EXCRETION OF MULTISPECIFIC ORGANIC ANION TRANSPORTER (MOAT) SUBSTRATES BY DOGFISH SHARK (*SQUALUS ACANTHIAS*) RECTAL GLAND TUBULES

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Marine animals, like mammalian tumor cells, exhibit the phenomenon of substrate-inducible, multixenobiotic resistance (Kurelec, *Environ. Hlth. Perspec.* 105(Suppl 4):855-860, 1997). Recent evidence indicates that marine animals also possess high levels of P-glycoprotein and a multispecific organic anion transporter (MOAT or Mrp2) in renal proximal tubule (Schramm et al., *Am. J. Physiol.* 268:F46-F52, 1995; Masereeuw et al., *Am. J. Physiol.* 40:F1173-F1182, 1996) and that these could contribute to a multixenobiotic resistance phenotype. However, at present, it is not clear which other organs are involved and which xenobiotic transporters they might use. In the present study, we used confocal microscopy and digital image analysis to demonstrate the excretory transport of fluorescent xenobiotics in secretory tubules of the dogfish shark (*Squalus acanthias*) rectal gland, an organ heretofore thought to function primarily in osmoregulation.

Secretory tubules were isolated by dicing rectal glands with a razor blade, followed by a digestion in collagenase (Valentich, J. D., *J. Tiss. Cult. Meth.* 13:149-162, 1991). Freshly isolated tubules were stored at 4°C in shark Ringer's (Valentich, *op. cit.*) until used. To measure transport, tubules were transferred to a Teflon chamber containing 1 ml of shark Ringer's containing fluorescent substrates. The chamber floor was a 4 x 4 cm glass cover slip, to which the tubules adhered lightly and through which the tissue could be viewed using a Noran Confocal Microscope (inverted) with a Nikon 40X Fluor oil immersion objective (NA 1.4). Illumination was provided by an Ar laser at 488 or 529 nm. To obtain an image, dye-loaded tubules in the chamber were viewed under transmitted light and a single tubule with well defined lumen and undamaged epithelium was selected, and examined with the plane of focus cutting through the center of the tubule lumen. In confocal fluorescence mode, 16 video frames were averaged. The confocal image (512x512x8 bits) was viewed on a high resolution monitor and saved to optical disk. Fluorescence intensities were measured from stored images using an Apple Power Macintosh 7100 computer and NIH Image (ver. 1.58) software (Miller, D. S., *Am. J. Physiol.* 269: R370-R379, 1995).

Fluorescent substrates for P-glycoprotein (NBDL-CSA, rhodamine 123 and daunomycin) and the renal organic cation transport system (rhodamine 123 and daunomycin) accumulated within the cells, but luminal fluorescence was always lower than cellular fluorescence. Fluorescein, a substrate for the organic anion system, did not accumulate within the cellular or luminal compartments. Substrates for a multispecific organic anion transporter in kidney (MOAT or Mrp2; Masereeuw et al., *op. cit.*), sulforhodamine 101 and fluorescein-methotrexate, did not accumulate to high levels within rectal gland cells, but luminal fluorescence was substantially higher than cellular or medium fluorescence. Of the six fluorescent substrates for renal xenobiotic transport systems, only those handled by MOAT showed concentrative secretion into the lumens of rectal gland tubules.

For tubules incubated to steady state in medium containing 5 μM sulforhodamine 101, luminal fluorescence was 5-8 times higher than medium fluorescence, which was 50-100% higher than cellular fluorescence. Increasing medium sulforhodamine 101 concentration over the range 5-20 μM resulted in less than proportional increases in luminal fluorescence; cellular fluorescence was a linear function of medium sulforhodamine 101 concentration. Addition of 0.1 mM KCN to the medium reduced luminal fluorescence by over 70%, but had little effect on cellular fluorescence. P-aminohippurate and tetraethylammonium, model substrates for the classical renal organic anion and organic cation transport systems, respectively, and verapamil, a substrate for both the renal organic cation transport system and P-glycoprotein, had no effect on sulforhodamine 101 uptake or distribution. In contrast, leukotriene C4 (100-3000 nM), cyclosporine A (1-5 μM) and chlorodinitrobenzene (5-25 μM), all inhibitors of renal and hepatic MOAT (Masereeuw et al., *op. cit.*), reduced luminal sulforhodamine 101 in a concentration-dependent manner; cellular sulforhodamine 101 was not affected. Thus, sulforhodamine 101 was secreted into the lumen of rectal gland tubules by a mechanism that was concentrative, saturable, energy dependent and specific. Transport into rectal gland cells was neither concentrative, energy-dependent nor specific.

The inhibitor profile described above indicated that sulforhodamine 101 transport from cell to lumen was mediated by a MOAT. To confirm this finding, we exposed frozen rectal gland sections with a polyclonal antibody to MOAT (a gift of D. Keppler; Konig, et al., *J. Biol. Chem.*, 271:15091-15098, 1996) and then stained with a fluorescent secondary antibody. Confocal micrographs showed strong and specific staining at the luminal plasma membrane and an absence of staining at the basolateral membrane or within the cells.

Finally, to demonstrate specific xenobiotic secretion in intact rectal glands, we perfused the vasculature with 1 μM sulforhodamine 101 and dye transport into duct fluid. When 25 μM chlorodinitrobenzene was also in the perfusate, steady state dye excretion was reduced by over 75%.

Taken together, the present results demonstrate that shark rectal gland not only actively excretes NaCl, but also actively excretes xenobiotics. Xenobiotic excretion is mediated by MOAT or Mrp2, an ATPase with wide specificity limits; P-glycoprotein does not appear to be involved. These results suggest that in fish nonrenal and nonhepatic transporting epithelia may contribute the multixenobiotic resistance phenotype and that their contributions may involve transporters other than P-glycoprotein.

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