Bull. Environ. Contam. Toxicol. (1989) 42:620–627

Environmental Contamination and Toxicology

Inhibition of Sodium-Dependent Taurine Transport in Red Blood Cells from the Marine Polychaete, *Glycera dibranchiata*, after Exposure to Mercury

Robert L. Preston[1,2,*] and Chang Wen Chen[1]

[1]Illinois State University, Normal, Illinois 61761, USA and [2]Mount Desert Island Biological Laboratory, Salsbury Cove, Maine 04672, USA

An earlier study (Chen and Preston, 1987) demonstrated that $HgCl_2$ at low concentrations (20 µM) inhibited 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 maintain an internal concentration of 190 mM taurine compared with an external concentration in the coelomic fluid of approximately 0.2 mM. This high gradient appears to be generated via a Na and Cl dependent active transport system which is specific for taurine and closely related analogues (Preston and Chen, 1986; Chen and Preston, 1986). This taurine transport system resembles other taurine transport systems observed in heart and kidney tissue (Awapara and Berg, 1985; Wolff et al, 1985). It appears that Glycera RBCs provide an excellent model system to study the mechanism of heavy metal inhibition of transport function. We proposed that one possible mechanism for the action of $HgCl_2$ is that this molecule binds to sulfhydryl groups in the Na binding site of the transport protein. In order to test this hypothesis we conducted a series of kinetic studies to analyze in more detail the interaction of Na and Hg with the transport system. In addition, we conducted experiments to determine whether the effects of $HgCl_2$ treatment could also be due to generalized changes in cellular permeability.

MATERIALS AND METHODS

Glycera RBCs were washed in artificial seawater (NaSW) and centrifuged to remove gametes and white cells. 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 conducted 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 the pellet was suspended in 0.36 ml of

*Correspondence and reprint requests

SW medium containing 0.1 mM ^{14}C-taurine and ^{3}H-polyethylene glycol (PEG) as an extracellular space (ECS) marker. After 5 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 ^{3}H and ^{14}C in the extract was evaluated using dual channel scintillation spectroscopy. Corrections were made for channel overlap and medium trapped in the ECS. A 50 µl aliquot of the RBC suspension was added to 5.0 ml Drabkins reagent and read at 540 nm to evaluate hemoglobin content which was directly proportional to RBC count. Data are usually expressed as mean ± standard error (S.E.) In plotted data if no error bars are present the S.E.s are smaller than the size of the points graphed. Unless otherwise indicated n = 3 for each point. Statistical comparisons, where noted, utilized Students t-test.

The effect of mercury on taurine transport was evaluated by preincubating the RBCs in medium containing various concentrations of $HgCl_2$. The RBCs were then washed 2 times in medium free of $HgCl_2$ before transport measurements were made. In efflux experiments, RBCs were incubated with ^{86}Rb in NaSW for 90 minutes. The RBCs were then washed in NaSW and treated with 10 µM to 30 µM $HgCl_2$ for 1 minute. The $HgCl_2$ containing medium was then replaced with NaSW and aliquots of this medium were sampled periodically to measure ^{86}Rb efflux.

RESULTS AND DISCUSSION

We have shown previously that 1 min incubation of Glycera RBCs with $HgCl_2$ inhibits taurine influx by 50% at about 20 µM $HgCl_2$ (Chen and Preston, 1987). Although this concentration is convenient for experimental purposes, the question can be asked whether substantially lower concentrations exposed to Glycera RBCs for longer time periods might also exhibit similar effects on taurine influx. Glycera RBCs were incubated with 1 µM $HgCl_2$ for various time periods up to 120 min and then ^{14}C-taurine influx (5 min flux period) was measured (Fig 1).

After 120 min taurine influx was inhibited by about 30%. The time course of inhibition was essentially linear during the exposure period. These data show that the membrane effects of $HgCl_2$ occur at much lower $HgCl_2$ concentrations (1 µM vs 30 µM) than we usually employ in these experiments. This suggests that at very low environmental Hg concentrations the cumulative effect of Hg interaction with cell membranes could be similar to the effects observed with short-term exposures to Hg at higher concentrations.

