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Regulation of a renal urea transporter with reduced salinity in a marine elasmobranch, *Raja erinacea*

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Summary

Marine elasmobranchs retain urea and other osmolytes, e.g. trimethylamine oxide (TMAO), to counterbalance the osmotic pressure of seawater. We investigated whether a renal urea transporter(s) would be regulated in response to dilution of the external environment. A 779 bp cDNA for a putative skate kidney urea transporter (SkUT) was cloned, sequenced and found to display relatively high identity with facilitated urea transporters from other vertebrates. Northern analysis using SkUT as a probe revealed three signals in the kidney at 3.1, 2.8 and 1.6 kb. Upon exposure to 50% seawater, the levels of all three SkUT transcripts were significantly diminished in the kidney (by 1.8- to 3.5-fold). In response to environmental dilution, renal tissue osmolality and urea concentration decreased, whereas water content increased. There were no significant differences in osmolyte and mRNA levels between the dorsal–lateral bundle and ventral sections of the kidney. Taken together, these findings provide evidence that the downregulation of SkUT may play a key role in lowering tissue urea levels in response to external osmolality.

Key words: osmoregulation, trimethylamine oxide, urea transporter gene, skate, *Raja erinacea*.

Introduction

Urea retention is the cornerstone of the marine elasmobranchs' osmoregulation strategy. A relatively high concentration of urea and trimethylamine oxide (TMAO) in the extracellular fluid allows these fishes to be slightly hyperosmotic to the marine environment (Smith, 1936). Urea produced in the liver is retained in the blood by the very low permeability of the gills and by reabsorptive mechanisms in the kidney (Boylan, 1967; Wood et al., 1995; Fines et al., 2001; Walsh and Smith, 2001). It has been calculated that approximately 90–96% of the urea in the glomerular filtrate is reabsorbed by the kidney tubule (Clark and Smith, 1932; Goldstein and Forster, 1971; Payan et al., 1973), ultimately resulting in only 4–7% of urea excretion to the environment (Payan et al., 1973; Wood et al., 1995). However, the exact site within the kidney and the mechanisms responsible for this reabsorption are still uncertain.

The elasmobranch nephron (Fig. 1) consists of five loops separated into two distinct regions – the dorsal–lateral bundle, which is enclosed by connective tissue, and the ventral 'mass', within which individual tubules meander convolutedly in blood sinuses (Lacy and Reale, 1985). The arrangement of the loops allows for a countercurrent system that may be involved in fluid regulation and the passive reabsorption of urea (Lacy et al., 1985). Hentschel et al. (1986) reported the presence of a lower urea concentration and a higher sodium concentration in the dorsal bundle zone compared to the ventral zone. The differences in the mean urea concentrations indicate a gradient between the two regions of the kidney. The presence of a gradient suggests that the countercurrent arrangement of the very early and late segments of single renal tubules supports the passive reabsorption of urea in the dorsal region.

It has been speculated that a carrier-mediated process is involved in elasmobranch renal urea absorption. It was first proposed that the reabsorption process is active, possibly connected to the reabsorption of sodium. Schmidt-Nielsen et al. (1972) found a 1:1.6 stoichiometry between urea and Na^+ reabsorption in the shark kidney. Recent evidence, however, supports the role of a facilitated urea transporter (UT) in the renal reabsorption process in elasmobranchs. A shark facilitated urea transporter has been isolated from the dogfish kidney, which is 66% homologous to the mammalian UT-A2 urea transporter (Smith and Wright, 1999). In the ureotelic gulf toadfish *Opsanus beta* and the Lake Magadi tilapia *Alcolapia grahami* urea transporter cDNA has been isolated from the gills and functional expression characterized (Walsh et al., 2000, 2001a). A homologue of the toadfish gill UT has been shown to exist in the gills of a wide range of marine teleost fishes (Walsh et al., 2001b). Interestingly, in the ammonotelic eel *Anguilla japonica*, a gill urea transporter has been isolated that is upregulated upon transfer from freshwater to seawater (Mistry et al., 2001). Regulation of renal UT mRNA expression in elasmobranchs has not been examined.

In euryhaline and marginally euryhaline elasmobranchs, dilution of the external salinity results in marked reductions of

