for individual oocytes by incubation in 200 μl of Barth’s solution containing 8 μCi/ml (1.3 mM) [14C]urea (NEN Life Science Products, Bos-
ton, MA) at room temperature. For the time series experiments, urea uptake was terminated between 30 s and 60 min by the addition of 2 ml of ice-cold Barth’s solution containing 1.4 mM deionized urea. For all other experiments, uptake was terminated after 90 s by the addition of 2 ml of ice-cold Barth’s solution containing 1.4 mM deionized urea. Individual oocytes were further washed three times with 2 ml of ice-cold Barth’s medium containing 1.4 mM deionized urea. Oocytes were solubilized in 10% SDS (0.5 ml) in 4 ml of scintillation fluid at 20°C for 1 h with repeated vortexing. [14C]urea uptake was determined by scintillation counting (Coulter LS6500, Beckman, Fullerton, CA).

Phloretin sensitivity of the urea transporter-mediated [14C]urea uptake was determined by preincubation of oocytes in Barth’s medium containing 0.5 mM phloretin for 20 min and then by incubation in the uptake solution containing radiolabeled urea and 0.5 mM phloretin.

To determine whether urea uptake was dependent on the presence of Na+ and/or Cl− in the external medium, oocytes were preincubated for 1 h in a modified Barth’s medium containing 8 μCi/ml (1.3 mM) [14C]urea in which the NaCl was replaced by sufficient mannitol to bring the osmolality of the solution to 200 mosmol/kgH2O (~180 mM mannitol). [14C]urea uptake by individual oocytes in this mannitol-Barth’s medium was determined by incubation for 90 s.

The effect of urea analogs on stingray urea transporter (strUT)-1-mediated [14C]urea uptake was determined by incubating oocytes in a modified mannitol-Barth’s uptake medium, where 150 mM mannitol was replaced with 150 mM urea or one the following urea analogs: acetamide, thiourea, methylurea, or 1,3-dimethylurea. The effect of trimethylamine oxide (TMAO) on [14C]urea uptake was also tested by replacement of the mannitol in the uptake buffer with 150 mM TMAO. All uptake solutions were adjusted to 210 mosmol/kgH2O using additional mannitol if required. Briefly, oocytes were held in mannitol-Barth’s medium for 1 h and then preincubated at room temperature for 3 min in the appropriate uptake solution. Uptake of radiolabeled urea by individual oocytes was determined after incubation in the appropriate uptake solution containing [14C]urea.

Statistical Analysis

Data from the functional characterization studies were not distributed normally (Kolmogorov-Smirnov test). Therefore, the data were logarithmically transformed before statistical analysis using one-way ANOVA. If data did not fit a normal distribution after transformation, one-way ANOVA on ranks was used to determine significance. Post hoc comparisons were tested using the Tukey-Kramer method (parametric) or Dunn’s multiple comparison test (nonparametric). Statistical significance was achieved when P < 0.05. Values are means ± SE.

RESULTS

We used RT-PCR and 5′/3′-RACE to obtain a full-length cDNA sequence for strUT-1. The strUT-1 cDNA is 2,670 bp long, with a putative open-reading frame (ORF) of 1,296 bp (73–1,368), a 5′-UTR of 72 bp, and a 3′-UTR including the poly(A)+ tail that is 1,302 bp long (Fig. 1; GenBank accession no. AF443781). Two sequential polyadenylation signals (AATAAA) at nucleotide positions 1250–1255 and 1258–1263 are located just upstream of the poly(A)+ tail.

The first ORF of the strUT-1 cDNA is the longest and encodes a putative protein 431 amino acids long (strUT-1; Fig. 2). The putative protein is 50 amino acids longer than the shUT; i.e., strUT-1 has a longer and unique COOH terminus. The molecular weight of strUT-1 was calculated to be 48,000. The putative protein is 71% identical to shUT and 53% identical to the frog kidney urea transporter (Fig. 2). However, if only the predicted amino acids 1–377 are considered, strUT-1 is 79% identical to shUT. Compared with the mammalian urea transporter isoforms, strUT-1 has a slightly higher sequence identity to rat UT-A2 (55%) and human UT-A2 (53%) than either rat UT-A3 (45%) or UT-A4 (47%) and shares 48% sequence identity with UT-B1.

