EFFECTS OF ANION SUBSTITUTION ON TAURINE TRANSPORT BY THE COELOMOCYTES OF THE MARINE POLYCHAETE, GLYCERA DIBRANCHIATA.

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In earlier studies we investigated the effects of mercuric chloride exposure and its mechanism of inhibition of the membrane transport of the amino acid, taurine, by the hemoglobin containing coelomocytes (red blood cells, RBCs) of the marine polychaete, Glycera dibranchiata (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). These RBCs maintain an internal concentration of about 190 mM taurine compared with a taurine concentration in the coelomic fluid of 0.2 mM. Our previous kinetic studies indicated that this high gradient appeared to be generated by a Na and Cl dependent active transport system which is specific for taurine and closely related analogues (Preston, R.L. and Chen, C.W., Bull.MDIBL 26: 129-132, 1986). This taurine transport system resembles other β-amino acid transport systems observed in heart, kidney and neural tissue (Awapara, J. and Berg, M. in Taurine, eds. Huxtable, R. and Barbeau, A., Raven Press, New York, pp 135-143, 1976; Wolff, N.A. et al., Bull. MDIBL 25: 90-93, 1985; Kanner, B.I., Biochim. Biophys. Acta 726: 293-316, 1983). The apparent Cl dependency of the Glycera RBC system has not been fully characterized previously and thus we conducted a series of experiments on the effects of replacement of chloride in the external medium with other anions.

Glycera RBCs were washed in artificial seawater (NaSW) and centrifuged to remove gametes and white cells. The NaSW had the following composition: 440 mM NaCl, 9 mM KCl, 9.3 mM $CaCl_2$, 23 mM $MgCl_2$, 26 mM $MgSO_4$, and 2.2 mM $KHCO_3$ (final pH 7.8). In some experiments other Na salts were substituted for NaCl to prepare Cl free medium and the Cl in the "minor components" (calcium, magnesium, and potassium salts) in NaSW was replaced with the equivalent nitrate or gluconate salts. D-Mannitol was also added to the medium in some cases to maintain constant osmotic pressure. Control experiments indicated that D-mannitol had no direct effects on taurine transport. The transport of 1 mM ^{14}C-taurine was measured at 12°C from artificial seawater medium (NaSW) containing ^{3}H-polyethylene glycol as an extracellular space marker (for methods see Chen, C.W. and Preston, R.L., Bull. Environ. Contam. Toxicol. 39: 202-208, 1987). The RBCs were separated from the incubation medium by centrifugation through dibutylphthalate, extracted with 2.5% TCA and the isotope in the extract measured with scintillation spectrometry. The usual incubation time for these experiments was 5 minutes.

Initial experiments indicated that taurine influx was Cl dependent when measured in a variety of media in which the chloride was replaced with other anions. Our preliminary results indicated that when NaSCN or $NaNO_3$ was used to replace NaCl, taurine influx was drastically reduced, the NaSCN medium reducing influx as much as 95%. As the proportion of NaCl was increased the taurine influx increased slowly at concentrations below 200 mM and then increased rapidly as the NaCl approached the control level of 440 mM (Fig 1). Taurine influx in $NaNO_3$ medium gave a similar nonlinear relationship as in Fig 1 but the basal influx in

chloride free media was several fold higher. This type of nonlinear dependence could imply that more than one chloride ion may be involved in the transport process. However, it is well known that anions that are relatively permeable to biological membranes such as SCN and NO_3 can influence membrane transport processes indirectly by inducing diffusion potentials or modifying the behavior of cellular proteins by lyotropic effects (Payne, J.A., Lytle, C. and McManus, T.J., J. Gen. Physiol. 90: 34a, 1987).