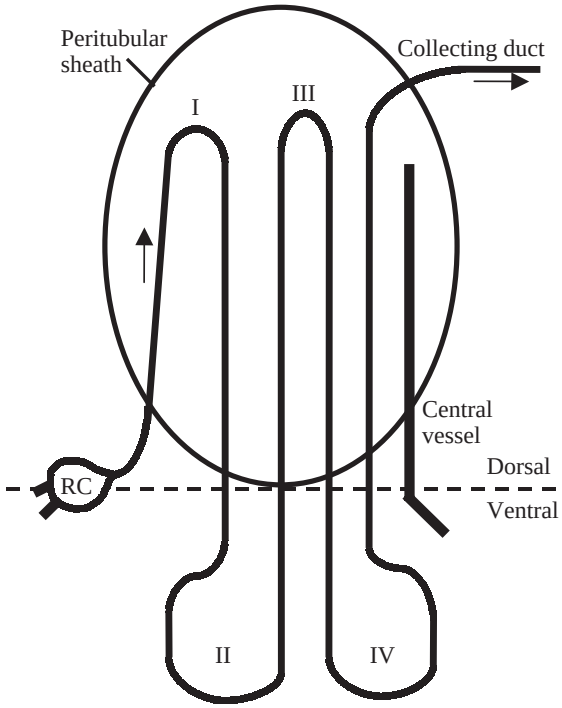

Fig. 1. Simplified drawing of the skate kidney nephron, confined within the peritubular sheath in the dorsal zone (dorsal–lateral bundle) and unconfined in the ventral zones in blood sinuses. Arrows indicate direction of tubular flow from the renal corpuscle (RC) to the collecting duct. Roman numerals indicate loop number. (After Lacy et al., 1985.)

urea concentrations in body fluids and, to a lesser extent, osmotic constituents such as TMAO and amino acids (Price and Creaser, 1967; Goldstein and Forster, 1971; Forster and Goldstein, 1976; Cooper and Morris, 1998; Sulikowski and Maginniss, 2001). During environmental dilution in the little skate, changes in urea permeability of gills are small, but renal urea excretion is sensitively attuned to osmoregulatory demands (Goldstein and Forster, 1971; Payan et al., 1973). Renal urea excretion is increased, resulting from increases in glomerular filtration rate, urinary flow, filtered load of urea, urea clearance, and in the percentage of urea excreted relative to filtered by the kidney (Goldstein and Forster, 1971; Payan et al., 1973). There is very little evidence, however, to suggest what mechanisms regulate the changes in renal urea reabsorption.

The focus of this study was twofold. Firstly, to examine the little skate *Raja erinacea* kidney for gradients of osmolality, urea and TMAO between the dorsal–lateral and ventral regions to help clarify the sites of renal urea reabsorption. Secondly, to identify skate renal urea transporters homologous to the shark facilitated UT (ShUT; Smith and Wright, 1999). A partial cDNA (779 bp) sequence of a urea transporter was isolated from the skate kidney to be used as a probe in northern analysis to measure SkUT: β-actin mRNA levels in skates exposed to 100% and 50% seawater. We predicted that SkUT mRNA levels would be lower in skates exposed to 50% seawater, based on previous reports of increased rates of renal excretion relative to urea reabsorption (Goldstein and Forster, 1971; Payan et al., 1973).

Materials and methods

Experimental animals

Little skates (*Raja erinacea* Mitchill 1825) were obtained by otter trawling in Passamaquoddy Bay, New Brunswick, Canada during the months of July and August, 2001, and maintained at the Huntsman Marine Science Center in 1000 litre outdoor tanks under natural photoperiod and supplied with filtered seawater (10°C). For some experiments, skates were transported to the Hagen Aqualab, University of Guelph, where they were maintained under natural photoperiod in artificial seawater (10°C). The skates were fed (filleted, chopped herring) on alternate days and were observed to feed.

Experiment protocol

Two groups of skates were transferred to 250 litre tanks with a flow-through, aerated system. One group was kept at 100% seawater (control, N=10); the other (N=10) was exposed to 75% salinity for 24 h, after which the salinity was decreased again (by 2‰ per hour) to 50% salinity and maintained for 5 days. Both groups were fasted for the duration of the experiment. At the end of the experimental period, skates were killed by a blow to the head, followed by severance of the spinal cord. The paired kidneys were then rapidly removed and dissected into dorsolateral and ventral sections, as described by Hentschel et al. (1986). The tissue was immediately frozen in liquid nitrogen and stored at –80°C for later analysis (within 3 months). Due to the small size of the skate kidney after separation into sections (approximately 1 g), half of the samples (5) were used for tissue analysis of urea, TMAO and osmolality, while the other samples were used for northern analysis of SkUT. All samples were used in water content determination.

Kidney tissue analysis

For urea and trimethylamine oxide (TMAO) determination, approximately 1 g (wet mass) of kidney tissue was homogenized (Euro Turrax T20b, IKA Labortechnik, Staufen, Germany), in 10 times (w/v) of 5% trichloroacetic acid (TCA). The samples were then centrifuged at 10 000 ***g*** for 10 min and the supernatant removed for the measurement of urea (Rahamatullah and Boyde, 1980) and TMAO (Wekell and Barnett, 1991). For osmolality determination, the method outlined by Schmidt-Nielson et al. (1983) was used. In brief, approximately 10 mg (wet mass) of tissue was added to a microcentrifuge tube containing 25 μl of dH_2O. This tube was tightly sealed and placed in boiling water for 3 min. After brief centrifugation, the samples were stored at 4°C for 24 h to allow diffusion. The samples were then briefly centrifuged and the osmolality of the supernatant analyzed (Vapro™ Vapour Pressure Osmometer 5520, Wescor, Logan, Utah, USA). To determine the final osmolality, a dilution factor was used, as follows:

$$\text{Dilution factor} = (25 + F_{H2O}m)/F_{H2O}m,$$

where m is the wet mass of the tissue (g), and F_{H2O} is the fractional water content.