The putative protein contained a repeated LP motif (aa 165–172 and 327–333), a feature characteristic of urea transporters. Furthermore, strUT-1 lacks the ALE domain, a feature present in UT-B1 and UT-B2 but absent from other urea transporters. Consensus site analysis of strUT-1 identified a single glycosylation site (NITW) at amino acids 203–206. Several protein kinase CK2 phosphorylation sites were also noted: TIVE (aa 6–9), TWPD (aa 205–208), and TYPE (aa 360–363). In contrast to shUT, strUT-1 contains a protein kinase C (PKC) phosphorylation site (SDK) at amino acids 129–131. As with other urea transporters from the lower vertebrates and rat UT-A3, strUT-1 contains a multicopper oxidase signature (GLWSSYNVLACIAVGGMFYAL, aa 276–296). Interestingly, rat UT-A2 has a number of amino acid changes in this region and thus does not contain this multicopper oxidase signature.

High-stringency Northern analysis of poly(A)+ RNA using a 5′-RACE riboprobe specific to strUT-1 detected four transcripts in kidney. In contrast, no transcripts were detected in other tissues (Fig. 3). The renal transcripts had molecular sizes of 2.8, 3.8, 4.5, and 5.5 kb. The 2.8-kb transcript was very close to the predicted size of strUT-1 was detectable only in kidney after 30 cycles (data not shown). However, after 35 cycles of PCR, a single product the same size as the product obtained for kidney was detected in all the extrarenal tissues examined and in erythrocytes (Fig. 4). PCR containing DNase I-treated total RNA that had not been reversed transcribed did not produce any detectable product (Fig. 4), indicating that the detection of strUT-1 in extrarenal tissues was not due to DNA contamination of any of the total RNAs. These findings indicate that strUT is expressed in extrarenal tissues but at markedly lower levels than in kidney.
Heterologous expression of strUT-1 in *Xenopus* oocytes induced a marked increase in \[^{14}\text{C}\]urea uptake (Fig. 5). \[^{14}\text{C}\]urea uptake by oocytes injected with 25 ng of strUT cRNA was logarithmic, with maximal uptake after 40 min (data not shown). Characterization of the strUT-1-induced \[^{14}\text{C}\]urea uptake was determined at the initial rate of urea uptake, i.e., 90 s after the oocytes were exposed to the uptake medium. Oocytes injected with strUT-1 cRNA exhibited a 33-fold elevation in \[^{14}\text{C}\]urea uptake over water-injected oocytes (68.1 \pm 5.7 vs. 2.1 \pm 1.3 pmol/oocyte \cdot \text{s}^{-1}, P < 0.05; Fig. 5). Preincubation with 0.5 mM phloretin completely attenuated strUT-1-induced \[^{14}\text{C}\]urea uptake (P < 0.05; Fig. 5). Replacement of the NaCl did not significantly alter \[^{14}\text{C}\]urea uptake by oocytes injected with 25 ng of strUT cRNA (Fig. 5). This finding indicates that strUT-1-induced urea uptake was independent of external Na\(^+\) and Cl\(^-\).

The \[^{14}\text{C}\]urea uptake induced by strUT-1 was not significantly inhibited by 150 mM urea, acetamide, or TMAO (Fig. 6). In contrast, the urea analogs thiourea, methylurea, and 1,3-dimethylurea at 150 mM markedly inhibited urea uptake by 75, 74, and 92%, respectively (Fig. 6).

