PRELIMINARY MOLECULAR STUDIES ON THE TAURINE TRANSPORT SYSTEM OF THE COELOMOCYTES OF THE MARINE POLYCHAETE, *GLYCERA DIBRANCHIATA*

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In prior studies, we have characterized mechanisms of mercury interaction with the taurine transporter in the hemoglobin containing coelomocytes (red blood cells, RBCs) of the marine polychaete, *Glycera dibranchiata*, (Preston, R. L. and Chen, C.W., Bull Environ. Contam. Toxicol. 42:620-627, 1989; Preston, R.L., Janssen, S.J., Lu, S. and Truong, T.T., Bull. MDIBL 29:74-77, 1990; Preston, R.L., Zimmermann, P.R., Kaleta, M.T. and Simokat, K.A., Bull. MDIBL 33:53-55, 1996). We have begun a series of investigations to identify and isolate the gene for the taurine transporter using *Xenopus laevis* oocyte expression and PCR techniques. Total RNA was isolated from *Glycera* RBCs by lysis with guanidinoisothiocyanate followed by adsorption to an oligo-dT affinity column (Qiagen Inc., Santa Clarita, CA.). The mRNA was eluted, concentrated by vacuum drying and stored at –80°C. *Xenopus* oocytes were surgically removed from the animals and treated with collagenase in amphibian Ringers solution. The oocytes were manually defolliculated and allowed to recover overnight in Ringers. Messenger RNA from *Glycera* (50 nl) was injected into the oocytes using a Narishige microinjector and allowed to incubate 3-6 days. Controls consisted of uninjected oocytes and oocytes injected with 50 nl of diethylpyrocarbonate (DEPC) treated water. Taurine influx was measured by incubating the oocytes with 0.1 mM ^{3}H-taurine for 30 to 120 minutes, To terminate the incubation the oocytes were transferred to a microfuge tube containing ice-cold Ringer solution and dibutylphthalate and then this tube was centrifuged at 14,000 x g for 5 min. The oocytes were extracted with 5% TCA, centrifuged for 5 min at 14,000 x g and the supernatant mixed with fluor for counting in a scintillation counter. In earlier preliminary studies, control expression experiments were also done using cRNA from a genetically modified human placental alkaline phosphatase (SEAP) that is secreted into the extracellular medium (Tate, S. S., Urade, R., Micanovic, R., Gerber, L. and Udenfriend, S., FASEB Journal 4: 227-231, 1990).

A second approach to identification of the taurine transport gene utilized screening *Glycera* cDNA for conserved sequences identified from other taurine transport systems that have been cloned. A comparison of six protein sequences (from human thyroid, retina and placenta, dog kidney, mouse and rat brain) revealed a high degree of homology in these proteins. Four degenerate primers were constructed that flanked the ends and two segments in the middle of the gene. RT-PCR was done using the Superscript/PCR Reagent System (GibcoBRL, Grand Island, NY.). The products were analyzed by agarose gel electrophoresis (2%), stained with ethidium bromide and photographed. Appropriate RT-PCR controls and a molecular weight standard ladder were included.

The *Xenopus* expression experiments have shown slight statistical support for positive expression. However, this result was not readily reproducible. We have shown that the basic methodology we employ is workable since we can get secretion of alkaline phosphatase following injection of using cRNA for this enzyme. The RT-PCR screening studies using degenerate primers (MTAU-FA, MTAU-ES) derived from conserved sequences for Na and Cl dependent taurine transporters in mammals yielded a weak band with *Glycera* cDNA produced from total mRNA. The calculated size of the region spanned by these primers in the mammalian cDNA was 1014 bp. The band we identified in *Glycera* was about 1100 bp. This band has not yet been sequenced but these data are consistent with the possibility that the *Glycera* transporter is related to the mammalian transporters. This is the first molecular evidence for this connection. (Tatiana Gott was suported by a NSF REU Fellowship DBI9531348. Drew Sommerville was supported by an American Heart Association Undergraduate Summer Fellowship. This work was also supported in part by NIEHS grant ESO3828-11 and its Core Facilities).