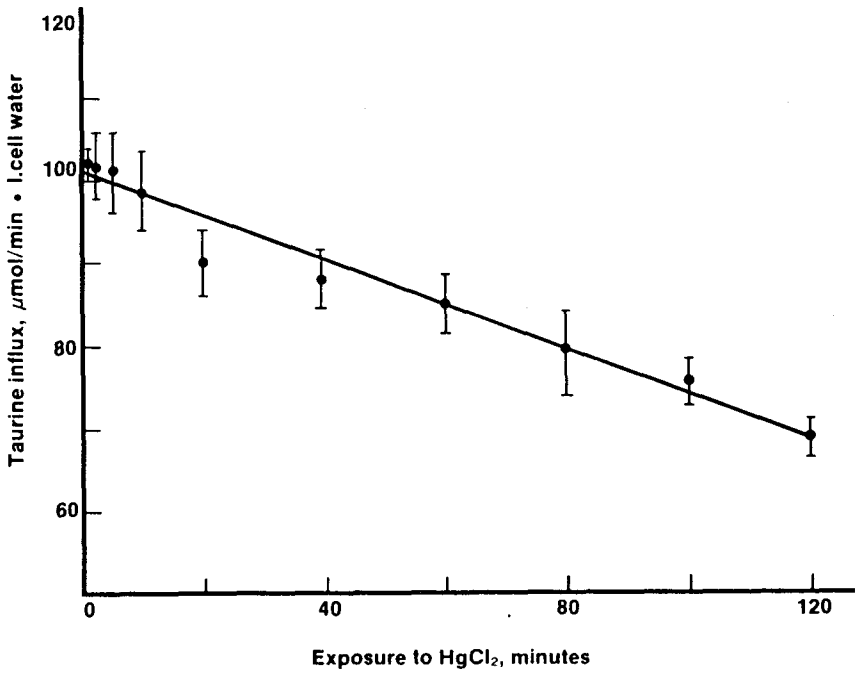

Figure 1. Inhibition of Taurine Influx after Exposure of Glycera RCBs to 1 μM $HgCl_2$. Red Cells were incubated with 1 μM $HgCl_2$ for various time periods, washed 2 times in NaSW and then incubated with ^{14}C-taurine in NaSW for 5 minutes. Indicated values are mean ± S.E. (n = 3).

The influx of 1 mM ^{14}C-taurine into Glycera RBCs was measured in media containing various concentrations of Na. In these experiments NaCl was replaced by choline chloride on an equimolar basis to maintain constant osmotic pressure in the incubation medium. As Na concentration was increased from 0 mM Na to about 270 mM Na, the taurine influx increased gradually from 80 ± 6 to 110 ± 11 μmol min^{-1} l. cell $water^{-1}$ ($p < 0.075$; Fig 2). Increasing the Na concentration further to 440 mM increases taurine influx much more rapidly to 194 ± 5 μmol min^{-1} l.cell $water^{-1}$ ($p < 0.005$; Fig 2). This nonlinear dependence on external Na concentration implies that more than one Na per taurine molecule may be required for each transport event.

The kinetic parameters for taurine transport by Glycera RBCs were evaluated at various Na concentrations (Table 1). Complete replacement of NaCl with choline chloride resulted in an increase ($p < 0.001$) in the K_t for taurine transport from 1.18 ± 0.07 mM (440 mM Na) to 4.85 ± 0.35 mM (0 mM Na). On the other hand, the J_{max} remains relatively constant throughout the range of Na concentrations. The K_t doubles between 380 mM Na and 0 mM Na and doubles again from 440 mM Na to 380 mM Na.

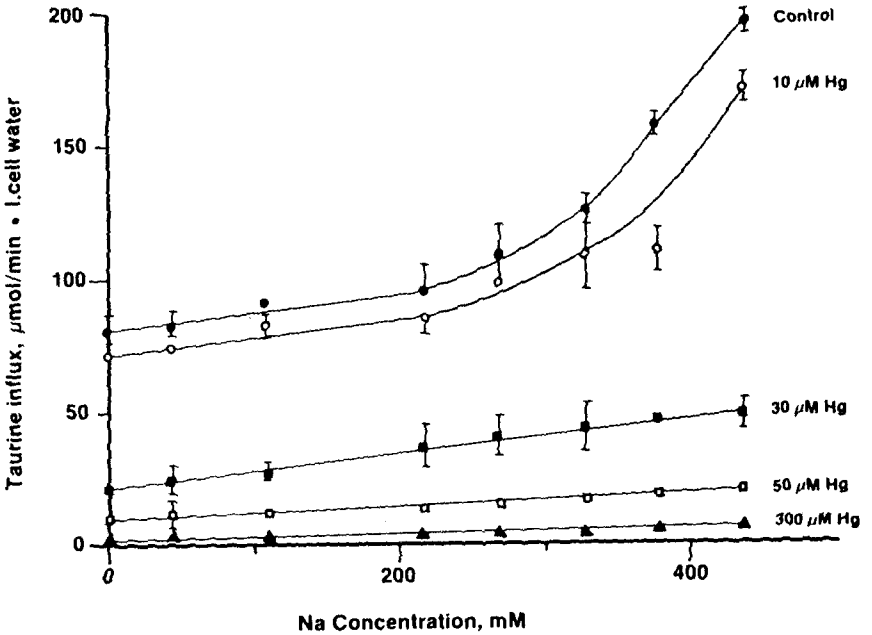

Figure 2. Effect of $HgCl_2$ on Na Dependency of Taurine Influx in Glycera RBCs. Red cells were incubated with various concentrations of $HgCl_2$ for 1 minute, washed 2 times in NaSW and then incubated with 1 mM ^{14}C-taurine for 5 minutes in media which contained various concentrations of Na. The Na concentration was adjusted by replacement of NaCl in the medium with equimolar choline chloride. Values indicated are mean ± S.E. ($n = 3$).

