REVERSAL OF MERCURIAL INHIBITION OF TAURINE TRANSPORT IN THE COELOMOCYTES OF THE MARINE POLYCHAETE, GLYCERA DIBRANCHIATA.

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The hemoglobin containing coelomocytes (red blood cells, RBCs) of the marine polychaete, Glycera dibranchiata, transport the amino acid, taurine, via a Na dependent system against gradients approaching 1000:1 (190 mM intracellular: 0.2 mM extracellular concentration). This transport system resembles the transport systems for taurine observed in heart, kidney and other tissues in that it is selectively inhibited by other ß-amino acid analogues and exhibits apparent coupling coefficients of 2 or 3 Na per taurine transported (Chen, C.W. and Preston, R.L., Bull. Environ. Contam. Toxicol. 39: 202-208, 1987; Preston, R.L. and Chen, C.W., Bull. Environ. Contam. Toxicol. 42: 620-627, 1989; Preston, R.L. and Chen, C.W., Bull.MDIBL 26: 129-132, 1986).

Earlier experiments have shown that this transport system is readily inhibited after exposure of the RBCs to low concentrations of $HgCl_2$ ($K_{1/2}$ for inhibition = 20 uM; Chen, C.W. and Preston, R.L., Bull. Environ. Contam. Toxicol. 39: 202-208, 1987). We have shown that one possible mechanism for the action of $HgCl_2$ was that it reacts with sulfhydryl groups that affect the Na binding site for this transport system leading to an increase in apparent Michaelis constant (K_t) which resembles a similar increase observed when NaCl in the medium is replaced with choline chloride (Preston, R.L. and Chen, C.W., Bull. Environ. Contam. Toxicol. 42: 620-627, 1989). We have also shown that the $HgCl_2$ inhibition of taurine transport can be readily reversed by subsequent exposure of these cells to reducing agents such as dithiothreitol (DTT) and that the kinetics of taurine transport by RBCs treated with $HgCl_2$ and then DTT were essentially normal (Chen, C.W. and Preston, R.L., Bull. Environ. Contam. Toxicol. 39: 202-208, 1987; Preston and Truong, unpublished data).

The present studies were conducted to examine the timecourse of this reversal process and the structure-activity relationships of a variety of other reducing agents. In addition, experiments were conducted to compare the effect of the organic mercurial, p-chloromercuriphenyl sulfonic acid (PCMBS), to $HgCl_2$ inhibition of taurine influx.

The transport of ^{14}C-taurine was measured at 12°C (the acclimation temperature) from artificial seawater medium (NaSW) containing ^{3}H-polyethylene glycol (^{3}H-PEG) as an extracellular space marker (see methods in Chen and Preston, 1987). The RBCs were separated from the incubation medium by centrifugation through dibutylphthalate (DBP). Uptake was linear for at least 7 minutes. The usual incubation time for kinetic analyses was 5 minutes. The standard conditions for treatment with mercurials were as follows: 1). Initial exposure of the red cells to the mercurial compound at various concentrations and/or incubation times. 2). The treated RBCs were then washed 2x (or more) with NaSW. 3). In reversal experiments, the Hg treated washed cells were then exposed to NaSW containing reversal agents. 4). Measurement of taurine influx in NaSW as described above. 5). Various reversal agents of

different molecular size were tested. Most experiments were repeated 3 times. Representative experiments are presented in this report.

The transport of taurine by Glycera RBCs was reduced to about 20% of the control fluxes after exposure to 30 uM $HgCl_2$ for 1 minute (Fig 1). This level of inhibition was maintained in these cells when they were further incubated in NaSW for periods up to 30 minutes. However, treatment of these cells with 10 mM DTT led to rapid reversal of the inhibition, returning close to control levels (88%) after about 6 minutes (Fig 1). The apparent half-time for reversal was less than 1 minute. These data suggest that the moieties sensitive to $HgCl_2$ are readily accessible to DTT, implying that they may lie in a location exposed to the medium or at least may be present in a region accessible to hydrophilic molecules such as DTT. The fact that the control cells did not show any indication of spontaneous reversal of inhibition when incubated in NaSW implies that cellular reducing agents such as glutathione or reduced cellular proteins may not have access to the site of mercurial interaction.

