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Evidence for Basolateral Uptake of Cadmium in the Kidneys of Rats

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In three separate sets of studies, the effects of ureteral ligation and coadministration of cadmium with cysteine or glutathione (GSH) (in either a 4:1 or 2:1 ratio of thiol to cadmium) on the renal disposition of cadmium were assessed in rats 1 h after the administration of cadmium. In all experiments, co-administration of cadmium with either cysteine or GSH caused the renal accumulation of cadmium to increase significantly (by approximately 60–70%) 1 h after injection. Moreover, in all experiments in which both ureters had been ligated in a rat prior to the administration of cadmium, the net total renal accumulation of cadmium was only about 20% less than that in control animals that had not undergone bilateral ureteral ligation when cadmium was administered as cadmium chloride. Furthermore, in animals in which only one ureter had been ligated, the net accumulation of cadmium in the kidney whose ureter had been ligated was between 25 and 30% less than that in the contralateral kidney. Coadministration of cadmium with cysteine or GSH also caused the net accumulation of cadmium to be increased in rats whose ureter(s) had been ligated. Overall, the present findings indicate that there is a significant basolateral component in the acute, *in vivo,* **renal tubular uptake of cadmium. Moreover, the findings indicate that the basolateral uptake of cadmium is enhanced when cadmium is coadministered with cysteine or GSH.** © 2000 Academic Press

The mechanisms by which cadmium ions gain entry into renal tubular epithelial cells are not yet well defined. Unfortunately, confusion and controversy regarding the mechanisms involved in the nephropathy induced by chronic exposure to cadmium has, to some degree, impeded the understanding of the mechanisms involved in the renal handling and transport of cadmium. It has been hypothesized, and widely accepted, that the nephropathy induced by cadmium is likely mediated through the renal tubular uptake of cadmium–metallothionein (cadmium–MT) (reviewed in Diamond and Zalups, 1998), which is premised on two factors. One, that cadmium-MT is released into the bloodstream following hepatocellular necrosis induced by cadmium, and two, that the cadmium-MT released into the blood (plasma) from dead hepatocytes is transported to the kidneys, is filtered freely into the luminal compartment of the proximal nephron, and then is taken up readily by the proximal tubular epithelium via endocytosis. It should be pointed out, however, that there are confounding data from various *in vitro* preparations of proximal tubules or their epithelial cells indicating that the rates of uptake of metals in the form of metal–MT complexes at the luminal membrane range from low to negligible (Zalups *et al.,* 1995; Liu *et al.,* 1994).

In the absence of any hepatocellular necrosis following exposure to a nontoxic dose of cadmium, it is not clear as to how cadmium gains entry into renal tubular epithelial cells. Since metallothionein does not contain a leader-sequence to promote its exocytosis from cells (especially from hepatocytes) into the extracellular (plasma) compartment, it is hard to conceive of a mechanism by which cadmium can gain entry into renal tubular epithelial cells as cadmium-MT following exposure to low nonhepatotoxic doses of cadmium. While it is certainly clear that cadmium-MT is a potent nephrotoxicant (Cain and Holt, 1983; Cherian *et al.,* 1976; Squibb *et al.,* 1984; Maitani *et al.,* 1988; Nordberg *et al.,* 1975; Zalups *et al.,* 1992), there is insufficient evidence implicating this complex in the renal tubular uptake of cadmium, especially under conditions where hepatic (or renal) injury are absent. Moreover, there appears to be some controversy regarding the potential role of cadmium-MT in the nephropathy associated with chronic exposure to cadmium. In a recent report, Liu and colleagues (1998) postulate that perhaps the nephropathy induced by acute administration of cadmium-MT is not a good model to study the nephropathy that is induced by chronic exposure to cadmium.

When considering alternate mechanisms in the renal tubular uptake of cadmium (i.e., those not involving the endocytosis of cadmium-MT), one must consider the possibility that cadmium may gain access to the intracellular compartment of renal tubular epithelial cells not only at the luminal plasma membrane, but also at the basolateral membrane. Unfortunately, very little attention has been given to the possibility that cadmium may be transported into renal tubular epithelial cells via a mechanism localized in the basolateral membrane, particularly those lining the three segments of the proximal tubule.

Since so much work over the years has focused on the renal transport and toxicity of cadmium–MT, little attention has been paid to alternate mechanisms by which cadmium ions may gain entry into renal epithelial cells and induce renal tubular injury *in vivo.* For instance, very little is known about the potential role that extracellular thiols, such as cysteine and glutathione (GSH), may play in the movement of cadmium ions into renal

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tubular epithelial cells. This is an important factor to assess, inasmuch as numerous studies have implicated clearly the direct and modulatory role of extracellular thiols in the renal tubular uptake of inorganic mercury (Zalups, 1995, 1998; Zalups and Barfuss, 1995b,c, 1998b), which is also a group IIB metal. Therefore, the potential role of extracellular thiols in the renal tubular uptake, accumulation, and intrarenal distribution of cadmium was studied in rats administered cadmium with or without cysteine or GSH.

The primary hypothesis tested in the present study, however, is that cadmium is taken up (with or without an extracellular thiol-ligand) into renal tubular epithelial cells by a basolateral mechanism. To test this hypothesis, the disposition of intravenously administered cadmium was studied in rats that had or had not undergone ureteral ligation. The protocol used for ureteral ligation is an established one in which there is strong evidence indicating that whole animal glomerular filtration rate is reduced to negligible levels, which allows one to isolate events occurring at the basolateral membrane.