RT–PCR

To clone a species-specific kidney urea transporter from the little skate, a reverse transcriptase/polymerase chain reaction (RT–PCR) approach was used. Total RNA was isolated from a little skate kidney by homogenization (Euro Turrax T20b) of approximately 1 g of tissue with 10 times (w/v) of phenol-guanidium thiocyanate (Trizol reagent, Gibco BRL, Burlington, ON, Canada) followed by standard chloroform extraction and isopropanol precipitation (Sambrook et al., 1989).

First-strand cDNA was synthesized by Moloney Murine Leukemia Virus (M-MuLV) reverse transcriptase and oligo(dT) primers (Amersham, Oakville, ON, Canada). Nested PCR was performed on this cDNA using specific primers designed from a consensus of DNA sequences from shark (ShUT), toadfish (tUT) and rat urea transporters. These were sense F1: 5′ACAAAATCCATTCATGGAGCA3′ (corresponding to bp 89–109 of ShUT) and F2: 5′TCAGGTGATGTTTGTCAA-CAA3′ (corresponding to bp 281–301 of ShUT); antisense R1: 5′CCAAGTGCATGCAGGTAATC3′ (corresponding to bp 1081–1100 of ShUT). HotStarTaq DNA polymerase (Qiagen, Mississauga, ON, Canada) was used for the PCR, and the conditions were 94°C for 30 s, 57.5°C for 30 s, 72°C for 60 s (40 cycles) followed by 72°C for 7.5 min. For nested PCR, 1 μl of the product from primers F1 and R1 was used in a second PCR reaction with primers F2 and R1.

PCR products were separated by gel electrophoresis (1.5% agarose gel in TAE buffer (40 mmol l^{-1} Tris-acetate, 1 mmol l^{-1} EDTA, pH 8.0), and the major band at 820 bp, corresponding to the band in the ShUT positive control, was gel-purified (Qiagen) and ligated into the plasmid vector pGEM-T Easy (Promega, Fisher, Mississauga, ON, Canada). The resultant plasmid was transfected by electrophoresis into One Shot TOP10 Electrocomp *E. coli* (Invitrogen, Burlington, ON, Canada). Standard blue/white screening on Luriabroth (LB) plates, containing 50 μg ml^{-1} ampicillin and 160 μg ml^{-1} X-Gal, identified positive colonies with potential inserts, which were then cultured in LB. The plasmid DNA was isolated by the alkaline lysis method (Qiagen). The insert DNA of several clones was sequenced and found to be homologous to the shark urea transporter (ShUT).

Northern blot analysis

Total RNA was extracted from kidneys of experimental skates as described above. In a separate analysis, total RNA was extracted from several skate tissues (kidney, liver, gill, brain, heart, skeletal muscle and intestine) to determine the presence of SkUT in different tissues. Equal amounts (10 μg) of total RNA were loaded onto a formaldehyde–agarose gel (1.5% agarose), electrophoresed and transferred to a nylon membrane (Hybond-N, Amersham) using standard methods (Sambrook et al., 1989). Hybridization of the membrane is based on the method of Church and Gilbert (1984). In general, membranes were pre-hybridized for 3 h at 65°C in hybridization solution, containing 0.5 mol l^{-1} phosphate buffer (Na_2HPO_4, pH 7.2), 7% (w/v) lauryl sulfate (SDS), 1 mmol l^{-1} EDTA, pH 8.0 and 1% (w/v) bovine serum albumin (BSA). The membrane was initially hybridized at 65°C in hybridization solution with ^{32}P-labeled skate urea transporter (SkUT) probe of the 779 bp fragment, followed by a ^{32}P-labeled skate β-actin probe as an invariant control gene. [^{32}P]-probe was produced by first amplifying the DNA clone using a PCR reaction, followed by purification using a PCR purification kit (Qiagen). The resulting probe was labeled with [α-^{32}P]-dCTP (Amersham) using the T7 QuickPrime kit (Pharmacia Biotech, Mississauga, ON, Canada) and purified with QiaQuick nucleotide removal kit (Qiagen). Final washes were performed at 65°C and the wash solution contained 40 mmol l^{-1} Na_2HPO_4, pH 7.2, 1% (w/v) SDS, 1 mmol l^{-1} EDTA, pH 8.0. After exposure on a PhosphorImager cassette (Molecular Dynamics, Sunnyvale, CA, USA), the image was scanned using a PhosphorImager SI (Molecular Dynamics) and densitometry was analyzed using the program ImageQuant 5.0 (Molecular Dynamics). The membrane was then stripped using Denhardt's solution (0.1×), 5 mmol l^{-1} Tris-HCl, pH 8.0 and 2 mmol l^{-1} EDTA, pH 8.0 at 65°C for 1 h and rehybridized with β-actin probe as described above.

