EFFECT OF MEDIA ANION COMPOSITION ON MERCURY INHIBITION OF TAURINE TRANSPORT BY THE COELOMOCYTES OF THE MARINE POLYCHAETE, GLYCERA DIBRANCHIATA

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Previous studies in our laboratory have shown that taurine transport by the hemoglobin containing coelomocytes (red blood cells, RBCs) of the marine polychaete, Glycera dibranchiata, is rapidly inhibited by exposure to micromolar concentrations of mercuric chloride (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). We have concluded that the probable site of action of mercuric chloride is the membrane transport carrier for taurine. It is also likely that mercuric chloride simultaneously modifies other cellular processes because of its high reactivity with sulfhydryl groups (e.g. glucose transport, Preston, R. L. et al., Bull. MDIBL 30:51-53, 1991). However, our evidence supports the notion that mercuric chloride acts directly on the transport carrier or associated moieties rather than by indirect effects (e.g. Preston, R. L. et al., Bull. MDIBL 29:78-81, 1990; Preston, R. L. and Chen, C.W., Bull Environ. Contam. Toxicol. 42:620-627, 1989).

The ionic state of mercury in solution depends on anion concentration (Webb, J.L. in Enzyme and Metabolic Inhibitors, Academic Press, N.Y., 1966). Mercury can exist in a variety of cationic, neutral or anionic forms depending on medium Cl^- concentration.

$$Hg^{++} \leftrightarrow HgCl^+ \leftrightarrow HgCl_2 \leftrightarrow HgCl_3^- \leftrightarrow HgCl_4^=$$

Increasing $[Cl^-]$ $\Longrightarrow$

In anion substitution studies in which we replaced Cl^- with gluconate, we showed that it is likely that the reactive form of mercury in our system is $HgCl_3^-$ (Preston, R. L. et al., Bull. MDIBL 33: 53-55, 1994). The chloride concentration (approx. 100 mM Cl^-) at which the $HgCl_3^-$ form is maximized correlates well with the concentration at which the inhibition of taurine transport is maximum. In the present set of experiments, we utilized other anion substitutes as well as gluconate to more rigorously test the hypothesis that $HgCl_3^-$ is the critical reactive form of mercury. We also noticed in preliminary studies that the usual pattern of mercury inhibition observed in gluconate and other media (maximum inhibition at 100 mM Cl^-) was not found in bromide and iodide media. Our present data will show that this anomalous behavior may be due to formation of less reactive mercury complexes in these media.

The concentration of Cl^- in incubation medium containing 20 µM mercuric chloride was varied by iso-osmotic replacement of NaCl with the Na salts of the following anions: gluconate, sulfamate, sulfate, nitrate, methylsulfate, isethionate, bromide, iodide and thiocyanate. In the case of sulfate, the medium contained D-mannitol as well to bring the solution to the correct osmotic prssure. Glycera RBCs were washed 2 times in artificial seawater (NaSW), washed 2 times in the appropriate anion substituted medium (without mercury) and then incubated in the mercury containing medium for 1 minute. This medium was then removed, the cells washed once in the appropriate anion substituted medium without mercury. In the controls, all conditions were identical except that the 1 minute incubation was done in mercury free medium.

Taurine influx was measured by incubating the RBCs at 12°C with 1 mM ^{14}C-taurine in NaSW for 5 minutes. The RBCs were then separated from the radioactive medium by centrifuging the cells through dibutylphthalate (Chen, C.W. and Preston, R. L., Bull Environ. Contam. Toxicol. 39:202-208, 1987). Trichloroacetic acid extracts of the RBCs were transferred to scintillation vials and isotope content determined by scintillation spectroscopy. The data were corrected for cell number by measuring hemoglobin content with Drabkin's reagent (Sigma Chemical Co., St. Louis) which is directly correlated with cell number and cell water content. Medium identified as 0 mM Cl^- medium in this study refers to medium in which no Cl^- salts were added. It should be recognized that low levels of contaminating Cl^- is probably present in the medium and cells suspensions (probably <1 mM).

Table 1: Effect of Various Anion Substituted Media on Mercury Inhibition of Taurine Transport.

