

Basolateral Uptake of Inorganic Mercury in the Kidney

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Renal disposition of administered inorganic mercury was studied in rats that had undergone an acute bilateral ureteral ligation shortly before being injected with a nontoxic 0.5- $\mu\text{mol/kg}$ iv dose of inorganic mercury with or without 2.0 $\mu\text{mol/kg}$ glutathione (GSH) or cysteine. Ureteral ligation and induction of "stop-flow" conditions were carried out to decrease glomerular filtration rate to negligible levels prior to the administration of inorganic mercury. The disposition of mercury was studied in the kidneys, liver, and blood 1 h after treatment. In rats given only mercuric chloride, the renal burden of mercury was approximately 20–25% of the administered dose of mercury, which is approximately 50% of the renal burden of mercury detected on average in normal rats. Coadministration of inorganic mercury with GSH or cysteine caused a significant increase in the renal uptake of mercury 1 h after treatment. The enhanced uptake of mercury in the kidneys was due to increased uptake of mercury in the renal cortex and outer stripe of the outer medulla. Pretreatment with *para*-aminohippuric acid caused significant reductions in the renal concentration and burden of inorganic mercury in all the rats administered inorganic mercury, regardless of whether the inorganic mercury was coadministered with GSH or cysteine. Overall, the findings from the present study provide additional evidence that there is basolateral uptake of inorganic mercury in the kidneys and that the primary mechanism involved in this basolateral uptake is dependent on the activity of the organic anion transporter. More importantly, the present findings also show that GSH and cysteine enhance the basolateral uptake of mercuric ions in the kidney when they are coadministered with inorganic mercury (presumably in the form of mercuric conjugates). On the basis of the present findings, one is led to believe that mercuric conjugates of GSH and cysteine are taken up at the basolateral membrane following exposure to inorganic forms of mercury. © 1998 Academic Press

There is a significant body of recent evidence indicating that the renal uptake of inorganic mercury involves both luminal and basolateral mechanisms, particularly along proximal portions of the nephron. The principal basolateral mechanism is localized along the proximal tubule and appears to be dependent on the activity of the organic anion transport system (Zalups, 1995, 1997; Zalups and Barfuss, 1995b, 1998a,b; Zalups and Lash, 1994; Zalups and Minor, 1995). Based on what we currently know about the renal uptake and transport of

inorganic mercury and the fact that mercuric ions have a high affinity for sulfhydryl groups (Zalups and Lash, 1994), it is likely that a significant fraction of the mercuric ions in plasma (following exposure to inorganic mercury) are bound to small-molecular-weight thiol(s) prior to being taken up at the basolateral membrane. What remains less clear at present is which thiols (found *in vivo*), when bound to mercuric ions, promote the uptake and transport of inorganic mercury at the basolateral membrane, specifically at the site of the organic transport system. Although there are several small-molecular-weight thiols in the body that can, and likely do, form conjugates with mercuric ions, glutathione (GSH) and cysteine appear to be the most likely candidates deserving initial investigation. The rationale for choosing GSH and cysteine is severalfold. Not only are these molecules found within all cells of the body in abundant quantities, but they are also present in the plasma at low micromolar concentrations (Lash and Jones, 1985a). Moreover, depletion of GSH by various means has been shown to have significant effects on the renal and hepatic disposition of inorganic mercury (Zalups and Lash, 1994). Thus, it seems logical to postulate that mercuric conjugates of GSH and/or cysteine are present in the blood after exposure to inorganic mercury and that these conjugates participate in the basolateral uptake of inorganic mercury along the proximal tubule.

Evidence supporting the notion that mercuric conjugates of GSH and/or cysteine can be potentially transported across the basolateral membrane of proximal tubular cells comes partly from several sets of *in vitro* findings (Dantzler *et al.*, 1995; Lash and Jones, 1985a; Lash and Anders, 1989), which show that certain organic conjugates of GSH or cysteine are transported across proximal tubular basolateral membranes. Moreover, it appears that the basolateral uptake of these organic conjugates of GSH and cysteine occurs at the site of the organic anion transporter (Dantzler *et al.*, 1995; Lash and Anders, 1989). An additional line of evidence implicating the basolateral transport of a mercuric conjugate of a small thiol-containing molecule comes from a recent *in vivo* study in which it was demonstrated that virtually all of the mercury taken up by the kidneys after administration of a mercuric conjugate of *N*-acteylcysteine was due almost exclusively to a basolateral mechanism (Zalups and Barfuss, 1998). Although there is strong support for the contention that a mercuric conjugate of cysteine is involved in the luminal uptake of

inorganic mercury along the proximal tubule (Berndt *et al.*, 1985; deCeaurriz *et al.*, 1994; Zalups, 1995; Zalups and Barfuss, 1995ab, 1996a, 1998; Zalups and Lash, 1994, 1997), it is less clear whether mercuric conjugates of GSH and/or cysteine are involved in the basolateral uptake of inorganic mercury along the proximal tubule.

To better understand the potential role of mercuric conjugates of GSH and/or cysteine in the basolateral uptake of inorganic mercury, we tested the hypothesis that the basolateral uptake of inorganic mercury occurs when it is administered as a mercuric conjugate of GSH or cysteine. In addition we tested the hypotheses that, if the uptake occurs, it is due to the activity of the *para*-aminohippuric acid (PAH)-sensitive organic anion transporter. In order to test these hypotheses, we evaluated the effects of pretreatment with PAH on the disposition of inorganic mercury 1 h after the injection of a 0.5 $\mu\text{mol/kg}$ iv nontoxic dose of inorganic mercury administered with or without 2.0 $\mu\text{mol/kg}$ GSH or L-cysteine in rats that had undergone an acute bilateral ureteral ligation prior to treatment.