MATERIALS AND METHODS

General experimental design. Three studies (studies 1–3) were carried out to evaluate the potential for basolateral uptake of cadmium in the kidneys as well as to determine the effects of coadministration of cadmium with cysteine or GSH on the renal accumulation of cadmium. In the first study, the renal, hepatic, and hematological disposition of cadmium was evaluated (in normal rats and rats that had undergone acute bilateral ureteral ligation) 1 h after the intravenous injection of a 10 μmol/kg dose of cadmium administered with or without 40 μmol/kg cysteine or GSH. The rationale for premixing and coadministering cadmium in a 1:4 ratio with either cysteine or GSH was to ensure formation of linear II coordinate covalent complexes between each atom of cadmium and two molecules of the respective thiol prior to injection (Rabenstein, 1989).

In the second study, the 1-h renal, hepatic, and hematological disposition of cadmium was evaluated in control rats and rats that had undergone acute bilateral ureteral ligation that were administered a 5-μmol/kg dose of cadmium with or without 10 μmol/kg cysteine or GSH. This study was carried out to assess the disposition of a lower, relatively nontoxic dose of cadmium (one that does not induce acute hepatic or renal injury), in the presence or absence of a 2:1 ratio cysteine or GSH in the injection solution.

To confirm basolateral uptake of cadmium in the kidneys, the disposition of cadmium in each of the two kidneys of rats that had undergone acute right-sided ureteral ligation was evaluated 1 h after the administration of a 10-μmol/kg dose of cadmium with or without 40 μmol/kg cysteine or GSH.

Mixing a 2:1 or 4:1 ratio of cysteine or GSH with cadmium prior to injection was implemented to provide a high probability for the formation of linear II coordinate covalent complexes involving each cadmium ion bonding to two molecules of the respective thiol. Although the binding affinity of thiolate anions (formed from reduced sulfhydryl groups on small thiols such as cysteine or GSH) for cadmium ions is not as great as it is for mercuric ions, there remains a high probability for the formation of linear II coordinate covalent complexes between small thiols and cadmium in aqueous solution (Rabenstein, 1989). The stability of the bonds between the cadmium ions and the sulfur atoms of the thiols, however, is not as great as it is between mercuric ions and thiols.

Animals and groups used. Six groups of four to five randomly selected male Sprague–Dawley rats (Harlan Sprague–Dawley, Indianapolis, IN), weighing 175–200 g were used in studies 1 and 2. Three of the six groups of rats used in these studies underwent acute bilateral ureteral ligation prior to any treatment. The other three groups served as sham-operated control groups. All animals were housed in animal care rooms for rats that were maintained at 22°C and 50% relative humidity. Prior to experimentation, animals were provided a water and a commercial laboratory diet for rats *ad libitum.*

In study 1, the 1-h disposition (levels) of cadmium (in kidneys, liver, and blood) was evaluated in one group of sham-control rats and one corresponding group of rats that had undergone acute bilateral ureteral ligation that received intravenously 10 μmol cadmium/kg in the form of cadmium chloride, 10 μmol cadmium/kg plus 40 μmol cysteine/kg simultaneously, or 10 μmol cadmium/kg plus 40 μmol GSH/kg simultaneously.

In study 2, the 1-h disposition (levels) of cadmium (in kidneys, liver, and blood) was evaluated in groups of rats that had undergone acute bilateral ureteral ligation that received intravenously 5 μmol cadmium/kg in the form of cadmium chloride, 5 μmol cadmium/kg plus 10 μmol cysteine/kg simultaneously, or 5 μmol cadmium/kg plus 10 μmol GSH/kg simultaneously.

In study 3, the 1-h renal disposition cadmium was evaluated in rats that had undergone acute unilateral ureteral ligation that received intravenously 10 μmol cadmium/kg in the form of cadmium chloride, 10 μmol cadmium/kg plus 40 μmol cysteine/kg simultaneously, or 10 μmol cadmium/kg plus 40 μmol GSH/kg simultaneously.

All injections in each of the three studies were administered in a 0.9% sodium chloride vehicle at a volume per kilogram body weight ratio of 2 ml/kg.

Surgical procedure for bilateral ureteral ligation. Both unilateral and bilateral ureteral ligation were carried out according to the protocols established previously 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 for bilateral ureteral ligation or on the right side for unilateral ureteral ligation. Once the appropriate ureters had been exposed with careful dissection, a sterile 2-0 silk suture was tied around the 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. In control rats, ureters were not ligated.

Experimental protocol. In order to study basolateral transport of cadmium 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-mmol/kg intravenous dose of mannitol (in 2 ml/kg normal saline) was administered to each animal five minutes prior to performing bilateral ureteral ligation (or sham-surgery). Mannitol was administered to promote the reduction of glomerular filtration by increasing the intraluminal osmotic pressure sufficiently to prevent any appreciable transport 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 into the tubular lumen and fluid is reabsorbed. Following ureteral ligation, the intraluminal hydrostatic pressure and osmotic pressure increase to a level where negligible reabsorption of water and solutes occurs and glomerular filtration is reduced to negligible levels.

It was estimated, 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, cadmium (with or without L-cysteine or GSH) was administered (in 2 ml/kg normal saline) into the right femoral vein while the rat was anesthetized with ether 75 min after surgery. 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 cadmium $^{109}Cd^{2+}$ (specific activity = 1.74 mCi/mg, NEN, Boston, MA) was added to the injection solution at a concentration to deliver approximately 3 μCi $^{109}Cd^{2+}$/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 cadmium. 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 cadmium.

