Biochemical Pharmacology, Vol. 53, pp. 1889–1900, 1997.

ISSN 0006-2952/97/$17.00 + 0.00
PII S0006-2952(97)00138-X

Binding of Mercury in Renal Brush-Border and Basolateral Membrane-Vesicles

IMPLICATION OF A CYSTEINE CONJUGATE OF MERCURY INVOLVED IN THE LUMINAL UPTAKE OF INORGANIC MERCURY IN THE KIDNEY

Rudolfs K. Zalups† and Lawrence H. Lash‡*

*DIVISION OF BASIC MEDICAL SCIENCES, MERCER UNIVERSITY SCHOOL OF MEDICINE, MACON, GA 31207; AND ‡DEPARTMENT OF PHARMACOLOGY, WAYNE STATE UNIVERSITY SCHOOL OF MEDICINE, DETROIT, MI 48201, U.S.A.

ABSTRACT. The influence of the thiols L-cysteine (CYS), glutathione (GSH), and 2,3-dimercapto-1-propanesulfonate (DMPS) on the binding and transport of inorganic mercury (Hg^{2+}) in luminal (brush-border) and basolateral membrane-vesicles isolated from the kidneys of rats was studied using radiolabeled mercury ($^{203}HgCl_2$). Membrane-vesicles were exposed to 1, 10, or 100 μM Hg^{2+} in the presence or absence of a 3:1 or 10:1 mole-ratio of CYS, GSH, or DMPS relative to Hg^{2+}. Equilibration of mercury with the membrane-vesicles occurred very rapidly, essentially being complete within 5 sec. By 60 sec, binding accounted for 87–97% of intravesicular Hg^{2+} in the absence of exogenous thiols. All three thiols significantly reduced the fraction of binding, with DMPS being the most effective agent. CYS enhanced the association of Hg^{2+} with luminal membrane-vesicles relative to that when Hg^{2+} was added alone, suggesting that conjugation of Hg^{2+} with CYS promotes the transport of low concentrations of Hg^{2+}. In contrast, an excess of either GSH or DMPS relative to Hg^{2+} interfered significantly with both the binding and transport of Hg^{2+} into either luminal or basolateral membrane-vesicles. In summary, the present study is the first to describe the association of Hg^{2+} with renal luminal and basolateral membrane-vesicles. Evidence was obtained for the involvement of a Hg^{2+}-CYS conjugate as a mechanism by which Hg^{2+} uptake and binding to luminal membranes occur and for an inhibitory effect of GSH and the chelator DMPS with regard to Hg^{2+} uptake and binding, demonstrating that extracellular thiols can modulate significantly the renal accumulation of Hg^{2+}. BIOCHEM PHARMACOL 53;12:1889–1900, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. inorganic mercury; kidney; brush-border membrane-vesicles; basolateral membrane-vesicles; cysteine; 2,3-dimercapto-1-propanesulfonate; glutathione

Despite countless studies over the years, the precise mechanisms involved in the renal tubular uptake and transport (as well as toxicity) of mercury have been very difficult to determine. This has been due, in part, to a lack of knowledge of the complex ligand–exchange interactions that inorganic and organic mercuric ions undergo *in vivo* when moving from one compartment to another and the factors that affect these interactions. Since mercuric ions have an especially high affinity for free sulfhydryl groups on biological molecules, the concentration and metabolism of intracellular and extracellular thiols seem to play an important role in the disposition and toxicity of mercury in various organs, including the kidney [1].

On the other hand, there are some encouraging findings indicating that we are getting closer to defining some of the mechanisms involved in the renal tubular uptake of inorganic mercury. Based on these findings, there appear to be at least two primary mechanisms involved in the uptake of inorganic mercury along the proximal tubule [2–5] (which is the primary site in the nephron where inorganic mercury is taken up [6–8]). One of these mechanisms involves the luminal membrane and at least one other involves the basolateral membrane [3, 4]. The luminal mechanism appears to be dependent on the activity of the γ-glutamyltransferase [2, 9–12], and the basolateral mechanism appears to involve the organic anion transport system [2–5].

Since mercury has to interact with, and pass through, the plasma membrane prior to entering into renal epithelial cells (particularly those along the proximal tubule), it is important to gain some understanding of the binding and transport characteristics of mercury in luminal (brush-border) and basolateral plasma membranes. Therefore, the primary aim of the present study was to begin characterizing the binding and transport of inorganic mercury in brush-border and basolateral membrane-vesicles isolated from the

† Corresponding author: Dr. Rudolfs K. Zalups, Division of Basic Medical Sciences, Mercer University School of Medicine, 1550 College St., Macon, GA 31207. Tel. (912) 752-2559; FAX (912) 752-5489.

§ *Abbreviations:* CYS, L-cysteine; GSH, glutathione; DMPS, 2,3-dimercapto-1-propanesulfonate; Hg^{2+}, inorganic mercury; and PMSF, phenylmethylsulfonyl fluoride.

Received 18 October 1996; accepted 2 January 1997.

kidneys of rats. One advantage of using isolated brush-border and basolateral membrane-vesicles is that they allow one to potentially dissect events occurring on different surfaces of renal epithelial cells in the absence of confounding intracellular processes.

A major component of the present study was to evaluate the binding and transport of inorganic mercury when it is conjugated to endogenous or exogenous compounds to which it binds avidly and that influence its renal tubular uptake. We chose to evaluate the binding and transport of mercury when it is conjugated to CYS§, GSH or the chelating agent DMPS. The rationale for studying the binding and transport of mercury when it is conjugated to CYS or GSH comes from evidence indicating that GSH and CYS conjugates of mercury are involved in a metabolic transport pathway associated with the luminal uptake of inorganic mercury [2, 3, 9–15]. DMPS, on the other hand, was chosen since it effectively reduces the renal burden of mercury after *in vivo* exposure to inorganic mercury, and it is transported by the organic anion transport system localized along proximal tubular epithelial cells [16–20]. Another rationale for studying the effects of these compounds is that the uptake of mercury in proximal tubular cells has also been studied when it has been conjugated to these compounds in other *in vitro* renal systems, such as in suspensions of proximal tubular fragments [21], in the isolated perfused tubule [22], and in cultured proximal tubular epithelial cells [23]. This is apparently the first study that has attempted to evaluate binding and transport of mercury in renal brush-border and basolateral membrane-vesicles.

MATERIALS AND METHODS

Materials

PMSF, triethanolamine hydrochloride, γ-glutamyl *p*-nitroanilide, GSH, CYS, DMPS, Percoll, Trizma base, HEPES, blue dextran, sucrose, ouabain, and mercuric chloride were purchased from the Sigma Chemical Co. (St. Louis, MO) and were of the highest purity available. All other chemicals used were reagent grade and were also purchased from Sigma. Radioactive inorganic mercury ($^{203}HgCl_2$, 2.0 mCi/mg) was purchased from the Buffalo Materials Corp. (Buffalo, NY). Metricel GN-6 25 mm nitrocellulose filters (0.45 μm pore-size) were purchased from Gelman Sciences (Ann Arbor, MI). Doubly distilled, deionized water was used for all assays and preparation of membrane-vesicles.

Animals

Male Sprague–Dawley rats weighing 200–225 g were used in the present study and were purchased from Harlan Sprague–Dawley (Indianapolis, IN). All animals were acclimated for several days in the climate-controlled vivarium at Mercer University School of Medicine prior to experimentation. During the acclimation period, the animals were provided water and a commercial laboratory diet for rodents *ad lib.*

Preparation of Brush-Border and Basolateral Membrane-Vesicles

On the morning of each day of experimentation, two to three rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). Both kidneys were then excised through a mid-line incision and placed quickly in a Na^+-free 10 mM triethanolamine/HCl buffer (pH 7.6) containing 250 mM sucrose and 0.1 mM PMSF (to inhibit proteolysis). A composite of renal tissue, incorporating the cortex and outer stripe of the outer medulla, was obtained from each kidney. The same buffer mentioned above was used throughout, with additions as indicated.