MATERIALS AND METHODS

General experimental design. In two separate sets of experiments (Experiments 1 and 2), we evaluated the effects of pretreatment with a 7.5-mmol/kg iv dose of PAH on the disposition of inorganic mercury 1 h after the injection of a 0.5- $\mu\text{mol/kg}$ iv dose of inorganic mercury administered with or without 2.0 $\mu\text{mol/kg}$ GSH or L-cysteine in rats that had undergone an acute bilateral ureteral ligation just prior to treatment.

Animals and groups used. Four groups of randomly selected male Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN), weighing 175–200 g, were used in Experiments 1 and 2. All rats used in Experiments 1 and 2 underwent an acute bilateral ureteral ligation prior to any treatment.

In Experiment 1, the disposition (levels) of mercury (in kidneys, liver, and blood) was evaluated in rats pretreated with PAH or normal saline (0.9% aqueous sodium chloride) that received the 0.5 $\mu\text{mol/kg}$ dose of inorganic mercury with or without 2.0 $\mu\text{mol/kg}$ GSH. In Experiment 2, the disposition (levels) of mercury (in kidneys, liver, and blood) was evaluated in rats pretreated with 7.5 mmol PAH/kg or normal saline (0.9% aqueous sodium chloride) that received the 0.5 $\mu\text{mol/kg}$ dose of inorganic mercury with or without 2.0 $\mu\text{mol/kg}$ cysteine.

Surgical procedure for bilateral ureteral ligation. Bilateral ureteral ligation was carried out on every animal according to the protocol established by Zalups and Minor (1995). After each animal had been anesthetized with a 50-mg/kg ip dose of sodium pentobarbital, a 2.5-cm flank incision was made through the skin and abdominal muscles on the left and right side of the body. Once the left and right ureters had been exposed with gentle dissection, a sterile 2-0 silk suture was tied around each ureter near the renal pelvis. Subsequently, the abdominal muscles were sewn together with sterile 4-0 silk suture and the skin was approximated with sterile 9-mm stainless steel wound clips.

Experimental protocol. In order to study basolateral transport of inorganic mercury following bilateral ureteral ligation, one must attain conditions in which glomerular filtration is reduced to negligible levels prior to the injection of inorganic mercury (Zalups and Minor, 1995; Malvin and Wilde, 1973). A 2.0-mmol/kg iv dose of mannitol (in 2.0 ml/kg normal saline) was administered to each animal 5 min prior to performing bilateral ureteral ligation. Mannitol was administered to promote the reduction of glomerular filtration by increasing the intraluminal osmotic pressure sufficiently high enough to prevent any appreciable reabsorption of solutes and water (Zalups and Minor,

1995; Malvin and Wilde, 1973). The principle behind using mannitol in association with ureteral ligation is that it is not reabsorbed by any portion of the nephron, and, thus, its concentration in the luminal fluid increases as it is filtered initially into the tubular lumen. With both ureters ligated, the intraluminal hydrostatic and osmotic pressures increases to a level at which negligible reabsorption of water and solutes occurs and a pressure equilibrium is reached, preventing any appreciable filtration from occurring at the site of the glomerular filtration barrier.

Five minutes prior to the injection of inorganic mercury and 70 min after bilateral ureteral ligation, a 7.5-mmol/kg iv dose of PAH (in 2.0 ml/kg normal saline) was administered into the left femoral vein. The 7.5-mmol/kg dose of PAH was chosen inasmuch as it has been shown to near maximally inhibit the renal (basolateral) uptake of inorganic mercury shortly after administration (Zalups and Barfuss, 1995b). It was assumed, on the basis of "stop-flow" hydrostatic pressures obtained in previous control experiments (Zalups and Minor, 1995), that glomerular filtration is reduced to negligible levels by 75 min after bilateral ureteral ligation. Therefore, the 0.5- $\mu\text{mol/kg}$ dose of mercuric chloride (with or without 2.0 $\mu\text{mol/kg}$ GSH or L-cysteine) was administered (in 2.0 ml/kg normal saline) into the right femoral vein while the rat was under light anesthesia induced by ether 75 min after bilateral ureteral ligation. This period of time after surgery was also sufficient to allow the animals to regain consciousness from anesthesia induced by the 50-mg/kg dose of sodium pentobarbital. Radioactive inorganic mercury $^{203}\text{Hg}^{2+}$ (specific activity = 15.0 mCi/mg, Buffalo Materials Corp., Buffalo, NY) was added to the infection solution at a concentration to deliver approximately 1 μCi $^{203}\text{Hg}^{2+}$ /animal. After the injection had been administered, the skin over the right femoral vein was approximated with sterile wound clips. Refer to Zalups and Minor (1995) for additional details on the protocol used to induce "stop-flow" conditions to promote significant reduction in glomerular filtration rate. Both experiments were terminated 1 h after the injection of inorganic mercury. At the termination of each experiment, all animals were anesthetized with a 100-mg/kg dose of sodium pentobarbital, and the acquisition of organs and tissues was carried out in order to determine the disposition of mercury.