Acquisition and handling of tissues and organs. At the end of an experiment, a 2-ml sample of blood was obtained from the inferior vena cava from each animal after it had been anesthetized deeply (as determined by corneal and tail reflexes) with a 100-mg/kg dose of sodium pentobarbital. One milliliter of this sample of blood was placed and sealed in a pre-weighed 12 × 75 mm polystyrene round-bottom tube for gamma-counting. The other 1 ml was spun down for 10 min at 20,000*g* 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 sample of blood had been drawn, the kidneys and liver were excised. Once removed from the body, the kidneys were cleared of fat and connective tissue and weighed. Each kidney 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. An approximate 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 cadmium 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 Compugamma CS deep-well gamma spectrometer with a 3″ sodium iodide crystal (Wallac, Gaithersburg, MD). The content of cadmium 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 ± SE for an *n* of 3–5. 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 analysis. This transformation takes the arcsine of the square root of the decimal fraction of the percent score.

In all three studies, 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 $p < 0.05$.

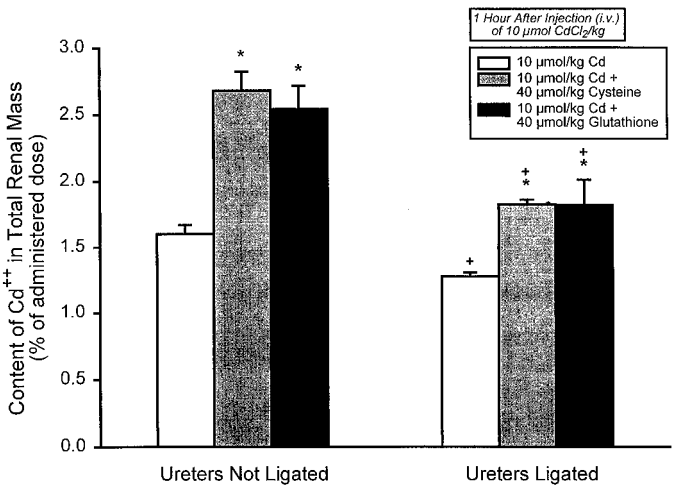

FIG. 1. Content of cadmium (percent of the administered dose) in the total renal mass 1 h after the intravenous administration of 10 μmol cadmium/kg with or without 40 μmol cysteine or glutathione/kg in rats that had, or had not, undergone an acute ureteral ligation prior to the administration of cadmium. Each value represents the mean ± SE obtained from four or five animals. *Significantly different ($p < 0.05$) from the mean for the corresponding control group (treated surgically in the same manner) that was administered 10 μmol cadmium/kg in the form of cadmium chloride. †Significantly different ($p < 0.05$) from the mean for the corresponding group that did not undergo acute ureteral ligation but was administered the same form of cadmium ($CdCl_2$, Cd + cysteine, or Cd + glutathione). The abbreviation of cadmium as Cd^{++}, in the *y* axis label, is used to designate the oxidation state, and not the valence, of the metal.

RESULTS

Study 1

Renal burden of cadmium. The average amount of cadmium in the total renal mass of the control sham-animals that received 10 μmol cadmium/kg iv in the form of cadmium chloride was 1.60% of the administered dose 1 h after the cadmium had been administered (Fig. 1). In the animals coadministered this same dose of cadmium along with a four times greater number of moles of cysteine or GSH, the amount of cadmium in the total renal mass was 68 or 59% greater, respectively, than that in the corresponding control sham-animals.

In the animals that underwent acute bilateral ureteral ligation and received 10 μmol cadmium/kg as cadmium chloride, the amount of cadmium in the total renal mass averaged 1.28% of the administered dose (Fig. 1). This amount is only about 20% less than that in the total renal mass of the corresponding group of rats that did not undergo acute ureteral ligation. The total renal burden of cadmium in the rats that had undergone acute bilateral ureteral ligation and were coadministered 10 μmol cadmium/kg along with 40 μmol cysteine or GSH/kg was 42% greater than that in the corresponding rats that had undergone acute bilateral ureteral ligation and that were treated with just cadmium chloride.

Between the corresponding paired groups of rats that were administered cadmium with cysteine or GSH, approximately 32 and 28% less cadmium, respectively, was found in the total renal mass of the rats that had undergone acute bilateral ureteral ligation than in the rats that had not undergone acute bilateral ureteral ligation (Fig. 1).

Intrarenal distribution of cadmium. Concentrations of cadmium in the renal cortex of the control sham-animals injected with cadmium chloride averaged 1.61% of the administered dose per gram tissue (Fig. 2A). In the corresponding control rats coadministered cadmium with cysteine or GSH, the mean concentration of cadmium in the renal cortex was 2.67 and 2.38% of the dose per gram tissue, respectively.

The concentration of cadmium in the renal cortex of the rats that had undergone acute bilateral ureteral ligation and that received cadmium without cysteine or GSH was approximately 40% less than that in the corresponding rats that did not undergo ureteral ligation (Fig. 2A). In the rats that underwent acute bilateral ureteral ligation and were administered cadmium with cysteine or GSH, the concentration of cadmium in the renal cortex was 50 and 55% less, respectively, than in the corresponding paired group of rats that did not undergo ureteral

