MRP2-LIKE TRANSPORT IN THE MALPIGHIAN TUBULE OF THE CRICKET, *ACHETA DOMESTICUS*

Karl. J. Karnaky, Jr.[1], Martina Sedmerova[1], David Petzel[2], Josh Bridges[3], Sarah Wade Boatwright[4], and David S. Miller[5]
[1]Dept. of Cell Biol. and Anat. and the Marine Biomed. and Environ. Sci. Prog., Med. Univ. of SC, Charleston, SC 29425
[2]Dept. Biomed. Sci., Creighton Univ. Sch. of Med., Omaha, NE, 68178
[3]Sumner Memorial High School, East Sullivan, ME 04607
[4]Mount Holyoke College, South Hadley, MA 10175
[5]Lab. of Pharmacol. and Chem., NIH/NIEHS, Res. Tri. Park, NC 27709

Our laboratories continue to investigate the special excretory transporter, the multispecific organic anion transporter (MOAT or multidrug resistance associated protein, mrp2) which has evolved to help organisms defend against xenobiotic molecules which invade their cells. Model systems have included kidney tubules of the bony fish, the killifish, *Fundulus heteroclitus* (Masereeuw et al. *Am. J. Physiol.*, 40:F1173-F1182, 1996), secretory tubules of the dogfish shark, *Squalus acanthias,* rectal gland (Miller et al., Am. J. Physiol. 275:R697-R705, 1998) and the Malpighian tubule (MT) of the common American cockroach, *Periplaneta americana* (Karnaky et al. *Bull. MDIBL* 39, 52-53, 2000). Insects possess from 4 to 200 of these tubules attached to their gut. These tubules are relatively easy to isolate and fluid and organic molecule transport rates can be measured utilizing microscopic, isotopic, chromatographic, or fluorescent methods. In this report we have examined the xenobiotic transport function of a putative multidrug resistance associated protein, mrp2, in the renal MT of a second insect, the brown domestic cricket, *Acheta domesticus.*

Animals were purchased from local pet shops and stored in small containers with slices of potato provided for food. Animals were anesthesized for 5-10 minutes in a freezer. Preparations were studied as a complete gut with all of the MT attached. Alternatively, we detached the ureter to which most MT are attached. These preparations were transferred to 0.10-1.0 ml of appropriate Ringer's solution (Lauf and Walz, *J. Exp. Biol.*, 202:729-738, 1999) with or without various transport substrates, including two fluorescent mrp2 substrates, Texas Red (TR: sulforhodamine 101; Masereeuw et al. *Am. J. Physiol.*, 40:F1173-F1182, 1996) and 5-chloromethylfluoroscein acetate (CMF) for 10-30 min. The tubules were viewed with a Biorad Confocal microscope (upright). Fluorescence intensities were measured from stored images using a Scion Image program on a Gateway PC computer. Transport of TR into the lumens of isolated tubules could also be viewed with dissecting and light microscopes. For the immunofluorescent localization of mrp2 frozen section of MT were fixed and stained with a polyclonal antibody made to sequence of rabbit mrp2 (a kind gift of D. Keppler).

Within thirty minutes after the beginning of exposure, luminal fluorescence of TR or CMF in the middle and proximal regions was always higher than cellular or medium fluorescence. Digital image analysis of confocal images showed that TR transport was inhibited 31% by 0.1 mM KCN and 33% by the mrp2 inhibitor, chlorodinitrobenzene, at a concentration (25 μM). To test the possibility that 25 μM CDNB was acting as a metabolic inhibitor, rather than a specific inhibitor of mrp2-like transport, we showed that this concentration of CDNB did not affect transport to the lumen of 25 μM chlorophenol red, a substrate for the classic organic anion transporter system (see Pritchard and Miller, *Physiol. Rev.* 73:765-796, 1993). Robust TR transport could also be detected with dissecting and light microscopes. This transport was inhibited by 25 μm CDNB, but not by para-aminohipppuric acid, a substrate of the organic anion transporter, by tetraethylylammonium chloride, a substate of the organic cation transporter, nor by verapamil, an inhibitor of the Pgp. Luminal cells were immunocytochemically stained with an antibody to a sequence of rat mrp2, further suggesting that a mrp2-like transporter is present in MT. When cricket MT were exposed

to the Pgp substrate, daunomycin, we observed the fluorescent molecule accumulating in cells, but no substrate in the lumen, consistent with observations on MT of the tobacco hornworm made by Gaertner and Morris (*Tissue & Cell.* 31:185-194, 1999).

Most of the MT in this species flow into a common duct which enters the gut through a ureter. This morphological arrangement should allow for cannulation and simultaneous collection of the combined excretion of over 100 MT. The robust transport detectable with the dissecting and light microscopes is especially interesting and might allow for the development of rapid screening methods to test large numbers of potential mrp2 substrates. Finally, the complete genome is now available for the fly. This gene database should greatly facilitate cloning the mrp2 transporter in this cricket.

In summary, the MT of this species is an excellent model system to study the mechanism and regulation of mrp2-like transport in insects and could lead to new strategies to overcome pesticide resistance observed in many insects. Supported by NIEHS-P30 ES03828 (MDIBL Center for Toxicity Studies, to KK), NIH DK49610 (to DP) and a MDIBL Dahlgren Fund (to DP). KK is a Senior Fellow of the Salisbury Cove Research Fund and was supported by a New Investigator Award. Publication No. 187 of the Grice Marine Biological Laboratory, University of Charleston. JB was supported by a Hancock County Young Scholars Award.