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Effect of Mercury on Taurine Transport by the Red Blood Cells of the Marine Polychaete, *Glycera dibranchiata*

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Ingestion of heavy metals such as mercury, cadmium and lead by man has been associated with a number of debilitating syndromes of medical importance. Multiple sites of organ damage have been identified including the nervous system, the kidneys, blood, and gastrointestinal tract (Chang et al. 1981; D'Itri 1972; Pfeiffer 1977; Samarawickrama 1979; Wood 1975). The major route for distribution of ingested heavy metals throughout the body is via the blood and therefore the behavior of heavy metals in the blood probably has a general impact on many other organ systems. Studies on the distribution of heavy metals in body tissues indicate that blood cells tend to accumulate heavy metals eventually establishing significantly higher levels in the cells compared with serum (Bremner 1979; D'Itri 1972). The extent of accumulation depends on whether the heavy metal is in organic or inorganic form, organic derivatives tending to accumulate more readily in cellular membranes. The fact that these metals as a rule act as sulfhydryl reagents suggests that the mechanism of toxicity of these metals is the result of chemical modification of cellular sulfhydryl groups. The logical initial site of action upon acute exposure to these substances is the cellular membrane. This concept has been framed formally as the "membrane theory of toxicity" by Kinter and Pritchard (1977). They suggest that changes in cell membrane permeability due to chemical modification of membrane proteins of ion and organic transport systems and passive permeability channels leads to disruption of normal cellular function.

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) of the marine polychaete, Glycera dibranchiata. Glycera has been used previously in studies on heavy metal absorption (Medeiros et al. 1980; Rice and Chen 1979). Glycera red cells (RBCs) were used for this study because they contain a high concentration of taurine (190 mM) which has been implicated as a major osmolyte in cellular volume regulation in marine invertebrates (Preston 1970; Costa and Pierce 1983; Gilles 1975; Mead and Preston 1979; Mead 1982). Taurine also appears to participate in osmoregulation of mammalian heart and brain tissue (Huxtable 1978; Sturman et al. 1978;

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Thurston 1981). The coelomic fluid bathing Glycera RBCs typically contains taurine at considerably lower concentrations (0.2 mM). The standing gradients (intracellular conc./extracellular conc.) for amino acids ranges from 50:1 for lysine to 950:1 for taurine. 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 (Chen and Preston 1986; Preston and Chen 1986). The fact that 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 taurine transport systems.

MATERIALS AND METHODS

Glycera RBCs were washed in artificial sea water (NaSW) and centrifuged repeatedly to remove contaminating gametes. The sea water used had the following composition: 440 mM NaCl, 9mM 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 choline chloride was substituted for NaCl to prepare Na free medium (CSW). Mercury treated cells were prepared by incubating RBCs in NaSW containing $HgCl_2$ (typically, 30 µM). The cells were then washed twice in NaSW to remove extracellular $HgCl_2$. In some experiments, the RBCs were subsequently incubated in dithiothreitol (DTT, 10 mM) before uptake measurements were made.

A typical uptake experiment was performed as follows: 0.2 ml of Glycera RBC suspension (10-20% hematocrit) was added to a 1.5 ml microfuge tube and washed 1 to 3 times (depending on the experiment) with 1.0 ml NaSW or CSW. The supernatant was removed and 0.36 ml of SW medium containing ^{14}C-taurine (usually 0.1 mM) and ^{3}H-polyethylene glycol ("PEG", as an extracellular space marker) added to the pellet. After the incubation period (usually 1 minute), 0.3 ml of the medium was placed in a microfuge tube containing 0.3 ml SW and 0.6 ml dibutylphthalate (DBP) prechilled in an ice bath. The tube was then centrifuged immediately at 10,000 x g for 1 minute. The RBCs were separated from the aqueous medium by sedimentation through the DBP layer. The pellet was then lysed with 0.5 ml 0.001% Triton X-100 in water. Then 0.5 ml 5% trichloroacetic acid (TCA) was added and the tube centrifuged for 4 minutes. The amount of radioisotope in the supernatant was evaluated using dual channel scintillation spectroscopy. Appropriate corrections were made for channel overlap and medium trapped in the extracellular space. A 0.05 ml aliquot of the RBC suspension was added to 5.0 ml Drabkins reagent and read at 540 nm after 2 hours to evaluate hemoglobin content which is proportional to RBC count.