Brush-border and basolateral membrane-vesicles were prepared by a modification of the Percoll density-gradient centrifugation method of Scalera *et al.* [24]. The composite renal tissue was first homogenized in a glass homogenizing vessel containing 30 mL of buffer with a serrated Teflon pestle (1200 rpm, 30 strokes, clearance of 0.15 to 0.23 mm; Arthur Thomas Co., Philadelphia, PA). An additional 20 mL of buffer was added to the homogenate, and then the total volume of homogenate was divided equally into two 50-mL polypropylene, round-bottom centrifuge tubes. The homogenate was centrifuged at 2500 *g* for 15 min at 4° in a Beckman Avanti 30 centrifuge using an F0650 rotor (Beckman Instruments, Inc., Fullerton, CA). Supernatants from the two centrifuge tubes were decanted into two new tubes and were centrifuged at 22,000 *g* for 15 min at 4° to obtain the fluffy layer (consisting of crude plasma membranes). This fluffy layer was resuspended in 30 mL of the sucrose buffer in a 55-mL glass homogenizing tube, and the membrane-fraction was homogenized with a Teflon pestle (20 strokes). Subsequently, 3.3 mL of Percoll was added to, and gently mixed with, 26.7 mL of this homogenate in a 38-mL polycarbonate centrifuge tube. Then this mixture was centrifuged at 48,000 *g* for 30 min at 4° in the Avanti 30 centrifuge using a Beckman F0630 rotor. The gradient was fractionated from the top of the centrifuge tube by pumping a 60% (w/v) aqueous sucrose solution, colored with blue dextran (3 mg/50 mL), to the bottom of the tube through an 18-gauge steel cannula fitted through the middle of a double-hole, rubber stopper affixed to the top of the tube. Fractions (1 mL) were collected into 12 × 75 mm polystyrene tubes.

Membrane fractions were assayed for activity of the enzymes γ-glutamyltransferase (EC 2.3.2.2) and (Na^+ + K^+)-stimulated ATPase (EC 3.6.1.3) to identify fractions enriched in brush-border or basolateral membranes. The activity of γ-glutamyltransferase was used as a marker of the brush-border (luminal) membrane (of proximal tubular epithelial cells) [25–28], while the activity of (Na^+ + K^+)-stimulated ATPase was used as a marker for the basolateral membrane [28–31]. The activity of γ-glutamyltransferase was measured by the method of Orlowski and

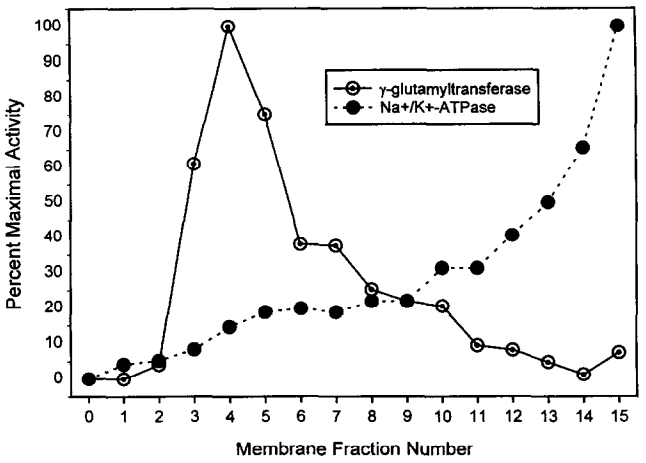

FIG. 1. A representative distribution of activities of γ-glutamyltransferase and (Na^+ + K^+)-stimulated ATPase in 15 fractions of renal plasma membranes isolated from the kidneys of rats. See Materials and Methods for details on isolation of plasma membranes. Fractions 3–7 contained the highest activity of γ-glutamyltransferase, while fractions 12–15 contained the highest activity of the (Na^+ + K^+)-stimulated ATPase. Based on the distribution of the activities of these two enzymes, brush-border membranes were presumed to be enriched in fractions 3–7 and basolateral membranes were presumed to be enriched in fractions 12–15.

Meister [32], with γ-glutamyl *p*-nitroanilide as the substrate and glycylglycine as the γ-glutamyl acceptor. The activity of (Na^+ + K^+)-stimulated ATPase was assessed by the method of Schoner *et al.* [33], and was calculated as the difference in activity found in the presence and absence of ouabain. Figure 1 shows the distribution of the activities of γ-glutamyltransferase and of (Na^+ + K^+)-stimulated ATPase in the 15 fractions obtained. On the basis of the distribution of the activities of these enzymes, fractions 3–7 were used for the isolation of brush-border membrane-vesicles and fractions 12–15 were used for the isolation of basolateral membrane-vesicles.

Fractions 3–7 and 12–15 were each placed and mixed in 26.3-mL polycarbonate centrifuge tubes and then were centrifuged at 100,000 *g* for 1 hr at 4°. The membrane-pellet in each of the two tubes was resuspended in 1.5 mL of sucrose buffer, and then the concentration of total protein in each resuspension was determined by using the Sigma Diagnostics Total Protein Kit (procedure No. 541; Sigma), that uses the biuret method.

Measurements of Binding and Uptake (Association) of Mercury in Membrane-Vesicles

Association of mercury with both brush-border and basolateral membrane-vesicles was studied at 5, 15, 30, 60, and 300 sec following exposure to inorganic mercury under several conditions. Inorganic mercury was presented to the membrane vesicles, in the extravesicular compartment, either by itself at 1, 10, or 100 μM or in a 1:3 or 1:10 ratio with CYS, GSH, or DMPS. The ratios were chosen to ensure the highest probability of conjugation of the inorganic mercury with each of the three thiols used. ^{13}C-NMR findings indicate that as little as a 2:1 ratio of GSH to inorganic mercury *in vitro* results in the formation of stable linear II coordinate covalent complexes between each mercuric ion in solution and two molecules of GSH [34].

At each concentration of inorganic mercury and at each time studied, 50 μL of either brush-border or basolateral membranes in sucrose buffer was incubated in 200 μL of an incubation solution that created the appropriate concentrations of inorganic mercury and thiol in the extravesicular compartment. In addition, the incubation solution was designed so that the initial extravesicular osmolality was 250 mOsmol/kg. This osmolality was generated by 100 mM sodium chloride (NaCl) and 50 mM sucrose. The NaCl was added to generate an extravesicular sodium-gradient for vesicular uptake. Prior to experimentation, the brush-border and basolateral membrane-vesicles were diluted with sucrose buffer so that the concentration of protein was 1 mg/mL. All incubations were carried out in 1.5-mL polypropylene microcentrifuge tubes.

After the appropriate incubation period had been completed, the extravesicular solution was removed and washed from around the membranes by vacuum filtration. This was accomplished by placing the membranes on a nitrocellulose filter fitted on a vacuum-filtration apparatus and then washing them with several 1.0-mL aliquots of sucrose buffer. To control for binding of mercury to nitrocellulose filters, 200 μL of each incubation solution was pipetted onto a nitrocellulose filter, which was then washed with sucrose buffer. The remaining radioactivity on the filter was subtracted from the radioactivity obtained from each experimental condition in which that incubation solution was used. In general, the percent binding of mercury to the filters when inorganic mercury was used without a thiol conjugate was less than 5% while it was less than 1% when inorganic mercury was in the presence of one of the three thiols.

Determination of the Percent Binding of Mercury to Membrane-Vesicles

In a separate set of experiments, the percent binding of mercury to each type of membrane-vesicle was estimated at 60 sec by evaluating the association of mercury when the extravesicular osmolality was 250 and 1000 mOsmol/kg H_2O. It has been demonstrated that intravesicular volume is reduced to near zero when the extravesicular osmolality is at 1000 mOsmol/kg H_2O [35, 36]. The extravesicular osmolality was adjusted with the addition of sucrose to the incubation solution. All data obtained when the extravesicular volume was 1000 mOsmol/kg were assumed to represent binding and were expressed as a percent of the amount of mercury associated at an extravesicular osmolality of 250 mOsmol/kg H_2O. Binding determinations were carried out with thiol conjugates only when the conjugates were in a ratio of 10:1 to inorganic mercury.

Determinations of the Amount of Mercury Associated with Membrane-Vesicles

At the end of each experiment, all filters used were placed individually in 12 × 75 mm polystyrene gamma-counting tubes. The amount of radioactivity on each filter was determined by counting them in a 1282 Compugamma CS deep-well gamma spectrometer with a 3-inch sodium iodide crystal (Pharmacia-LKB, Gaithersburg, MD) operating at a counting efficiency of approximately 50% for $^{203}Hg^{2+}$. The content of mercury in each sample was calculated by dividing the activity (cpm) in the sample by the specific activity (cpm/nmol) of mercury in standards. The amount of mercury associated with the membrane-vesicles was expressed as nanomoles mercury per milligram protein. The phrase "amount of mercury associated with" is used in the present manuscript to denote the sum of the total amount of binding of mercury to, plus the amount of mercury transported into, the respective membrane-vesicles studied. Due to the complexity of interactions between mercuric ions and binding sites on plasma membranes, there is an inherent difficulty in assessing only transport.