Acquisition and handling of tissues and organs. A 2.0-ml sample of blood was obtained from the inferior vena cava from each animal after it had been anesthetized (as determined by corneal and tail reflexes) with a 100-mg/kg dose of sodium pentobarbital. One milliliter of blood was placed and sealed in a preweighed 12 \times 75-mm polystyrene round-bottom tube for gamma-counting. The other 1 ml was spun down for 10 min at 20,000g to separate the cellular fraction of blood from the plasma. Both plasma and cellular fractions were placed individually and sealed in counting tubes. After the 2.0-ml sample of blood was obtained, the kidneys and liver were excised and were quickly cleared of fat and connective tissue and weighed. Each of the two kidneys from every animal was cut in half along the transverse plain. One half of each kidney was placed and sealed in a preweighed polystyrene counting tube. A 3-mm section of the remaining half of the left kidney was also obtained. From this section of tissue, samples of cortex, outer and inner stripes of the outer medulla, and inner medulla were obtained and were placed in preweighed counting tubes. A 1 g section of liver was also obtained and placed and sealed in a preweighed counting tube. The total volume of blood in each animal was estimated to be 6% of body weight.

Determinations of the content of mercury in tissues and organs. The amount of radioactivity in the samples of tissues, organs, and injection solutions (standards) was determined by counting the samples in a 1282 Compu-gamma CS deep-well gamma spectrometer with a 3-in sodium iodide crystal (Wallac, Gaithersburg, MD) operating at a counting efficiency of approximately 50% for ^{203}Hg . The content of mercury in each sample was calculated by dividing the activity (dpm) in the sample by the specific activity (dpm/nmol) of the injection solution.

Statistical analysis. All values presented are means \pm SE unless otherwise stated. Since data expressed as a percent of a total do not fit a normal or Gaussian distribution, all data expressed as a percent were first normalized using the arcsine transformation prior to applying any parametric statistical

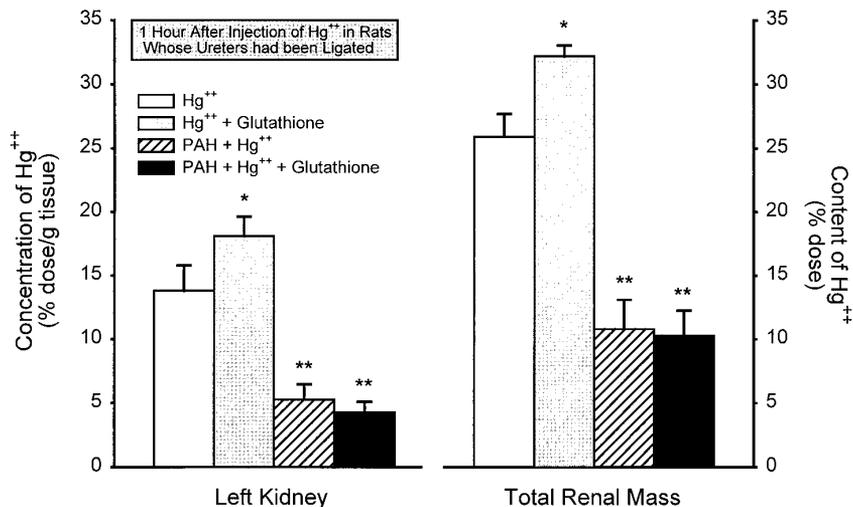


FIG. 1. Concentration of mercury (% of the administered dose/g tissue) in the left kidney and the content of mercury (% of administered dose) in the total renal mass 1 h after treatment. Values represent means \pm SE for four animals. *Significantly different ($p < 0.05$) from the mean for the corresponding group of control rats treated with 0.5 μmol inorganic mercury (Hg^{2+})/kg. **Significantly different ($p < 0.05$) from the mean for the corresponding group of control rats treated only with 0.5 μmol Hg^{2+} /kg and the mean for the corresponding group of rats treated with 0.5 μmol Hg^{2+} /kg plus 2.0 μmol glutathione/kg. PAH, pretreatment with 7.5 mmol *para*-aminohippurate/kg.

analysis. This transformation takes the arcsine of the square root of the decimal fraction of the percent score.

In both Experiments 1 and 2, evaluation of differences between means for any set of data was carried out by applying a two-way analysis of variance (ANOVA) followed by Tukey's protected *t*-test. The level of significance for all statistical analyses was chosen *a priori* to be $p < 0.05$.

RESULTS

Experiment 1

Renal Disposition of Mercury

Renal concentration and content of mercury. The concentration of mercury in the left kidney (% dose/g tissue) and the total renal burden of mercury (%dose) were significantly greater in the group of rats coadministered 0.5 μmol /kg *iv* inorganic mercury plus 2.0 μmol /kg GSH than in the control rats that received the 0.5 μmol /kg dose of mercuric chloride alone 1 h after treatment (Fig. 1). In both groups of rats that received pretreatment with PAH, the concentration of mercury in the left kidney and the total renal burden of mercury were significantly less than those in the corresponding groups not pretreated with PAH. In fact, both the renal concentration of mercury and the total renal burden of mercury in the rats that were pretreated with PAH were less than 50% of those in the corresponding rats not pretreated with PAH.