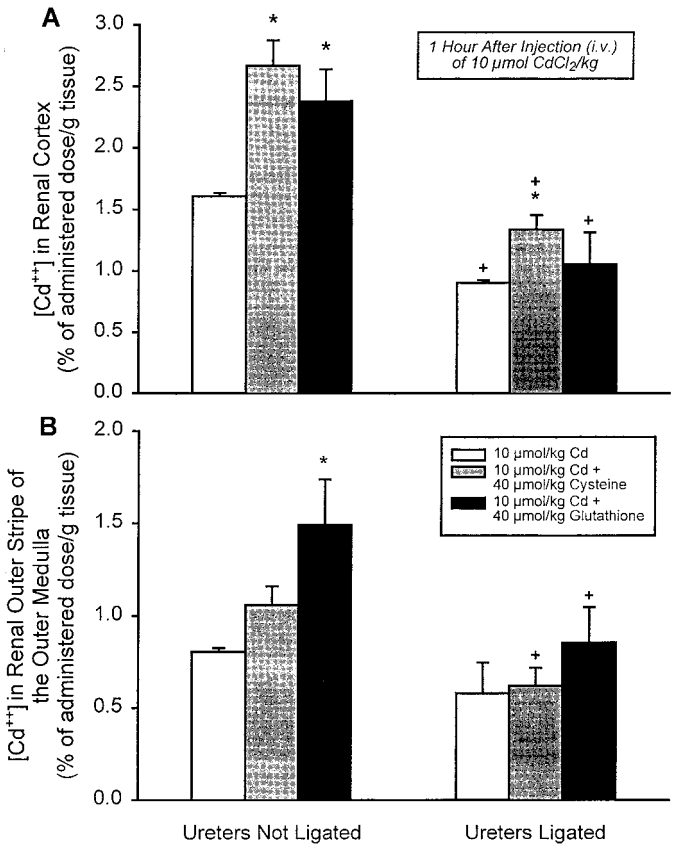

FIG. 2. Concentration of cadmium (percent of the administered dose per gram tissue) in the renal cortex (A) and outer stripe of the outer medulla (B) 1 h after the intravenous administration of 10 μmol cadmium/kg with or without 40 μmol cysteine or glutathione/kg in rats that had, or had not, undergone an acute ureteral ligation prior to the administration of cadmium. Each value represents the mean ± SE obtained from four or five animals. *Significantly different ($p < 0.05$) from the mean for the corresponding control group (treated surgically in the same manner) that was administered 10 μmol cadmium/kg in the form of cadmium chloride. †Significantly different ($p < 0.05$) from the mean for the corresponding group that did not undergo acute ureteral ligation but that was administered the same form of cadmium ($CdCl_2$, Cd + cysteine, or Cd + glutathione). The abbreviation of cadmium as Cd^{++}, in the *y* axis label, is used to designate the oxidation state, and not the valence, of the metal.

ligation but that was treated in the same manner. Among the three groups of rats that underwent bilateral ureteral ligation, the only significant difference in the concentration of cadmium in the renal cortex was detected between the group treated with cadmium plus cysteine and the group treated with cadmium chloride.

In the outer stripe of the outer medulla of the control sham-animals, the concentration of cadmium was approximately 0.8% of the dose per gram tissue (Fig. 2B). Only in the corresponding group of rats treated with cadmium and GSH was the concentration of cadmium in the renal outer stripe of the outer medulla significantly different from that in the control rats. It was approximately 84% greater than that in the control rats.

There was no statistically significant difference in the concentration of cadmium in the outer stripe of the outer medulla between the two groups of rats treated with the 10 μmol/kg dose of cadmium chloride (Fig. 2B). By contrast, there were significant differences in the concentration of cadmium in the outer stripe of the outer medulla between the two groups treated with cadmium and cysteine and the two groups treated with cadmium plus GSH. In the groups treated with cadmium plus cysteine or GSH, the concentration of cadmium in the outer stripe of the outer medulla in the rats that underwent bilateral ureteral ligation was 42% less than that in the corresponding group that did not undergo ureteral ligation.

Hepatic burden of cadmium. There were no statistically significant differences in the hepatic burden of cadmium among the six groups of rats used in study 1. Approximately one half of the administered dose of cadmium was present in the liver 1 h following the administration of cadmium.

Disposition of cadmium in the blood. In all six groups of rats in study 1, less than 1% of the administered dose of cadmium was present in the estimated total blood volume. There were no significant differences in the estimated amount of cadmium in blood among the six groups of rats in this study.

Significant differences in the distribution of cadmium in blood, however, were detected among the six groups of rats in this study (Fig. 3). Among the three groups of rats that did, or did not, undergo bilateral ureteral ligation, the fraction of cadmium present in the plasma was significantly greater in the rats coadministered cadmium with cysteine or GSH than in the corresponding rats treated with just cadmium chloride.

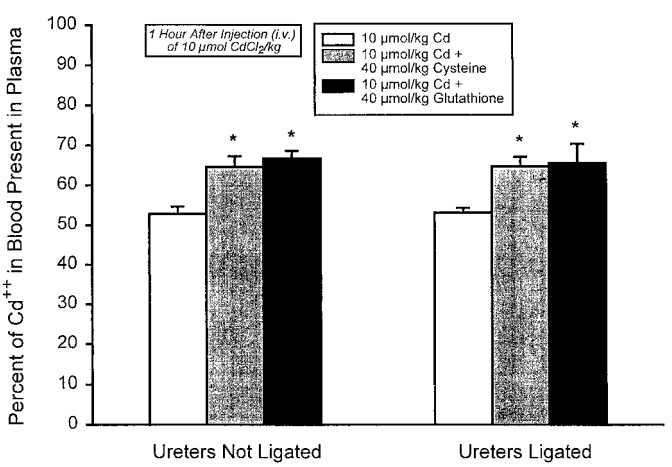

FIG. 3. Percent of the cadmium in blood present in the plasma 1 h after the intravenous administration of 10 μmol cadmium/kg with or without 40 μmol cysteine or glutathione/kg in rats that had, or had not, undergone an acute ureteral ligation prior to the administration of cadmium. Each value represents the mean ± SE obtained from four or five animals. *Significantly different ($p < 0.05$) from the mean for the corresponding control group (treated surgically in the same manner) that was administered 10 μmol cadmium/kg in the form of cadmium chloride. The abbreviation of cadmium as Cd^{++}, in the *y* axis label, is used to designate the oxidation state, and not the valence, of the metal.