Statistical Analysis

Values are expressed as means ± SEM for 3–4 measurements. Differences between means at each time studied were evaluated statistically by using a one-way analysis of variance following by Tukey's multiple comparison test. The level of significance for all statistical analyses was chosen *a priori* to be $P < 0.05$.

RESULTS

Disposition of Mercury after Exposure to 1 μM Hg^{2+}

When luminal (brush-border) membrane-vesicles (Fig. 2A) or basolateral membrane-vesicles (Fig. 2B) were exposed to 1 μM inorganic mercury (Hg^{2+}), association of the mercury with the vesicles occurred very rapidly. In fact, near maximal rates of association were attained during the first 5 sec of exposure. A subsequent plateau effect in the rate of association occurred soon after the first 5 sec. Very little change in the association of mercury with either the luminal or basolateral membrane-vesicles occurred between 5 and 300 sec after exposure. The amount of mercury associated per milligram of membrane protein was much greater in the basolateral membrane-vesicles than in the luminal membrane-vesicles.

Disposition of Mercury after Exposure to 1 μM Hg^{2+} plus 3 μM CYS, GSH, or DMPS

Perhaps one the most important findings of the present study is the fact that significant changes in the rates and magnitude of association of mercury occurred when luminal membrane-vesicles were exposed to Hg^{2+} and CYS. At all times studied, except at 30 sec, the amount of mercury

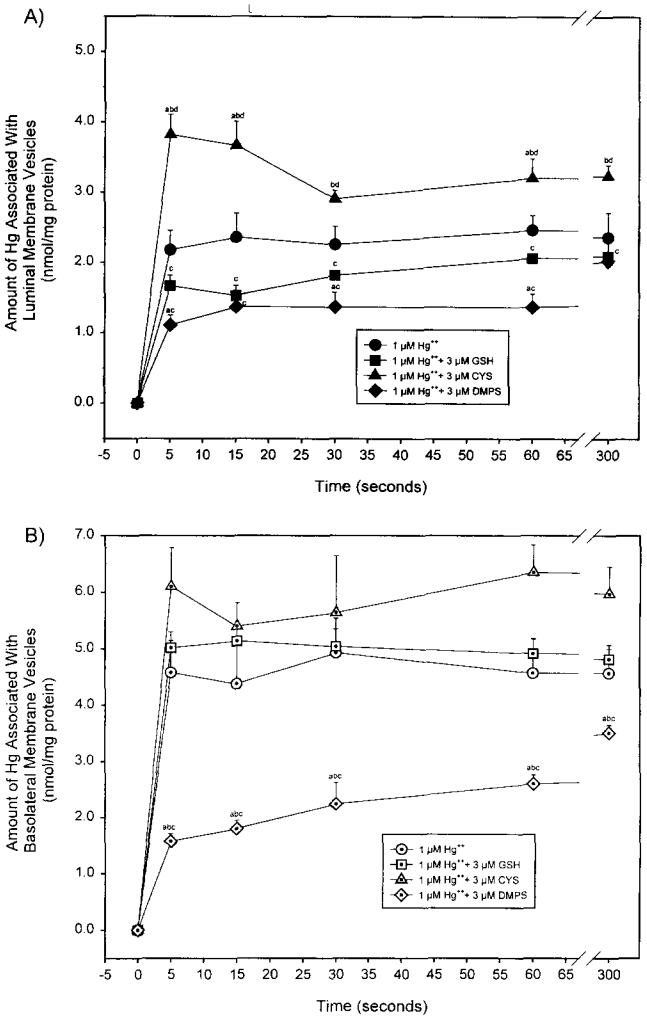

FIG. 2. Association of 1 μM mercury with luminal (brush-border) and basolateral membrane-vesicles isolated from the kidneys of rats in the absence or presence of 3-fold molar excess of thiols. Both luminal (A) and basolateral (B) membrane-vesicles were exposed to 1 μM inorganic mercury (Hg^{2+}) alone or in combination with 3 μM GSH, 3 μM CYS or 3 μM DMPS in the extracellular medium for 5, 15, 30, 60, or 300 sec. Sodium chloride at a concentration of 100 mM was also in the extracellular medium to generate a sodium gradient to promote sodium-driven transport. Values represent means ± SEM for 3–4 experiments. Key: (a) significantly different ($P < 0.05$) from the mean for the corresponding vesicles exposed to 1 μM Hg^{2+} for the same period of time; (b) significantly different ($P < 0.05$) from the mean for the corresponding vesicles exposed to 1 μM Hg^{2+} + 3 μM GSH for the same period of time; (c) significantly different ($P < 0.05$) from the mean for the corresponding vesicles exposed to 1 μM Hg^{2+} + 3 μM CYS for the same period of time; and (d) significantly different ($P < 0.05$) from the mean for the corresponding vesicles exposed to 1 μM Hg^{2+} + 3 μM DMPS for the same period of time.

associated with the membranes (on a per milligram protein basis) was significantly greater when they were exposed to the combination of 1 μM Hg^{2+} plus 3 μM CYS than when they were exposed to 1 μM Hg^{2+} alone or in combination with 3 μM GSH or 3 μM DMPS (Fig. 2A). This potentiation effect, which was statistically significant, was not

detected in basolateral membrane-vesicles, although mean values tended to be greater after exposure to 1 μM Hg^{2+} plus 3 μM CYS (Fig. 2B).

The magnitude of association of mercury in luminal membrane-vesicles (Fig. 2A) tended to be lower (at all times studied) when they were exposed to 1 μM Hg^{2+} plus 3 μM GSH than when they were exposed 1 μM Hg^{2+} alone, although the differences were not statistically different, due to the variance between samples. In basolateral membrane-vesicles, GSH had no apparent effect on the association of Hg^{2+} with the vesicles. By contrast, exposure of luminal or basolateral membrane-vesicles to 1 μM Hg^{2+} plus 3 μM DMPS caused a significant decrease in the association of mercury relative to corresponding membrane-vesicles exposed to 1 μM Hg^{2+} alone. In the basolateral membrane-vesicles exposed to 1 μM Hg^{2+} plus 3 μM DMPS, the magnitude of association of mercury at each time studied was significantly lower than that detected with any of the other three forms of exposure to Hg^{2+}.

Disposition of Mercury after Exposure to 1 μM Hg^{2+} *plus* 10 μM CYS, GSH, or DMPS

When luminal membrane-vesicles were exposed to 1 μM Hg^{2+} plus 10 μM CYS, the amount of mercury associated with these vesicles at each time studied was significantly greater than that detected after exposure to 1 μM Hg^{2+} alone or in combination with 10 μM GSH or 10 μM DMPS (Fig. 3A). The magnitude of association of mercury after exposure of the luminal vesicles to 1 μM Hg^{2+} plus 10 μM CYS tended to be greater than that after exposure to 1 μM Hg^{2+} plus 3 μM CYS, particularly at the later times studied (Fig. 2A). In basolateral membrane-vesicles, the level of association of mercury after exposure to 1 μM Hg^{2+} plus 10 μM CYS was consistently greater than that detected after exposure to 1 μM Hg^{2+} alone, although the differences were not statistically significant using a one-way analysis of variance due to a large amount of variance between samples (Fig. 3B). These differences may prove to be statistically significant in experiments where the number of trials is increased.

Exposure of both luminal and basolateral membrane-vesicles to 1 μM Hg^{2+} plus 10 μM GSH caused the amount of mercury associated with the vesicles to be lower than that detected after exposure to 1 μM Hg^{2+} alone. However, once again, the differences were not statistically significant, except for 5 sec after exposure in the basolateral membranes.

In basolateral membrane-vesicles, significantly less mercury was associated with the membranes after exposure to 1 μM Hg^{2+} plus 10 μM DMPS than after exposure to 1 μM Hg^{2+} alone at each time studied, except for 300 sec after exposure.

