EFFECT OF HYPO-OSMOTIC MEDIA ON TAURINE FLUX IN POLYCHAETE RED BLOOD CELLS.

Robert L. Preston[1], Sarah J, Janssen[1], Karen S. Peterson[1],
Kristin A. Simokat[2] and Kristi L. McQuade[3]
[1]Department of Biological Sciences,
Illinois State University, Normal, IL. 61761.
[2]Southwest Harbor, Maine, 04679
[3]Department of Chemistry, McKendree College, Lebanon, Il. 62254

In a series of studies in this laboratory, we have shown that taurine transport by the hemoglobin containing coelomocytes (red blood cells, RBCs) of the marine polychaete, Glycera dibranchiata, is readily inhibited by low concentrations of mercuric chloride (Chen and Preston, Bull. Environ. Contam. Toxicol. 39:202-208, 1987; Preston and Chen, Bull. Environ. Contam. Toxicol. 42:620-627, 1989; Preston et al., Bull. MDIBL 26:129-132, 1990). The influx of taurine is inhibited 50% after a 1 min exposure to seawater containing 20 uM mercuric chloride. We have concluded that the probable site of action of mercuric chloride is the membrane transport carrier for taurine or some closely associated moiety. However, mercuric chloride is not specific for this carrier since it is highly reactive with sulfhydryl groups present in most proteins. To evaluate the other effects of mercuric chloride in these cells we have also studied mercurial inhibiton of D-glucose transport in Glycera RBCs (Preston et al., Bull. MDIBL 30:51-53, 1990) and have begun a series of studies on the effects of mercury on cell volume regulation by these cells.

In dilute media, the red cells of Glycera display a regulatory volume decrease (RVD) mediated, in part, by the release of amino acids (in particular, taurine) and potassium (Costa and Pierce, J. Comp. Physiol. 151:133-144, 1983). These two solutes are present at high concentrations in the RBCs, each being about 200 mM. The data presented here describe some of our preliminary results on the effect of osmotically dilute media on the influx and efflux of taurine by Glycera RBCs.

The basic techniques used in this study have been described in detail elsewhere (Chen and Preston, Bull. Environ. Contam. Toxicol. 39:202-208, 1987). Briefly, the transport of ^{14}C-taurine was measured using an artificial seawater medium (NaSW) at 12^{o}C. In some experiments, the Na in the medium was replaced on an equimolar basis with choline Cl to provide a Na free seawater (CSW). The RBCs were separated from the incubation medium by centrifuging the cells through dibutylphthalate (DBP). Influx (5 min incubation period) was measured in RBCs exposed to NaSW or CSW diluted up to 50% with deionized water. The RBCs were pre-equilibrated with the dilute medium 5 min before influx measurements were made. In some experiments, influx was measured in the presence of the competitive inhibitors, beta-alanine, gamma-aminobutyric acid and hypotaurine. Efflux measurements were made by preloading the RBCs with ^{14}C-taurine for 60 min, washing the cells in 100% NaSW and then exposing the labelled RBCs to 100% or 50% NaSW for various time periods. The amount of ^{14}C-taurine in the aqueous upper layer after the RBCs were centrifuged through DBP was taken as a measure of taurine released by the cells.

Taurine influx in media ranging from 50% to 100% NaSW did not change (Table 1). This result was somewhat unexpected in that earlier experiments

(Preston and Chen, Bull. Environ. Contam. Toxicol. 42:620-627, 1989) have shown that as NaCl in NaSW was replaced with choline Cl influx dramatically decreased to about 25% of the control value in 100% NaSW. Assuming that influx would decrease solely in response to decreased Na concentration in 50% NaSW the predicted influx should be less than 50% of the value in 100% NaSW. The fact that there is no significant change in the rate of influx suggests that some compensatory stimulation of influx may be occurring activated by low osmotic pressure. Taurine influx was also measured in CSW diluted from 100% to 50% on the same batch of RBCs (Table 1). The Na-independent component of taurine influx approximately doubled as the medium is diluted from 100% to 50% CSW. Deducting the Na-independent flux from the Na-dependent flux, it is apparent that the Na-dependent component has decreased about 50% and the Na-independent component has doubled (Table 1). These data are consistent with the presence of a volume activated component to taurine flux that has been observed elsewhere (e.g. Fincham, et al. J. Membrane Biol. 96:45-56, 1987).