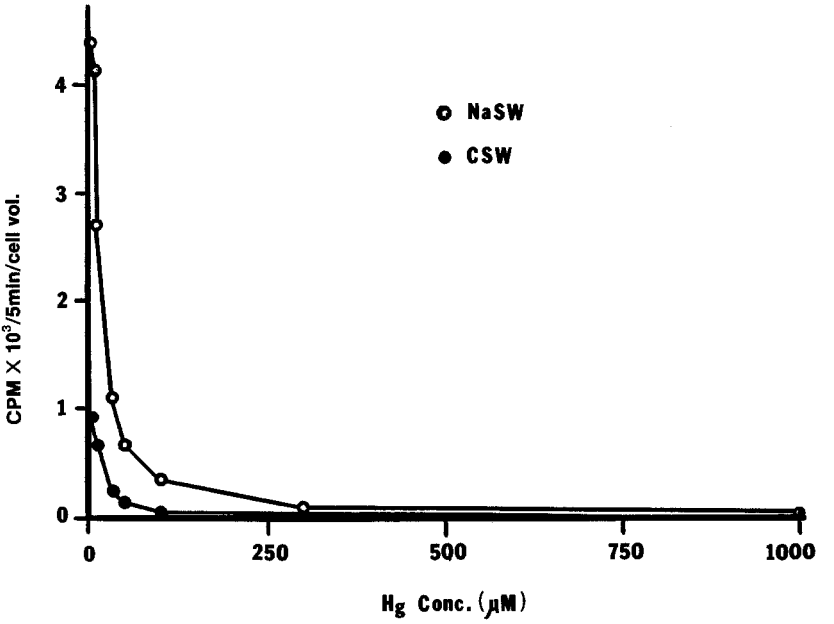

Figure 1. Inhibition of taurine transport in Glycera RBCs after exposure to various concentrations of $HgCl_2$ for one minute.

RESULTS AND DISCUSSION

An earlier study on Glycera RBCs showed that taurine uptake occurred via a specific Na and Cl dependent transport system (Preston and Chen, 1986; Chen and Preston, 1986). Kinetic analysis revealed that the Michaelis constants for taurine transport in NaSW were: $K_t = 0.99 \pm 0.08$ mM and $J_{max} = 0.18 \pm 0.01$ mmol. min^{-1} liter cell $water^{-1}$ (n=5). Competitive inhibition studies showed that only closely related β-amino acids such as β-alanine, γ-aminobutyric acid (GABA) and hypotaurine were good substrates for this transport system. Ion substitution experiments demonstrated that taurine transport was both Na and Cl dependent. As Na or Cl concentrations were increased taurine influx rose rapidly (and nonlinearly) which suggested that more than one Na or Cl may be cotransported with taurine. As part of these earlier studies experiments were also conducted on the effects of preincubation of red cells with 1 mM $CdCl_2$, $PbNO_3$, 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. Because of the sensitivity of this transport process to $HgCl_2$ further experiments reported here centered on this compound.

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% inhibitory concentration ($K_{1/2}$) was about 20 μM (Fig 1). A parallel set of experiments in CSW also showed a similar $K_{1/2}$ although the absolute flux rates were about 1/4 of the corresponding fluxes in NaSW (Fig 1). These data suggest that

sulfhydryl groups associated with the transport protein are readily accessible to $HgCl_2$ and that the Na independent component of taurine transport is susceptible to inhibition. It is possible that modification of other membrane channels influence taurine transport indirectly. Most Na dependent neutral amino acid transport systems are also dependent to some extent on transmembrane electrical potential as well as the Na gradient as a driving force for amino acid accumulation (Crane 1977; Murer and Kinne 1980; Schultz 1977). Mercury treatment could be collapsing Na or potential gradients which subsequently inhibit taurine uptake. For example, Rothstein (1970) observed that the cation permeability of human red cells increased 20 fold after treatment with p-chloromercuribenzoic acid (PCMB). It has also been observed that the Na/K ATPase which is the primary pump maintaining cellular K and Na gradients is inhibited by mercurials (Rothstein 1970; Kinter and Pritchard 1977; Nechay and Saunders 1978). In order to evaluate the possibility that large changes in Na and K gradients were occurring, the Na and K ratios in Glycera RBCs were measured before and after $HgCl_2$ treatment (30 µM, 10 min preincubation) by flame photometry. In the control cells the ratio was 9.4 $\pm$ 0.4 (intracellular K/intracellular Na, n=3). In the $HgCl_2$ treated cells the ratio was 10.5 $\pm$ 0.4 (n=4). The expected ratio would approach that in the external NaSW (11.2 mM K/440 mM Na = 0.025) as a limiting value if the cell membrane permitted complete equilibration with the extracellular fluid. It is therefore unlikely under the conditions chosen for these experiments that changes in the cellular cation gradients can account for the inhibition of taurine transport. The fact that inhibition of transport also occurs without Na in the medium suggests that the site of action of $HgCl_2$ is directly on the carrier protein rather than on the cation gradients.