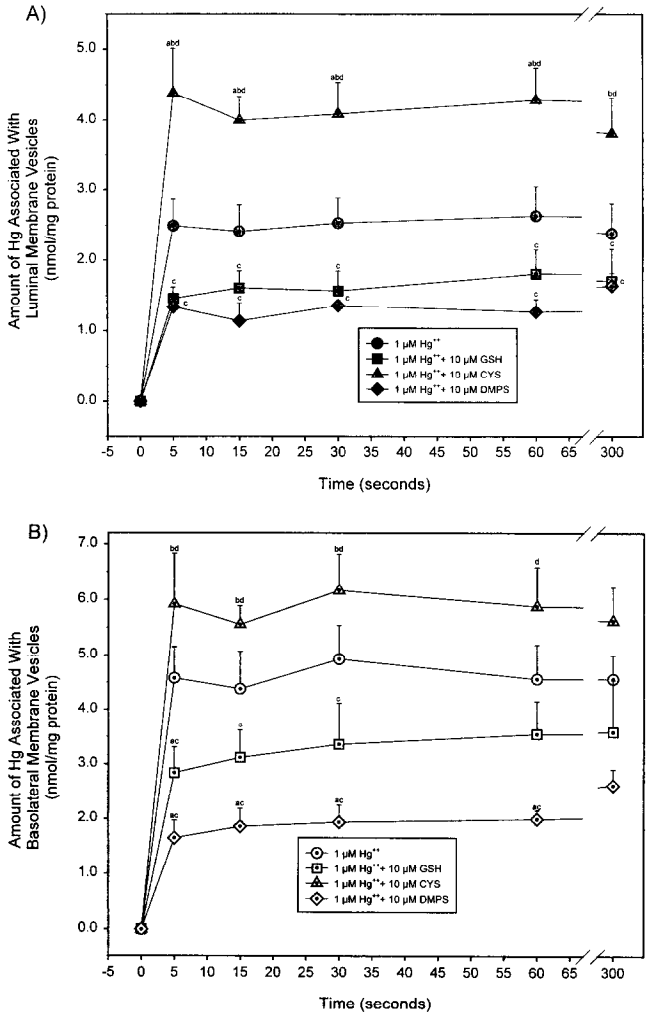

FIG. 3. Association of 1 μM mercury with luminal (brush-border) and basolateral membrane-vesicles isolated from the kidneys of rats in the absence or presence of 10-fold molar excess of thiols. Both luminal (A) and basolateral (B) membrane-vesicles were exposed to 1 μM inorganic mercury (Hg^{2+}) alone or in combination with 10 μM GSH, 10 μM CYS or 10 μM DMPS in the extracellular medium for 5, 15, 30, 60, or 300 sec. Sodium chloride at a concentration of 100 mM was also in the extracellular medium to generate a sodium gradient to promote sodium-driven transport. Values represent means ± SEM for 3–4 experiments. Key: (a) significantly different ($P < 0.05$) from the mean for the corresponding vesicles exposed to 1 μM Hg^{2+} for the same period of time; (b) significantly different ($P < 0.05$) from the mean for the corresponding vesicles exposed to 1 μM Hg^{2+} + 10 μM GSH for the same period of time; (c) significantly different ($P < 0.05$) from the mean for the corresponding vesicles exposed to 1 μM Hg^{2+} + 10 μM CYS for the same period of time; and (d) significantly different ($P < 0.05$) from the mean for the corresponding vesicles exposed to 1 μM Hg^{2+} + 10 μM DMPS for the same period of time.

Disposition of Mercury after Exposure to 10 μM Hg^{2+}

As was the case after exposure to 1 μM Hg^{2+}, the rate of association of mercury in both luminal and basolateral membrane-vesicles was fast during the initial 5 sec after exposure to 10 μM Hg^{2+} (Fig. 4, A and B). The establish-

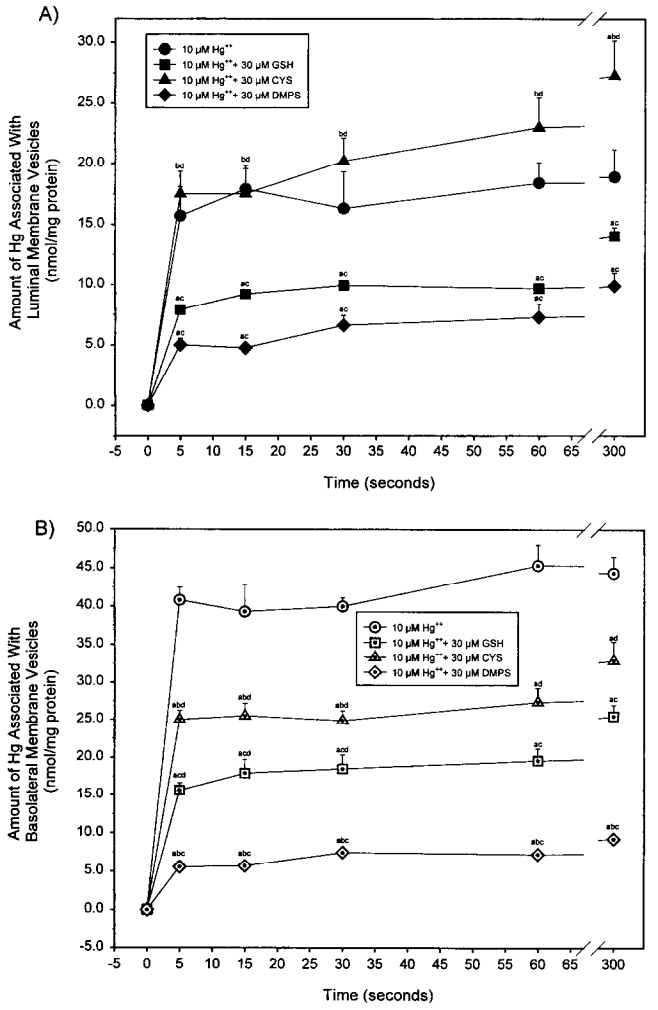

FIG. 4. Association of 10 μM mercury with luminal (brush-border) and basolateral membrane-vesicles isolated from the kidneys of rats in the absence or presence of 3-fold molar excess of thiols. Both luminal (A) and basolateral (B) membrane-vesicles were exposed to 10 μM inorganic mercury (Hg^{2+}) alone or in combination with 30 μM GSH, 30 μM CYS, or 30 μM DMPS in the extracellular medium for 5, 15, 30, 60, or 300 sec. Sodium chloride at a concentration of 100 mM was also in the extracellular medium to generate a sodium gradient to promote sodium-driven transport. Values represent means ± SEM for 3–4 experiments. Key: (a) significantly different ($P < 0.05$) from the mean for the corresponding vesicles exposed to 10 μM Hg^{2+} for the same period of time; (b) significantly different ($P < 0.05$) from the mean for the corresponding vesicles exposed to 10 μM Hg^{2+} + 30 μM GSH for the same period of time; (c) significantly different ($P < 0.05$) from the mean for the corresponding vesicles exposed to 10 μM Hg^{2+} + 30 μM CYS for the same period of time; and (d) significantly different ($P < 0.05$) from the mean for the corresponding vesicles exposed to 10 μM Hg^{2+} + 30 μM DMPS for the same period of time.

ment of a plateau phase for association of mercury was evident after 5 sec of exposure. In addition, more mercury was associated with the basolateral membrane-vesicles than the luminal membrane-vesicles, when expressed on a per milligram protein basis.

Disposition of Mercury after Exposure to 10 μM Hg^{2+} plus 30 μM CYS, GSH, or DMPS

At each time studied, exposure of luminal membrane-vesicles to 10 μM Hg^{2+} plus 30 μM GSH or DMPS caused significantly less mercury to become associated with the vesicles than when the vesicles were exposed to 10 μM Hg^{2+} either alone or in combination with 30 μM CYS (Fig. 4A). Addition of 30 μM CYS to 10 μM Hg^{2+} did not have a significant effect on the association of mercury in luminal membrane-vesicles relative to that detected following exposure to 10 μM Hg^{2+} alone. There were no significant differences between the effects of 30 μM GSH and 30 μM DMPS on the level of association of mercury in luminal membrane-vesicles.

In basolateral membrane-vesicles, exposure to 10 μM Hg^{2+} plus 30 μM CYS, GSH or DMPS resulted in significantly less mercury being associated with the vesicles at each time studied relative to that caused by 10 μM Hg^{2+} alone (Fig. 4B). Exposure to 10 μM Hg^{2+} plus 30 μM DMPS had the greatest inhibitory effect on the association of mercury with the basolateral membrane-vesicles. At each time studied, the amount of mercury associated with the vesicles was significantly less than that detected after exposure to any of the other three treatment conditions. While co-exposure to Hg^{2+} plus DMPS had the greatest inhibitory effect on the association of mercury with the basolateral membrane-vesicles, the co-exposure to 10 μM Hg^{2+} and 30 μM CYS had the least inhibitory effect.