Statistical analysis

All data are presented as means ± standard error of the mean (S.E.M.). A one-way analysis of variance (ANOVA) was used to establish differences between the control and skates exposed to a dilute environment. Tukey–Kramer tests and Student *t*-tests were used to determine where differences were significant ($P<0.05$).

Results

Exposure to dilute environment

After exposure to 50% seawater, there was a significant increase (+6%) in the kidney tissue % water content, and a corresponding decrease (from –30% to –38%) in the osmolality of the kidney tissue (Table 1). If the water content is expressed as ml g^{-1} dry mass, then the changes are more pronounced (1.6-fold; Table 1). In both the dorsal and ventral sections of the kidney there was a significant decrease (–35%) in the tissue concentration of urea (Fig. 2); however, there was no corresponding change seen in the tissue concentration of TMAO (Fig. 3).

Cloning the skate urea transporter (SkUT)

Using PCR, a 779 bp piece of cDNA was isolated (accession no. AY161305; Fig. 4) with high homology to published sequences of urea transporters (UTs), specifically the shark kidney urea transporter (ShUT) (Smith and Wright, 1999) and the gill urea transporters of the toadfish (tUT) and Magadi tilapia (mtUT) (Walsh et al., 2000, 2001a). The longest open reading frame (ORF) begins at nucleotide 2 and extends to the end of the fragment. This encodes for a 259-amino-acid-

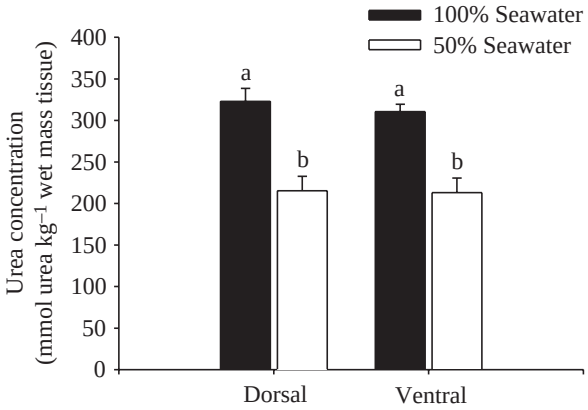

Fig. 2. Urea concentrations in the dorsal and ventral regions of the kidney of the little skate *Raja erinacea* exposed to 100% and 50% seawater for 5 days. Values are means ± S.E.M., *N*=5. Values sharing the same letter are not significantly different (Tukey test, $P<0.05$).

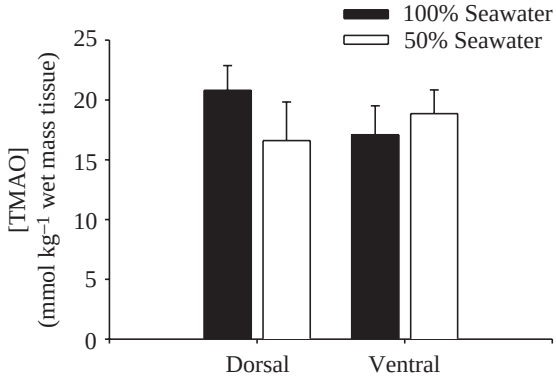

Fig. 3. Trimethylamine oxide (TMAO) concentrations in the dorsal and ventral regions of the kidney of the little skate *Raja erinacea* exposed to 100% and 50% seawater for 5 days. Values are means ± S.E.M., *N*=5.

residue partial protein, which was named 'SkUT' (skate UT). This sequence is approximately 68% complete when compared to the ShUT amino acid sequence.

Northern blot analysis

High-stringency northern analysis of different tissues revealed hybridization of SkUT cDNA to mRNA in the kidney, with a strong signal at 3.1 kb and slightly weaker signals at 2.8 kb and 1.6 kb (Fig. 5). Of the other tissues studied (gill, liver, intestine, heart, muscle and brain), only brain showed a weak signal at 3.1 kb; the other signals observed in the kidney were not present in the brain. Examination of the relative expression levels in kidney mRNA from skates exposed to 50% seawater indicated that there was a significant decrease of 1.8- to 3.5-fold in all three bands in the dorsal region and in the 3.1 kb and 1.6 kb bands in the ventral region (Fig. 6), indicating a downregulation of the SkUT mRNA.

Discussion

Response to environmental dilution

Elasmobranchs are remarkable in the complexity of the kidney nephron structure. Detailed histological examinations have revealed the sequence and structure of the nephron tubules, but there is little information on the mechanisms of urea reabsorption (Lacy and Reale, 1999). Although it was predicted that solute gradients would exist between the dorsal and ventral kidney sections, there were no differences detected in urea or TMAO levels, either before or after environmental dilution. Neither was there a difference in osmolality, suggesting that overall there is no osmotic gradient between the two sections.