Table 1: Taurine Influx in Hypo-osmotic Seawater

Medium	Percent Seawater Concentration	Influx $\pm$ S.E. (n = 3) (μmol·min^{-1}·l.cell water^{-1}	J_{dil}/J_{con}	p
NaSW	100	214 $\pm$ 2	-	
	90	213 $\pm$ 10	0.99	N.S.
	70	211 $\pm$ 4	0.98	N.S.
	50	197 $\pm$ 16	0.92	N.S.
CSW	100	67 $\pm$ 3	-	
	90	75 $\pm$ 1	1.12	<0.1
	70	107 $\pm$ 1	1.60	<0.001
	50	126 $\pm$ 2	1.88	<0.001
Na Dependent Component (NaSW-CSW)	100	147		
	90	138		
	70	104		
	50	71		

Glycera RBCs were pre-equilibrated in the media above for 5 min and then incubated with 1 mM ^{14}C-taurine for 5 min. J_{dil}/J_{con} = ratio of taurine influx in the dilute medium to the influx of taurine in 100% NaSW or 100% CSW. Student's t-test was used to compare the statistical significance (p) of the experimental conditions to the control. N.S. = no statistical significance.

Competition experiments in 100% and 50% NaSW and CSW indicated that there was little significant change in the ability of the competitors beta-alanine, hypotaurine and gamma-amino butyric acid to inhibit influx. For example, the ratio (J_I/J_o) of ^{14}C-taurine influx in the presence (J_I) and absence (J_o) of beta-alanine was 0.10 (100% NaSW), 0.10 (50% NaSW), 0.08 (100% CSW) and 0.07 (50% CSW). This suggests the selectivity of the influx pathway in dilute media resembles that in control medium. These data contrast with that of Fincham et al. (J. Membrane Biol 96:45-56, 1987) in fish erythrocytes that show a decrease in selectivity for substates in dilute media.

The logical role for this Na-independent influx component in these cells might be as a pathway for taurine efflux during RVD. To directly address this possibility, we measured efflux from RBCs preloaded with ^{14}C-taurine and then exposed to 100% and 50% NaSW and CSW for 60 min (Table 2). The data indicate that taurine efflux is stimulated in 50% NaSW compared with 100% NaSW (49%) and in 50% CSW compared with 100% CSW (175%). These data are consistent with the hypothesis that the swelling of Glycera RBCs hypo-osmotic medium activates a volume sensitive Na-independent pathway that may be involved in RVD in these cells. As an operational hypothesis, we propose that this efflux pathway may be identical with or similar to the Na-independent influx pathway for taurine. Further work is being conducted to confirm or disprove this possibility.

Table 2: Taurine Efflux in Hypo-osmotic Seawater

Medium	Percent Seawater Concentration	Efflux $\pm$ S.E. (n = 3) (CPM·h^{-1}·cell vol^{-1})	J_{dil}/J_{con}	p
NaSW	100	1540 $\pm$ 224	-	
	50	2296 $\pm$ 59	1.49	<0.05
CSW	100	1313 $\pm$ 52		
	50	3606 $\pm$ 370	2.75	<0.005

Glycera RBCs were preloaded with 0.1 mM ^{14}C-taurine for 5 min in 100% NaSW. The RBCs were then washed in 100% NaSW and then transferred to control or dilute seawater as indicated above. Efflux is expressed as CPM. The total CPM in an aliquot of cells equivalent to that used for the efflux measurements was about 120,000 CPM. J_{dil}/J_{con} = ratio of taurine influx in the dilute medium to the efflux of taurine in 100% NaSW or 100% CSW. Student's t-test was used to compare the statistical significance (p) of the experimental conditions to the control. N.S. = no statistical significance.

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