QUINIDINE INHIBITS TAURINE TRANSPORT BY THE COELOMOCYTES OF THE MARINE POLYCHAETE, GLYCERA DIBRANCHIATA

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Earlier investigations 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). It is probable that mercuric chloride reacts with sulfhydryl groups associated with the membrane transport carrier for taurine since under the conditions employed in these experiments there are no apparent changes in ion gradients or membrane potential that might influence taurine transport indirectly (Preston, R. L. and Chen, C.W., Bull Environ. Contam. Toxicol. 42:620-627, 1989; Preston, R. L., Truong, T. T., Lu, S. and Janssen, S. J., Bull. MDIBL 29:78-81, 1990; Wondergem, personal communication). Recent experiments have also shown that the reactive form of mercury is most likely the $HgCl_3^-$ complex (Preston, R. L., Zimmermann, P. R., Kaleta, M. T. and Simokat, K. A., Bull. MDIBL 33:53-55, 1994). The fact that the anionic form of mercury is most effective suggests that inhibitors of anion channels might also have some effect on taurine transport. Quinine and its analogue, quinidine, have been shown to inhibit anion channels involved in volume regulation (Banderali, U. and Roy, G. J. Membr. Biol. 126:219-234, 1992; Sanchez Olea, R. Pasantes-Morales, H. Lazaro, A. and Cereijido, M., J. Membr. Biol. 121:1-9, 1991). In this series of experiments, we show that quinidine rapidly and irreversibly inhibits taurine transport by Glycera RBCs.

Glycera RBCs were removed from the animals, washed in artificial seawater (NaSW) and separated from gametes and other coelomocytes by differential centrifugation. All experiments were done at 12°C. Quinidine (0.01 mM - 5 mM) was dissolved in NaSW which was then preincubated with Glycera RBCs for 5 min. Controls were incubated in medium containing only NaSW. Taurine influx was measured by incubating the RBCs 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 which is directly correlated with cell number and cell water content. In some experiments, RBCs were also incubated with 20 μM $HgCl_3^-$ for 1 min and or 1 mM quinidine for 1 min to determine possible additive effects of these agents. All experiments reported here have been repeated in triplicate at a minimum.

Preliminary experiments in which Glycera RBCs were incubated with 1 mM quinidine for times ranging from 10 sec to 5 min showed that the inhibition of taurine influx occurred quickly (< 1 min), reaching a plateau from 1 min through 5 min of about 50% inhibition. Other experiments also showed that the effect of quinidine was not readily reversible with repeated washing. Therefore, in most subsequent experiments the RBCs were treated with quinidine, washed in NaSW and then incubated with ^{14}C-taurine for flux measurement.

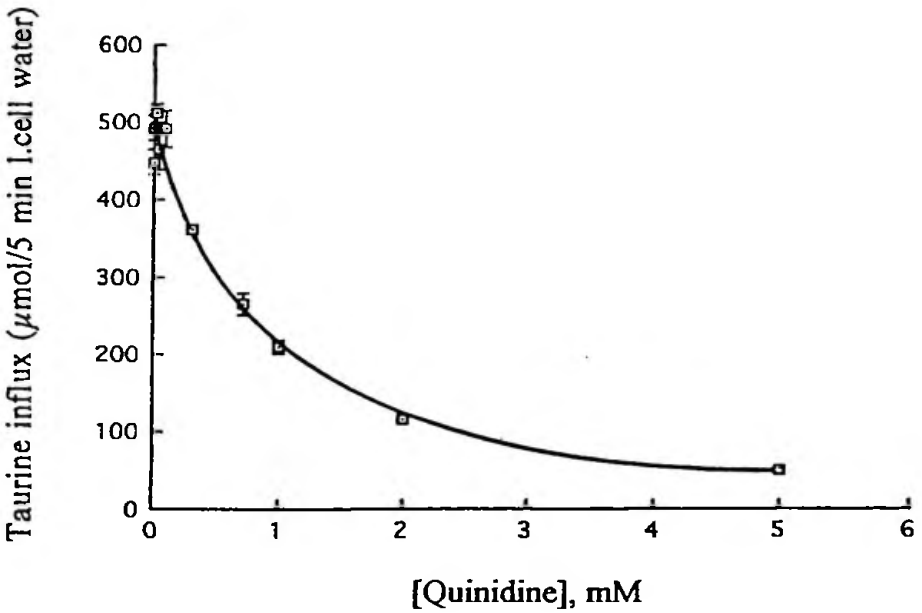

Fig 1. Quinidine Inhibition of Taurine Transport.
^{14}C-Taurine transport by Glycera RBCs was measured in NaSW after they were preincubated with quinidine at various concentrations for 5 min.