The counter-current model of Boylan (1972) predicts a zone of low urea concentration around the terminal segment (i.e. collecting duct) of the elasmobranch nephron. A lower urea concentration and higher water content was reported in the dorsal bundle zone compared to the ventral zone in the kidney of *R. erinacea*, but the actual amounts of urea were identical in the two zones (Hentschel et al., 1986). In the present study, urea and water content were not significantly different between the renal dorsal and ventral sections, leading to the same conclusion. Thus, there is no evidence for urea zonation between the two major sections in the skate kidney, unlike the situation between the cortex and inner medulla in the mammalian kidney (Bankir, 1995).

The peritubular sheath appears to segregate the dorsal tubule bundle of a single nephron from other tubule bundles (Fig. 1).

Table·1. *Comparison of water content and osmolality in the dorsal and ventral regions of the kidney of the little skate* Raja erinacea *exposed to 50% seawater for 5 days*

	Dorsal		Ventral		
	100%	50%	100%	50%	*N*
Water content (ml·g^{-1}·dry·mass)	81.91±1.02	87.86±0.65*	82.14±0.93	87.71±0.74†	10
	4.69±0.32	7.47±0.48*	4.74±0.32	7.40±0.50†	10
Osmolality (Osmol·kg^{-1})	913.81±21.60	635.22±8.99*	981.73±29.04	608.71±23.51†	5

100% Seawater = 910·mmol·kg^{-1}; 50% Seawater = 494·mmol·kg^{-1}.
Values are means ± S.E.M. (*N* = number of fish).
*Significantly different from 100% SW Dorsal; †significantly different from 100% SW Ventral (Tukey test, $P<0.05$).

