ORGANIC AND INORGANIC MERCURIAL INHIBITION OF TAURINE TRANSPORT BY THE COELOMOCYTES OF THE MARINE POLYCHAETE, GLYCERA DIBRANCHIATA.

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The transport of the amino acid, taurine, in the hemoglobin containing coelomocytes (red blood cells, RBCs) of the marine polychaete, Glycera dibranchiata occurs via a specific Na-dependent system against gradients approaching 1000:1 (190 mM intracellular: 0.2 mM extracellular concentration; 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). This transport system is readily inhibited after exposure of the RBCs to low concentrations of $HgCl_2$ ($K_{1/2}$ for inhibition = 20 uM). We have also shown that the $HgCl_2$ inhibition of taurine transport can be readily reversed by subsequent exposure of these cells to sulfhydryl reducing agents (Preston et al., Bull. MDIBL 29: 78-81, 1990). The ability of a variety of these agents to reverse mercury inhibition appeared to be correlated with the molecular weight of the agents implying that the moieties of the transport carrier that interact with mercuric chloride were partially occluded within the membrane. In this study we compared the effect of $HgCl_2$ treatment of RBCs in Na-free medium with that in Na medium to determine whether mercurial inhibition of the Na-independent component of taurine influx resembles the inhibition of the Na-dependent component. We also have investigated the effect of the organic mercurial, p-chloromercuriphenyl sulfonic acid (PCMBS) on inhibition of taurine influx and measured the effects of reducing agents on reversal of PCMBS inhibition of taurine influx.

The transport of ^{14}C-taurine and was measured at 12°C from artificial seawater (NaSW) or in Na-free seawater (choline Cl substituted for NaCl) containing ^{3}H-polyethylene glycol as an extracellular space marker. The RBCs were separated from the incubation medium by centrifugation through dibutylphthalate (Chen, C.W. and Preston, R.L., Bull. Environ. Contam. Toxicol. 39: 202-208, 1987). The incubation time with ^{14}C-taurine for most experiments was 5 minutes. 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.

Treatment of Glycera RBCs with $HgCl_2$ for 1 min inhibited taurine influx 50% at a concentration of about 15-20 uM in both choline seawater (CSW) and NaSW. Competitive inhibition experiments (1 mM ^{14}C-taurine, 20 mM inhibitor) in both NaSW and CSW gave the following values for taurine and analogues (percent inhibition in parentheses for NaSW and CSW respectively): taurine (90%, 79%), B-alanine (92%, 85%),

γ-aminobutyric acid (GABA, 92%, 73%) and hypotaurine (79%, 64%). Other neutral amino acids were weak inhibitors (<25%) or noninhibitory. Treatment of Glycera RBCs with 30 uM $HgCl_2$ followed by incubation with the reducing agent dithiothreitol (DTT) at 10 mM resulted in rapid reversal of inhibition in both CSW and NaSW (half-reversal times, 0.5 - 1 min). These data show that the properties of $HgCl_2$ inhibition of taurine influx is very similar in both Na and Na-free media. These results support the conclusion that taurine transport in CSW and NaSW is mediated by the same transport carrier whose affinity for taurine is modified by Na (Preston, R.L. and Chen, C.W., Bull. Environ. Contam. Toxicol. 42: 620-627, 1989) rather than by separate Na-dependent and Na-independent systems.

A series of experiments with the organic mercurial, PCMBS, indicated that this compound was a much less effective inhibitor of taurine influx than $HgCl_2$. The half-inhibition concentration for PCMBS (1 min incubation) was about 500 uM (Table 1). At 30 uM PCMBS the half-inhibition time was about 30 min and at 500 uM PCMBS the half-inhibition time was about 1.5 min. In comparison, the half-inhibition time for 30 uM $HgCl_2$ was <0.5 min. Treatment of RBCs with 1 mM PCMBS for 1 min followed by 10 mM DTT resulted in a half-reversal time of about 20 min for inhibition of taurine influx. This compares with a half-reversal time of 1 min for RBCs treated with 30 uM $HgCl_2$ for 1 min and then 10 mM DTT. Dose-response experiments for DTT reversal (10 min exposure) of PCMBS (1 mM, 1 min) treated cells indicated that higher DTT concentrations were more effective in reversal of PCMBS inhibition ($\geq$ 10 mM) although DTT by itself may depress taurine influx somewhat (19% inhibition at 50 mM DTT, Table 1). Reversal of PCMBS (1 mM, 1 min) treated RBCs with other reducing agents (50 mM, 10 min) showed the following pattern (listed in order of effectiveness: B-mercaptoethylamine, L-cysteine, DTT > DL-penicillamine, DL-homocysteine. N-Acetylcysteine and reduced glutathione had no effect. These data show that the organic mercurial PCMBS has less effect than $HgCl_2$ on taurine transport, possibly due to less accessibility to the sensitive reactive moieties in the membrane. This is also consistent with the fact that PCMBS is less lipid soluble than $HgCl_2$.