Disposition of Mercury after Exposure to 10 μM Hg^{2+} plus 100 μM CYS, GSH, or DMPS

Exposure of both luminal and basolateral membrane-vesicles to 10 μM Hg^{2+} plus 100 μM CYS, GSH, or DMPS caused a significant reduction in the association of mercury with the vesicles, relative to that which occurred after exposure to 10 μM Hg^{2+} alone (Fig. 5, A and B). Combined exposure of 10 μM Hg^{2+} plus 100 μM GSH or DMPS had the greatest inhibitory effect on the association of mercury in luminal and basolateral membrane-vesicles. Although the combined exposure to 10 μM Hg^{2+} and 100 μM CYS caused less mercury to become associated with luminal membrane-vesicles during the initial 15 sec than exposure to 10 μM Hg^{2+} alone, no significant differences between the two treatments at the level of association of mercury with the vesicles were detected after 30 sec of exposure.

Disposition of Mercury after Exposure to 100 μM Hg^{2+}

Much as at the other two concentrations of Hg^{2+}, the rate of association of mercury in both luminal and basolateral membrane-vesicles exposed to 100 μM Hg^{2+} was very rapid during the first 5 sec. A plateau was attained as early as 5

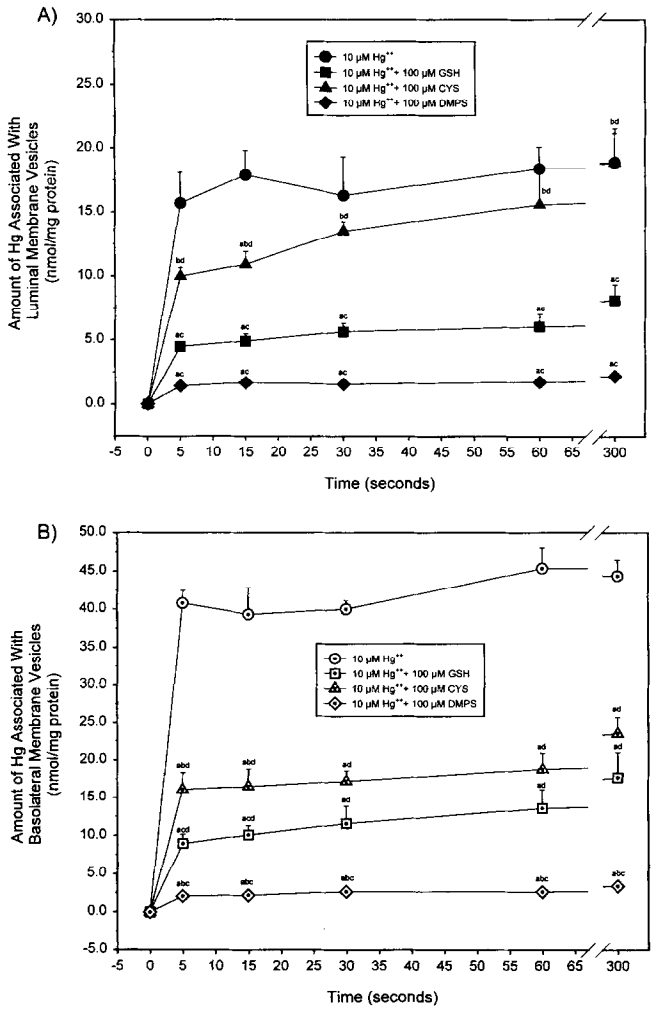

FIG. 5. Association of 10 μM mercury with luminal (brush-border) and basolateral membrane-vesicles isolated from the kidneys of rats in the absence or presence of 10-fold molar excess of thiols. Both luminal (A) and basolateral (B) membrane-vesicles were exposed to 10 μM inorganic mercury (Hg^{2+}) alone or in combination with 100 μM GSH, 100 μM CYS, or 100 μM DMPS in the extracellular medium for 5, 15, 30, 60, or 300 sec. Sodium chloride at a concentration of 100 mM was also in the extracellular medium to generate a sodium gradient to promote sodium-driven transport. Values represent means ± SEM for 3–4 experiments. Key: (a) significantly different ($P < 0.05$) from the mean for the corresponding vesicles exposed to 10 μM Hg^{2+} for the same period of time; (b) significantly different ($P < 0.05$) from the mean for the corresponding vesicles exposed to 10 μM Hg^{2+} + 100 μM GSH for the same period of time; (c) significantly different ($P < 0.05$) from the mean for the corresponding vesicles exposed to 10 μM Hg^{2+} + 100 μM CYS for the same period of time; and (d) significantly different ($P < 0.05$) from the mean for the corresponding vesicles exposed to 10 μM Hg^{2+} + 100 μM DMPS for the same period of time.

sec after exposure. Once again, more mercury was associated with the basolateral membrane-vesicles than the luminal membrane-vesicles when expressed per milligram of membrane protein.

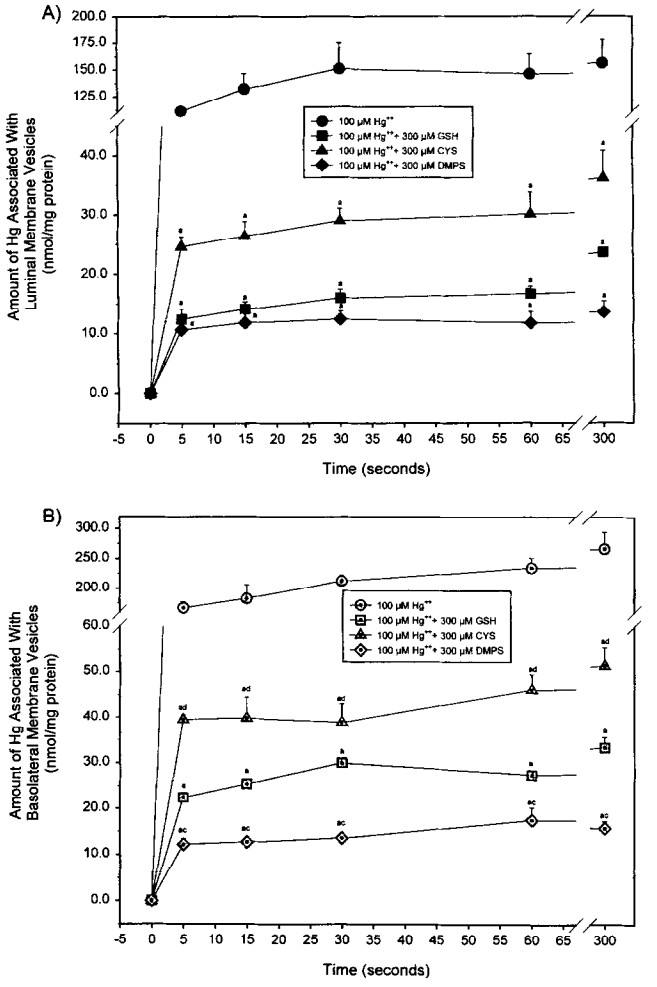

FIG. 6. Association of 100 μM mercury with luminal (brush-border) and basolateral membrane-vesicles isolated from the kidneys of rats in the absence or presence of 3-fold molar excess of thiols. Both luminal (A) and basolateral (B) membrane-vesicles were exposed to 100 μM inorganic mercury (Hg^{2+}) alone or in combination with 300 μM GSH, 300 μM CYS, or 300 μM DMPS in the extracellular medium for 5, 15, 30, 60, or 300 sec. Sodium chloride at a concentration of 100 mM was also in the extracellular medium to generate a sodium gradient to promote sodium-driven transport. Values represent means ± SEM for 3–4 experiments. Key: (a) significantly different ($P < 0.05$) from the mean for the corresponding vesicles exposed to 100 μM Hg^{2+} for the same period of time; (c) significantly different ($P < 0.05$) from the mean for the corresponding vesicles exposed to 100 μM Hg^{2+} + 300 μM CYS for the same period of time; and (d) significantly different ($P < 0.05$) from the mean for the corresponding vesicles exposed to 100 μM Hg^{2+} + 300 μM DMPS for the same period of time.

Disposition of Mercury after Exposure to 100 μM Hg^{2+} *plus* 300 μM CYS, GSH, *or* DMPS

The association of mercury with either luminal or basolateral membrane-vesicles exposed to 100 μM Hg^{2+} plus 300 μM CYS, GSH, or DMPS was markedly lower than that detected in the absence of any of the three sulfhydryl-containing compounds at all times studied (Fig. 6, A and B).

No significant differences were detected in the level of

association of mercury between luminal membrane-vesicles exposed to any the three sulfhydryl-containing compounds, although the association of mercury tended to be greater in the membrane-vesicles exposed to both 100 μM Hg^{2+} plus 300 μM CYS (Fig. 6A). In basolateral membrane-vesicles, however, the association of mercury was significantly greater after exposure to 100 Hg^{2+} plus 300 μM CYS than after exposure to 100 μM Hg^{2+} plus 300 μM GSH or 300 μM DMPS (Fig. 6B).

