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Influence of Exogenous Thiols on Inorganic Mercury-Induced Injury in Renal Proximal and Distal Tubular Cells from Normal and Uninephrectomized Rats[1]

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ABSTRACT

Inorganic mercury (Hg^{2+}) induced time- and concentration-dependent cellular injury in freshly isolated proximal tubular (PT) and distal tubular (DT) cells from normal (control) rats or uninephrectomized (NPX) rats. PT cells from NPX rats were more susceptible than PT cells from control rats, and DT cells were slightly more susceptible than PT cells to cellular injury induced by Hg^{2+} (not bound to a thiol). Preloading cells with glutathione increased Hg^{2+}-induced cellular injury in PT cells from control rats. However, coincubation of PT or DT cells from control or NPX rats with Hg^{2+} and glutathione (1:4) provided significant protection relative to incubations with Hg^{2+} alone. No support was obtained for a role for γ-glutamyltransferase in glutathione-dependent protection. However, the organic anion carrier does appear to play a role in accumulation and toxicity of mercuric conjugates of cysteine in PT cells from control, but not NPX, rats. Coincubation with Hg^{2+} and cysteine (1:4) had little effect on, or slightly enhanced, Hg^{2+}-induced cellular injury at low concentrations of Hg^{2+} in all cells studied. Coincubation with Hg^{2+} and albumin (1:4) markedly protected PT and DT cells from control and NPX rats at all concentrations except the highest concentration of Hg^{2+} in DT cells from NPX rats. 2,3-Dimercapto-1-propanesulfonic acid protected cells both when preloaded or added simultaneously with Hg^{2+}. Thus, renal cells from NPX rats are more susceptible to Hg^{2+}-induced injury, PT and DT cells respond differently to exposure to Hg^{2+}, and thiols can significantly modulate the toxic response to Hg^{2+}.

The kidneys are the primary organs that accumulate inorganic mercury (Hg^{2+}) and exhibit toxicity after in vivo exposures to Hg^{2+} (Zalups, 1993a). Hg^{2+} accumulates selectively along the three segments of the proximal tubule (Zalups, 1991a,b). Freshly isolated proximal tubular (PT) and distal tubular (DT) cells (a nontarget renal cell population) from rats accumulate comparable levels of Hg^{2+} after exposure to $HgCl_2$ (Lash et al., 1998). Hence, although PT cells are the primary in vivo target cells that accumulate, and are intoxicated by, Hg^{2+}, other renal cell populations can accumulate Hg^{2+} in an in vitro model.

Protein and nonprotein thiols bind Hg^{2+} with high affinity and are the major extracellular and intracellular ligands for Hg^{2+} (Zalups and Lash, 1994). Alterations in the cellular content of thiols, particularly glutathione (GSH), modulate the intracellular uptake and accumulation of Hg^{2+} (Berndt et al., 1985; Baggett and Berndt, 1986; Girardi and Elias, 1993; Burton et al., 1995; Zalups and Lash, 1997a). Furthermore, coadministration of GSH or L-cysteine (Cys) with Hg^{2+} both in vivo (de Ceaurriz et al., 1994; Zalups and Barfuss, 1995a,b; Zalups, 1998) and in vitro (Lash et al., 1998; Zalups and Barfuss, 1998a) alters the rate of renal tubular uptake and accumulation of Hg^{2+}. Data are consistent with mercuric conjugates of GSH and/or Cys being physiological transport forms of Hg^{2+} (Tanaka et al., 1990; Zalups, 1995, 1998; Zalups and Barfuss, 1995a,b, 1998b; Zalups and Minor, 1995; Zalups and Lash, 1997b). Luminal transport of mercuric conjugates of GSH appears to require activity of renal γ-glutamyltransferase (GGT; Tanaka et al., 1990; Zalups and Barfuss, 1995a; Zalups and Minor, 1995; Zalups and Lash, 1997a,b; Lash et al., 1998). Mercuric conjugates of Cys may thus be the primary luminal transport form because mercuric conjugates of GSH are likely processed to the corresponding Cys conjugates before the uptake of Hg^{2+}. The organic anion transporter on the basolateral membrane and Na^+-dependent and -independent amino acid transporters on the luminal membrane are involved in the uptake of mercuric

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ABBREVIATIONS: Hg^{2+}, inorganic mercury; PT, proximal tubular; acivicin, L-(αS,5S)-α-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid; Cys, L-cysteine; DMPS, 2,3-dimercapto-1-propanesulfonic acid; DT, distal tubular; GGT, γ-glutamyltransferase; GSH, glutathione; LDH, lactate dehydrogenase; NPX, uninephrectomized; PAH, *p*-aminohippurate.

conjugates of Cys (Zalups, 1995, 1998; Zalups and Barfuss, 1995a,b, 1998a,b; Zalups and Minor, 1995; Zalups and Lash, 1997b; Lash et al., 1998; V. T. Cannon, D. W. Barfuss, and R.K.Z., unpublished observations).