Preliminary measurements were also conducted on Na/K ATPase activity in Glycera RBC membrane. These data suggest that this activity is rather more sensitive to $HgCl_2$ inhibition (<<20 uM) than taurine transport or D-glucose transport (see Preston et al, this issue). Wondergem and Sisson (this issue) made measurements of the transmembrane potential with microelectrodes in control and $HgCl_2$ treated Glycera RBCs. They found that under conditions that nearly completely inhibits taurine transport (20 uM $HgCl_2$, exposure $\geq$ 6 min) no significant change in membrane potential was detected. These data support the hypothesis that a major site of action of mercury on cell function is the cell membrane and that transport processes are particularly sensitive to mercurials. These data also support the notion that the inhibitory effects of $HgCl_2$ on each transport system are unique to the specific structural and functional characteristics of these systems and are not the result of nonspecific changes in membrane properties, membrane potential or membrane permeability. The fact that mercurial inhibition of membrane transport may be readily reversed with DTT but not other

larger molecular weight reducing agents suggests that the reactive moieties (presumably SH groups) of the transport carriers may be partially occluded by the cell membrane.

Table 1: Effect of PCMBS on Taurine Transport

Concentration of PCMBS, uM	Influx ± S.E. (n = 3) (umol·min^{-1}·l.cell water^{-1})	J_i/J_o	p
Control	223 ± 5	-	-
30	203 ± 4	0.91	<0.05
100	190 ± 2	0.85	<0.005
300	132 ± 4	0.59	<0.001
500	108 ± 3	0.48	<0.001
1000	79 ± 3	0.35	<0.001
3000	8 ± 3	0.04	<0.001
Reversal Experiment:			
Control	443 ± 12	-	-
1000 uM PCMBS	159 ± 8	0.36	<0.001
0.1 mM DTT only	410 ± 8	0.92	<0.1
1 mM DTT only	422 ± 5	0.95	<0.2
10 mM DTT only	404 ± 5	0.91	<0.05
20 mM DTT only	399 ± 3	0.90	<0.025
50 mM DTT only	360 ± 8	0.81	<0.005
1000 uM PCMBS + 0.1 mM DTT	171 ± 1	0.42*	<0.001
1000 uM PCMBS + 1 mM DTT	202 ± 2	0.48*	<0.001
1000 uM PCMBS + 10 mM DTT	281 ± 5	0.70*	<0.001
1000 uM PCMBS + 20 mM DTT	289 ± 6	0.72*	<0.001
1000 uM PCMBS + 50 mM DTT	296 ± 14	0.82*	<0.025

Glycera RBCs were treated with PCMBS in NaSW at the specified concentrations for 1 min and then the cells were washed 2x in NaSW. In the reversal experiments the RBCs were then incubated with DTT at the concentrations indicated for 10 min. In the control, RBCs were incubated only in NaSW and not treated with PCMBS or DTT. RBCs were also incubated with DTT alone to measure direct effects of DTT on influx. ^{14}C-Taurine influx was measured for 5 min at 1 mM taurine. J_i/J_o = ratio influx in PCMBS or in DTT ("DTT only") treated RBCs to influx in control cells. *In PCMBS + DTT treated cells J_o was taken as influx value for the "DTT only" condition. Student's t-test was used to compare the statistical significance (p) of the experimental conditions with the control. N.S. = no statistical difference.

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