	Taurine influx, μmol. 5 min^{-1} l.cell $water^{-1}$ (± S.E., n = 3)*							
		Incubated with 20μM Hg for 1 min						
	**Control	0 Cl^-	(J_I/J_0)	100 Cl^-	(J_I/J_0)	514 Cl^-	(J_I/J_0)
Gluconate	1233 ± 71	1206 ± 71	0.98	168 ± 9	0.14	362 ± 52	0.29
Isethionate	1174 ± 26	1380 ± 50	1.18	276 ± 13	0.24	415 ± 18	0.35
Methysulfate	1094 ± 47	1104 ± 18	1.01	173 ± 17	0.16	339 ± 8	0.31
Nitrate	94 ± 46	696 ± 60	0.78	117 ± 9	0.13	376 ± 50	0.42
Sulfamate	867 ± 28	938 ± 85	1.08	172 ± 28	0.20	418 ± 36	0.48
Sulfate	841 ± 26	712 ± 28	0.85	96 ± 13	0.11	294 ± 17	0.35
Bromide	974 ± 26	884 ± 29	0.91	783 ± 36	0.80	412 ± 31	0.42
Iodide	366 ± 22	437 ± 10	1.19	443 ± 19	1.21	415 ± 44	1.13

* Data from two separate experiments were combined in this table.
** Control fluxes were determined on cells washed in the appropriate anion substituted medium (0 mM Cl^-) but were not exposed to mercuric chloride. Na salts of the anion listed were substituted iso-osmotically for NaCl. D-Mannitol was added to sulfate medium to adjust the osmotic pressure. The Cl^- concentrations are mM. (J_I/J_0) = inhibition ratio where J_I = taurine influx after exposure to mercury; J_0 = control taurine influx.

Table 1 shows the results for inhibition of taurine transport by Glycera RBCs after 1 min incubation with various anion substituted media containing 20 μM mercuric chloride. The results for gluconate are typical: Little or no inhibition occurred in 0 Cl^- medium compared with the control which was not exposed to mercury (J_I/J_0 = 0.98; where J_I = taurine influx after exposure to mercury and J_0 = control taurine influx). At 100 mm Cl^- mercury inhibited taurine influx ≥85% (J_I/J_0 = 0.14). In 514 mM Cl^- (the normal NaSW concentration) mercury inhibited taurine influx by ≥70% (J_I/J_0 = 0.29). This pattern reflects the shift from the cationic mercury forms to the anionic forms as Cl^- concentration in the medium increases and is consistent with the hypothesis that $HgCl_3^-$, which is in maximum relative concentration at about 100 mM Cl^-, is the form that reacts with the taurine transporter. Although 3 Cl^- concentrations were used in this study for screening purposes, more detailed studies using intermediate Cl^- concentrations are entirely consistent with this hypothesis. A similar pattern of inhibition is seen for isethionate, methylsulfate, nitrate, sulfamate and sulfate media. For these media as a group, the inhibition ratios (J_I/J_0) for 0 mM Cl^- ranged from J_I/J_0 = 0.78 for nitrate to J_I/J_0 = 1.18 for isethionate, with most values close to

1.0; for 100 mM Cl^- J_I/J_0 = 0.11 for sulfate to J_I/J_0 = 0.24 for isethionate; and for 514 mM Cl^- J_I/J_0 = 0.31 for methylsulfate to J_I/J_0 = 0.48 for sulfamate.

In contrast, mercury was substantially less effective in inhibiting taurine transport in 100 mM Cl^- medium in which bromide or iodide were used as anion replacements (J_I/J_0 = 0.80 for bromide and J_I/J_0 = 1.21 for iodide). One possible explanation for this may be that both bromide and iodide have higher affinity constants for complexation with mercury and thus would preferentially form $HgBr_n^x$ or HgI_n^x complexes rather than $HgCl_3^-$ (where n may range from 1 to 4 and x from +1 to -2). In addition, one must assume that the bromide and iodide complexes are less permeable to membrane and/or less reactive with taurine transport protein. If this is true, we would predict that adding low concentrations of Br^- or I^- in the presence of Cl^- should lessen the inhibitory effect of mercury. This hypothesis was tested by incubating RBCs with mercury in 100 mM Cl^- medium with and without Br^- or I^- added at concentrations ranging from 0.01 mM to 10 mM (Table 2).