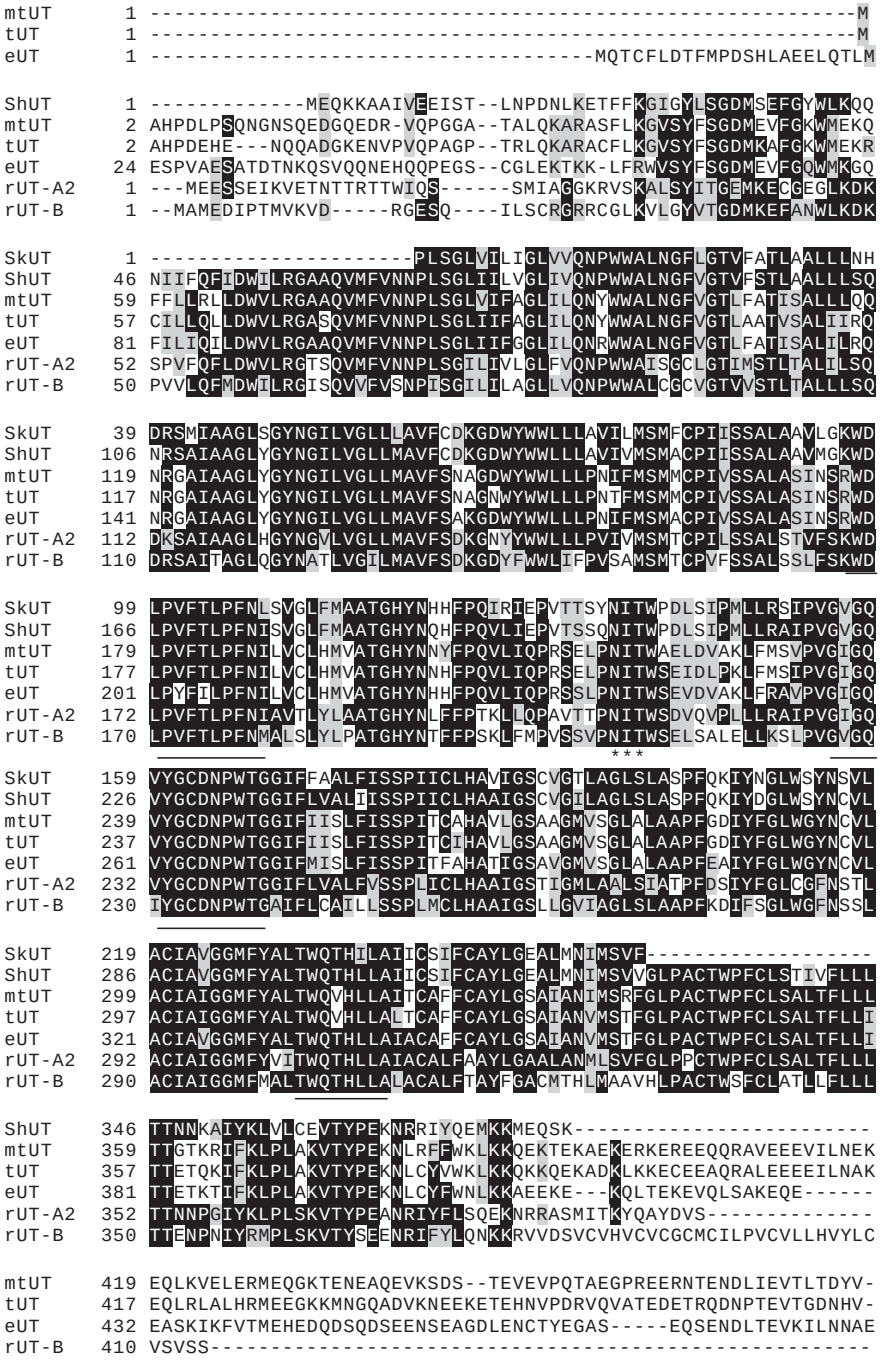

Fig. 4. Alignment of SkUT with other urea transporter (UT) proteins. Abbreviations and accession numbers are as follows: ShUT, shark UT, AF257331; mtUT, Magadi tilapia UT, AF278537; tUT, toadfish UT, AF165893; eUT, eel UT, AB049726; rUT-A2, rat UT-A2, U09957; rUT-B, rat UT-B, X98399. Black boxes denote identical amino acid residues, grey boxes denote conservative substitutions, and clear areas denote non-identity. *Indicates possible *N*-glycosylation site; underline indicates conserved amino acid motif.

Internal osmotic pressure in the skate is slightly higher relative to the external environment. This results in a net uptake of water, which was evident in our study by the increased water content of the kidney tissue in skates exposed to a gradual reduction in salinity over 2 days and then maintained in 50% seawater for 5 days. This elevation in body mass, which persists after 5 days, is characteristic of partial-osmoregulators transferred to 50% seawater, such as *Trygonoptera testacea* (7%) and *Heterodontus portusjacksoni* (15%; Cooper and Morris, 1998). The ability to reverse initial water uptake and return body mass to near pre-dilution levels is considered a criterion of osmoregulation for marine elasmobranchs, such as *Dasyatis sabina*, that tolerate wide variations in environmental salinity (Goldstein and Forster, 1971; De Vlaming and Sage, 1973).

During environmental dilution of varying degrees, it has been demonstrated in several elasmobranch species that the decline in plasma urea concentration was greater than that of sodium or chloride concentration (Cooper and Morris, 1998; Sulikowski and Maginniss, 2001). As predicted, skates in dilute seawater retained less urea in the kidney. The significant decrease in osmolarity and urea concentrations in the skate kidney at 50% seawater is comparable to other studies on marine skates where changes in muscle, plasma and erythrocyte urea levels have been determined (Goldstein and Forster, 1971; Payan et al., 1973; Forster and Goldstein, 1976; Boyd et al., 1977). For example, plasma osmolarity and urea decreased by 25% (100% SW, 965 mmol kg^{-1} to 50% SW, 719 mmol kg^{-1}; Forster and Goldstein, 1976) and 44% (100% SW, 396 mmol l^{-1} to 50% SW, 220 mmol l^{-1}; Goldstein and Forster, 1971) after 1 week in a dilute environment.

Payan et al. (1973) reported a slight, but significant, increase in total body urea clearance in the little skate on the fifth day

The presence of tight junctions between the sheath cells suggests that they act as a barrier so that the environment of solutes and water is different inside relative to outside the bundle (Lacy and Reale, 1985). This suggests that the dorsal part of a nephron surrounded by its peritubular sheath forms its own counter-current exchange and that there is a urea gradient from the tip of the bundle to the ventral end present within each bundle (Hentschel et al., 1998). In order to examine this hypothesis, fine scale sampling (e.g. micropuncture) of tubule sections would be needed, but complexity of the elasmobranch nephron makes this extremely difficult.

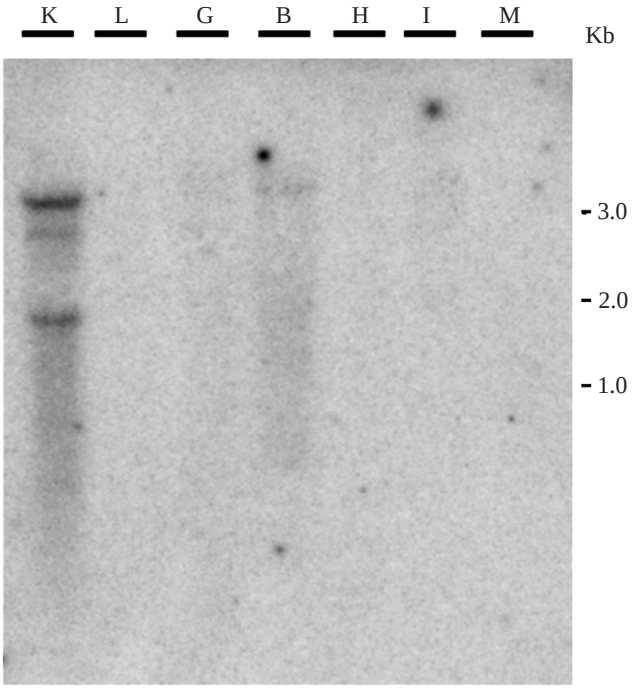

Fig. 5. Tissue distribution of SkUT using high-stringency northern analysis. Membranes were probed using ^{32}P-labelled random-primed SkUT cDNA probe and hybridized at 65°C. Each lane contained 10 μg of total RNA. K, kidney; L, liver; G, gill; B, brain; H, heart; I, intestine; M, muscle. The positions of kb markers are shown.