Intrarenal distribution of mercury. In the rats treated with inorganic mercury plus GSH, the concentrations of mercury in the renal cortex and outer stripe of the outer medulla were significantly greater than those in the rats administered inorganic mercury alone (Fig. 2). Moreover, the concentra-

tions of mercury in the cortex and outer stripe of the outer medulla were significantly less in the rats pretreated with PAH than in the corresponding groups not pretreated with PAH. The effect of pretreatment with PAH on the disposition of mercury in the renal cortex and outer stripe of the

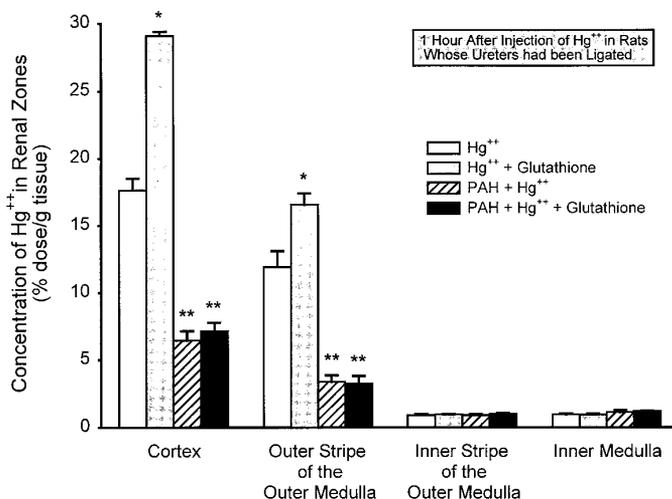


FIG. 2. Concentration of mercury (% of the administered dose/g tissue) in the renal cortex, outer stripe of the outer medulla, inner stripe of the outer medulla, and inner medulla 1 h after treatment. Values represent means \pm SE for four animals. *Significantly different ($p < 0.05$) from the mean for the corresponding group of control rats treated with 0.5 μmol inorganic mercury (Hg^{2+})/kg. **Significantly different ($p < 0.05$) from the mean for the corresponding group of control rats treated only with 0.5 μmol Hg^{2+} /kg and the mean for the corresponding group of rats treated with 0.5 μmol Hg^{2+} /kg plus 2.0 μmol glutathione/kg. PAH, pretreatment with 7.5 mmol *para*-aminohippurate/kg.

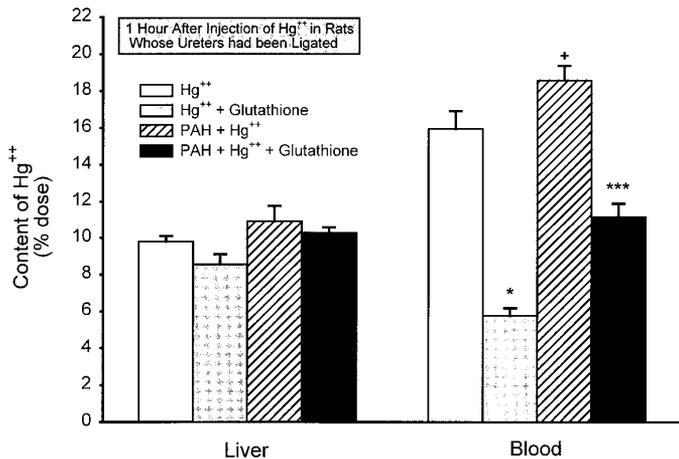


FIG. 3. Content of mercury (% of the administered dose) in the liver and blood 1 h after treatment. Values represent means \pm SE for four animals. *Significantly different ($p < 0.05$) from the mean for the corresponding group of control rats treated with 0.5 μmol inorganic mercury (Hg^{2+})/kg. ***Significantly different ($p < 0.05$) from the means for all of the other corresponding groups. +Significantly different ($p < 0.05$) from the mean for the corresponding group treated with 0.5 μmol Hg^{2+} /kg plus 2.0 μmol glutathione/kg. PAH, pretreatment with 7.5 mmol *para*-aminohippurate/kg.

outer medulla was substantial in the rats treated with inorganic mercury alone or in combination with GSH. No statistically significant differences in the concentration of mercury were detected in either the inner stripe of the outer medulla or inner medulla among the four groups of rats in this experiment.

Disposition of mercury in the liver and blood. Among the four groups in Experiment 1, no significant differences were detected in the content of mercury in the liver 1 h after treatment (Fig. 3).

Co-administration of inorganic mercury with GSH did cause marked changes in the content of mercury in the blood when compared to the content of mercury in the blood of control rats (Fig. 3). Between the two groups of the rats pretreated with PAH, the content of mercury in the total estimated volume of blood in the rats coadministered inorganic mercury plus GSH was significantly greater than that in the corresponding PAH-pretreated rats that were administered inorganic mercury alone.

In both groups of rats treated with inorganic mercury plus GSH, approximately 87–89% of the mercury in blood was present in the plasma, while in the two groups of rats treated with inorganic mercury alone, only approximately 33–44% of the mercury in the blood was present in the plasma (Fig. 4). There was a slight, but significant, difference in the percent of mercury in blood that was present in plasma between the two groups of rats treated with inorganic mercury alone.

Experiment 2

Renal Disposition of Mercury

Renal concentration and content of mercury. In the group of rats coadministered 0.5 μmol inorganic mercury/kg iv plus 2.0 μmol cysteine/kg. The concentration of mercury in the left kidney and the total renal burden of mercury were significantly greater than those in the control rats that received inorganic mercury alone (Fig. 5). The concentration of mercury in the left kidney and the total renal burden of mercury in both groups of rats pretreated with PAH were significantly less than those in the corresponding groups not pretreated with PAH. In the rats given inorganic mercury alone, pretreatment with PAH caused an approximate 52% decrease in the total renal burden of mercury 1 h after treatment. Between the two groups of rats treated with inorganic mercury plus cysteine, the renal burden of mercury in the rats pretreated with PAH was approximately 69% less than that in the corresponding group not pretreated with PAH. No significant difference in the renal concentration or burden of mercury was detected between the two groups of rats pretreated with PAH.