In vivo administration of, or in vitro exposure to, GSH (Lash and Zalups, 1992; de Ceaurriz et al., 1994; Burton et al., 1995), Cys (Lash and Zalups, 1992; Zalups and Barfuss, 1996), serum albumin (Lash and Zalups, 1992), or 2,3-dimercapto-1-propanesulfonic acid (DMPS; Zalups et al., 1991, 1998; Lash and Zalups, 1992; Zalups, 1993b) protects PT cells from the toxic effects induced by Hg^{2+}. Although serum albumin and DMPS virtually completely protect from the toxic effects induced by Hg^{2+} (which correlates with their ability to nearly completely inhibit renal cellular uptake and accumulation of Hg^{2+}), GSH and Cys protect to varying degrees (which appears to correlate with their influence on renal cellular uptake and accumulation of Hg^{2+}). Moreover, at relatively low concentrations, coincubation in vitro with Cys stimulates renal cellular uptake and accumulation of Hg^{2+} and coincubation in vitro with GSH inhibits renal cellular uptake and accumulation of Hg^{2+} to a much lesser degree than coincubation with serum albumin or DMPS (Zalups and Barfuss, 1995a, 1998a,b; Zalups and Lash, 1997b; Lash et al., 1998; Zalups, 1998).

Compensatory renal growth, which occurs after reductions in renal mass, is another major factor that influences the disposition and nephrotoxicity of Hg^{2+}. Remnant renal tissue undergoes rapid changes after uninephrectomy, and the acute hemodynamic, functional, and biochemical effects in rodents are nearly complete within 7 to 10 days after surgery (Fine, 1986). These changes include increased renal cellular synthesis and content of GSH and metallothionein (Zalups and Lash, 1990; Zalups and Lash, 1994), increased activities of several GSH-dependent enzymes (Lash and Zalups, 1994), and increased renal cellular uptake and accumulation of Hg^{2+} (Zalups et al., 1987; Zalups and Diamond, 1987; Zalups and Lash, 1990; Zalups, 1997a) compared with kidneys or renal tissue from control animals. The nephropathy and the in vitro cytotoxicity induced by Hg^{2+} are also increased (Houser and Berndt, 1986; Lash and Zalups, 1992; Zalups, 1997b).

In the present study, we used freshly isolated PT and DT cells from control and uninephrectomized (NPX) rats to determine: 1) the dose and time dependence of Hg^{2+}-induced renal cellular injury, 2) the influence of albumin and low-molecular-weight thiols on Hg^{2+}-induced renal cellular injury, and 3) the influence of modulating GSH-conjugate metabolism and the organic anion transporter in the renal cellular injury induced by mercuric conjugates of GSH or Cys.

Experimental Procedures

Materials. Percoll, collagenase (type I), BSA (fraction V), acivicin L-(α*S*,5*S*)-α-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid], *p*-aminohippurate (PAH), and DMPS were purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals were of the highest purity available and were purchased from commercial sources.

Animals and Surgical Procedures. Male Sprague-Dawley rats (175–200 g at time of surgery; Harlan Sprague-Dawley, Indianapolis, IN) were used in the present study. Animals were housed in the Wayne State University vivarium, were allowed access to laboratory chow and water ad libitum, and were kept in a room on a 12-h light/dark cycle. The rats were divided into two surgical groups: one that underwent uninephrectomy (NPX rats) and nonsurgical control rats. Animals were anesthetized with i.p. injections of sodium pentobarbital (50 mg/kg b.wt.) before surgery. Uninephectomies were performed by removal of the right kidney as described previously (Zalups and Lash, 1990). Previous studies show that there is no difference in cells isolated from untreated or sham-operated rats; hence, nonsurgically treated rats were used as controls.