**DISCUSSION**

Whether marine elasmobranchs are in the ocean or in estuaries or rivers, renal reabsorption of urea appears to be one of the central mechanisms underpinning their osmoregulatory strategy (i.e., to maintain body fluid osmolality higher than that of the surround-
The mechanisms involved in and the tubular site(s) of elasmobranch renal urea reabsorption have yet to be identified. Several mechanisms have been proposed by which urea can be reabsorbed from the tubular fluid of elasmobranchs. These mechanisms have involved passive reabsorption of urea down localized concentration gradients via facilitated urea transporters and/or active reabsorption via Na⁺/H⁺ urea cotransporters (3, 18, 34, 46).

We hypothesized that if phloretin-sensitive facilitated urea transporters played a role in tubular urea reabsorption in the elasmobranch kidney, urea transporters homologous to that identified in the kidney of S. acanthias would also be present in the kidneys of D. sabina, a myliobatiform marine elasmobranch that exploits habitats across a remarkable range of environmental salinities. Using 5′-RACE techniques, we identified a novel urea transporter from the kidney of the Atlantic stingray. We designated this transporter strUT-1. This urea transporter was functional, displaying phloretin-inducible urea transport comparable to that observed for other lower vertebrate and mammalian urea transporters (1, 9, 20, 36, 39, 44, 45, 49).

The stingray urea transporter strUT-1 was predicted to be 431 amino acids long, making it 51 amino acids longer than the shark urea transporter. The difference in length is due to an extension of the hydrophilic COOH-terminal region of strUT-1. This COOH-terminal sequence was predicted to be 431 amino acids long, showing a high degree of sequence identity to shUT (71%) and fUT (53%) but has a longer and distinct COOH-terminal sequence.
nal extension represents a unique sequence not present in other lower vertebrate or mammalian urea transporters. Inasmuch as no putative consensus regulatory sites are predicted to reside within this COOH-terminal extension, its functional significance remains to be determined.

Except for the COOH-terminal extension, the stingray and shark urea transporters have a high degree of sequence identity. Furthermore, several consensus regulatory sites (glycosylation, protein kinase CK2, and multicopper oxidase) are located at similar positions in both transporters. However, there are some differences between the two transporters in the number of consensus regulatory sites.
The stingray urea transporter has a putative protein kinase CK2 regulatory site close to the NH$_2$ terminus, a feature that is absent from the shark urea transporter. The shark urea transporter has two potential glycosylation sites in the NH$_2$-terminal region that are absent from strUT-1. Interestingly, the stingray urea transporter contains a PKC consensus site within its NH$_2$ terminus, again a feature that is absent from the shark renal urea transporter. The presence of a PKC consensus site in strUT-1 leads us to propose that PKC may act to directly alter the function of the stingray, but not the shark, urea transporter.

Recent analysis of the mammalian urea transporter genes indicates that they have developed by a series of duplications of a common ancestral module that have resulted in two genes [UT-A (Slc14a2) and UT-B (Slc14a1)] (11, 26, 27). Four UT-A protein isoforms are expressed in the medullary tubular epithelia of the kidney (33). Interestingly, each isoform is generated by several splice variants (2). Transcription of three of the isoforms (UT-A1/A3/A4) is under the control of one promoter, whereas the other (UT-A2) is under the control of another promoter (26).

The gene(s) encoding urea transporters from the lower vertebrates has yet to be sequenced. However, comparison of the cDNAs of strUT-1 and shUT provides some insight into the elasmobranch urea transporter gene. Although _D. sabina_ and _S. acanthias_ are representative of phylogenetically distinct marine elasmobranchs, there is a high degree of sequence identity within the ORFs of the cDNAs for strUT-1 and shUT (with the exception of the most 3' region of the ORF). Thus we propose that strUT-1 and shUT are orthologous products of a common elasmobranch urea transporter gene and that transcription of these products may be under the control of a single promoter (based on similarities in the sequence and length of the 5'-UTRs).