Intrarenal distribution of mercury. Greater concentrations of mercury were detected in the renal cortex and outer stripe of the outer medulla of the rats treated with inorganic mercury plus cysteine than in the rats treated with inorganic mercury alone (Fig. 6). In the rats pretreated with PAH, the concentrations of mercury in the cortex and outer stripe of the outer medulla were significantly less than those in the corresponding groups not pretreated with PAH. Pretreatment with PAH caused the concentrations of mercury in the renal cortex and

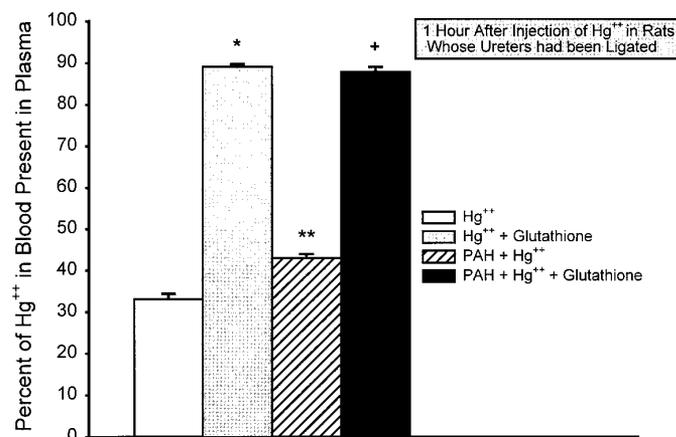


FIG. 4. Percent of mercury present in plasma 1 h after treatment. Values represent means \pm SE for four animals. *Significantly different ($p < 0.05$) from the mean for the corresponding group of control rats treated with 0.5 μmol inorganic mercury (Hg^{2+})/kg. **Significantly different ($p < 0.05$) from the mean for the corresponding control group of rats treated only with 0.5 μmol Hg^{2+} /kg and the mean for the corresponding group of rats treated with 0.5 μmol Hg^{2+} /kg plus 2.0 μmol glutathione/kg. +Significantly different ($p < 0.05$) from the mean for the corresponding group pretreated with PAH that received 0.5 μmol Hg^{2+} /kg. PAH, pretreatment with 7.5 mmol *para*-aminohippurate/kg.

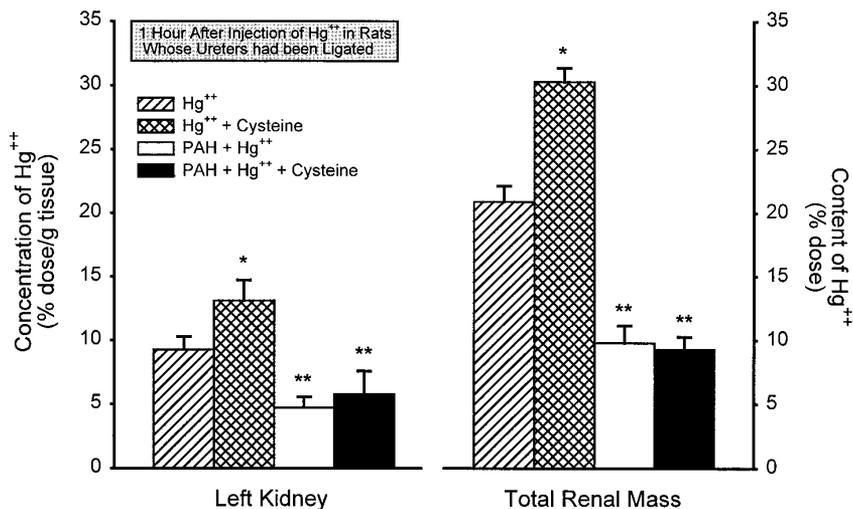


FIG. 5. Concentration of mercury (% of the administered dose/g tissue) in the left kidney and the content of mercury (% of administered dose) in the total renal mass 1 h after treatment. Values represent means \pm SE for four animals. *Significantly different ($p < 0.05$) from the mean for the corresponding group of control rats treated with $0.5 \mu\text{mol}$ inorganic mercury (Hg^{2+})/kg. **Significantly different ($p < 0.05$) from the mean for the corresponding group of control rats treated only with $0.5 \mu\text{mol}$ Hg^{2+} /kg and the mean for the corresponding group of rats treated with $0.5 \mu\text{mol}$ Hg^{2+} /kg plus $2.0 \mu\text{mol}$ cysteine/kg. PAH, pretreatment with 7.5 mmol *para*-aminohippurate/kg.

outer stripe of the outer medulla to be approximately 45 and 52% lower, respectively, in the rats that received inorganic mercury alone. By comparison, pretreatment with PAH caused the concentrations of mercury in the renal cortex and outer stripe of the outer medulla to be approximately 73 and 78%