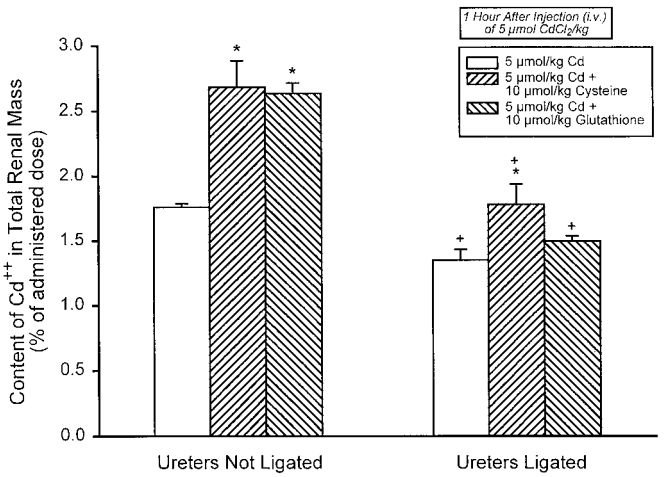

FIG. 4. Content of cadmium (percent of the administered dose) in the total renal mass 1 h after the intravenous administration of 5 μmol cadmium/kg with or without 10 μmol cysteine or glutathione/kg in rats that had, or had not, undergone an acute ureteral ligation prior to the administration of cadmium. Each value represents the mean ± SE obtained from four or five animals. *Significantly different ($p < 0.05$) from the mean for the corresponding control group (treated surgically in the same manner) that was administered 5 μmol cadmium/kg in the form of cadmium chloride. †Significantly different ($p < 0.05$) from the mean for the corresponding group that did not undergo acute ureteral ligation but that was administered the same form of cadmium ($CdCl_2$, Cd + cysteine, or Cd + glutathione). The abbreviation of cadmium as Cd^{++}, in the *y* axis label, is used to designate the oxidation state, and not the valence, of the metal.

Study 2

The findings for the renal burden of cadmium (Fig. 4), the intrarenal distribution of cadmium (Fig. 5A and 5B), the hepatic burden of cadmium (Fig. 6), and the disposition of cadmium in the blood and plasma (Figs. 7 and 8) in the animals treated with 5.0 μmol cadmium/kg with or without 10 μmol GSH or cysteine/kg were similar to the corresponding findings obtained in study 1 (refer to appropriate figures for details).

Study 3

Renal disposition of cadmium. In the rats that underwent unilateral ureteral ligation and were treated with 10 μmol cadmium/kg, the content of cadmium in the left kidney (whose ureter had not been ligated) averaged 0.93% of the administered dose 1 h after the injection of cadmium (Fig. 9). The content of cadmium in the left kidney of the rats treated with cadmium and cysteine or GSH was 88 or 118% greater, respectively, than that in the control rats treated with cadmium chloride.

The content of cadmium in the right kidney (whose ureter had been ligated) of the control rats treated with cadmium chloride was approximately 31% less than that in the left kidney. Moreover, in the rats treated with cadmium plus cysteine or GSH, the content of cadmium in the right kidney was 26 or 43% less, respectively, than that in the left kidney. Furthermore, the content of cadmium in the right kidney of the rats treated with cadmium plus cysteine or GSH was approximately 102 or 80% greater, respectively, than that in the right kidney of the rats treated with cadmium chloride.

DISCUSSION

Data from all three sets of experiments in the present study indicate clearly that intravenous pretreatment with mannitol combined with either acute unilateral or bilateral ureteral ligation causes the net renal accumulation of cadmium to be reduced significantly during the initial hour after the administration of a 5- or 10-μmol/kg dose of cadmium in the form of cadmium chloride. On the surface, this would seem to be an expected result, especially in light of the prevailing notion that

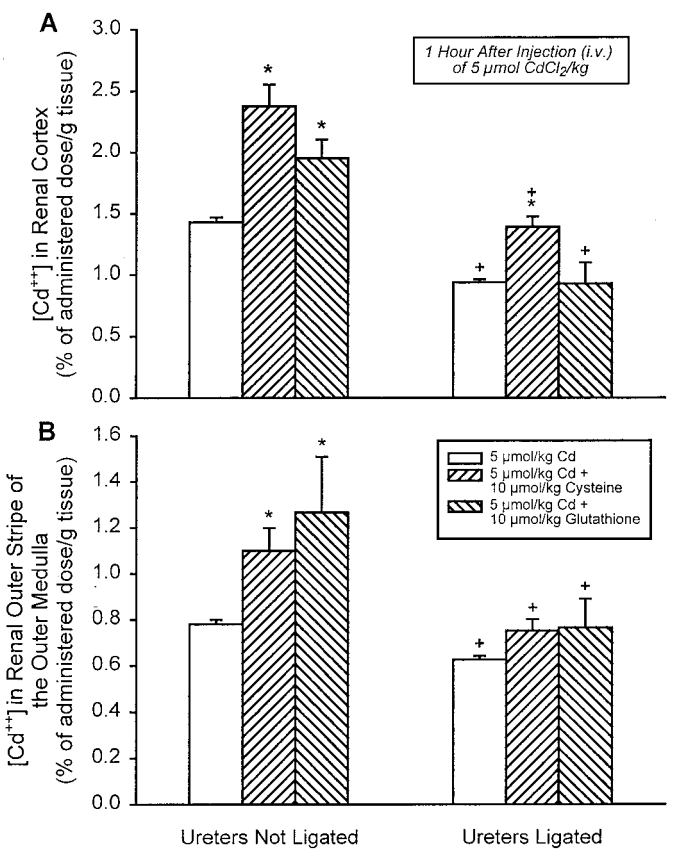

FIG. 5. Concentration of cadmium (percent of the administered dose per gram tissue) in the renal cortex (A) and outer stripe of the outer medulla (B) 1 h after the intravenous administration of 5 μmol cadmium/kg with or without 10 μmol cysteine or glutathione/kg in rats that had, or had not, undergone an acute ureteral ligation prior to the administration of cadmium. Each value represents the mean ± SE obtained from four to five animals. *Significantly different ($p < 0.05$) from the mean for the corresponding control group (treated surgically in the same manner) that was administered 5 μmol cadmium/kg in the form of cadmium chloride. †Significantly different ($p < 0.05$) from the mean for the corresponding group that did not undergo acute ureteral ligation but that was administered the same form of cadmium ($CdCl_2$, Cd + cysteine, or Cd + glutathione). The abbreviation of cadmium as Cd^{++}, in the *y* axis label, is used to designate the oxidation state, and not the valence, of the metal.