Elucidation of the cDNA and putative protein sequences for the urea transporters from the lower vertebrates has provided preliminary information about the possible links between the lower vertebrate and mammalian genes. On the basis of the observations that the dogfish shark urea transporter had a slightly higher putative protein sequence identity to UT-A2 than to UT-B2 and lacked the ALE domain (44, 45), we propose that the urea transporters from elasmobranch and teleost fish and the mammalian UT-A2s are derived from a common ancestral form. A corollary of this proposal is that, of the mammalian urea transporter genes, the UT-A2 component of the mammalian UT-A gene is the most representative of the common ancestral form.

Preliminary evidence from our laboratory indicates that a shUT-like isoform may be present in the kidneys of other elasmobranchs (14). Thus shUT appears to be representative of a common urea transporter isoform present in the kidneys of phylogenetically distinct elasmobranch groups. In contrast, it has yet to be determined whether an isoform equivalent to strUT-1 is expressed in the kidneys of elasmobranchs other than the Atlantic stingray. The renal urea transporters appear to play an important role in the regulation of tubular urea reabsorption, which, in turn, appears to contribute to the maintenance of water homeostasis after movement between habitats of different salinities. The identification of those elasmobranch species that express the strUT-1 isoform may help clarify the role of this urea transporter. It is possible that this isoform may be unique to _D. sabina_. However, it is interesting to speculate that the strUT-1-like isoform may be expressed in the kidneys of euryhaline elasmobranchs but not in the kidneys of the stenohaline and/or marginally euryhaline elasmobranchs.

In the mammalian kidney, the various products of the UT-A gene have specific tubular locations and specific functions in the urinary concentrating process: urea reabsorption from the lumen of the medullary collecting duct or recycling between the medullary interstitium and the tubular fluid (32). The complex distribution of cell types in the elasmobranch nephron, the bundling of proximal and distal nephron segments in a countercurrent arrangement within a peritubular sheath, the meandering arrangement of proximal and distal nephron segments in the sinus zone (23, 24), and zones of differing interstitial urea concentration within the kidney (18) indicate that urea reabsorption may occur at several sites along the nephron. Thus it is possible that different urea transporter isoforms may have specific tubular locations or play different roles in urea reabsorption by the elasmobranch kidney.

A number of urea analogs (at 150 mM) have been found to inhibit facilitated urea transporter-mediated urea uptake by oocytes (9, 36–38, 49). The most potent inhibitors, thiourea and 1,3-dimethylurea, markedly attenuate facilitated urea transporter-mediated urea uptake, including that induced by strUT-1 in the present study. However, there are differences among the urea transporter isoforms in the effectiveness of acetamide blockade of urea uptake. Acetamide inhibits UT-A3-induced urea uptake (37) and partially inhibits the uptake of urea induced by the gill urea transporter from the teleost fish, the Lake Magadi tilapia (44), but does not alter urea uptake induced by UT-A2 (49). Because acetamide did not alter strUT-1-induced urea uptake, the stingray urea transporter shares a similar functional characteristic with UT-A2.
Northern analysis indicated that strUT-1 as well as homologous transcripts appeared to be almost exclusively expressed in kidneys of Atlantic stingrays. Only in liver, under low-stringency conditions, was a low-molecular-weight transcript detected. This limited tissue expression of urea transporter transcripts detectable by Northern analysis contrasts with that observed for the dogfish shark, where transcripts homologous to shUT were detected in the kidney and brain of S. acanthias by high-stringency analysis and in all the tissues surveyed by low-stringency Northern analysis (39). Therefore, we further examined the apparent renal-specific strUT expression by utilizing the more sensitive technique of RT-PCR to determine whether strUT-1 or homologous transcripts were expressed in extrarenal tissues as well as in the kidney of *D. sabina*. Our findings indicated that strUT message was expressed in all extrarenal tissues examined, but at markedly lower abundance than in the kidney. If we assume that the expression of urea transporter protein parallels transcript abundance, then the extrarenal expression of strUT message has interesting implications for the role of strUT-like urea transporters in a number of tissues. Expression of strUT transcripts in liver and erythrocytes would indicate that urea movement across hepatocyte and erythrocyte membranes could occur, in part, through a strUT-1 or similar facilitation across hepatocyte and erythrocyte membranes.

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