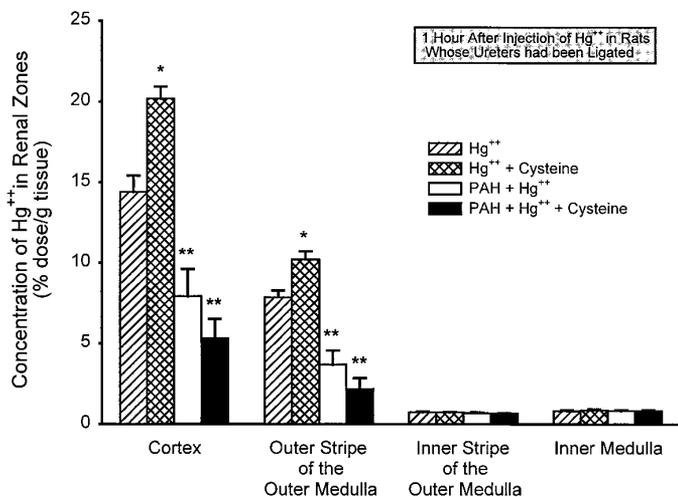


FIG. 6. Concentration of mercury (% of the administered dose/g tissue) in the renal cortex, outer stripe of the outer medulla, inner stripe of the outer medulla, and inner medulla 1 h after treatment. Values represent means \pm SE for four animals. *Significantly different ($p < 0.05$) from the mean for the corresponding group of control rats treated with $0.5 \mu\text{mol}$ inorganic mercury (Hg^{2+})/kg. **Significantly different ($p < 0.05$) from the mean for the corresponding group of control rats treated only with $0.5 \mu\text{mol}$ Hg^{2+} /kg and the mean for the corresponding group of rats treated with $0.5 \mu\text{mol}$ Hg^{2+} /kg plus $2.0 \mu\text{mol}$ cysteine/kg. PAH, pretreatment with 7.5 mmol *para*-aminohippurate/kg.

lower, respectively, in the rats that received inorganic mercury plus cysteine. There were no significant differences in the concentrations of mercury in the cortex and outer stripe of the outer medulla between the two groups of rats pretreated with PAH. No statistically significant differences in the concentration of mercury in either the inner stripe of the outer medulla or inner medulla were detected among the four groups of rats in this experiment.

Disposition of Mercury in the Liver and Blood

No significant differences in the hepatic burden of mercury were detected among the four groups of animals in this experiment (Fig. 7).

Coadministration of inorganic mercury plus cysteine had significant effects of the disposition of mercury in the blood (Fig. 7). The content of mercury in the estimated total blood volume was approximately 67% lower 1 h after treatment in the rats coadministered inorganic mercury plus cysteine than in the control rats that were administered inorganic mercury without cysteine. In addition, the content of mercury in the blood of the PAH-pretreated rats that received inorganic mercury plus cysteine was approximately 35% less than that in the corresponding PAH-pretreated rats treated only with inorganic mercury. Pretreatment with PAH caused the content of mercury in the rats coadministered inorganic mercury plus cysteine to be approximately 106% greater than that in the corresponding control rats coadministered inorganic mercury with cysteine. No significant difference in the content of mercury in the blood was detected between the two groups of rats administered inorganic mercury only.

In the rats treated with inorganic mercury plus cysteine,

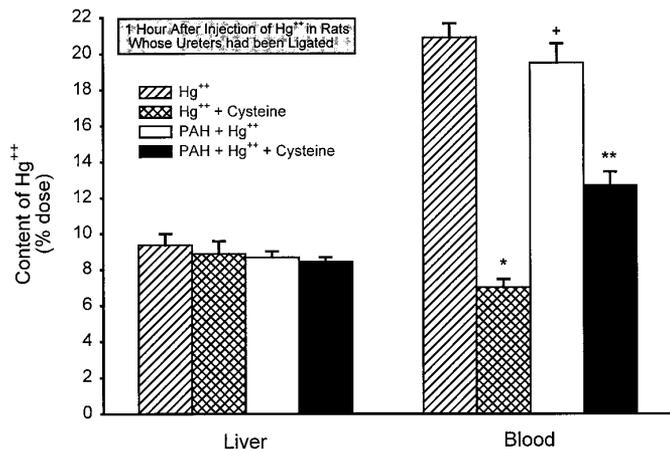


FIG. 7. Content of mercury (% of the administered dose) in the liver and blood 1 h after treatment. Values represent means \pm SE for four animals. *Significantly different ($p < 0.05$) from the mean for the corresponding group of control rats treated with 0.5 μmol inorganic mercury (Hg^{2+})/kg. **Significantly different ($p < 0.05$) from the mean for the corresponding control group of rats treated only with 0.5 μmol Hg^{2+} /kg and the mean for the corresponding group of rats treated with 0.5 μmol Hg^{2+} /kg plus 2.0 μmol cysteine/kg. +Significantly different ($p < 0.05$) from the mean for the corresponding group treated with 0.5 μmol Hg^{2+} /kg plus 2.0 μmol L-cysteine/kg. PAH, pretreatment with 7.5 mmol *para*-aminohippurate/kg.

approximately 71–75% of the mercury in blood was present in the plasma. By contrast, in the rats treated with inorganic mercury alone, only approximately 27–40% of the mercury in the blood was present in the plasma, depending on whether the animals were pretreated with PAH (Fig. 8). In fact, between the two groups of rats treated only with inorganic mercury, the fraction of blood-mercury in the plasma was significantly greater in the group of rats pretreated with PAH treated than in the group not pretreated with PAH.