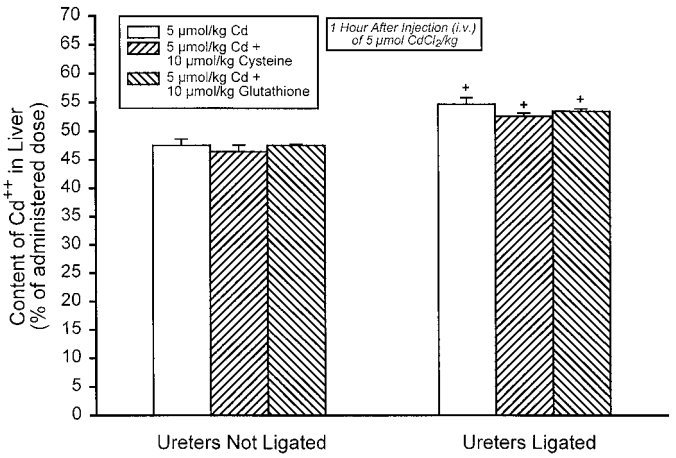

FIG. 6. Content of the cadmium (percent of the administered dose) in the liver 1 h after the intravenous administration of 5 μmol cadmium/kg with or without 10 μmol cysteine or glutathione/kg in rats that had, or had not, undergone an acute ureteral ligation prior to the administration of cadmium. Each value represents the mean ± SE obtained from four to five animals. †Significantly different ($p < 0.05$) from the mean for the corresponding group that did not undergo acute ureteral ligation but that was administered the same form of cadmium ($CdCl_2$, Cd + cysteine, or Cd + glutathione). The abbreviation of cadmium as Cd^{++}, in the *y* axis label, is used to designate the oxidation state, and not the valence, of the metal.

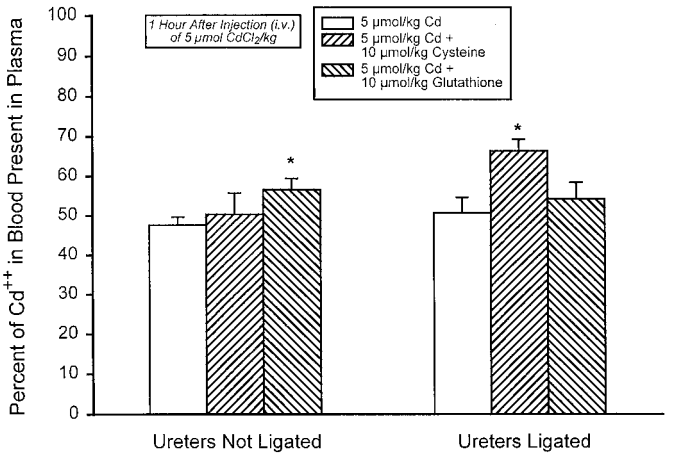

FIG. 8. Percent of the cadmium in blood present in the plasma 1 h after the intravenous administration of 5 μmol cadmium/kg with or without 10 μmol cysteine or glutathione/kg in rats that had, or had not, undergone an acute ureteral ligation prior to the administration of cadmium. Each value represents the mean ± SE obtained from four to five animals. *Significantly different ($p < 0.05$) from the mean for the corresponding control group (treated surgically in the same manner) that was administered 5 μmol cadmium/kg in the form of cadmium chloride. The abbreviation of cadmium as Cd^{++}, in the *y* axis label, is used to designate the oxidation state, and not the valence, of the metal.

a major route by which cadmium gains entry into renal tubular epithelial cells is via a luminal mechanism (Robinson *et al.*, 1993; Felley-Bosco and Diezi, 1987, 1989; and reviewed in Diamond and Zalups, 1998). What is surprising, however, is that the net accumulation of cadmium in the kidneys whose ureters had been ligated decreased by only 20 to 30% of the normal burden of cadmium detected in the absence of ureteral ligation. These findings have a number of important implica-

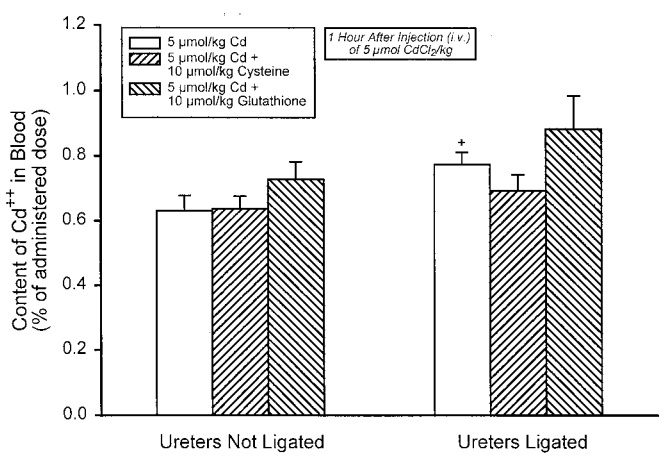

FIG. 7. Estimated content of the cadmium (percent of the administered dose) in the total volume of blood 1 h after the intravenous administration of 5 μmol cadmium/kg with or without 10 μmol cysteine or glutathione/kg in rats that had, or had not, undergone an acute ureteral ligation prior to the administration of cadmium. Each value represents the mean ± SE obtained from four to five animals. †Significantly different ($p < 0.05$) from the mean for the corresponding group that did not undergo acute ureteral ligation but that was administered the same form of cadmium ($CdCl_2$, Cd + cysteine, or Cd + glutathione). The abbreviation of cadmium as Cd^{++}, in the *y* axis label, is used to designate the oxidation state, and not the valence, of the metal.