Isolation of Rat Renal PT and DT Cells. Renal cortical cells were isolated by collagenase perfusion, and enriched populations of PT and DT cells were then obtained by Percoll density-gradient centrifugation (Lash and Tokarz, 1989). Briefly, cortical cells (5 ml, $5–8 \times 10^6$ cells/ml) were layered on 35 ml of 45% (v/v) isosmotic Percoll solution in 50-ml polycarbonate centrifuge tubes and were centrifuged at 4°C for 30 min at 20,000*g* in a Sorvall RC2B centrifuge in an SS34 rotor. Fractions were collected, and cell types of origin were identified by the use of marker enzymes and cell type-specific respiratory responses (Lash and Tokarz, 1989). Based on enzymology and morphology, the renal PT cell preparation contains cells derived from both convoluted and straight segments and is estimated to be at least 97% pure; the renal DT cell preparation contains cells derived from the distal convoluted tubule, the cortical collecting duct, and connecting tubules, but not the thick ascending limbs, and is estimated to have less than 10% contamination from PT cells, with a purity of approximately 88%.

Experimental Design. Before incubations, cells were diluted 5-fold with Krebs-Henseleit buffer, pH 7.4, containing 25 mM HEPES and metabolic substrates (5 mM glucose, 5 mM glutamine), washed to remove Percoll, and then resuspended in fresh buffer at a concentration of 1.2×10^6 cells/ml. Cell concentrations were determined in the presence of 0.2% (w/v) trypan blue using a hemacytometer, and cell viability was estimated by either trypan blue exclusion or by the release of lactate dehydrogenase (LDH) activity from the cells (Lash and Tokarz, 1989). All buffers were equilibrated with 95% O_2/5% CO_2, and cells were stored on ice in 25-ml polyethylene Erlenmeyer flasks until used. Incubations were performed in 25-ml polyethylene Erlenmeyer flasks in a 37°C Dubnoff metabolic shaking incubator (60 cycles/min) under an atmosphere of 95% O_2/5% CO_2.

Incubation of isolated cells with Hg^{2+} in the absence of thiol ligands represents nonphysiological conditions because renal cells in the intact kidney are never exposed to free Hg^{2+} ions. Examination of Hg^{2+}-induced cellular injury under such conditions, however, is important because it provides a baseline so the effects of thiol ligands can be studied.

To assess the effect of increases in intracellular content of GSH or DMPS, both PT and DT cells were preincubated with 5 mM GSH or 5 mM DMPS for 15 min before the addition of Hg^{2+}. Previous studies have shown that preincubation of renal PT cells with 5 mM GSH significantly increases intracellular content of GSH over this time course (Visarius et al., 1996). Free DMPS is transported into the renal proximal tubule by the basolateral organic anion carrier (Klotzbach and Diamond, 1988; Zalups et al., 1998), so the isolated cells should accumulate DMPS under these incubation conditions. The organic anion transporter is not present in distal cell populations. Thus, it is very unlikely that a highly polar molecule like DMPS is transported into any distal cell population. However, the DT cells were preincubated with DMPS to provide a parallel experimental design with measurements performed with PT cells.

To assess the influence of exogenous thiols on the severity of the cellular injury induced by Hg^{2+}, PT and DT cells were coincubated with 5, 10, or 100 μM Hg^{2+} and a 4-fold molar excess of either GSH, Cys, BSA, or DMPS. Hg^{2+} forms 1:2 linear II coordinate complexes with thiols (Rabenstein, 1989). A 4-fold molar excess of the thiols was used to ensure that all the Hg^{2+} was in the form of a mercuric-thiol conjugate consisting of two molecules of a thiol bound to a single mercuric ion. In two experiments, cells were coincubated with the indicated concentrations of Hg^{2+} and either 5 mM GSH or 5 mM

DMPS to parallel the preloading experiments with GSH and DMPS described above.

To assess the role of activity of the brush-border membrane enzyme GGT in the toxic effects of mercuric conjugates of GSH, cells were preincubated for 15 min with 0.25 mM acivicin to inhibit GGT activity before the addition of the mercuric conjugates of GSH. Acivicin would be expected to decrease toxicity if metabolism of the glutathionyl moiety is required for uptake and subsequent cytotoxicity of the mercuric conjugates of GSH. Pretreatment of PT cells with this concentration of acivicin inhibits more than 95% of GGT activity (Visarius et al., 1996).

To assess the role of the organic anion carrier in the cellular uptake and subsequent cytotoxicity of mercuric conjugates of Cys, cells were coincubated with 1 mM PAH and the mercuric conjugates of Cys. PAH, which is a substrate for the organic anion carrier, would be expected to decrease toxicity if function of this carrier is required for the uptake and subsequent cytotoxicity of mercuric conjugates of Cys.

