

## Localization of Facilitated Urea Transporters to Tubular Segments in the Bundle and Sinus Zones of the Kidney of the Euryhaline Stingray, *Dasyatis sabina*.

Adair Dempsey<sup>1</sup>, Eric Lacy<sup>2</sup>, Michael Janech<sup>3</sup>, Donald Miller<sup>4</sup>, David Ploth<sup>3</sup>, and Wayne Fitzgibbon<sup>3</sup>

<sup>1</sup> Grice Marine Laboratory, College of Charleston, <sup>2</sup> Marine Biomedicine and Environmental Sciences Center, MUSC, <sup>3</sup> Division of Nephrology, MUSC, <sup>4</sup> Cell and Molecular Pharmacology and Experimental Therapeutics, MUSC

The principle component of the osmoregulatory strategy of marine elasmobranchs is the maintenance of high concentrations of urea in their body fluids. The reabsorption of filtered urea by the renal tubules is the primary mechanism underlying the retention of urea. Urea movement across the renal tubular epithelium occurs, at least in part, via specific phloretin-sensitive, facilitated transport proteins. We have identified two members of a urea transporter (UT) family from the kidneys of the Atlantic stingray, *Dasyatis sabina*. To clarify the role of these UTs, we utilized immunohistochemistry to identify the tubular sites at which they are expressed. Stingrays were maintained in harbor water (850 mOsmol/kg H<sub>2</sub>O) and fed a diet of shrimp for at least 2 weeks prior to study. They were anesthetized with MS-222 in buffered harbor water and perfused with elasmobranch Ringer's followed by 4% paraformaldehyde. The kidneys were blocked in paraffin. Five-micron sections were incubated with an affinity-purified antiserum generated to a sequence common to the 2 UTs (strUT-1 and strUT-2). Localization of UT expression was visualized using DAB stain. The specificity of the signal was confirmed by incubation of adjacent sections with the antiserum preincubated with the immunizing peptide. We also examined the expression of 2 other membrane transporters. Tubular segments were identified from the criteria reported by Lacy and Reale (1985). Numerous positively stained tubular segments were observed in both bundle and sinus zones in the presence of strUT. In the sinus zone, strong immunoreactive signal was observed in the Proximal-III tubular segment and in the Intermediate VI tubular segment. Weak staining was found in the Intermediate I tubular segment. In the bundle zone, immunoreactive signal was observed in Neck I and II, Proximal I, and Distal-I segments. In contrast, Na<sup>+</sup>-K<sup>+</sup>-ATPase was localized to Neck I and II as well as Proximal I and II in the bundle zone. In the sinus zone, staining was found in both Intermediate I and VI. The Intermediate segments in the bundle zone still require further study to determine their immunoreactivity to both antibodies. Our findings indicate that the strUTs are expressed in tubular segments in both the bundle and sinus zones. The expression of UTs in the bundle zone supports a role for countercurrent exchange in urea reabsorption. The mechanism(s) by which urea is reabsorbed via UTs in segments in the sinus zone remain to be identified.