MERCURY BINDING AND INHIBITION OF TRANSPORT SYSTEMS IN PLASMA MEMBRANES ISOLATED FROM RECTAL GLAND OF *SQUALUS ACANTHIAS*

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Previous studies have shown that chloride secretion is strongly inhibited by mercury and that the Na,K,2Cl cotransporter [E. Kinne-Saffran and R.K.H. Kinne, Bull. MDIBL 33:93-94, 1994] as well as the Na,K-ATPase are potential targets of the heavy metal. In order to elucidate the correlation between binding of mercury to the membranes and its inhibitory potency on the membrane bound transporters a 'bioassay' was established to determine the amount of mercury associated with plasma membranes at various concentrations of the heavy metal.

Plasma membranes were isolated from dogfish (*Squalus acanthias*) rectal glands by differential centrifugation as described elsewhere [J. Eveloff et al., Pflügers Arch. 378:87-92, 1978]. Na,K-ATPase activity was determined by measuring the amount of inorganic phosphate released from the substrate ATP in the absence and in the presence of 2 mM ouabain during incubation of the membranes at 25°C. The assay medium contained 100 mM NaCl, 20 mM KCl, 6 mM $MgCl_2$, 3 mM Tris-ATP, and 20 mM HEPES, pH 7.4. The binding capacity of rectal gland plasma membranes for mercury was determined by incubating the membranes with known concentrations of mercuric chloride for 30 seconds at 15°C. The membranes were then separated by centrifugation at 4°C for 1 hour at 20,000 rpm (Sorval SS34 rotor). Aliquots of the supernatant were monitored for their free mercuric chloride concentration by evaluating the inhibition of Na,K-ATPase activity in untreated plasma membranes from rectal gland. These data were compared with a standard inhibition curve of Na,K-ATPase incubated with known concentrations of mercuric chloride (bioassay). The apparent K_i for mercuric chloride obtained under these conditions was 1.4 x 10^{-6} M.

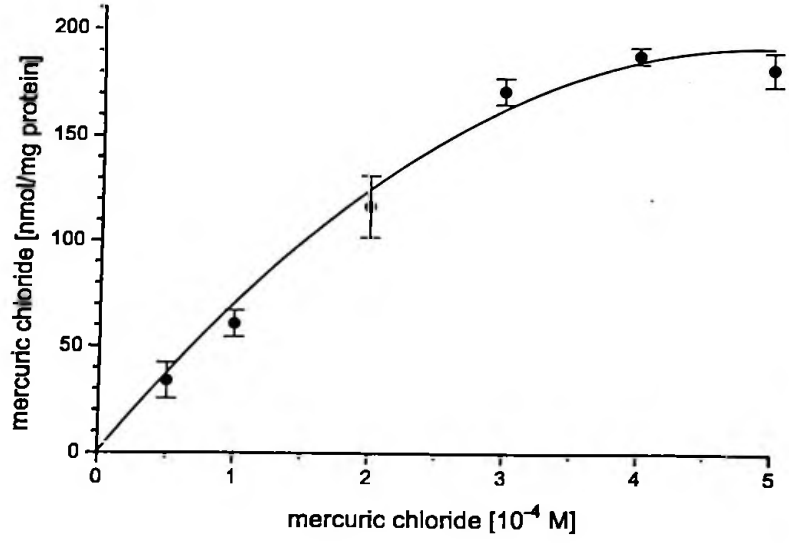

Figure 1. Binding of Hg^{2+} to plasma membrane

As shown in figure 1 binding of mercuric chloride to rectal gland plasma membranes is almost linear in the range from 0.5×10^{-4} M to 3×10^{-4} M. At higher concentrations saturation is reached. In table 1 the I_{50} for inhibition of Na,K-ATPase and Na,K,2Cl cotransporter by mercuric chloride are compared with the I_{50} of mercury binding to the membranes.

Table 1. Comparison of inhibition of transporters and mercury binding

Studied systems	Amount	Sensitivity to Mercury
Na,K-ATPase	0.01 nmoles/mg protein*	$I_{50} = 1.4 \times 10^{-6}$ M$^{\diamond}$
Na,K,2Cl-cotransport	0.10 nmoles/mg protein$^{\blacktriangle}$	$I_{50} = 2.0 \times 10^{-5}$ M$^{\blacklozenge}$
Mercury binding	200 nmoles/mg protein$^{\diamond}$	$I_{50} = 1.5 \times 10^{-4}$ M$^{\diamond}$

Na,K-ATPase activity was determined in lyophilized plasma membranes from rectal gland in the presence of varying concentrations of mercuric chloride. The activity of the Na,K,2Cl cotransport was determined by measuring the chloride-dependent uptake of ^{86}Rb into membrane vesicles. Binding of mercury to plasma membranes was studied as described in this paper. * [P. Silva et al., J. Membrane Biol. 75:105-114, 1983]; $^{\diamond}$ [this paper]; $^{\blacktriangle}$ [J. Hannafin et al., 75:73-83, 1983]; $^{\blacklozenge}$ [E. Kinne-Saffran and R.K.H. Kinne, Bull. MDIBL 33:93-94, 1994]

With respect to the question whether mercuric chloride is bound to the membranes and/or taken up into the membrane vesicles the size of the intravesicular space has to be taken into consideration. According to previous studies the intravesicular space of rectal gland plasma membranes can be assumed to be in the range of 2 µl/mg protein [J. Hannafin et al., J. Membrane Biol. 75:73-83, 1983]. At a concentration of 5×10^{-4} M the mercury in the intravesicular space - assuming simple equilibration - would amount to 10×10^{-10} moles/mg protein. This number is much lower than the maximum binding actually observed. A large amount of mercury thus binds to negative charges of phospholipids and glycoproteins which are abundantly present at the surface of plasma membranes. It is also noteworthy that the apparent affinity of mercuric chloride binding to membranes is much lower than the I_{50} values determined for the two transport systems. With regard to the mechanisms of action of mercury these results suggest that the inhibitory effect of mercury is not due to unspecific modifications of the plasma membranes in which the transporters are embedded but due to a direct interaction with the transporters at specific sites.

With regard to toxicological aspects these studies demonstrate that the plasma membrane acts as an initial scavenger when cells are acutely exposed to mercury thus protecting the cell interior from potential deleterious actions of the heavy metal.

This study was supported in part by NSF grant NFS ESI-9452682 (P. Pruett) and NIE EHS-P30-ES03828 to the Center for Membrane Toxicity Studies