DISCUSSION

The findings from the present study indicate that within 1 h after the intravenous injection of a nontoxic dose of mercuric chloride, approximately 20–25% of the administered dose of inorganic mercury is taken up by the total renal mass of rats that have undergone an acute bilateral ureteral ligation just prior to the administration of the mercury. These findings confirm the existence of a basolateral mechanism in the renal tubular uptake of inorganic mercury (Zalups, 1995, 1997; Zalups and Barfuss, 1995a,b; 1996a; 1998a,b; Zalups and Lash, 1994). Considering that approximately 40% of the dose is normally taken by the total renal mass during the initial hour after the intravenous injection of a nontoxic dose of inorganic mercury (Zalups, 1997; 1998; Zalups and Barfuss, 1995a,b; 1996a,b; 1998a,b; Zalups and Lash, 1994), the present findings indicate that approximately 50% of the normal renal burden of mercury (1 h after injection) can be attributed to a basolateral mechanism.

We have implicated previously the activity of the organic anion transport system as a primary mechanism involved in the basolateral uptake of inorganic mercury (Zalups, 1995, 1997; Zalups and Barfuss, 1995, 1996, 1998a,b; Zalups and Lash, 1994). Specifically, we showed that pretreatment with PAH (which is a specific inhibitor of the renal organic anion transporter [Roch-Ramel, 1992] inhibits the renal tubular uptake of administered inorganic mercury in both normal rats and in rats whose ureters had been ligated prior to the administration of inorganic mercury (Zalups, 1995, 1997; Zalups and Barfuss, 1995, 1996; Zalups and Lash, 1994). More recently, we also showed that small aliphatic dicarboxylic acids, such as glutaric or adipic acid, also inhibit the renal (basolateral) uptake of inorganic mercury by acting at the site of the organic anion transporter and/or dicarboxylic acid transporter, which affects indirectly the activity of the organic anion transporter (Zalups and Barfuss, 1998b). In the present study, pretreatment with PAH also inhibited the renal uptake of mercury (after administration of mercuric chloride) such that the renal burden of mercury was decreased by greater than 50% in rats whose ureters had been ligated. These findings serve as additional evidence for the involvement of the organic anion transport system in the basolateral uptake of administered inorganic mercury in the kidney.

In general, when normal animals are exposed to, or injected with, inorganic mercury in the form of mercuric chloride, virtually all of the mercuric ions in systemic circulation are bound to various endogenous ligands. Of the mercuric ions present in the plasma, the majority of them are bound to albumin and other large proteins (Zalups and Lash, 1994).

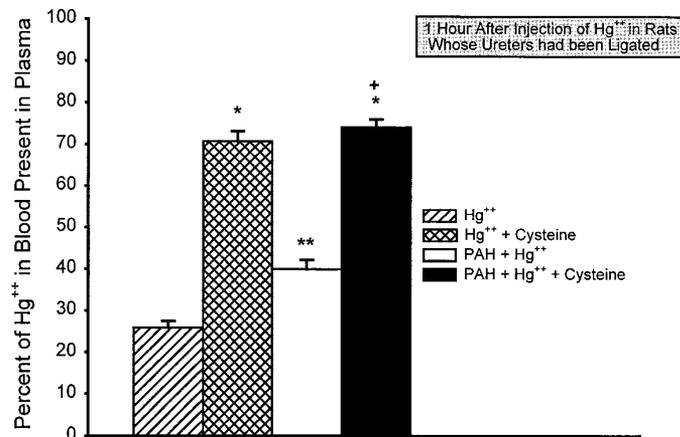


FIG. 8. Percent of mercury present in plasma 1 h after treatment. Values represent means \pm SE for four animals. *Significantly different ($p < 0.05$) from the mean for the corresponding group of control rats treated with 0.5 μmol inorganic mercury (Hg^{2+})/kg. **Significantly different ($p < 0.05$) from the mean for the corresponding group of control rats treated only with 0.5 μmol Hg^{2+} /kg and the mean for the corresponding group of rats treated with 0.5 μmol Hg^{2+} /kg plus 2.0 μmol cysteine/kg. +Significantly different ($p < 0.05$) from the mean for the corresponding group pretreated with PAH that received 0.5 μmol Hg^{2+} /kg. PAH, pretreatment with 7.5 mmol *para*-aminohippurate/kg.

Since the organic anion transporter does not transport large proteins, small molecular conjugates of mercury are likely transported. Two conjugates of inorganic mercury that likely form in the body and that may be transported by the organic anion transport system are mercuric conjugates of GSH and/or cysteine.

Formation of mercuric conjugates of GSH and/or cysteine does occur within other organs, such as the liver (Ballatori and Clarkson, 1985). Some of these conjugates likely enter systemic circulation and are carried in the plasma to the kidneys. A link between the liver and some aspects of the renal uptake of inorganic mercury has been demonstrated recently (Zalups and Barfuss, 1996b). Specifically, it was demonstrated that ligation of the bile duct prior to the intravenous administration of a nontoxic dose of mercuric chloride results in a significant decrease in the renal uptake of inorganic mercury. The formation of mercuric conjugates of GSH and/or cysteine in blood also seems probable since the concentrations of GSH and cysteine in the plasma (of rats) are around 10 μM (Lash and Jones, 1985a). These concentrations of GSH and cysteine in plasma provide a significant pool of nonprotein thiols to which mercuric ions can bind. Recent unpublished data from our laboratory also indicate that biliary ligation causes a marked reduction in the pool of both reduced GSH and cysteine in the plasma within 1 h after ligation and that this reduction is linked to the decreased uptake of mercury in the kidneys.

