

**REGULATION OF UREA TRANSPORT IN DORSAL AND VENTRAL  
SECTIONS OF THE LITTLE SKATE KIDNEY (*RAJA ERINACEA*) –  
RESPONSE TO CHANGING EXTERNAL SALINITY**

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**EXTENDED ABSTRACT ONLY – DO NOT CITE**

**Abstract**

The retention of high concentrations of urea in the tissues of marine elasmobranchs is the key to their osmoregulation strategy. During environmental dilution, changes in the mechanisms of retention in the kidney result in the reduction of urea concentrations in the body fluids. In the kidney of the little skate, *Raja erinacea*, the cDNA of a renal urea transporter (UT) was isolated, with high homology to that of the shark kidney UT (SkUT). This UT was down regulated during environmental dilution, indicating its importance in the ability of the renal tubule to reabsorb urea. Facilitated urea transport was characterized also in brush-border membrane vesicles of renal tubules from the dorsal and ventral sections of the kidney. Although facilitated transport was demonstrated in vesicles from both kidney sections, there were significant differences in inhibitor, analogue and ion responses. These findings provide evidence for the role of facilitated urea transporters in urea retention by the skate kidney.

## **Introduction**

Osmoregulation in marine elasmobranchs is mainly accomplished by the retention of high levels of urea in the body fluids. The kidney is important in this retention because it reabsorbs almost all (>90%) of the urea in the glomerular filtrate (Kempton, 1953). The arrangement of the elasmobranch nephron allows for a counter-current system that may be involved in the passive reabsorption of urea, however it has been speculated that a carrier-mediated process is also present. However, the exact site and mechanisms responsible for urea reabsorption are uncertain. In euryhaline elasmobranchs, dilution of the external salinity results in a marked reduction of urea concentrations in body fluids. During this adaptation, urea excretion is increased, while urea biosynthesis and renal tubule urea reabsorption rates are decreased (Goldstein and Forster 1971). The mechanisms involved in reducing renal urea reabsorption are not known. The purpose of this study was to examine the role of renal urea transporters in urea retention in a marine elasmobranch, the little skate, *Raja erinacea*.

## **Materials and Methods**

Skates were exposed to 100% seawater (control) and 50% seawater (treatment) in a flow-through system for 5 days. The skates were sacrificed, the paired kidneys removed and dissected into dorsal-lateral and ventral sections as described by Hentschel et al. (1986). Using total RNA prepared from the kidney, a partial sequence of a skate urea transporter (SkUT) was isolated using primers from a consensus of fish urea transporter sequences. Relative expression of SkuT mRNA to  $\beta$ -actin mRNA in skates was determined using Northern blot analysis.

In a separate experiment, brush-border membrane vesicles (BBMV) were isolated from dorsal-lateral and ventral sections of the skate kidney using the method of Kipp et al. (1997). Characteristics of  $^{14}\text{C}$ -urea uptake by the BBMV were determined using a rapid filtration method (Fines et al., 2000).

## **Results**

A skate kidney urea transporter (SkUT) was isolated, which has 88% homology with the shark kidney urea transporter (ShUT; Smith and Wright, 1999). High stringency Northern analysis revealed the presence of three SkUT bands at 3.1, 2.8 and 1.6 kb. In skates exposed to 50% seawater, there was a significant

decrease in the relative expression of SkUT in all band in the dorsal segment and in the 3.1 and 1.6 kb bands in the ventral segment (Fig. 1).

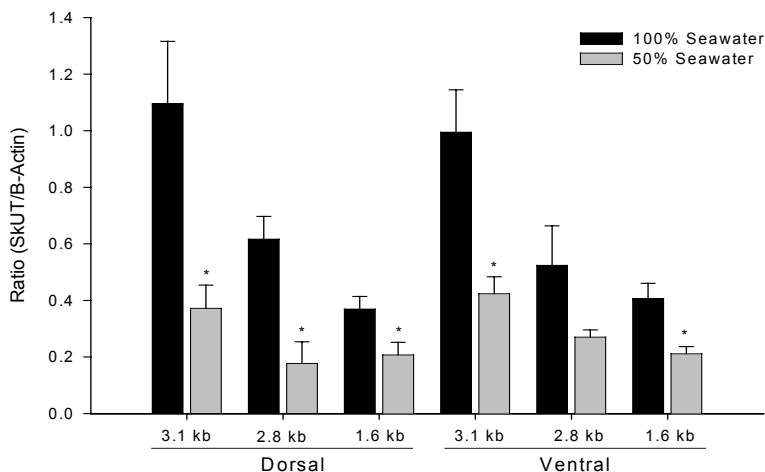


Fig. 1. Expression of skate urea transporter (SkUT) mRNA relative to *B-Actin* mRNA. Means  $\pm$  SE,  $n = 4$ . \* Significant difference to respective control (Student t-test,  $P < 0.05$ ).

There was a linear relationship between urea uptake and urea concentration in both dorsal and ventral BBMVs at high urea concentrations (5–370 mM), whereas saturation kinetics was observed at lower urea concentrations (0.2–2 mM). This uptake was inhibitable by phloretin and mercury chloride, and in dorsal BBMVs by the urea analog, NPTU. There was no effect on urea uptake with the addition of ATP. In the ventral but not dorsal BBMVs, uptake was significantly increased in the presence of a sodium gradient, while there was no effect in the presence of a potassium gradient.

### Conclusions

In the skate kidney, the presence of urea transporter mRNA suggests that it may have a role in urea retention. During environmental dilution, SkUT mRNA is down regulated, which may decrease the reabsorption of urea by the renal tubule resulting in a greater rate of urea excretion. The presence of three SkUT bands suggests that there are different isoforms of the urea transporter gene, much like that seen in mammalian UT families. Further work is necessary to complete the molecular analyses of these possible isoforms. Our physiological data using BBMVs indicates that the characteristics of urea uptake differ between the

dorsal and ventral renal sections. There is evidence for the presence of two transporters, one of which is linked to Na<sup>+</sup> transport. Clearly, the mechanisms of urea reabsorption are complex in the elasmobranch kidney, not surprising in the most structurally complex kidney among the vertebrates.

### References

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