Cytotoxicity Assay. Cell viability after incubations with Hg^{2+} or Hg^{2+} in the presence of various thiols could not be measured by LDH release from cells because of direct inhibition of LDH activity by Hg^{2+} (Lash and Zalups, 1992). Total LDH activity (extracellular plus intracellular), however, correlated with trypan blue uptake (Lash and Zalups, 1992) and was used to demonstrate and quantify toxicity due to Hg^{2+}.

Data Analysis. Results are expressed as mean ± S.E. values of measurements from the indicated number of separate cell preparations. Significant differences among selected mean values were first assessed by a one- or two-way ANOVA. When significant F values were obtained with ANOVA, the Fisher's protected least significant difference t test was performed to determine which mean values were significantly different from each other with two-tailed P values of $<.05$ considered significant.

Results

Time and Concentration Dependence of Hg^{2+}-Induced Cellular Injury. Time courses of total cellular LDH activity in PT and DT cells incubated with buffer or 0.5, 1, 10, or 100 μM Hg^{2+} confirmed previous results (Lash and Zalups, 1992) that in the absence of BSA in the extracellular buffer, PT cells from NPX rats exhibited greater susceptibility to Hg^{2+}-induced cellular injury and higher LDH activity than did PT cells from control rats (Fig. 1, A and B). Furthermore, in control PT cells, a threshold effect was observed such that no significant decrease in LDH activity was observed with Hg^{2+} concentrations of 10 μM and below, whereas 100 μM Hg^{2+} produced a 97% decrease in LDH activity after 2 h of incubation. In contrast, 10 μM Hg^{2+} produced significant decreases in LDH activity after 1 and 2 h of incubation in PT cells from NPX rats (19 and 42% decrease, respectively).

Similar to previous results (Lash and Zalups, 1994), DT cells exhibited higher LDH activity than PT cells. Hg^{2+}-induced cellular injury in DT cells from control rats (Fig. 1C) was greater than that in PT cells from control rats, with 10 μM Hg^{2+} producing significant decreases in LDH activity (18 and 31% decrease after 1 and 2 h of incubation, respectively). In contrast to results with PT cells, compensatory renal growth had no effect on Hg^{2+}-induced cellular injury in DT cells (Fig. 1D).

Modulation of Hg^{2+}-Induced Cellular Injury by GSH. To assess the role of GSH in Hg^{2+}-induced cytotoxicity, three experiments were performed, involving preloading of cells with GSH to increase the intracellular content of GSH, simultaneous incubation of cells with Hg^{2+} and GSH to form the mercuric conjugates of GSH, and alteration of the metabolism of the mercuric conjugates of GSH.

Preloading of PT cells from control rats with GSH significantly increased cellular injury induced by 10 μM Hg^{2+} (Fig. 2A). In contrast, PT cells from NPX rats (Fig. 2B) or DT cells from control (Fig. 2C) or NPX (Fig. 2D) rats were protected by preloading with GSH. Also note that in this set of experiments, 10 μM Hg^{2+} produced a small but statistically significant decrease in LDH activity in PT cells from control rats (Fig. 2A). This contrasts with results presented in Fig. 1A. However, in these experiments, the relative decrease in LDH activity was still smaller in PT cells from control rats than in PT cells from NPX rats (cf. Fig. 2A with Fig. 2B).

Simultaneous incubation of PT or DT cells from either control or NPX rats with 10 μM Hg^{2+} and 5 mM GSH protected cells from decreases in LDH activity in all cases (Fig. 3). Thus, although preloading of PT cells from control rats with 5 mM GSH enhanced Hg^{2+}-induced cellular injury (cf. Fig. 2A), simultaneous incubation of these cells with 5 mM GSH protected cells (Fig. 3A).

Simultaneous incubation of PT or DT cells from control or NPX rats with Hg^{2+} and a 4-fold molar excess of GSH provided virtually complete protection from Hg^{2+}-induced cytotoxicity at all Hg^{2+} concentrations and in both cell types and surgical groups, except at 100 μM Hg^{2+} in DT cells from NPX rats, where GSH was only partially protective (Fig. 4).

Pretreatment of PT cells with acivicin significantly reduced LDH inactivation in the absence of GSH in PT and DT cells from NPX rats but had no effects on cellular injury induced by mercuric conjugates of GSH (Fig. 5).