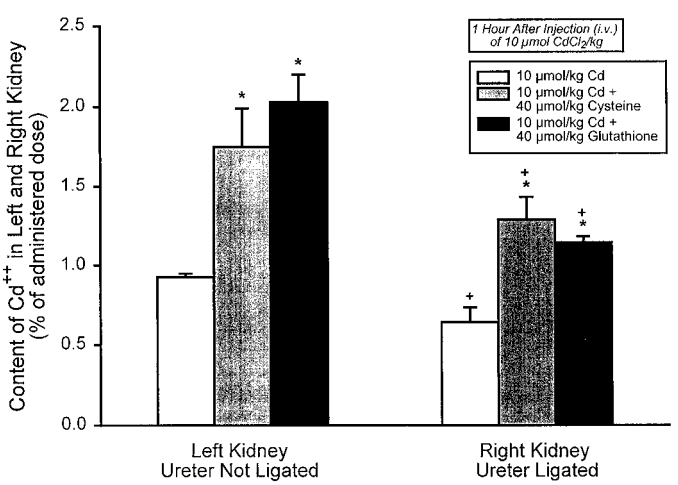

FIG. 9. Content of cadmium (percent of the administered dose) in the left and rights kidneys 1 h after the intravenous administration of 10 μmol cadmium/kg with or without 40 μmol cysteine or glutathione/kg in rats that had undergone an acute right-sided ureteral ligation prior to the administration of cadmium. Each value represents the mean ± SE obtained from four or five animals. *Significantly different ($p < 0.05$) from the mean for the corresponding control group (treated surgically in the same manner) that was administered 10 μmol cadmium/kg in the form of cadmium chloride. †Significantly different ($p < 0.05$) from the corresponding mean obtained from the left kidney of the same animal. The abbreviation of cadmium as Cd^{++}, in the *y* axis label, is used to designate the oxidation state, and not the valence, of the metal.

tions (assuming that glomerular filtration rate had been reduced to negligible levels and that there was minimal to no paracellular leak of cadmium into the lumen following ureteral ligation). First and foremost, the findings indicate that between 70 and 80% of the acute renal uptake and accumulation of cadmium following exposure to a low dose of cadmium chloride likely occurs as a result of a basolateral mechanism. Second, inasmuch as significant effects of ureteral ligation on the renal disposition of cadmium were demonstrated in both the cortex and outer stripe of the outer medulla, it is likely that at least some of the apparent basolateral uptake of cadmium occurs along the entire proximal tubule. Third, the present findings argue against luminal endocytosis of hepatically synthesized cadmium-MT being the predominant mechanism involved in the proximal tubular uptake of cadmium (at least following acute exposure to low doses of cadmium), which has been a proposed mechanism involved in the renal tubular uptake of cadmium (reviewed by Diamond and Zalups, 1998). It is inconceivable that significant amounts of cadmium-MT could have been synthesized and released into the blood from hepatocytes during the 1 h of the present study, especially in the animals given the 5-μmol/kg dose of cadmium. This dose of cadmium has not been shown to induce acute hepatocellular injury in rats (R. H. Zalups, unpublished findings). Also, since the endocytotic machinery involved in the uptake of proteins along the nephron is localized predominantly at the apical pole of proximal tubular epithelial cells, the data also argue against an endocytotic mechanism being involved in the basolateral uptake of cadmium.

It should be mentioned that the data in the present study do not exclude the possibility that some fraction of cadmium may gain entry into proximal tubular epithelial cells as a result of endocytosis of cadmium-MT in situations where chronic exposure to cadmium leads to hepatic and renal injury. Taking the findings from the present study into account, the renal uptake and disposition of cadmium under conditions where chronic exposure of cadmium leads to renal (and hepatic) injury are likely complex, involving multiple factors and mechanisms.

The data from the present investigations apparently serve as the first real substantive line of *in vivo* evidence implicating a basolateral mechanism in the renal tubular uptake of cadmium in the rat. A potential basolateral mechanism in the renal uptake of cadmium had been suggested by Foulkes (1974), based on indirect findings from single-pass experiments in the rabbit. The potential for a basolateral mechanism in the renal tubular uptake of cadmium had also been suggested by Diamond and colleagues (1986), who demonstrated that occlusion of the ureter did not decrease significantly the net accumulation of cadmium in isolated kidneys (from rats) perfused with 1 μM cadmium chloride in a protein-free buffer. Although the conditions in the experiments utilizing the isolated perfused kidney do not reflect conditions found *in vivo*, the data collected most closely resemble the findings obtained in the present study.