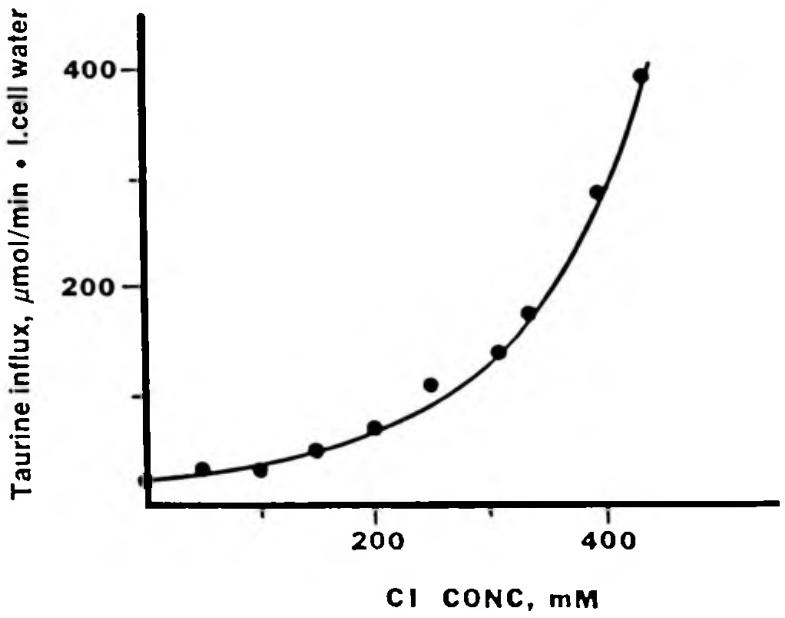

Fig. 1: Taurine Influx as a Function of Chloride Concentration in SCN Medium. NaCl in NaSW was replaced on an equimolar basis with NaSCN. The taurine concentration was 1 mM. Points represent mean (n=3), errors being smaller than the size of the points.

In order to investigate this possibility we measured taurine influx from media containing the Na salts of a variety of other anions (Fig 2). Taurine influx is inhibited little or not at all in Na media containing sulfate, sulfamate, gluconate or isethionate compared with NaCl controls. Other replacement media resulted in reduction of taurine influx to the following levels (expressed as percent control flux remaining): Na methylsulfate (78%), NaBr (68%), $NaNO_3$ (42%), NaI (11%) and NaSCN (2%). On the basis of the flux measurements made after substitution of chloride with sulfate and other organic anions, one would conclude that taurine transport is chloride independent. On the other hand, flux measurements made in media containing the other replacement anions and in particular NaSCN and $NaNO_3$ lead to the opposite conclusion that taurine transport may be chloride dependent.

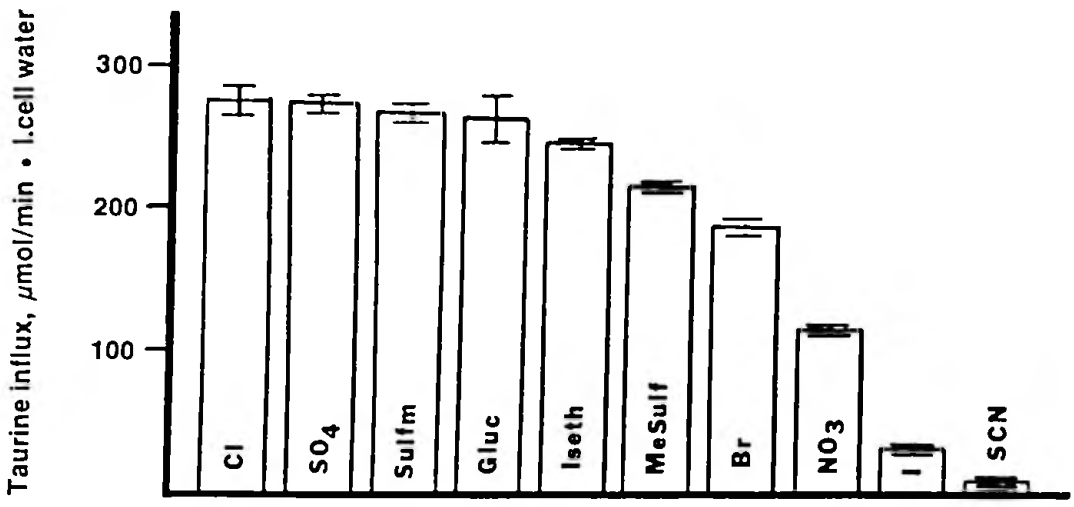

Fig. 2: Taurine Transport in Media in which Chloride has been Replaced with Other Anions. The Na concentration was 440 mM for all conditions. Taurine concentration was 1 mM. Bars indicate mean ± S.E. (n=3). Abbreviations: Sulfm = sulfamate; Gluc = gluconate; Iseth = isethionate; MeSulf = methylsulfate.