Disposition of Mercury after Exposure to 100 μM Hg^{2+} plus 1000 μM CYS, GSH, or DMPS

Exposure of both luminal and basolateral membrane-vesicles to 100 μM Hg^{2+} plus 1000 μM CYS, GSH, or DMPS caused a tremendous decrease in the association of mercury with the vesicles relative to that in the absence of any of the three thiols (Fig. 7, A and B). The association of mercury with either luminal or basolateral membrane-vesicles also tended to be greater in the presence of 1000 μM CYS than in the presence of either 1000 μM GSH or DMPS. Much as in most of the other experiments, addition of DMPS to the extravesicular medium had the greatest effect on diminishing the association of mercury with either luminal or basolateral membrane-vesicles.

Estimation of the Amount of Binding of Hg^{2+} to Luminal and Basolateral Membrane-Vesicles

Estimates of the percent binding of mercury to luminal and basolateral membrane-vesicles following 60 sec of exposure to 1, 10, or 100 μM Hg^{2+}, in the presence or absence of a 10-fold higher concentration of CYS, GSH, or DMPS, were made by incubating vesicles in a 1000 mOsmol/kg medium, where intravesicular volume is assumed to be zero (Table 1). The estimated percent binding of mercury in luminal and basolateral membrane-vesicles, respectively, was approximately 96 and 97% at 1 μM Hg^{2+}, 95 and 96% at 10 μM Hg^{2+} and 87 and 93% at 100 μM Hg^{2+}. Administering Hg^{2+} with one of the three thiols used in the study reduced the amount of binding considerably. However, in most cases, the calculated level of binding accounted for more than 60% of the amount of mercury associated with the membrane-vesicles. An important point that requires some consideration is how much of what became bound to the membrane-vesicles was first transported into the vesicles prior to becoming bound. This is particularly relevant when considering the binding of mercury when the vesicles were exposed to both Hg^{2+} and one of the three thiol-containing compounds.

DISCUSSION

When luminal (brush-border) or basolateral membrane-vesicles were exposed to an extravesicular concentration of Hg^{2+} of 1 μM, there was a very rapid association of mercury with the vesicles. More Hg^{2+} became associated with the

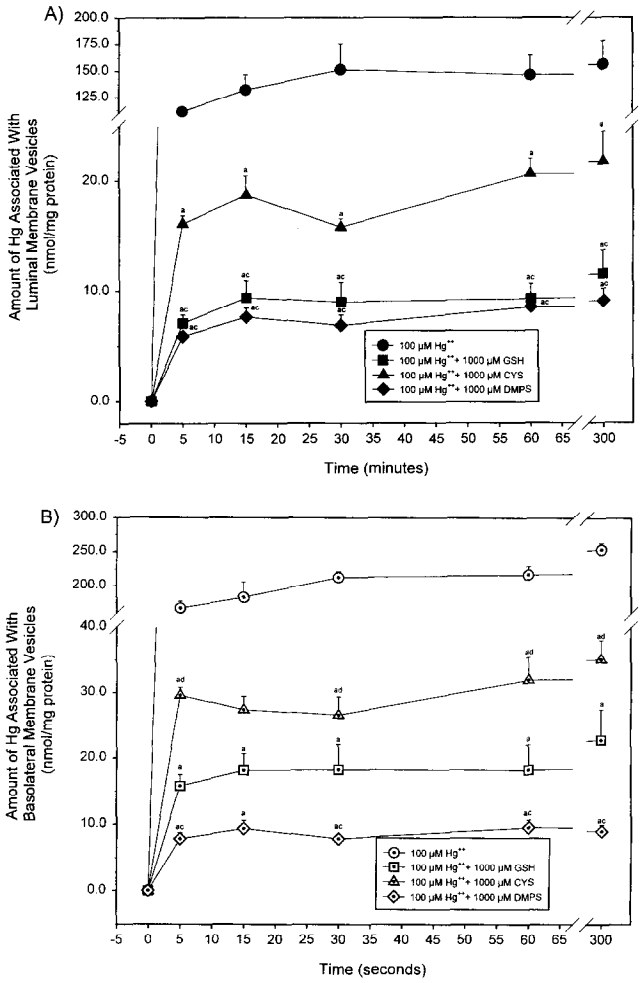

FIG. 7. Association of 100 μM mercury with luminal (brush-border) and basolateral membrane-vesicles isolated from the kidneys of rats in the absence or presence of 10-fold molar excess of thiols. Both luminal (A) and basolateral (B) membrane-vesicles were exposed to 100 μM inorganic mercury (Hg^{2+}) alone or in combination with 1000 μM GSH, 1000 μM CYS, or 1000 μM DMPS in the extracellular medium for 5, 15, 30, 60, or 300 sec. Sodium chloride at a concentration of 100 mM was also in the extracellular medium to generate a sodium gradient to promote sodium-driven transport. Values represent means ± SEM for 3–4 experiments. Key: (a) significantly different ($P < 0.05$) from the mean for the corresponding vesicles exposed to 100 μM Hg^{2+} for the same period of time; (c) significantly different ($P < 0.05$) from the mean for the corresponding vesicles exposed to 100 μM Hg^{2+} + 1000 μM CYS for the same period of time; and (d) significantly different ($P < 0.05$) from the mean for the corresponding vesicles exposed to 100 μM Hg^{2+} + 1000 μM DMPS for the same period of time.

basolateral membrane-vesicles than the luminal membrane-vesicles. An apparent near maximal rate and level of association were attained during the first 5 sec of exposure. Subsequently, a plateau in the level of association was attained, which was maintained (on average) throughout 300 sec of exposure. It is not clear how much of the association of mercury during the initial 5 sec of exposure was the result of very rapid movement of mercuric ions into

TABLE 1. Percent binding of mercury to luminal and basolateral membrane-vesicles after 60 sec of treatment

Type of treatment	Type of membrane-vesicles	Amount of binding of Hg^{2+} to membranes (%)
1 μM Hg^{2+}	Brush border	96
	Basolateral	97
1 μM Hg^{2+} + 10 μM CYS	Brush border	79
	Basolateral	76
1 μM Hg^{2+} + 10 μM GSH	Brush border	74
	Basolateral	62
1 μM Hg^{2+} + 10 μM DMPS	Brush border	81
	Basolateral	70
10 μM Hg^{2+}	Brush border	95
	Basolateral	96
10 μM Hg^{2+} + 100 μM CYS	Brush border	82
	Basolateral	73
10 μM Hg^{2+} + 100 μM GSH	Brush border	75
	Basolateral	65
10 μM Hg^{2+} + 100 μM DMPS	Brush border	69
	Basolateral	63
100 μM Hg^{2+}	Brush border	87
	Basolateral	93
100 μM Hg^{2+} + 1000 μM CYS	Brush border	79
	Basolateral	80
100 μM Hg^{2+} + 1000 μM GSH	Brush border	92
	Basolateral	56
100 μM Hg^{2+} + 1000 μM DMPS	Brush border	47
	Basolateral	90

The amount of binding was evaluated by measurement of $^{203}Hg^{2+}$ accumulation after a 60-sec incubation of membrane vesicles in a 1000 mOsmol/kg medium, as described in Materials and Methods. Results are averages of duplicate determinations.

the membrane-vesicles with subsequent binding to the intravesicular surface of the membranes. As mentioned earlier, the complexity of the rapid chemical interactions between mercuric ions and the membrane-vesicles makes it very difficult to determine accurately the actual rates of extravesicular to intravesicular transport of Hg^{2+}. This is due to the difficulty, if not impossibility, of clearly separating transport events from binding events at both intravesicular and extravesicular surfaces of the plasma membranes. However, on the basis of estimates of binding, it appears that more than 95% of the mercury associated with the luminal membrane-vesicles was bound to the membranes by the end of 60 sec after exposure to 1 μM Hg^{2+}.

At the concentrations of Hg^{2+} studied, the association of Hg^{2+} was always greater in basolateral membrane-vesicles than in luminal membrane-vesicles. Although the precise content of free sulfhydryl groups per unit of protein on luminal or basolateral membrane proteins is currently unknown, a higher content of free sulfhydryl groups on proteins in the basolateral membrane could explain the greater association of Hg^{2+} with vesicles derived from these membranes. Very little is also known about the interactions between mercuric ions and the free sulfhydryl groups on membrane proteins. However, there is some evidence indicating that interactions between mercuric ions and intracellular thiols is very complex [34]. Clearly, further studies on the binding characteristics of mercuric ions to the sulfhydryl groups on membrane proteins is needed to better understand the mechanisms by which Hg^{2+} enters into renal or other cell types.