Table 1. Kinetics of Na Dependency

Na Conc., (mM)	K_t, mM (n = 3)	J_{max}, μmol min^{-1} l.cell $water^{-1}$ (n = 3)
0	4.85 ± 0.35	336 ± 18
110	4.09 ± 0.25	341 ± 15
270	2.71 ± 0.25	321 ± 20
380	2.24 ± 0.22	380 ± 24
440	1.18 ± 0.07	365 ± 12

This nonlinear behavior parallels that observed in Fig. 2. The data also demonstrate that the kinetic effect of Na is to modify the apparent affinity of the transport carrier for taurine. In

this regard, this transport system appears to resemble the neutral amino acid transport system in rabbit intestine which has been extensively characterized by Curran, Schultz and coworkers (Curran et al., 1967; Preston et al., 1974). They proposed a model in which the carrier molecule contains separate binding sites for both the amino acid and Na. The binding of Na appears to induce an allosteric change in the carrier protein which then translocates the amino acid to the cytoplasmic face of the membrane. Schaeffer et al. (1973) showed that the Na binding site in this intestinal transport system was sensitive to inhibition by the mercurial p-chloromercuriphenyl sulfonic acid (PCMBS). We tested this possibility in Glycera RBCs by examining the effect of $HgCl_2$ treatment on the kinetic parameters for taurine transport.

The influx of ^{14}C-taurine in media containing various amounts of Na was measured in RBCs treated with 10 µM to 300 µM $HgCl_2$ for 1 minute (Fig 2). At $HgCl_2$ concentrations of 30 µM or greater there was an apparent decrease in the stimulation of taurine transport by increasing concentrations of Na. This supports the hypothesis that $HgCl_2$ interferes in some way with the binding of Na to the transport carrier. If the primary kinetic effect of decreasing Na on taurine transport is to increase the K_t (Table 1), one would predict that $HgCl_2$ treatment should also raise the apparent K_t for taurine transport.

Table 2. Effect of Hg on the Kinetics of Taurine Transport

$HgCl_2$, (M)	K_t, mM (n = 3)	J_{max}, µmol. min^{-1} l.cell $water^{-1}$ (n = 3)
0	1.70 ± 0.33	116 ± 13
10	1.72 ± 0.35	83 ± 10
20	3.23 ± 0.32	64 ± 4
30	6.29 ± 1.55	40 ± 7

The kinetic parameters for taurine transport were measured in NaCl medium at 10 µM, 20 µM and 30 µM $HgCl_2$ (Table 2). As $HgCl_2$ concentration was increased the apparent K_t for taurine transport increased from 1.70 ± 0.33 mM in control RBCs to 6.29 ± 1.55 mM in cells treated with 30 µM $HgCl_2$ ($p < 0.05$). The J_{max}, on the other hand, decreased from 116 ± 13 µmol min^{-1} l.cell $water^{-1}$ in the control to 40 ± 7 mol min^{-1} l.cell $water^{-1}$ ($p < 0.01$). These results agree with the prediction that $HgCl_2$ treatment should increase K_t and hence one effect of $HgCl_2$ may be to interfere with Na interaction with the transport carrier. However, the decrease in J_{max} suggests that there are also additional effects of $HgCl_2$ which inactivates the carrier protein in some other way. Presumably this could reflect another site of action which may be distinct from the site which modifies the Na binding effects.

These data generally agree with our earlier observations (Chen and Preston, 1987) that one effect of $HgCl_2$ treatment is to decrease J_{max}. However, our previous data suggested that the K_t for taurine transport was also decreased by treatment with $HgCl_2$. The source of this discrepancy is unclear, although the experiments presented here were designed specifically with a larger number of points in the sensitive concentration range and thus may be assumed to more accurately measure K_t values for taurine transport. We have also noted variability in tissue from experiment to experiment. It is possible that the acclimation status of Glycera is important since these animals can occur in diverse environments ranging from ocean sediments to estuarine mudflats in which the salinities may be as low as 50% that in open ocean.

In addition to directly inhibiting taurine transport by chemical modification of the carrier, mercurials could also influence Na cotransport systems indirectly by changing cation permeability and collapsing ion gradients, thus reducing the driving force for substrate transport (Rothstein, 1970). We reported earlier (Chen and Preston, 1987) that the intracellular/extracellular K/Na ratios (measured by flame photometry) were not changed in Glycera RBCs after 10 min incubation with 30 μM $HgCl_2$. In an attempt to confirm that K permeabilities are not significantly modified by $HgCl_2$ treatment we measured the efflux of ^{86}Rb from ^{86}Rb preloaded cells before and after 1 min incubation with 30 μM $HgCl_2$ (Table 3).