Payne, Lytle and McManus (J. Gen. Physiol. 90: 34a, 1987) noted that NO_3, I, and SCN media induced an immediate and significant water loss (up to 10%) in human RBCs. Methanesulfonate and sulfamate had little effect on water content. They suggested that the binding of lyotropic anions to cellular colloids (presumably hemoglobin) resulted in an apparent increase in fixed intracellular charge. They were able to confirm this hypothesis by showing that the cell isoelectric point declined from 6.93 in normal NaCl medium to 5.70 in SCN. The lyotropic sequence of anions generally follows a classical Hofmeister series (Collins, K. D. and Washabaugh, M. W., Quart. Rev. Biophys. 18: 323-422, 1985): $SO_4 = HPO_4 > F > Cl > Br > I > SCN$. Anions following chloride are generally regarded as "chaotropes" or water-structure breakers which destabilize proteins. This is the same general sequence that we have noted in our chloride replacement data (Fig 2).

Carter-Su and Kimmich (Am. J. Physiol. 237: C67-C74, 1979) noted that diffusion potentials established using various Na-anion gradients stimulated 3-O-methylglucose in ATP-depleted isolated chicken intestinal cells in the same sequence as the expected relative membrane permeability of the anions, SCN > Cl > isethionate > SO_4 (see also Wright, E.M. and Diamond, J.M., Physiol. Rev. 57: 109-157, 1977). If taurine transport was influenced by a similar mechanism, it might be expected that taurine influx should be stimulated rather than inhibited. Our data therefore seem to be more reasonably explained by other

mechanisms, although more complex interactions of these anions with transmembrane potentials cannot be completely eliminated.

It is possible that other anions could be cotransported with taurine in Na medium as effectively as chloride, resulting in no apparent inhibition of transport and leading to the mistaken conclusion that taurine transport is chloride independent in ion replacement experiments. However, if other anions were cotransported equally well as chloride, it might be expected that the smaller membrane permeable anions would be selected rather than the bulkier organic anions. In our data, the reverse is true, the larger organic anions have no effect on taurine transport whereas the smaller anions cause inhibition.

In order to test the hypothesis that the inhibitory effect of NaSCN is due to physical or chemical effects on Glycera RBCs rather than its role as a chloride replacement, we conducted an experiment in which the NaCl concentration was kept constant at 240 mM while the SCN concentration was increased from 0 to 200 mM. The experimental conditions and results are shown in Table 1. Taurine influx decreases from 245 $\pm$ 3 umol·min^{-1}liter cell water^{-1} to 6 $\pm$ 0.2 umol·min^{-1}liter cell water^{-1} as the SCN concentration is raised from 0 mM (control) to 200 mM. Since Na and Cl concentrations were held constant, taurine influx should remain constant. There is a possibility that SCN competes with Cl binding for the carrier and is not cotransported with taurine. However, because gluconate, sulfate, isethionate and sulfamate fail to inhibit taurine influx (Fig 2), it seems more likely that the effect of SCN may be due to lyotropic or chemical effects on the RBCs. These data point to the possibility that the inhibitory capacity of these anions is correlated with their chaotropic behavior and that the apparent chloride dependence observed in our experiments may, in fact, be due to other chemical or physical inhibitory effects on cellular membrane properties or cellular proteins.

Table 1: Effect of SCN of Taurine Transport at Constant Sodium and Chloride Concentrations

[NaCl] mM	[ChCl]* mM	[Mann]* mM	[NaSCN] mM	Taurine influx** umol·min^{-1}l·cell water^{-1}
240	0	400	0	109 $\pm$ 7
190	50	300	50	88 $\pm$ 2
140	100	200	100	28 $\pm$ 2
90	150	100	150	12 $\pm$ 1
40	200	0	200	6 $\pm$ 1

* ChCl is choline chloride; Mann is D-mannitol

** The final ion concentrations were: [Na] = 240 mM, [Cl] = 240 mM, D-mannitol was added to maintain osmotic balance, osmotic pressure including "minor components" was 925 mOsM.

This work was supported by NIEHS grant 2P30ES0382-04, NIH grant 1R15DK38901-01 and an American Heart Association, Maine Affiliate Grant-in-Aid. Sarah Janssen was the recipient of a Summer Fellowship of the American Heart Association, Maine Affiliate.