DOGFISH SHARK RENAL UREA TRANSPORTER (ShUT) mRNA EXPRESSION IN BUNDLE ZONE NEPHRONS OF *SQUALUS ACANTHIUS*

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Plasma of the dogfish shark is nearly isotonic to seawater (approximately 1000mOsmol/kg water) with urea a major osmolyte (approximately 350 mM or more), whereas urinary urea is less than 100 mM. Renal urea reabsorption likely occurs via a phloretin-sensitive, facilitated urea tranporter (ShUT; Smith, C.P. and Wright, P.A., Am. J. Physiol. 276, R622-R626,1999) that exhibits homology to the mammalian UT-A2 urea transporter. For much of its length, the shark nephron lies in the venous portal system (mesial zone); however, a relatively short segment lies within a closed peritubular sheath (bundle zone). The nephron forms 2 hairpin loops within the bundle zone, with these loops resembling the architecture of the mammalian inner medullary countercurrent system. Proximal segment III (diluting segment) and proximal segment V within the bundle zone, and the collecting tubule outside the peritubular sheath exhibit at least minimal urea transport, and when tested on proximal V, phloretin reduced urea transport 75-80% (Friedman, P.A. and Hebert, S.C., Bull. MDIBL 25, 24-26,1985). We have investigated expression of the shark urea transporter mRNA in isolated nephron segments using RT-PCR. Localization of this transporter was undertaken in order to understand the feasibility of the countercurrent mechanism for urea reabsorption.

Kidneys were placed into shark Ringers, then cut into 2mm cubic sections and transferred to a solution of 890 mM glucose/10 mM HEPES, pH 7.4 for tubule isolation. Tubule lengths were determined with an ocular micrometer. Tubule segments, in a volume of 2 µl glucose/HEPES solution, were transferred into a PCR tube. Reverse transcription (RT) of messenger RNA and amplification of first strand cDNA by polymerase chain reaction (PCR) were carried out directly with intact, Triton X-100-permeabilized tubules as we have previously described for isolated rat thin limbs of Henle (Pannabecker, T.L. et al., Am. J. Physiol. 278, F202-F208, 2000), with the forward primer 5'- GCT TGT TCA TGG CTG CA - 3' and reverse primer 5'-GTT GTT AGT GGT CAG CA-3'. Thirty-five PCR cycles were carried out with the following conditions: 95° C for 30 s; 55° C for 30 s; 72° C for 1 min; followed by a final extension at 72° C for 10 min. PCR products were ethanol-precipitated overnight, size-fractionated on 1.5% agarose gel, stained with ethidium bromide, and sequenced by the MDIBL DNA Sequencing Center. In order to control for genomic contamination, reverse transcriptase was omitted during the cDNA synthesis step from renal messenger RNA in control samples, and these reactions showed no reaction product.

The reaction products for seven sets of isolated tubule segments from one shark kidney are shown in Figure 1. Products for sets of tubules from the mesial zone were absent or weakly expressed (lanes 1-3), whereas variable but strong expression was observed for sets of tubules from the bundle zone (lanes 4-7). A similar pattern of expression was seen for kidneys from three sharks. The nucleotide sequence of the PCR product from lane 7 was identical to that of the cloned dogfish shark urea transporter ShUT. These observations demonstrate that ShUT mRNA is present within epithelial nephron segments of the dogfish shark kidney and indicate that

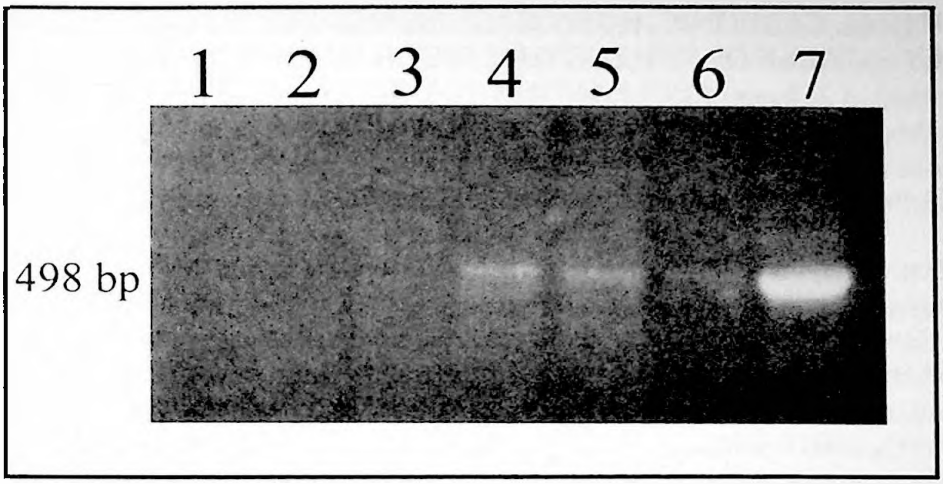

Figure 1. RT-PCR reaction products for shark urea transporter (ShUT) from tubule segments of dogfish shark kidney mesial zone (lanes 1-3) and bundle zone (lanes 4-7). Total lengths (mm) of segments included in reactions shown for lanes 1-7 respectively: 4.0, 5.0, 9.0, 3.6, 1.3, 3.2, 2.3.

tubules of the bundle zone may express significantly higher levels of ShUT than tubules of the mesial zone. Further work must be carried out to determine whether or not ShUT-expressing segments of the bundle zone correspond to bundle segments previously shown to exhibit phloretin-sensitive, facilitated urea transport.

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