Inasmuch as both GSH and cysteine have been implicated as being endogenous ligands to which mercuric ions bind to prior to being taken up in the kidney, it was deemed important to determine if there are differences in the basolateral uptake of mercury when inorganic mercury is administered with GSH and cysteine. Interestingly, coadministration of inorganic mercury with GSH or cysteine had virtually identical effects on the renal disposition of mercury. In particular, coadministration of either thiol with inorganic mercury caused an increase in the (apparent) basolateral uptake of mercury, relative to that detected when inorganic mercury was administered as mercuric chloride. In addition, the effects of PAH on the renal disposition of mercury were also comparable when inorganic mercury was coadministered with GSH or cysteine. On the basis of these findings, there do not appear to be any significant differences in the basolateral handling of inorganic mercury in the kidney between the conditions created when inorganic mercury is coadministered with GSH and those created when it is coadministered with cysteine. Thus, one is led to believe that perhaps both mercuric conjugates of GSH and cysteine are taken up at the basolateral membrane by the organic anion transport system. Alternatively, it is possible that, once the presumed mercuric conjugates of GSH and/or cysteine are delivered into systemic circulation, some form of thiol substitution occurs with the molecules of GSH and/or cysteine that then allows for the uptake of inorganic mercury at the basolateral membrane. This latter hypothesis seems less likely at present due to data from a recent study indicating that the

mercuric conjugates of *N*-acetylcysteine in the plasma seem to remain intact during the initial hour after intravenous administration (Zalups and Barfuss, 1998a).

In this study mentioned above (Zalups and Barfuss, 1998a), we showed that the total renal burden of mercury in rats that had undergone bilateral ureteral ligation and had been treated subsequently with inorganic mercury plus *N*-acetylcysteine was approximately 40% of the administered dose 1 h after treatment. What is remarkable about this finding is that the amount of mercury present in the kidneys is equivalent to the entire renal burden of mercury present in a normal rat given the same dose of inorganic mercury as mercuric chloride (Zalups and Barfuss, 1998a). We speculated that the negative charge on the molecules of *N*-acetylcysteine promoted the rapid transport of mercuric conjugates of *N*-acetylcysteine into proximal tubular cells at the site of the organic anion transporter.

Since there is a net negative charge on molecules of GSH and since there is evidence for the transport of organic conjugates of GSH across renal basolateral membranes (Lash and Jones, 1985b), it seems plausible that mercuric conjugates of GSH are also transported at the basolateral membrane. The findings from the current study support this notion. Despite the fact that cysteine has a net neutral charge at physiological pH, there is *in vitro* evidence indicating that some organic conjugates of cysteine are in fact taken up at the basolateral membrane of proximal tubular epithelial cells by the organic anion transporter (Dantzler *et al.*, 1995; Lash and Anders, 1989). One line of recent evidence indicating that mercuric conjugates of cysteine may also be taken up at the basolateral membrane comes from *in vitro* data obtained with basolateral membrane vesicles isolated from the kidneys of rats. These data show that rates of association and transport of inorganic mercury in basolateral membrane vesicles tends to be greater when the vesicles are exposed to mercuric conjugates of cysteine than when they are exposed to mercuric chloride (Zalups and Lash, 1997). The hypothesis that basolateral uptake of mercuric conjugates of cysteine occurs is also supported indirectly by our previous (Zalups and Barfuss, 1998a) and present data obtained from rats whose ureters had been ligated and who were treated with inorganic mercury plus cysteine. These data show that bilateral ureteral ligation causes an approximate one-half reduction in the renal burden of mercury in both control rats treated with 0.5 $\mu\text{mol/kg}$ inorganic mercury alone and in the rats coadministered this dose of inorganic mercury with 2.0 $\mu\text{mol/kg}$ cysteine. Additionally, the findings show that the renal uptake of mercury is enhanced in the animals treated with inorganic mercury plus cysteine, relative to animals treated with inorganic mercury alone, while the relative intrarenal distribution of mercury remains similar in both groups of rats. The most reasonable explanation for these findings is that, by injecting mercuric conjugates of cysteine, more of these conjugates than normally are formed when inorganic mercury is administered alone were made available at the site of the organic anion transporter (and possibly other basolateral trans-

porters) to promote the uptake of mercury. In the present study, PAH pretreatment also caused a significant decrease in the renal uptake of mercury in rats (whose ureters had been ligated) administered inorganic mercury plus cysteine. This finding indicates that the organic anion transporter is likely involved in the basolateral uptake of mercuric conjugates of cysteine.

The dispositional data obtained from blood tend to support the presumption that much of the mercuric ions coadministered with GSH or cysteine remained in the form of some coordinate covalent complex, presumably consisting of one mercuric ion bound to two molecules of GSH or cysteine, after intravenous administration. The data show the content of mercury in the blood was significantly lower and that partitioning of mercury between the plasma and cellular fractions of blood was greatly different in the animals coadministered mercury with GSH or cysteine relative to that in the animals given inorganic mercury alone. Additional support for this notion comes from ^{13}C nuclear magnetic resonance data on the formation constants and thermodynamic stability of thiol conjugates of mercury in aqueous solutions (Rabenstein, 1989).

In conclusion, the findings of the present study indicate that the organic anion transport system is involved in the basolateral uptake of inorganic mercury in the kidney when it is coadministered *in vivo* with GSH or cysteine. The implications of the current findings are that basolateral uptake of mercuric conjugates of GSH and cysteine occurs along the proximal tubule at the site of the organic anion transport system following exposure to inorganic mercury.

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