Table 2A,B: Low Concentrations of Bromide and Iodide Reduce Mercury Inhibition of Taurine Transport in 100 mM Chloride Medium.

A:*	Taurine influx, μmol. 5 min^{-1} 1.cell water^{-1} (± S.E., n = 3)** Incubated with 20μM Hg for 1min				
Control***	0 mM Br^-	0.01 mM Br^-	0.1 mM Br^-	1mM Br^-	10 mM Br^-
1250 ± 31 (J_I/J_0)	156 ± 5 (0.12)	131 ± 7 (0.10)	139 ± 15 (0.11)	505 ± 25 (0.40)	679 ± 53 (0.54)
B:*					
Control***	0 mM I^-	0.01 mM I^-	0.1 mM I^-	1mM I^-	10 mM I^-
1250 ± 31 (J_I/J_0)	156 ± 5 (0.12)	339 ± 20 (0.27)	1268 ± 52 (1.01)	1001 ± 35 (0.80)	1158 ± 22 (0.93)

* The 1 minute incubation of the RBCs with 20 μM mercuric chloride was conducted in 100 mM Cl^- medium with Na gluconate replacing the remaining Cl^- iso-osmotically. Br^- or I^- was added in additon to the 100 mM Cl^- present in the medium.

** Taurine influx was measured in NaSW which had a Cl^- concentration of 514 mM (see methods).

*** Control fluxes were determined on cells washed in 100 mM Cl^- medium but were not exposed to mercuric chloride. (J_I/J_0) = inhibition ratio where J_I = taurine influx after exposure to mercury; J_0 = control taurine influx.

The data in Table 2A show that 20 μM mercuric chloride in 100 mM Cl^- medium (gluconate replacement) strongly inhibited taurine transport ($J_I/J_0 \cong$ 0.10) in the range of 0 mM - 0.1 mM added Br^-. However, at 1 mM and 10 mM Br^- the effect of mercury was considerably lessened ($J_I/J_0 \cong$ 0.40 and 0.54 respectively). A similar experiment with added I^- (Table 2B) shows that the effect of mercury is lessened at the lowest I^- concentration tested (0.01 mM, J_I/J_0 = 0.27) compared with the control value of (J_I/J_0 = 0.12) . At higher I^-

concentrations (0.1 mM to 10 mM), 20 μM mercury had little if any effect (J_1/J_0 = 0.80 to 1.01). These data should also reflect, in a general way, the relative affinities of the halides for mercury in relation to Cl^- and OH^-. As an index of what the relative affinities of halides for mercury may be, one can use the values published by Webb (Webb, J.L. in Enzyme and Metabolic Inhibitors, Academic Press, N.Y., 1966) for dissociation constants for the equilibrium $K_1 = [Hg^{++}][A^-]/[HgA^+]$, (see below, units in parentheses are -log dissociation constants).

I^- (12.9) < OH^- (10.3) < Br^- (9.05) < Cl^- (6.74) < gluconate$^-$ (?)

The data in Table 2 are generally consistent with this pattern of affinitites. Iodide is effective at concentrations that are, perhaps, 1/100 to 1/1000 that of bromide. Bromide at least partially prevents mercury inhibition at concentrations 1/10 to 1/100 that of Cl^-. The dissociation constant for gluconate was not available in Webb's data but we would predict that gluconate, as well as the other anion substitutes employed in Table 1, would have a substantially lower dissociation constant for mercury complexes. Other explanations for this behavior are possible, but we feel these data are quite consistent with the notion that inactive complexes are formed with Br^- and I^-. If other tissues (intestinal epithelia, for example) resemble Glycera RBCs in general transport characteristics and sensitivity to mercury, one might speculate that low concentrations of bromide or iodide in the diet might substantially reduce the reactivity of inorganic mercury with membrane transporters. This may be another approach to amelioration of mercurial toxicity in some systems.

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