Na-INDEPENDENT TAURINE TRANSPORT BY THE COELOMOCYTES OF THE MARINE POLYCHAETE, *GLYCERA DIBRANCHIATA*

Robert L. Preston[1], Drew Sommerville[1], Sun Lu[2] and Tatianna Gott[3]
[1]Department of Biological Sciences
Illinois State University, Normal, IL. 61790-4120
[2]FDA/CBER, Bethesda, MD. 20892
[3]Cornell University, Ithaca, NY

Previous work in our laboratory has focused on the mechanisms of mercury inhibition of taurine transport by the hemoglobin containing coelomocytes (red blood cells, RBCs) of the marine polychaete, *Glycera dibranchiata*, (Preston, R.L., Zimmermann, P.R., Kaleta, M.T. and Simokat, K.A., Bull. MDIBL 33:53-55, 1996). As part of preliminary kinetic characterization of the taurine transport system, we measured transport in media in which cations and anions were replaced other organic and inorganic ions (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., Janssen, S.J., Lu, S. and Truong, T.T., Bull. MDIBL 29:74-77, 1990). We concluded that taurine transport by *Glycera* RBCs was Cl-independent and Na-dependent. We recently had occasion to re-examine the question of ion dependency in this system and have confirmed that it is Cl-independent. However, in media in which sodium was replaced choline chloride and other cations, it became apparent that choline chloride, directly inhibited taurine transport. These data suggest that taurine transport is Na-independent.

Glycera RBCs were washed in artificial seawater (NaSW) and separated from other cell types by differential centrifugation. All experiments were done at 12°C. The composition of the NaSW used for control taurine influx measurements was 440 mM NaCl, 9 mM KCl, 2.2 mM $KHCO_3$, 9.3 mM $CaCl_2$, 23 mM $MgCl_2$ and 26 mM $MgSO_4$. The composition of "mannitol seawater" (MSW) was identical except that the NaCl was replaced with isotonic mannitol. *Glycera* RBCs were washed in MSW or NaSW three times before unidirectional influx measurements (5 min incubation period)were made in these media containing 1 mM ^{14}C-taurine and ^{3}H-polyethylene glycol (as an extracellular space marker). In some experiments, amino acid analogues (20 mM; β-alanine, γ-aminobutyric acid (gaba), hypotaurine, D-alanine and L-alanine)were added to measure specificity of the taurine transport system. The RBCs were then separated from the radioactive medium by centrifuging the cells through dibutylphthalate. Trichloroacetic acid extracts of the RBC pellets 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 which is directly correlated with cell number and cell water content. Correction was also made for ^{14}C-taurine trapped in the extracellular space. All experiments were repeated in triplicate at a minimum.

Table 1A shows data for taurine influx in Na-free mannitol seawater compared with NaSW. The fluxes in the two media are essentially the same although in this set of experiments there was a slight apparent increase in influx ($J_I/J_0 = 1.12$) in MSW. Taurine transport also shows nearly identical patterns of inhibition in NaSW and MSW in the presence of competitive β-amino acid analogues (β-alanine, gaba, hypotaurine) and noncompetitive α-amino acid analogues(D-alanine, L-alanine). Detailed kinetic analyses (not reported here) also show that taurine transport has virtually identical kinetic constants in NaSW (0.88 $\pm$ 0.06 mM; Jmax = 355 $\pm$ 16 μmol · min^{-1} l.cell water^{-1}) and MSW (0.82 $\pm$ 0.03 mM; Jmax = 334 $\pm$ 13 μmol min^{-1} l.cell water^{-1}). These data strongly support the hypothesis that the same transport system mediates taurine influx in NaSW and in Na-free MSW. Our earlier conclusion that the system was Na-dependent was based primarily on the fact that when choline chloride was used as a salt to replace NaCl, taurine influx decreased. Choline chloride is perhaps the most common Na-substitute and is used by most investigators for this type of study. The assumption is that choline chloride has no "toxic" physiological

effects, but it is apparent that for taurine transport there must be direct inhibition. Table 1B shows experiments in which taurine influx was measured in 340 mM NaCl with added Na replacement compounds present at 100 mM (for salts) or 200 mM (for mannitol). This did not change osmotic pressure across the experimental conditions used in these experiments except for the unsubstituted 340 mM NaCl control. The "minor" ionic components of the seawaters was the same as controls (see above).

Table 1: Taurine Transport by *Glycera* RBCs in NaSW and Na-free Mannitol Seawater in the Presence of Competitive Inhibitors and Cation Substitutes

Incubation Conditions	Taurine influx[1] NaSW	MSW	(J_I/J_0)[2] NaSW	MSW
PART A:				
Control	1552 ± 23	1740 ± 19	-	(1.12)[3]
+β-alanine	132 ± 1	142 ± 4	0.08	0.08
+gaba	158 ± 4	132 ± 1	0.10	0.08
+hypotaurine	319 ± 4	310 ± 4	0.21	0.18
+D-alanine	1344 ± 20	1529 ± 78	0.86	0.88
+L-alanine	1502 ± 5	1615 ± 31	0.97	0.93
PART B:				
440 mM NaCl	604 ± 28		-	
340 mM NaCl[4]	596 ± 41		0.98	
+ KCl	530 ± 50		0.88	
+ LiCl	587 ± 28		0.92	
+ NMG-Cl	592 ± 42		0.98	
+ mannitol	621 ± 21		1.03	
+ choline Cl	310 ± 16		0.51**	

[1] μmol. 5 min^{-1} l.cell $water^{-1}$ (± S.E., n = 6)

[2] (J_I/J_0) = inhibition ratio where J_I = taurine influx in the presence of 20 mM amino acid analogues in NaSW or MSW; J_0 = control taurine influx in NaSW or MSW.

[3] This value is the control flux in NaSW/Control flux in MSW

[4] The salts listed below are added to 340 mM at a final concentration of 100 mM to maintain constant osmotic pressure (confirmed by direct measurement of these solutions using vapor pressure osmometry). Mannitol was present at 200 mM. The control at 340 mM NaCl was not osmotically compensated and thus was somewhat hypotonic.
NMG-Cl = N-methyl glucamine Cl.

** The inhibition of taurine transport by choline Cl was statistically significant (Student's t-test, $p<0.05$, n = 6).

These data show that choline chloride has direct effects on taurine transport whereas other ion replacement salts have little effect with perhaps the exception a small inhibition (12%) by KCl. The inhibition is probably not due to general "toxicity" to these RBCs because choline chloride has no effects on D-glucose transport (Preston, R.L., McQuade, K.L., Janssen, S.J., and Lu, S., Bull. MDIBL 30:51-53, 1991). Choline media also do not increase cell lysis. We conclude that taurine transport by *Glycera* RBCs is Na-independent. Furthermore, transport studies using choline Cl as a Na-replacement should be done with caution and should recognize that direct inhibition of transport is possibility. (Supported in part by NIEHS grant ESO3828-11. Tatianna Gott was recipient of a Judy and Stan Fellowship.)