Modulation of Hg^{2+}-Induced Cellular Injury by Cys. Simultaneous incubation of PT cells from control rats with Hg^{2+} and a 4-fold molar excess of Cys had no effect on LDH inactivation at 5 and 10 μM Hg^{2+} but almost completely protected at 100 μM Hg^{2+} (Fig. 6A). In contrast, simultaneous incubation with Cys had no significant effect on Hg^{2+}-induced LDH inactivation at 5 μM Hg^{2+} but almost completely protected PT cells from NPX rats from Hg^{2+}-induced cellular injury at 10 and 100 μM Hg^{2+} (Fig. 6B). Simultaneous incubation of DT cells from control rats with Hg^{2+} and a 4-fold molar excess of Cys partially protected cells from Hg^{2+}-induced cellular injury at 100 μM Hg^{2+} (Fig. 6C), whereas the same experiment in DT cells from NPX rats showed partial protection at both 10 and 100 μM Hg^{2+} (Fig. 6D).

To assess the role of the organic anion carrier in the uptake and subsequent cellular injury induced by mercuric conjugates of Cys, an excess amount of PAH (a competitive substrate for the carrier) was included in the incubation medium. The addition of PAH significantly attenuated the cytotoxicity of mercuric conjugates of Cys in PT cells from both control and NPX rats, had a modest effect in DT cells from control rats, and had no effect in DT cells from NPX rats (Fig. 7).

Modulation of Hg^{2+}-Induced Cellular Injury by BSA. Simultaneous incubation of PT cells from control rats with a 4-fold molar excess of BSA completely protected the cells from Hg^{2+}-induced LDH inactivation at all concentrations of Hg^{2+} tested (Fig. 8A). In contrast, BSA completely protected PT cells from NPX rats at 5 and 10 μM Hg^{2+} but only partially protected at 100 μM Hg^{2+} (Fig. 8B). DT cells from