In a more recent study utilizing LLC-PK_1 cells (which are derived from porcine proximal tubular cells) in trans-well cultures, Liu and colleagues (1994) demonstrated significant association of cadmium with these cells when they were exposed to cadmium, in the form of $CdCl_2$ or cadmium-MT, on their basal surface. A number of other studies have also examined basolateral uptake and/or binding of cadmium *in vitro* using LLC-PK_1 (Bruggeman *et al.*, 1992; Prozialeck *et al.*, 1993; Prozialeck and Lamar, 1993; Kimura *et al.*, 1996). Findings from some of these studies indicate that basolateral uptake of cadmium in LLC-PK_1 cells grown on a permeable membrane insert is greater than the luminal uptake of cadmium (Bruggeman *et al.*, 1992; Prozialeck *et al.*, 1993; Prozialeck and Lamar, 1993). Unfortunately, the findings from some of these studies are clouded by documentation of cellular intoxication induced by the cadmium (Prozialeck *et al.*, 1993; Prozialeck and Lamar, 1993). Although the aforementioned findings tend to indicate that cadmium can bind to, and/or be taken up into, cultured proximal tubular epithelial cells at both luminal and basolateral membranes, it is not clear whether the findings reflect the manner by which cadmium is taken up at the basolateral membrane *in vivo*, particularly in light of the current findings obtained with the use of GSH and cysteine.

When 5 or 10 μmol cadmium/kg was administered intravenously, to rats in the present study, in a 1:2 or 1:4 ratio with either cysteine or GSH, the net renal accumulation of cadmium increased by at least 50% relative to that detected in animals given cadmium as cadmium chloride. The magnitude of the response appears to be governed in part on the dose of cadmium and the ratio of thiol to cadmium administered. Conjugation of cysteine or GSH with cadmium prior to the injection of cadmium appears to have provided more of a transportable form of cadmium to the renal tubular epithelium during the initial hour after the injection of cadmium. Moreover, data obtained from rats that had undergone acute ureteral ligation indicate that the predominant fraction of the cadmium that accumulates in the kidneys when the cadmium is coadministered with cysteine of GSH comes from basolateral uptake. The precise mechanism(s) that is/are responsible for the basolateral uptake of cadmium along the nephron (with presumably most of it occurring along the proximal tubule) under conditions generated when cadmium is administered as cadmium chloride or a conjugate of cysteine of GSH are not known. Possible transporters that could be involved include, but are not limited to, the organic anion transport system and basolateral amino acid transporters.

Whether cadmium is actually transported into renal epithelial cells as a conjugate of cysteine and/or GSH *in vivo*, the current data indicate rather convincingly that the presence of an excess amount of the small thiols cysteine or GSH in the presence of cadmium prior to exposure to cadmium promotes the renal uptake of cadmium. Moreover, since the patterns for the effects of ureteral ligation on the disposition of cadmium in the renal cortex and outer stripe of the outer medulla were somewhat similar among the three treatment groups of rats, these data tend to support the notion that addition of cadmium

thiol complexes provide an increased amount of an actual transportable species of cadmium that is formed *in vivo.* Alternate mechanisms may also be involved in the increased renal tubular uptake and/or accumulation of cadmium when the metal is coadministered with cysteine or GSH.

When considering the notion of cadmium being transported into renal epithelial cells as a conjugate of cysteine or GSH, it should be brought to light that there is a significant body evidence indicating that inorganic mercury (another group IIB metal) is taken up by renal tubular epithelial cells (specifically those lining the proximal tubule) in the form of mercuric–thiol conjugates at both the luminal and basolateral mechanisms (Zalups, 1995; Zalups and Barfuss, 1995a, 1998a; Zalups and Minor, 1995). On the basolateral side, there is a very strong evidence implicating the activity of the organic anion and dicarboxylic acid transport systems in the uptake of inorganic mercury (Zalups and Barfuss, 1995a, 1998a; Zalups and Minor, 1995). On the luminal side, current evidence is implicating at least two amino acid transporters in the uptake and transport of inorganic mercury, particularly in the form of a mercuric conjugate of cysteine (Zalups, 1995). Recent unpublished findings from our laboratory indicate that one of the transporters is sodium-dependent and the other sodium-independent.

Coadministration of inorganic mercury with a fourfold greater amount of cysteine or GSH has also been shown to cause significant increases the renal uptake and net accumulation of mercury 1 h after treatment in both normal rats and rats that have undergone an acute bilateral ureteral ligation prior to the injection of mercury (Zalups, 1998; Zalups and Barfuss, 1995b,c, 1998b). The similarities in the effects of coadministering cysteine or GSH with cadmium or inorganic mercury on the renal disposition of the respective metal lead one to suggest that perhaps there are potential common mechanisms involved in the renal tubular uptake of cadmium and inorganic mercury, despite the fact that the overall disposition (especially the renal disposition) of injected cadmium is greatly different from that of injected inorganic mercury.

As mentioned above, both cadmium and mercury are group IIB metals and both have a strong affinity for sulfhydryl groups, especially small molecules such as cysteine, GSH, and MT. On the basis of these similarities and those mentioned in the paragraph above, it is not unreasonable to hypothesize that one or more of the mechanisms participating in the renal tubular uptake of cadmium involves either the transport of thiol–cadmium complex (such as a cysteine or GSH conjugate of cadmium) or the interaction of cadmium–thiol complex at the luminal and/or basolateral plasma membrane to release cadmium to be taken up by another mechanism, such as a calcium channel. It should be stressed that a significant amount of research is required to test this hypothesis. In addition, the basic premise of this hypothesis does not preclude the potential for there being some separate independent mechanisms involved in the renal uptake and accumulation of cadmium that are dissimilar from those in the renal uptake and accumulation of inorganic mercury. This is probably the case, especially under conditions generated during chronic exposure to cadmium that lead to the induction of hepatic and renal cellular injury.

In summary, the findings from the present study provide substantive evidence for a basolateral mechanism being involved in the *in vivo* renal tubular uptake and accumulation of cadmium. Moreover, the findings indicate that the renal uptake and accumulation of cadmium are increased significantly when cadmium is coadministered with cysteine or GSH. It is likely that coadministration of cadmium with cysteine or GSH provides an increased amount of a transportable form of cadmium to the renal tubular epithelial cells that take up and accumulate cadmium, namely proximal tubular epithelial cells.

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