Table 3. ^{86}Rb from $HgCl_2$ Treated RBCs

$HgCl_2$ Conc, (μM)	Efflux period, min	^{86}Rb efflux, CPM (n=3)
0	1.0	301 ± 13
0	5.0	557 ± 34
0	10.0	625 ± 15
0	30.0	1565 ± 39
30	1.0	309 ± 22
30	5.0	609 ± 27
30	10.0	846 ± 48
30	30.0	1614 ± 30

Glycera RBCs were preloaded for 90 min with 11 mM ^{86}Rb, washed in NaSW and split into two aliquots. One aliquot was incubated for 1 min in NaSW containing 30 μM $HgCl_2$ and then washed 2 times and suspended in NaSW for efflux measurements. The control was treated identically but $HgCl_2$ was deleted from the medium. After the various efflux periods the cells were separated from the medium by centrifugation, a 1 ml aliquot of the medium sampled and the ^{86}Rb measured by scintillation spectroscopy.

It appears that $HgCl_2$ treatment has relatively little effect on ^{86}Rb efflux ($p > 0.2$), except possibly a small enhancement of efflux at 10 min. Other replicate experiments show essentially the same results although sometimes either the control or $HgCl_2$ treated cells show slightly greater efflux. In no case, however, does it appear that ^{86}Rb efflux is drastically modified as might be expected from the powerful effects $HgCl_2$ has on taurine influx.

These data show that Glycera RBCs exposed to 1 μM $HgCl_2$ for 120 min causes substantial (30%) inhibition of taurine influx. This suggests that low environmental concentrations produce physiological effects similar to those observed at higher concentrations which are more practical experimentally. The mechanism of $HgCl_2$ inhibition of taurine transport is in part due to modification of the carrier affinity for taurine (K_t effect). The data are consistent with the hypothesis that reduction of taurine affinity is due to a decrease in Na binding to the transport protein (Fig 2, Tables 1, Table 2). However, there is also an additional effect of $HgCl_2$ treatment which may be due to other types of interaction of $HgCl_2$ with the carrier (J_{max} effects). Measurement of ^{86}Rb efflux supports the notion that under the conditions chosen for these experiments, $HgCl_2$ does not drastically alter K gradients or K membrane permeability (Table 3). We feel that these data provide a promising beginning toward analysis of the mechanism of interaction of Hg with the taurine Na dependent cotransport system.

Acknowledgments. This work was supported by a Grant-in-Aid from the American Heart Association, Maine Affiliate, and NIEHS grant #1P30ES03828-01 awarded to MDIBL, NSF grant #DCB-8501665.

REFERENCES

Awapara J, Berg M (1976) Uptake of taurine by slices of rat heart and kidney. In: Huxtable R, Barbeau A (ed) Taurine. Raven Press, New York, pp 135-143

Chen CW, Preston RL (1986) Taurine transport by the coelomocytes of the marine polychaete, Glycera dibranchiata. Am Zool 26:74A

Chen CW, Preston, RL (1987) Effect of mercury on taurine transport by the red blood cells of the marine polychaete, Glycera dibranchiata. Bull Environ Contam Toxicol 39:202-208

Curran PF, Schultz SG, Chez RA, Fuisz RE (1967) Kinetic relations of Na-amino acid interaction at the mucosal border of intestine. J Gen Physiol 50:1261-1286

Preston RL, Chen CW (1986) Effect of heavy metals on taurine transport by the coelomocytes of the marine polychaete, Glycera dibranchiata. The Bulletin. Mount Desert Island Biological Laboratory 26:129-132

Preston RL, Schaeffer JF, Curran PF (1974) Structure-affinity relationships of substrates for the neutral amino acid transport system in rabbit ileum. J Gen Physiol 64:443-467

Rothstein A (1970) Sulfhydryl groups in membrane structure and function. In: Bronner F, Kleinzeller A (eds) Current Topics in Membranes and Transport Vol 1. Academic Press, New York, pp 135-176.

Schaeffer JF, Preston RL, Curran PF (1974) Inhibition of amino acid transport in rabbit intestine by p-chloromercuriphenyl sulfonic acid. J. Gen Physiol 62:131-146

Wolff NA, Kinne R, Elger B, Schoelermann B, Scheutz H, Goldstein L. (1985) Taurine secretion in toadfish (Opanus tau) kidney: Transport in the intact kidney and in brush border membrane vesicles. The Bulletin. Mount Desert Island Biological Laboratory 25:90-93

Received June 8, 1988; accepted August 26, 1988.