The concentration dependence of quinidine inhibition is shown in Fig 1. The estimated half-inhibition concentration is about 0.8 mM. This concentration is similar to that used by Banderali and Roy (1 mM; J. Membr. Biol. 126:219-234, 1992) to block Cl^- channels in MDCK cells. However, they found that the quinidine can be washed out and the inhibitory effect is reversible in their system.

The fact that quinidine inhibits taurine transport and that the anionic form of mercury, $HgCl_3^-$, is most effective in inhibition of taurine transport suggests it is possible that one or the other of these compounds could "protect" or block access of the other to reactive sites on the transporter. To test this hypothesis, Glycera RBCs were incubated sequentially with 20 μM mercuric chloride followed by 1 mM quinidine. In another set of samples the order of incubation was reversed. In addition, controls were included for untreated cells and for each agent incubated alone with RBCs. Table 1 shows the results of this experiment.

If mercuric chloride and quinidine act independently, one would expect that their inhibitory effects would be additive. This prediction seems to be borne out in the case of mercury treatment followed by quinidine (Hg/Quin, Table 1). The inhibition ratio compared with the NaSW control (J_I/J_0) for quinidine and mercuric chloride alone was 0.56 and 0.30 respectively. The predicted J_I/J_0 after treatment to both agents should therefore be 0.56 x 0.30 = 0.17. The observed value for J_I/J_0 was 0.18. However, if the order of exposure was reversed (Quin/Hg) the observed value for J_I/J_0 was 0.28. This is not significantly different from the inhibition due to mercuric chloride alone. These data suggest that prior treatment of the RBCs with quinidine may partially prevent mercurial inhibition of taurine transport. If quinidine

Table 1: Effect of Order of Exposure to Quinidine and Mercuric Chloride on Taurine Transport by Glycera RBCs.

Preincubation	Taurine influx*	(J_I/J_0)**	p
NaSW	1118 ± 55	-	
Quinidine (1 mM)	626 ± 34	0.56	<0.001
$HgCl_2$ (20 µM)	342 ± 23***	0.30	<0.001
Quin/Hg	311 ± 24	0.28	<0.001
Hg/Quin	206 ± 9	0.18	<0.001

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

** (J_I/J_0) = inhibition ratio where J_I = taurine influx after exposure to mercury, quinidine or both; J_0 = control taurine influx in NaSW. Quin/Hg = 1 mM quinidine incubation for 1 min followed by 20 µM $HgCl_2$ incubation for 1 min. The order of exposure was reversed in the Hg/Quin condition. Cells were then washed and taurine influx measured.

*** $HgCl_2$ treatment is not significantly different than the Quin/Hg treatment ($p > 0.4$). All other comparisons are significantly different ($p \leq 0.005$). Student's t-test was used to compare the statistical significance (p) of the experimental conditions to the control.

completely blocked mercuric chloride inhibition then J_I/J_0 should equal 0.56 as in the quinidine control. The fact that the reactive form of mercury appears to be $HgCl_3^-$ suggests that the ability of quinidine to block anion channels extends to blocking anionic mercury as well. Perhaps this involves blocking access of mercuric chloride to reactive sites directly or allosteric changes in the transport protein following quinidine binding at occludes site reactive with mercury. It is also possible that this interaction occurs by more indirect means since both quinidine and especially mercuric chloride are somewhat nonspecific in their reactivity. For the present, at least, these data are consistent with the notion that the taurine transporter in Glycera RBCs has some characteristics that resemble an anion channel or anion selective transport system. Future investigations will test this hypothesis further.

Supported in part by NIEHS grant P30-ES03828. Paula Zimmermann was supported by a NOAA/Sea Grant awarded to RLP. Marianne Kaleta was a recipient of an Undergraduate Research Fellowship from the American Heart Association, Maine Affiliate. Keith Katsma and Kristin Simokat were recipients of Grass Fellowships. Graciana Lapetina was a recipient of an NSF-REU Undergraduate Research Fellowship.