The effect of Hg on the kinetics of taurine transport and the extent of Hg reversibility after treatment with the reducing agent dithiothreitol (DTT) were also investigated. Red cells were treated with 30 µM $HgCl_2$ in NaSW for 1 min. The cells were then washed 3 times in NaSW. An aliquot of these cells was then incubated with 10 mM DTT for 5 minutes and then washed in NaSW. Controls were done in identical fashion except that $HgCl_2$ was not present. The kinetic constants were measured in NaSW medium. In the untreated control, DTT treated control cells and DTT treated $HgCl_2$ inhibited cells, the K_ts and J_{max}s were not significantly different among the three conditions (Fig 2). The values were respectively: K_t = 1.11, 0.79, 1.13 mM and J_{max} = 192, 190, 182 µmol min^{-1} liter cell $water^{-1}$. In comparison, the K_t for the $HgCl_2$ inhibited cells was 0.27 mM (1/4 the control K_t) and the J_{max} was 13 µmol min^{-1} liter cell $water^{-1}$ (1/15 the control J_{max}). These data indicate that the effects of acute exposure of red cell membranes to $HgCl_2$ are readily reversible with reducing agents such as DTT. From the standpoint of taurine transport, the cells behave normally after DTT treatment.

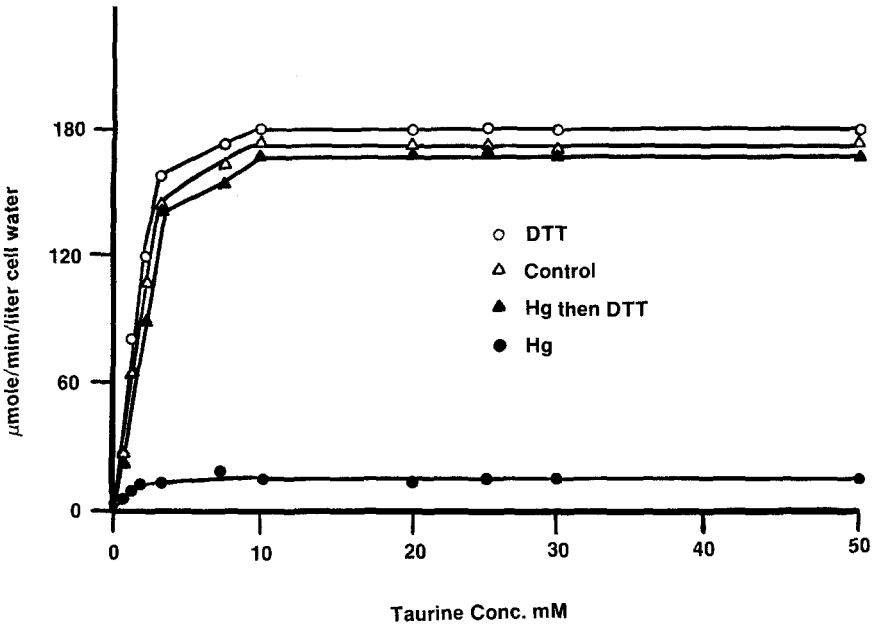

Figure 2. Kinetics of taurine transport in Glycera RBCs treated with 30 μM $HgCl_2$ for 1 min and 10 mM dithiothreitol (DTT) for 5 min.

Whether $HgCl_2$ treatment altered other membrane or intracellular processes as well is not known. At the short $HgCl_2$ treatment times employed in these experiments it is logical to assume that the primary effect of $HgCl_2$ treatment was on the cell membrane and that with longer exposure mercurials should react with other regions of the cell in addition to the membrane.

Reversal of the inhibition of amino acid transport by mercurials has been observed previously. Studies on rabbit intestine by Schaeffer et al. (1974) demonstrated that p-chloromercuriphenyl-sulfonic acid (PCMBS) strongly inhibited the Na dependent neutral amino acid transport by substantially reducing the Na sensitivity of the transport carrier without radically changing the capability of this system to passively transport amino acids. They also demonstrated that the effects of short term exposure to PCMBS were reversible after incubation of the tissue with DTT. The effects after long term exposure to PCMBS were not completely reversible with DTT.

Inhibition of amino acid transport systems leads to decreases in the intracellular amino acid pool size. This has several consequences: Since amino acids usually represent a major osmotic constituent of the cellular cytoplasm, loss of amino acids may lead to potentially damaging cell volume changes. Amino acids are also essential substrates for protein metabolism, can be utilized as energy supplies in cellular metabolism, and are precursors for a

number of important metabolic intermediates. It is clear that radical decreases in the cellular free amino acid pool potentially could have global effects on cell function.

The long term effects of heavy metals on cell function can be quite complex due to their reactivity with -SH groups and the fact that almost all cellular proteins contain -SH groups (Webb 1979). The experiments presented here suggest that by selecting the appropriate experimental conditions (short treatment times) it may be possible to dissect the effects of heavy metals on membrane function from other sites of action.

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