EVIDENCE FOR THE MULTIDRUG TRANSPORTER, P-GLYCOPROTEIN, IN THE KILLIFISH, FUNDULUS HETEROCLITUS

Karl J. Karnaky, Jr.[1], Kyle Suggs[1], Stacey French[2], and Mark C. Willingham[3]
[1]Department of Anatomy and Cell Biology, and the Marine Biomedical and Environmental Sciences Program, Medical University of South Carolina, Charleston, SC 29425
[2]University of Maine at Farmington, Farmington, Maine, 04938
[3]and the Department of Pathology and Laboratory Medicine, Medical University of South Carolina, Charleston, SC 29425

In humans an ATP-dependent drug transporter, termed P-glycoprotein, has been postulated to protect the body by excreting certain xenobiotic molecules. P-glycoprotein is located on the bile canalicular face of hepatocytes and on the apical membranes of intestinal cells and kidney proximal tubule cells, as well as in several other "exit" locations in the body.

We have asked the question: Do P-glycoproteins exist in the bony fishes? Fish are subject to pollutants in their aqueous environments and P-glycoproteins would be highly advantageous to the animal. Because P-glycoprotein is an ATPase, and thus requires a source of ATP, we chose to examine the opercular epithelium of the killifish, Fundulus heteroclitus. This epithelium has a large population of mitochondria-rich chloride cells (Karnaky et al, J. Exptl. Zool. 199:355-364, 1977). Because of its large population of mitochondria and location between the blood and the external environment of the fish, the chloride cell is a cell type that might exhibit this transport protein.

Specimens of Fundulus heteroclitus adapted to 100% seawater were used for this study. We mounted opercular epithelia in Ussing chambers and allowed them to come to a steady state under short-circuit conditions (Karnaky et al., Science 195:203-205, 1977; Karnaky In: Cell-Cell Interactions [ed. by B. R. Stevenson, W. Gallin, and D. Paul], IRL/Oxford University Press. Oxford, England. 1992, pp. 257-274). Each fish provided two opercular epithelia, one for efflux studies and one for influx studies. We then placed 10^{-8} M rhodamine 123, one of the substrates for P-glycoprotein (Neyfakh, Exp. Cell Res. 174:168-176, 1988), on either the basolateral or apical side. After a period of 30 min we sampled the opposite side of the chamber at 30 min intervals. We measured the appearance of the rhodamine 123 in the chamber bath using spectrofluorometric techniques.

Beginning at the first 30 time sample point, unidirectional fluxes were linear for a period of 90 min. Significantly, in five experiments, the efflux rate was 4 times the influx rate. In two experiments we added reserpine (10^{-6} M, basolateral side addition), a competitive inhibitor of P-glycoprotein, and measured unidirectional fluxes for an additional two hours. In both cases, this reserpine addition lowered the efflux rate to the same rate as the influx rate, but had no effect on the influx rate. The net flux rate of rhodamine 123 was approximately 1 pmoles x cm^{-2} x h^{-1}. This is 5 times higher than the net flux rate of ^{3}H-vinblastine across MDCK cells (Hunter et al., Biochem. Biophys. Res. Comm. 181:671-676, 1991).

These data are consistent with the presence of a P-glycoprotein transport system in the chloride-cell rich opercular epithelium. Rhodamine 123 is a fluorescent dye which quickly stains mitochondria in chloride cells of the opercular epithelium, so there is no doubt that this dye penetrates into the cytoplasm of this cell type. The chloride cell is the most likely candidate to exhibit the P-glycoprotein. However, in a single attempt to immunocytochemically localize this protein with monoclonal antibodies C219, C494, and JSB-1, we failed to observe any epithelial cell staining. Interestingly, Valentich (Bull. MDIBL 30:67-68, 1991) reported the possible presence of a P-glycoprotein in dogfish shark rectal gland cells using rhodamine 123 as visualized by fluorescence microscopy. When these cells are grown in culture in wells such that rhodamine 123 can be added to either the apical or basolateral surface, the cells stain only from the basolateral side. It was suggested that the P-glycoprotein was pumping apically-administered rhodamine 123 out of the cells so rapidly that it could not reach the mitochondria at the base of the cells. However, if reserpine was added to the medium, rhodamine 123 did stain from the apical side, presumably because is was not pumped out fast enough. The most compelling conclusion is that the rectal gland possesses P-glycoprotein.

Some implications of the presence of the P-glycoprotein in the bony fishes are clear. This transport system would help the fish excrete certain lipophilic xenobiotic molecules which would gain easy access to the fish through its permeable body membranes. Given the well-known biological variability in animals, one would expect some species of fish to possess more of this transport ability more than others. It should be possible to test different species of fish to determine which one(s) exhibited the greatest transport ability. In the future it may be possible to genetically engineer this capability into a species of fish which has many other ideal characteristics, but which lacked strong drug transport capabilities.

Supported in part by NIH EHS-P30-ESO3828 to the Center for Membrane Toxicity Studies and grants from the University Research Committee of MUSC and the Cystic Fibrosis Foundation. Stacey French was a recipient of a Hearst Student Fellowship. Publication No. 108 of the Grice Marine Biological Laboratory.