Interestingly, the association of mercury with the luminal vesicles was enhanced significantly when the vesicles were exposed to 1 μM Hg^{2+} plus 3 or 10 μM CYS (relative to that which occurred with 1 μM Hg^{2+}), particularly during the first 5 and 15 sec of exposure. In addition, there was a trend for increased association of mercury with luminal membrane-vesicles after exposure to 10 μM Hg^{2+} plus 30 μM CYS, particularly at the later times studied. These findings strongly suggest that conjugation of Hg^{2+} with CYS promotes the transport of low concentrations of Hg^{2+} across the luminal plasma membrane, perhaps by the cotransport of Hg^{2+} with CYS by one of the sodium-dependent amino acid transporters. By the end of 60 sec of exposure, it appeared that slightly less than 80% of the mercury in the vesicles was bound to the membranes, indicating that some of the Hg^{2+} in the vesicles was probably in the form of a CYS conjugate. Since the majority of the luminal membrane-vesicles are oriented right-side-out, the implication of the current findings is that some of the luminal uptake of mercury by proximal tubular epithelial cells (which is where Hg^{2+} is taken up

primarily along the nephron [6–8]) occurs by a mechanism involving the uptake of a CYS conjugate of Hg^{2+}.

Experimental findings from some *in vivo* studies support this hypothesis. One set of findings indicates that when the activity of renal γ-glutamyltransferase (located on the luminal membrane of proximal tubular cells) is inhibited (by acivicin) prior to exposure or administration of Hg^{2+}, there is a subsequent decrease in the renal uptake of Hg^{2+} and an increase in the urinary excretion of Hg^{2+} [2, 9–12] and GSH [9]. Consequently, one can conclude that enzymatic cleavage of the γ-glutamylcysteine bond on molecules of GSH, which are present in the tubular lumen and which are presumably bound to Hg^{2+}, is a required step in the uptake of Hg^{2+} across the luminal membrane. It is likely that most of the molecules of cysteinylglycine formed from the enzymatic cleavage of the γ-glutamylcysteine bond of GSH are broken down rapidly to cysteine and glycine by an abundance of dipeptidase activity on the luminal membrane. This would then leave a mercuric S-conjugate of CYS to interact with the luminal membrane, and result in the uptake of the mercuric ion. Enzymatic degradation of GSH to cysteine can also occur while a mercuric ion remains bound to the sulfur atom on the cysteinyl residue [37]. Another set of *in vivo* findings indicates that when Hg^{2+} is co-administered with CYS, there is enhanced uptake of mercury in the kidneys [14, 15], and there is an increased severity in the nephropathy induced by Hg^{2+} [15]. Thus, on the basis of the current *in vitro* findings and previous *in vivo* findings, there is a strong indication that the uptake of Hg^{2+} across the luminal membrane of proximal tubular epithelial cells is accomplished by a mechanism involving CYS, presumably in the form of a CYS conjugate of Hg^{2+}, and occurs by transport across the luminal plasma membrane by one of the sodium-dependent amino acid transporters.

After basolateral membrane-vesicles were exposed to 1 μM Hg^{2+} and 3 or 10 μM CYS, trends for increased association of mercury (relative to that detected with 1 μM Hg^{2+} alone) became apparent, although statistical significance between mean values was not achieved. This was particularly the case when the basolateral membrane-vesicles were exposed to 1 μM Hg^{2+} and 10 μM CYS. Slightly more variability occurred from one experiment to the next when the basolateral membrane-vesicles were exposed to 1 μM Hg^{2+}, which probably accounts for the lack of statistical significance in differences detected. Future studies need to be carried out to determine if, in fact, CYS may play a role in the basolateral uptake of Hg^{2+}. There are findings from recent studies that implicate the organic anion transport system in the basolateral uptake of Hg^{2+} along the proximal tubule [2, 4]. Whether the organic anion transport system can also transport a CYS conjugate of Hg^{2+} is not known at present. However, there is evidence of the participation of the organic anion transport system in the transport of other CYS conjugates into renal proximal tubular cells [38]. This evidence supports the possibility that the organic anion transport system could play a role in the basolateral uptake of a CYS conjugate of Hg^{2+}. An additional mechanism that could participate in the basolateral uptake of a CYS conjugate of Hg^{2+} might involve the enzymatic degradation of a GSH conjugate of Hg^{2+} at the basolateral membrane. There is histochemical evidence that γ-glutamyltransferase is also localized, albeit to a much lesser extent, on the basolateral membrane of proximal tubular epithelial cells [39].

Trends for decreased association of mercury (relative to that detected with 1 μM Hg^{2+}) were detected in luminal membrane-vesicles exposed to 1 μM Hg^{2+} plus 3 or 10 μM GSH or DMPS. In basolateral membrane vesicles, the association of mercury was diminished significantly at most times studied when they were exposed to 1 μM Hg^{2+} plus 3 or 10 μM DMPS. When either luminal or basolateral membrane-vesicles were exposed to 10 or 100 μM Hg^{2+} plus a 3:1 or especially a 10:1 ratio of CYS (except for luminal membrane-vesicles exposed to 10 μM Hg^{2+} plus 30 μM CYS), GSH or DMPS, the association of mercury with the membrane-vesicles was diminished from that detected in the same membrane-vesicles exposed to the corresponding concentration of Hg^{2+} alone. These findings indicate that the presence of an excess of GSH or DMPS relative to Hg^{2+} interferes significantly with the binding and transport of Hg^{2+} in luminal and basolateral membrane-vesicles. The fact that CYS caused a significant decrease in the uptake of mercury in luminal membrane-vesicles at higher concentrations of mercury may reflect an effect of saturation of the amount of Hg^{2+} that can be transported into the vesicles, or even membrane toxicity, particularly at the 100 μM concentration of Hg^{2+}. The inhibition of the association of Hg^{2+} with luminal and basolateral membrane-vesicles by GSH and DMPS may explain the mechanism by which these thiols protect rat kidney proximal tubular cells from Hg^{2+}-induced cytotoxicity [40].

Diminished association or uptake of Hg^{2+} when it is at an extracellular concentration of 10 μM or greater and is present with CYS or GSH in a ratio of less than 1:2 with these ligands has also been detected in other *in vitro* preparations. In suspensions of isolated proximal tubular fragments from the kidneys of rabbits, addition of 40 μM CYS or GSH to a medium containing 10 μM Hg^{2+} caused the uptake of mercury to be decreased by about 85–95% of levels detected with Hg^{2+} alone during the first hour of exposure [21]. Co-perfusion of 80 μM CYS or GSH with 18.4 μM Hg^{2+} through the lumen of isolated S1, S2, or S3 segments of the proximal tubule resulted in significant decreases in the luminal uptake and transepithelial transport of mercury [22]. In the latter case, however, more mercury was transported into, and a greater level of toxicity of mercury was present in, the isolated perfused S1 segments when 80 μM CYS was in the lumen than when 80 μM GSH was in the lumen.

The effect of DMPS in basolateral membrane-vesicles was a bit surprising, particularly at the low concentrations of Hg^{2+}, inasmuch as DMPS has been shown to be transported by the organic anion transport system [17–19].

It was postulated that DMPS might increase the uptake of mercury into the basolateral membrane-vesicles at low concentrations of mercury. However, this was not the case. Since the rate of association of mercury in basolateral membrane-vesicles tended always to be lower when DMPS was used, it appears that DMPS significantly inhibits the binding of and/or movement of Hg^{2+} into basolateral or luminal membrane-vesicles. By the same token, it appears that the binding of Hg^{2+} to DMPS interferes with the basolateral transport of DMPS across the basolateral membrane. Preliminary findings indicate that mercuric conjugates of DMPS are not transported across the basolateral membrane of isolated perfused proximal tubules (Zalups RK and Barfuss DW, unpublished findings), which is consistent with the present findings.

In summary, the present study is the first to directly describe the association of Hg^{2+} with luminal and basolateral membrane-vesicles isolated from the renal cortex and outer stripe of the outer medulla of the rat. Evidence was obtained that supports the hypothesis that a mercuric-CYS conjugate is involved as part of the mechanism in the luminal uptake of mercury along the renal proximal tubule. In addition, we showed that GSH and DMPS significantly inhibited the uptake and/or binding of Hg^{2+} in both luminal and basolateral membrane-vesicles, indicating that certain extracellular thiols can presumably modulate significantly the uptake and accumulation of Hg^{2+} in the epithelial cells lining the proximal tubule.