after introduction to a dilute environment, the net change between an increase in renal urea excretion and a reduction in branchial urea excretion. It is possible that, upon the initial exposure to a dilute environment (e.g. 0–24 h), a more pronounced increase in renal urea excretion occurs prior to a decrease in branchial urea excretion (Goldstein and Forster, 1971; Payan et al., 1973), and the net effect is a higher rate of total body urea loss to the environment, which, in turn, would have an impact on tissue urea levels. This putative large initial increase in renal urea excretion would be partially due to a decrease in fractional urea reabsorption (Goldstein and Forster, 1971; Payan et al., 1973), which could be related, in part, to the downregulation of urea transport proteins such as SkUT (see below). It would be very interesting to compare the time course of the downregulation of SkUT mRNA and protein levels with the changes in renal urea excretion rates. A rapid decrease in the rate of urea biosynthesis may also play a role in reducing internal urea levels (Goldstein and Forster, 1971), but further research on the activity of the ornithine–urea cycle enzymes during environmental dilution is needed.

The other key osmoregulatory solute in elasmobranchs is trimethylamine oxide (TMAO). During environmental dilution, however, the kidney tissue levels of TMAO remained unchanged. Similar findings have been observed in muscle tissue, whereas plasma and erythrocyte concentrations significantly decreased (Goldstein and Forster, 1971; Forster and Goldstein, 1976). This indicates that in little skate kidneys, at least, TMAO is not critically important in short-term osmoregulatory adjustments. The TMAO concentration of kidney tissue in this study is similar to that reported in skate plasma and liver in other studies (S. Steele and P. Wright, unpublished data), but is considerable lower than that found in dogfish *Squalus acanthias* plasma or muscle tissue (for a review, see Ballantyne, 1997). This species difference is probably due to the higher levels of other organic osmolytes in skate tissues (but not in plasma) in comparison with the dogfish shark (Boyd et al., 1977). Indeed, in very recent studies we discovered that in skate liver and muscle tissue these ‘other osmolytes’ (e.g. β-alanine, betaine, inositol, sarcosine, taurine) have a combined concentration that is 1.7- and 3.7 fold, respectively, higher relative to TMAO concentrations (S. Steele, P. Yancey and P. Wright, manuscript submitted). Hence, further work is necessary to elucidate the physiological role of methylamines and other osmolytes in renal mechanisms of osmoregulation in skates.

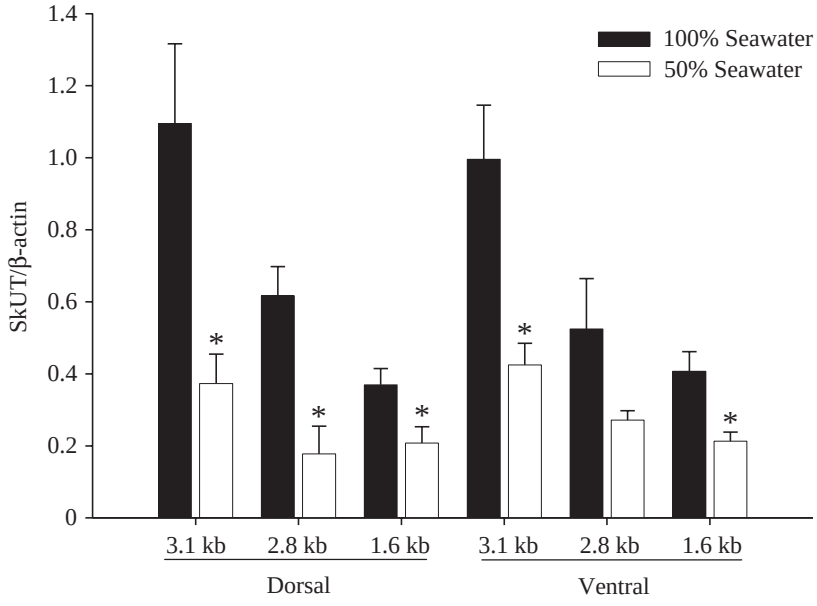

Fig. 6. Expression of SkUT mRNA relative to β-actin mRNA in the dorsal and ventral regions of the kidney of the little skate, *Raja erinacea*, exposed to 50% seawater for 5 days. The signal of each band was measured by densitometry and presented as a ratio (SkUT/β-actin) (see Materials and methods). Values are means ± S.E.M., *N*=4. *Significant difference to respective control (Student’s *t*-test, $P<0.05$).

