EFFECT OF HEAVY METALS ON TAURINE TRANSPORT BY THE COELOMOCYTES OF THE MARINE POLYCHAETE, GLYCERA DIBRANCHIATA.

Robert L. Preston and Chang Wen Chen, Department of Biological Sciences, Illinois State University, Normal, Illinois 61761.

Introduction

The objective of this study was to characterize the effects of heavy metal exposure on the transport of the amino acid, taurine, by the hemoglobin containing coelomocytes (red blood cells, RBCs) of the marine polychaete, Glycera dibranchiata. These red cells contain a high concentration of taurine (190 mM) which has been implicated as an important osmolyte in cellular volume regulation (Preston, unpublished data; Costa and Pierce, J. Comp. Physiol. 151: 133-144, 1983). The coelomic fluid bathing these cells typically contains taurine at considerably lower concentrations (0.2 mM). Preliminary experiments demonstrated that the maintenance of the large standing gradient for taurine was apparently due to the presence of a specific Na and Cl dependent taurine transport system in these cells. One of the initial sites of action of heavy metals is the cell membrane (Kinter and Pritchard, In: Handbook of Physiology Section 9. Reactions to Environmental Agents. Ed. D. H. K. Lee, American Physiological Society, New York, pp 563-576, 1977). Typically these agents inhibit transport activity of a large variety of transport systems including nonelectrolyte transport systems. The fact the Glycera RBCs actively maintain large taurine gradients suggests that this tissue should be an excellent one to use in analysis of the mechanisms of heavy metal interaction with amino acid transport systems.

Methods:

Glycera RBCs were washed in artificial seawater (NaSW) and centrifuged repeatedly to remove gametes. The NaSW had the following composition: 440 mM NaCl, 9 mM KCl, 9.3 mM $CaCl_2$, 23 mM $MgCl_2$, 26 mM $MgSO_4$, and 2.2 mM $KHCO_3$ (final pH 7.8). In some experiments other salts were substituted for NaCl to prepare Na or Cl free medium. A typical uptake experiment was performed as follows: 0.2 ml of Glycera RBC suspension (10-20% hematocrit) was placed in a 1.5 ml microfuge tube and rapidly washed 3 times with 1.0 ml NaSW or an appropriate ion substituted seawater. The supernatant was removed and 0.36 ml of SW medium containing 0.1 mM ^{14}C-taurine and ^{3}H-polyethylene glycol (PEG) as an extracellular space (ECS) marker added to the pellet. After 1 min incubation (12°C), 0.3 ml of the medium was placed in a microfuge tube containing 0.3 ml SW and 0.6 ml dibutylphthalate (DBP). The tube was then centrifuged (10,000 x g, 1 minute) to separate the RBCs from the aqueous medium by sedimentation through the DBP layer. The pellet was extracted with 1.0 ml 0.001% Triton X-100/2.5% trichloroacetic acid and then centrifuged for 4 minutes. The amount of radioisotope in the extract was evaluated using dual channel scintillation spectroscopy. Corrections were made for channel overlap and medium trapped in the ECS. A 0.05 ml aliquot of the RBC suspension was added to 5.0 ml Drabkins reagent and read at 540 nm to evaluate hemoglobin content which is proportional to RBC count. The effects of heavy metals on taurine transport were evaluated by preincubating the RBCs in medium containing various concentrations (up to 1 mM) of $CdCl_2$, $PbNO_3$, or $HgCl_2$. The RBCs were then washed 2 times in medium free of heavy metal before transport measurements were made.

Preliminary Kinetic Analysis:

The transport of ^{14}C-taurine at medium concentrations of 0.1 mM and 1 mM was linear for at least 10 minutes in both NaSW and in seawater (CSW) in which choline chloride was substituted for NaCl. Consequently, 5 minutes was chosen as the usual incubation period for measurements of initial velocity of uptake in other experiments. Kinetic analysis of the taurine transport system was conducted to provide a basis for comparison and interpretation of the results of the experiments in which the red cells were pretreated with heavy metals. In NaSW using a substrate concentration which ranged from 2 μM to 50 mM, the kinetics of uptake appeared to fit that which would be expected for a single transport system with an added diffusion component. The K_t, estimated from Eadie-Hofstee plots, was 1.06 ± 0.08 mM and J_{max} was 174 ± 10 μmol. min-1 1.cell water-1 (data from 6 experiments).