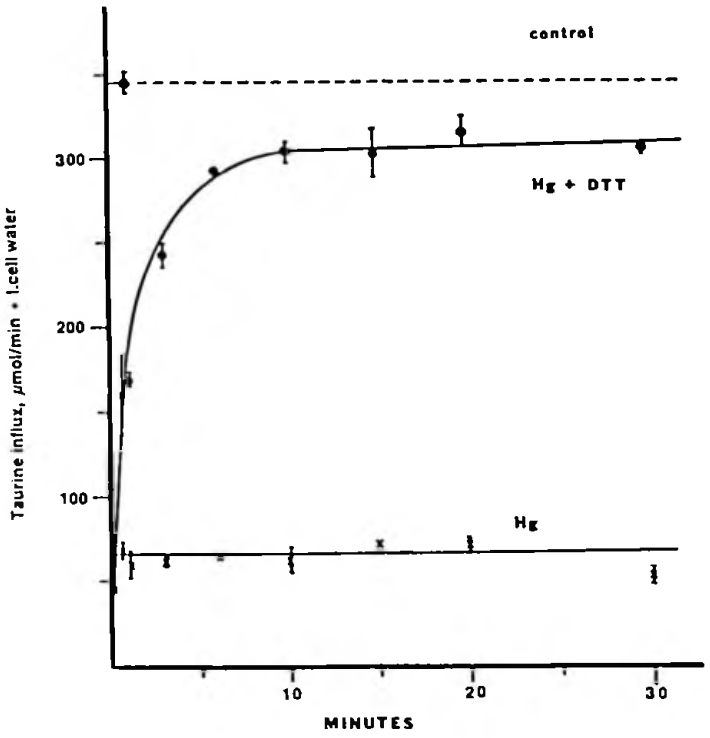

Fig. 1: Timecourse of Reversal of $HgCl_2$ Inhibition of Taurine Influx. Glycera RBcs were incubated with 30 uM $HgCl_2$ for 1 minute. The cells were washed and transferred to NaSW (x) or NaSW containing 10 mM DTT (o). The control indicates flux measurements in cells exposed only to NaSW not $HgCl_2$. The taurine concentration was 1 mM. Points indicate mean $\pm$ S.E. (n=3). Where S.E. are not shown errors are smaller than the points.

To investigate further whether the site of the sensitive sulfhydryl groups are accessible to reducing agents of different size (and therefore presumably differing membrane permeability), a series of organic thiol compounds were tested for their ability to reverse $HgCl_2$ inhibition of taurine transport (Table 1). The following agents nearly completely reversed (> 90%) taurine inhibition by $HgCl_2$ at 10 mM concentration incubated for 10 minutes: DTT, D-penicillamine, β-mercaptoethylamine and DL-homocysteine. Partial reversal occurs with L-cysteine and L-cysteine ethyl ester. No reversal occured with glutathione (GSH), N-acetyl cysteine, N-(2-mercaptopropionyl)glycine. These data suggest that the reactive sulfhydryl on the carrier is accessible to reversal agents with low molecular weight but molecules of the size of dipeptides or tripeptides did not readily have access to this site. GSH (10 mM) did not reverse inhibition to any significant extent after exposure to $HgCl_2$ treated RBCs for 60 minutes. Sometimes stimulation of influx above control values is observed with DTT

Table 1: Reversal of $HgCl_2$ Inhibition of Taurine Transport by Various Reducing Agents

Condition	Taurine Influx umol.5 min^{-1}1.cell $water^{-1}$ ± S.E. (n = 3)	J_i/J_o
Control (NaSW only)	562 ± 15	-
Control (30 uM $HgCl_2$ only)	191 ± 19	0.34
Dithiothreitol	784 ± 17	1.40
D-Penicillamine	556 ± 29	0.99
β-Mercaptoethylamine	536 ± 41	0.95
DL-Homocysteine	604 ± 10	1.07
N-Acetyl cysteine	179 ± 16	0.32
N-(2-Mercaptopropionyl)glycine	298 ± 19	0.53
Control (NaSW only)	1339 ± 132	-
Control (30 uM $HgCl_2$ only)	500 ± 81	0.37
Dithiothreitol	1545 ± 58	1.15
L-Cysteine	888 ± 85	0.66
L-Cysteine ethyl ester	934 ± 56	0.70
Glutathione	479 ± 85	0.36

All fluxes were measured for 5 min at 1 mM ^{14}C-taurine in NaSW. In the experimental conditions the RBCs were first exposed to 30 uM $HgCl_2$ in NaSW for 1 min, washed 2 times in NaSW and then resuspended in NaSW containing 10 mM thiol reducing agents for a period of 10 min. These cells were then washed in NaSW and flux measurements conducted as indicated above. J_i/J_o is the influx in the experimental conditions divided by the control influx. The data below the dotted line represent those obtained in a separate experiment.

treatment (J_i/J_o = 1.15 to 1.4; Table 1) but this is not always observed in every experiment or in control experiments where RBCs are exposed to DTT without prior $HgCl_2$ treatment (Table 2).

PCMBS inhibited taurine influx (1 min exposure) in RBCs more slowly than $HgCl_2$, requiring higher concentrations (1 mM) to obtain inhibition equivalent to that 30 uM $HgCl_2$ (J_i/J_o approx. 0.3). Presumably this reflects lower accessibility to and/or lower reactivity with the sensitive sulfhydryls involved with taurine transport. PCMBS inhibition of taurine influx was also partially reversible with DTT (10 mM, 20 min exposure; Table 2).

Table 2: PCMBS Inhibition of Taurine Transport

Condition	Taurine Influx umol.5 min^{-1}1.cell $water^{-1}$ $\pm$ S.E. (n = 3)	J_i/J_o
Control (NaSW only)	992 $\pm$ 21	-
Control (1 mM PCMBS)	230 $\pm$ 10	0.23
DTT only (20 min)	882 $\pm$ 39	0.89
PCMBS then DTT (20 min)	504 $\pm$ 57	0.51

The experimental conditions were the same as in Table 1 except that the RBCs were first exposed to 1 mM PCMBS in NaSW for 1 min, washed 2 times in NaSW and then resuspended in NaSW containing 10 mM DTT for a period of 20 min.

These data are consistent with the hypothesis that the sulfhydryl groups essential for taurine transport are located in an environment which is accessible to sulfhydryl reducing agents of low molecular weight. This implies that the RBC membrane shields these groups to some extent. The fact that $HgCl_2$ inhibition does not slowly spontaneously reverse in NaSW suggests that intracellular reducing potential is not available to these extracellular sites. The inhibition of taurine transport by PCMBS requires higher concentrations than $HgCl_2$ and is more slowly reversed by DTT. In part, this may be because the bulky PCMBS molecule does not easily penetrate the membrane to the sensitive sulfhydryl groups of this transport system.

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