Skate kidney urea transporter

Overall, SkUT has 88%, 66%, 68%, 67% and 64% amino acid identity with ShUT, tUT, mtUT, eUT (eel UT) and rUT (rat UT-A3), respectively (Fig. 4). This homology indicates a greater identity with ShUT, while the rest approximates the level of identity between ShUT and mammalian urea transporters (Smith and Wright,

1999). Several signature sequences of characteristic domains of UTs (Walsh et al., 2001a), e.g. the NIT potential *N*-glycosylation site (residues 137–139) and the amino acid motifs: WDLPVFTLPFN (residues 97–107), PVGVGQVYGCDNPW (residues 153–166) and TWQTHILA (residues 232–239) (Mistry et al., 2001), are highly conserved (Fig. 4). Notably, the ALE domain, which is a signature of UT-B proteins (residues 215–217 in rat UT-B), is absent from SkUT, strongly suggesting that SkUT is an UT-A-like protein. SkUT has slightly higher identity (66–68%) with the toadfish (tUT), Magadi tilapia (mtUT) and eel (eUT) gill UTs than with the mammalian kidney UT families (Walsh et al., 2000, 2001a; Mistry et al., 2001). This similarity suggests that the piscine UTs constitute a phylogenetically ancestral form of the mammalian UT families.

High-stringency northern analysis of mRNA from skate kidney revealed three bands of 3.1, 2.8 and 1.6 kb. This pattern of multiple bands has often been reported. Smith and Wright (1999) detected three bands when various dogfish tissues were probed with ShUT under low-stringency conditions. In the gulf toadfish *Opsanus beta*, two bands were reported in gill tissue (Walsh et al., 2001a). Northern analysis of mammalian kidney mRNA has revealed as many as five bands when hybridized with different UT-specific probes (Karakashian et al., 1999). Each of these five bands in the mammalian kidney is a functional urea transporter, created by alternative splicing of the UT-A1 gene (Fenton et al., 2002). UT-A1 is the complete isoform while the other four are truncated versions. This suggests that the bands seen in the skate kidney belong to three isoforms of a urea transporter family, with the longest (3.2 kb) as the full-length sequence and the shorter two as differently spliced isoforms. It is interesting to note that the 3.1 kb band appears to be more sensitive to salinity changes than the 1.6 kb band. Further investigation of the full-length sequence and the function of each transcript will be necessary, however, to show if a family of urea transporter proteins exists in the kidney of elasmobranchs.

In skates exposed to environmental dilution, as predicted, there was a downregulation (1.8- to 3.5-fold) in the relative expression of SkUT in kidney mRNA. Very few researchers have demonstrated a change in UT expression during environmental modification. Mistry et al. (2001) reported the induction of an eel *Anguilla japonica* gill UT during acclimation from freshwater to seawater. However, the functional significance of this induction is not clear. In fish, changes in the external environment can directly influence kidney function (e.g. Wood et al., 1999), whereas in mammals the same sort of changes can be linked to variations in the internal environment (e.g. Wright et al., 1992). In rat kidney, Wang et al. (2002) demonstrated a decrease in the abundance of UT-A1 and UT-A3 during extracellular fluid (ECF) volume expansion (i.e. increased water content). Associated with the downregulation of UT-A1 and UT-A3 was a decrease in serum urea concentration and an increase in urea clearance. It was hypothesized that the decrease in the abundance of the UTs would counterbalance the ECF volume expansion by stimulating an increase in water excretion (Wang et al., 2002). Despite the fact that mammals and elasmobranchs have very different renal osmoregulatory strategies, a similar situation occurs in the skate during environmental dilution, where renal tissue water content is elevated (Table 1). The downregulation of SkUT mRNA levels would presumably decrease SkUT protein levels and may reduce the renal capacity for tubular urea reabsorption. Interestingly, in a companion study on urea transport kinetics of skate kidney brush-border membranes (Morgan et al., 2003), we present evidence for the possible involvement of renal aquaporins as well in water and urea movement. In contrast to the mammalian kidney, very little is known about the relationship between urea and water reabsorption in the elasmobranch kidney. As a first step in developing a model, the relative permeabilities of water and urea along different nephron segments need to be determined in the kidney of marine elasmobranchs.

In conclusion, we found no evidence for solute gradients between the ventral and dorsal bundle zone of the skate kidney. Upon exposure to a dilute environment, urea and osmolality decreased, whereas water content increased in both zones of the kidney. A partial cDNA of a skate urea transporter (SkUT) was cloned, and the physiological changes were correlated with significant decreases in SkUT mRNA levels. Taken together, our data provide evidence that urea transporter(s) in the skate kidney play a role in urea retention.

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