This research was supported by National Institute of Environmental Health Sciences Grant 5 R01-ES05157 and -ES05980. L.H.L. is the recipient of a Research Career Development Award from the National Institute of Diabetes and Digestive and Kidney Diseases (Grant K04-DK02090).

References

1. Zalups RK and Lash LH, Advances in understanding the renal transport and toxicity of mercury. *J Toxicol Environ Health* **42:** 1–44, 1994.
2. Zalups RK, Organic anion transport and action of γ-glutamyltranspeptidase in kidney linked mechanistically to renal tubular uptake of inorganic mercury. *Toxicol Appl Pharmacol* **132:** 289–298, 1995.
3. Zalups RK, Enhanced renal outer medullary uptake of mercury with uninephrectomy: Implication of a luminal mechanism. *J Toxicol Environ Health*, **50:** 173–194, 1997.
4. Zalups RK and Minor KH, Luminal and basolateral mechanisms involved in the renal tubular uptake of inorganic mercury. *J Toxicol Environ Health* **46:** 73–100, 1995.
5. Zalups RK and Barfuss DW, Pretreatment with *p*-aminohippurate inhibits the renal uptake and accumulation of injected inorganic mercury in the rat. *Toxicology* **103:** 23–35, 1995.
6. Zalups RK and Barfuss DW, Accumulation of inorganic mercury along the renal proximal tubule of the rabbit. *Toxicol Appl Pharmacol* **106:** 245–253, 1990.
7. Zalups RK, Method for studying the *in vivo* accumulation of inorganic mercury in segments of the nephron in the kidneys of rats treated with mercuric chloride. *J Pharmacol Methods* **26:** 89–104, 1991.
8. Zalups RK, Autometallographic localization of inorganic mercury in the kidneys of rats: Effect of unilateral nephrectomy and compensatory renal growth. *Exp Mol Pathol* **54:** 10–21, 1991.
9. Berndt WO, Baggett JM, Blacker A and Houser M, Renal glutathione and mercury uptake by kidney. *Fundam Appl Toxicol* **5:** 832–839, 1985.
10. Tanaka T, Naganuma A and Imura N, Role of γ-glutamyltranspeptidase in renal uptake and toxicity of inorganic mercury in mice. *Toxicology* **60:** 187–198, 1990.
11. Tanaka-Kagawa T, Naganuma A and Imura N, Tubular secretion and reabsorption of mercury compounds in mouse kidney. *J Pharmacol Exp Ther* **264:** 776–782, 1993.
12. de Ceaurriz J, Payan JP, Morel G and Brondeau MT, Role of extracellular glutathione and γ-glutamyltranspeptidase in the disposition and kidney toxicity of inorganic mercury in rats. *J Appl Toxicol* **14:** 201–206, 1994.
13. Zalups RK, Accumulation and handling of inorganic mercury in the kidney after co-administration with glutathione. *J Toxicol Environ Health* **44:** 385–399, 1995.
14. Zalups RK, Renal disposition of mercury in rats after intravenous injection of inorganic mercury and cysteine. *J Toxicol Environ Health* **44:** 401–413, 1995.
15. Zalups RK and Barfuss DW, Nephrotoxicity of inorganic mercury co-administered with L-cysteine. *Toxicology* **109:** 15–29, 1996.
16. Aposhian HV, DMSA and DMPS—water soluble antidotes for heavy metal poisoning. *Annu Rev Pharmacol Toxicol* **23:** 193–215, 1983.
17. Stewart JR and Diamond GL, Renal tubular secretion of the alkanesulfonate 2,3-dimercapto-1-propanesulfonate. *Am J Physiol* **252:** F800–F810, 1987.
18. Stewart JR and Diamond GL, *In vivo* renal tubular secretion and metabolism of the disulfide of 2,3-dimercapto-1-propanesulfonate. *Drug Metab Dispos* **16:** 189–195, 1988.
19. Klotzbach JM and Diamond GL, Complexing activity and excretion of 2,3-dimercapto-1-propane sulfonate in rat kidney. *Am J Physiol* **254:** F871–F878, 1988.
20. Zalups RK, Influence of 2,3-dimercaptopropane-1-sulfonate (DMPS) and *meso*-2,3-dimercaptosuccinic acid (DMSA) on the renal disposition of mercury in normal and uninephrectomized rats exposed to inorganic mercury. *J Pharmacol Exp Ther* **267:** 791–800, 1993.
21. Zalups RK, Knutson KL and Schnellmann RG, *In vitro* analysis of the accumulation and toxicity of inorganic mercury in segments of the proximal tubule isolated from the rabbit kidney. *Toxicol Appl Pharmacol* **119:** 221–227, 1993.
22. Zalups RK, Robinson MK and Barfuss DW, Factors affecting inorganic mercury transport and toxicity in the isolated perfused proximal tubule. *J Am Soc Nephrol* **2:** 866–878, 1991.
23. Aleo MD, Taub ML and Kostyniak PJ, Primary cultures of rabbit renal proximal tubule cells. III. Comparative cytotoxicity of inorganic and organic mercury. *Toxicol Appl Pharmacol* **112:** 310–317, 1992.
24. Scalera V, Huang Y-K, Hildmann B and Murer H, A simple isolation method for basal-lateral plasma membranes from rat kidney cortex. *Membr Biochem* **4:** 49–61, 1981.
25. George SG and Kenny J, Studies on the enzymology of purified preparations of brush border from rabbit kidney. *Biochem J* **134:** 43–57, 1973.
26. Glenner GG and Folk JE, Glutamyl peptidases in rat and guinea pig kidney slices. *Nature* **192:** 338–340, 1961.
27. Glossman H and Neville DM Jr, γ-Glutamyltransferase in kidney brush border membranes. *FEBS Lett* **19:** 340–344, 1972.
28. Liang CT and Sacktor B, Preparation of renal cortex basal-lateral and brush border membranes: Localization of adenylate

cyclase and guanylate cyclase activities. *Biochim Biophys Acta* **466:** 474–487, 1977.
29. Heidrich HG, Kinne R, Kinne-Saffran E and Hannig K, The polarity of the proximal tubule cell in rat kidney. Different surface charges for the brush-border microvilli and plasma membranes from the basal infoldings. *J Cell Biol* **54:** 232–245, 1972.
30. Kinne R, Schmitz J-E and Kinne-Saffran E, The localization of the Na^+-K^+-ATPase in the cells of rat kidney cortex. A study on isolated plasma membranes. *Pflugers Arch* **329:** 191–206, 1971.
31. Schmidt U and Dubach UC, Na K stimulated adenosinetriphosphatase: Intracellular localization within the proximal tubule of the rat nephron. *Pflugers Arch* **330:** 265–270, 1971.
32. Orlowski M and Meister A, γ-Glutamyl-*p*-nitroanilide: A new convenient substrate for determination and study of L- and D-γ-glutamyltranspeptidase activities. *Biochim Biophys Acta* **73:** 679–681, 1963.
33. Schoner W, von Ilberg C, Kramer R and Seubert W, On the mechanism of Na^+- and K^+-stimulated hydrolysis of adenosine triphosphate. I. Purification and properties of a Na^+- and K^+-activated ATPase from ox brain. *Eur J Biochem* **1:** 334–343, 1967.
34. Rabenstein DL, Metal complexes of glutathione and their biological significance. In: *Glutathione: Chemical, Biochemical and Medical Aspects* (Eds. Dolphin D, Auramovic O and Poulson R), Vol. 3, pp. 147–186. Wiley, New York, 1989.
35. Murer H and Kinne R, The use of isolated membrane vesicles to study epithelial transport processes. *J Membr Biol* **55:** 81–95, 1980.
36. Sachs G, Jackson RL and Rabon EC, Use of plasma membrane vesicles. *Am J Physiol* **238:** G151–G164, 1980.
37. Naganuma A, Oda-Urano N, Tanaka T and Imura N, Possible role of hepatic glutathione in transport of methylmercury into mouse kidney. *Biochem Pharmacol* **37:** 291–296, 1988.
38. Lash LH and Anders MW, Uptake of nephrotoxic *S*-conjugates by isolated rat renal proximal tubular cells. *J Pharmacol Exp Ther* **248:** 531–537, 1989.
39. Spater HW, Poruchynsky MS, Quintana N, Inoue M and Novikoff AB, Immunocytochemical localization of γ-glutamyltransferase in rat kidney with protein A-horseradish peroxidase. *Proc Natl Acad Sci USA* **79:** 3547–3550, 1982.
40. Lash LH and Zalups RK, Mercuric-chloride-induced cytotoxicity and compensatory hypertrophy in rat kidney proximal tubular cells. *J Pharmacol Exp Ther* **261:** 819–829, 1992.