In order to define the specificity of this transport system over 20 amino acid analogues were tested as competitive inhibitors (at 2 mM concentration) of taurine influx at 0.1 mM. Hypotaurine, β-alanine, and γ-aminobutyric acid which are structurally similar to taurine were good inhibitors decreasing taurine influx 40% or more. On the other hand, L-proline, L-Glutamic acid, L-lysine, L-alanine, and glycine were poor inhibitors which decreased taurine flux by 5% or less.

The ion dependency of taurine transport was evaluated by replacing NaCl in the medium with other salts. It was found that in a series of cation replacements (Cl salts) that taurine flux decreased in the following sequence: Na > K > Li > Rb > choline. The flux remaining in choline chloride medium was 20% of that in NaSW. In anion replacement experiments (Na salts), it was found that SCN substitution inhibited taurine influx 95%. Other anions also decreased influx but to a lesser degree in the following sequence: acetate > SO_4 > HCO_3 > Br > I > NO_3 > SCN. It is possible that a component of the inhibition of influx was connected with cell volume changes after ion replacement. Although this cannot be ruled out in all cases, preliminary measurements of cell volume in CSW using capillary hemocytometry indicated no significant differences compared with NaSW controls (Preston, unpublished data). Kinetic analysis of the effect of Na replacement revealed that the major effect of substitution of choline Cl for NaCl is to decrease J_{max} without significantly changing K_t. It was also shown that at 0.1 mM taurine influx rose rapidly (and nonlinearly) as Na or Cl concentrations were increased. This suggested that coupling coefficients for Na and Cl for taurine influx were greater than unity for this system.

Heavy Metal Effects:

Initial experiments were conducted on the effects of preincubation of red cells with 1mM $CdCl_2$, $PbNO_3$, and $HgCl_2$ for periods up to 60 minutes. Neither $CdCl_2$ nor $PbNO_3$ significantly affected taurine uptake after 60 minutes. However, $HgCl_2$ very rapidly inhibited taurine influx 95% within 1 minute of exposure (Table 1). This very clear disparity in sensitivity to these heavy metals has also been observed in other systems (Kinter and Pritchard, In: Handbook of Physiology Section 9. Ed. D. H. K. Lee, American Physiological Society, New York, pp563-576, 1977). Because of the sensitivity of this transport process to $HgCl_2$ further experiments centered primarily on this compound.

Table 1: Inhibition of taurine influx by Glycera coelomocytes by heavy metals

Heavy Metal (1 mM)	Influx ($\mu mol.min^{-1}l.cell\ water^{-1}$) ± S.E. (n = 3)				
	Time of exposure to 1 mM heavy metal				
	1 min	5 min	10 min	30 min	60 min
$CdCl_2$	171 ± 11	181 ± 7	191 ± 5	186 ± 13	166 ± 4
$PbNO_3$	150 ± 5	163 ± 5	179 ± 5	195 ± 18	154 ± 14
$HgCl_2$	2.8 ± 1.3	4.9 ± 1.4	5.2 ± 0.3	---	---

(Control influx for untreated cells, 174 ± 4 µmol/l.cell water). One mM heavy metal salts in artificial seawater were incubated with the coelomocytes for the times indicated. The cells were then washed in NaSW and incubated for 10 min in medium containing 0.1 mM ^{14}C-taurine and ^{3}H-PEG as an extracellular space marker. Cell lysis occurred after 30 min incubation with 1 mM $HgCl_2$.

Table 2: Effect of $HgCl_2$ and dithiothreitol on the kinetics of taurine transport.

