PRELIMINARY STUDIES OF PASSIVE UREA TRANSPORTERS IN SEVERAL MARINE SPECIES

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Excretion of nitrogen in aquatic animals is ensured by diffusion of ammonia out of the gills into the surrounding water. In terrestrial vertebrates, nitrogen is mostly excreted in the form of uric acid (reptiles and birds) or urea (mammals) (Jorgensen, *Comp. Biochem. Physiol.* 117A:161-70, 1994; Shoemaker et al, *Ann. Rev. Physiol.* 39:449-71, 1977). However, some lower vertebrates (elasmobranchs, a few fish, and some frogs) excrete urea in spite of the high energetic cost of its synthesis (3 ATP per molecule). In these species, urea is not only an end product of nitrogen metabolism but also serves to maintain water balance (Schmidt-Nielsen In: Transport mechanisms in epithelia, edited by Ussing, H. H., and N. A. Thorn, Academic Press, 1973, p. 608-621). In elasmobranchs, the urea concentration in plasma (up to 400 mM) serves to counteract the osmotic pressure of seawater. In some frogs, urea is used to store fresh water in the body (that can be reabsorbed from the urinary bladder) during times spent out of the water. In mammals, urea is recycled in the renal medulla, contributing both to water conservation and urea excretion in the form of a concentrated urine. Urea is thus coupled to water balance in all vertebrates in which this compound is the major form of nitrogen excretion.

Several urea transporters (UT) have recently been cloned in mammals and are abundantly expressed in kidney and erythrocytes (Sands et al, *Am. J. Physiol.* 273:F321-F339, 1997. These transporters enable facilitated diffusion of urea through the basolateral and luminal membranes of some nephron segments permitting intrarenal movements of urea related to water conservation. The goal of the present preliminary studies was to initiate experiments to clone and sequence urea transporters in lower species including elasmobranchs.

Degenerate PCR primers, based on the sequence of known passive UTs were kindly provided by Craig Smith (Manchester, England). Total RNA from shark kidney and rectal gland (*Squalus acanthias*), little skate kidney, lamprey and flounder kidney, hagfish gill and frog kidney and bladder (*Rana catesbeiana*) was extracted with Total RNA extraction kit (United States Biochemical REX). Reverse-transcription (Invitrogen cDNA cycle kit) and PCR was then performed with the following cycles: 4' at 94°C, 30 or 35 cycles with 1' at 94°C, 1' at 50°C, and 1' at 72°C, followed by a final 7' at 72°C.

We obtained PCR products of the approximate expected length from shark and skate kidneys and a twofold longer product from the shark rectal gland. No product was amplified from the frog, lamprey, flounder and hagfish tissues.

PCR products from skate kidney and shark kidney were cloned into the pCR2.1 cloning vector (Invitrogen) and bi-directionally sequenced at the University of Maine sequencing center and yielded 482 and 481 bp, respectively. The nucleotide sequence of these two PCR products shares a strong homology with the rat facilitated urea transporter (Hediger et al., *Kid. Intern.* 49:1615-23,

1996; Smith et al., *J. Clin. Invest.* 96:1556-63, 1995) (60% and 64%, respectively). The band from shark rectal gland was sequenced and had an open reading frame with high identity to mouse oligosaccharyl transferase.

Figure 1. Second PCR amplification with degenerate urea transporter primers. Shark kidney cDNA PCR re-amplified (lane 1) and skate kidney cDNA PCR re-amplified (lane 3). Negative controls without cDNA were run in duplicate (lanes 2 and 4).

These first preliminary results will serve as a starting point for more extensive work on UTs in aquatic (marine and fresh water) lower vertebrates. They show that UTs with relatively high homology to mammalian forms are present in several elasmobranchs but may not be present in the other species studied. The shark kidney transporter may be the same as that already cloned by Craig Smith and colleagues (Smith et al., *J. Physiol.* 504:139, 1997). This will be confirmed when the sequence of this transporter is released (Smith et al., *Am. J. Physiol.* In press, 1999). The fact that no PCR product of the expected length was found in the shark rectal gland suggests, but does not establish, that this gland may not possess a facilitated urea transporter, in agreement with the observation that it exhibits a relatively low permeability to urea.

This work was supported by NIH grants DK 34208 and NIEHS P30-ES 3828 (Center for Membrane Toxicology Studies), and a Grant in Aid from the American Heart Association, Maine Affiliate.