Condition	K_t, mM	J_{max}, $\mu mol.min^{-1}l.cell\ water^{-1}$
Control	1.1 mM	192
DTT (10 mM) treated	0.8 mM	191
Hg (30 µM) treated	0.3 mM	13
Hg (30 µM) treated followed by DTT (10 mM)	1.1 mM	182

The medium for all preincubations was NaSW to which was added the compounds indicated. Coelomocytes were preincubated in NaSW (control condition), 10 mM dithiothreitol (DTT) for 5 min, 30 µM $HgCl_2$ for 1 min, or 30 µM $HgCl_2$ for 1 min followed by 10 mM DTT for 5 min. The kinetic parameters for ^{14}C-taurine uptake (5 min flux period) were then determined in NaSW using 11 taurine concentrations ranging from 30 µM to 50 mM.

A dose response curve using $HgCl_2$ preincubated with the red cells for 1 minute at concentrations from 1 µM to 1 mM indicated that the 50% inhibititory concentration ($K_{0.5}$) was about 20 µM. A parallel set of experiments in CSW also showed a similar $K_{0.5}$ although the absolute flux rates were considerably reduced.

Additional experiments were conducted on the effect of Hg on the kinetics of taurine transport and the extent of Hg reversibility after treatment with the reducing agent dithiotreitol (DTT). Red cells were pretreated with 30 μM $HgCl_2$ in NaSW for 1 minute. The cells were then washed 3 times in NaSW. Aliquots of these cells were then treated with 10 mM DTT for 5 minutes and then washed in NaSW. Controls were done in parallel without $HgCl_2$ being present but otherwise identical. The kinetic constants were then measured in these cells in NaSW medium. In the control condition, DTT treated control cells and DTT treated $HgCl_2$ preincubated cells, the K_ts and J_{max}s were essentially the same (Table 2). These data suggest that Hg inhibition of taurine transport is readily reversible with DTT treatment and that the "reversed" tissues display essentially normal kinetic behavior. The effect of Hg seems to be primarily on J_{max} although the K_t is also decreased noticeably.

Discussion:

The transport of taurine by Glycera coelomocytes occurs via a Na and Cl dependent transport system specific for β-amino acids. The kinetic properties of this taurine transport system resemble those observed in other tissues such as cardiac tissue (Awapara and Berg, In: Taurine, Ed. Huxtable, R. and Barbeau, A., Raven Press, New York, pp 135-143, 1976) and fish kidney (Wolff et al., Bull. MDIBl, 25: 90-93, 1985).

There was essentially no effect after treatment (60 minute exposure) of these cells with 1 mM $PbNO_3$ or $CdCl_2$. On the other hand, $HgCl_2$ at 20 μM will inhibit taurine flux 50% in 1 minute. This suggests that the sites at which $HgCl_2$ acts in the membrane are likely to be less reactive with or accessible to cadmium and lead. $HgCl_2$ treated cells had a K_t approximately 1/4 that in control cells and J_{max} 1/16 that of control cells. The decrease in K_t suggests that the residual taurine flux (which was about 1/10 of the control flux) behaves as if taurine has enhanced affinity for the transport system. Whether this is a direct effect of $HgCl_2$ on the transport system or a result of indirect effects on other membrane properties is uncertain. The drastic decrease in J_{max} supports the idea that a major effect of $HgCl_2$ exposure is to inactivate the transport carrier protein. $HgCl_2$ inhibition (at short exposure times) is completely reversible after treatment with 10 mM DTT for 5 minutes. Kinetic characterization of the DTT "reversed" red cells indicated that the taurine transport by these cells was essentially normal. This suggests that the reactive moieties on the carrier protein (probably -SH groups) are readily accessible to polar reducing agents such as DTT.

There is a possibility that $HgCl_2$ modifies ion permeabilities resulting in changes in membrane potential. We cannot at present eliminate this possibility since direct measurement of ion permeabilities and membrane potential have not been made in $HgCl_2$ treated cells. However, preliminary measurements of cellular Na and K by flame photometry indicated that standing ion gradients seem unaffected by $HgCl_2$ treatment (30 μM $HgCl_2$, 10 min. incubation; Chen and Preston, unpublished data). The rapid reversibility of inhibition by DTT is consistent with the notion that perturbations of ion gradients are not directly responsible for inhibition of influx.

This work was supported by NIEHS grant #1P30ESO3828-01 awarded to MDIBL and NSF grant # DCB-8501665.