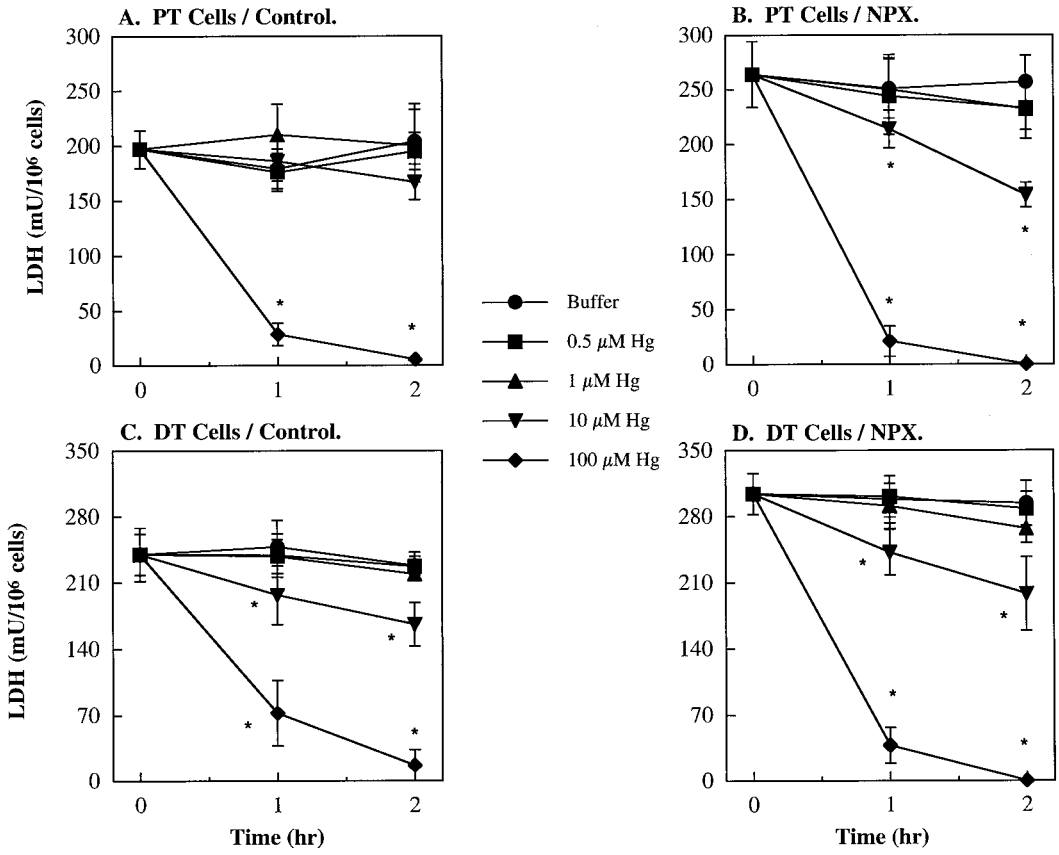

Fig. 1. Time and concentration dependence of Hg^{2+}-induced decreases in LDH activity. Suspensions of PT (A and B) or DT (C and D) cells (2×10^6 cells/ml) isolated from control (A and C) or NPX (B and D) rats were incubated with the indicated concentrations of Hg^{2+}. After 1 or 2 h, aliquots were removed, and LDH activity in the presence of Triton X-100 was measured. Results are the mean ± S.E. values of measurements from separate cell preparations from three control and three NPX rats. *, significantly different ($P < .05$) from sample incubated with buffer at same time point.

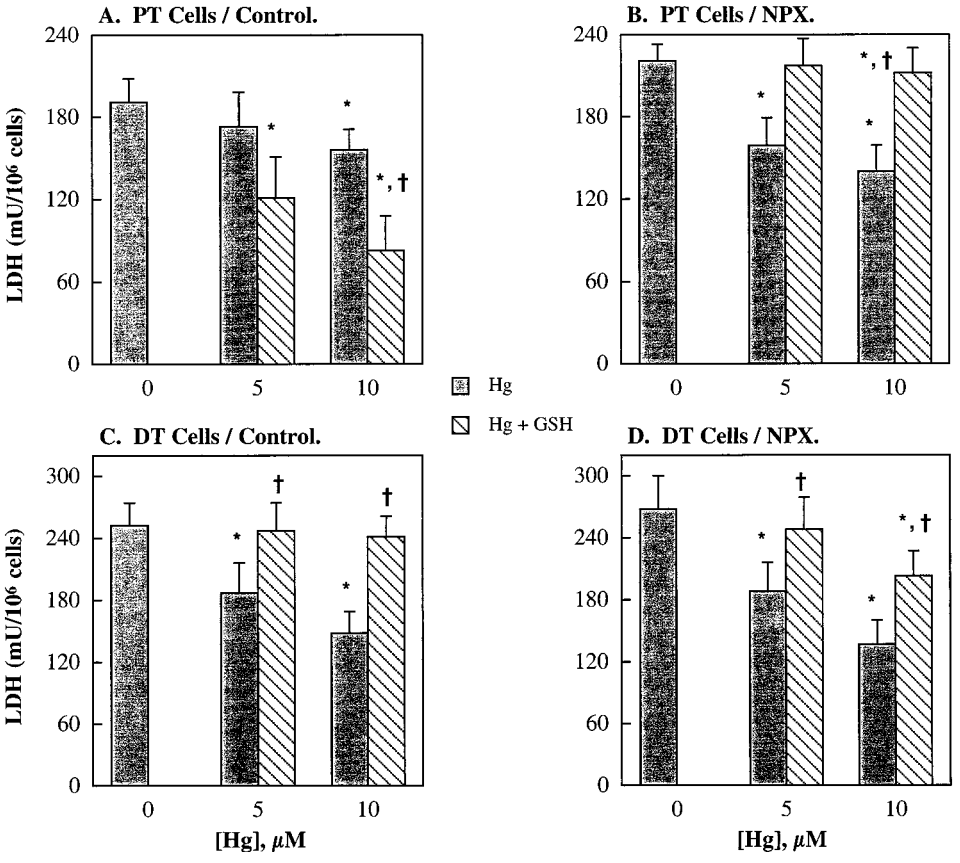

Fig. 2. Effect of preloading with 5 mM GSH on Hg^{2+}-induced decreases in LDH activity. Suspensions of PT (A and B) or DT (C and D) cells (2×10^6 cells/ml) isolated from control (A and C) or NPX (B and D) rats were preincubated for 15 min with either buffer or 5 mM GSH. Cells were then incubated with either 0, 5, or 10 μM Hg^{2+} for up to 2 h. After 1- or 2-h incubations, aliquots were removed, and LDH activity in the presence of Triton X-100 was measured. Data for 2-h incubations are shown, and results are the mean ± S.E. values of measurements from separate cell preparations from three control and three NPX rats. *, significantly different ($P < .05$) from sample incubated with buffer at same time point. †, significantly different ($P < .05$) from corresponding sample incubated without GSH.

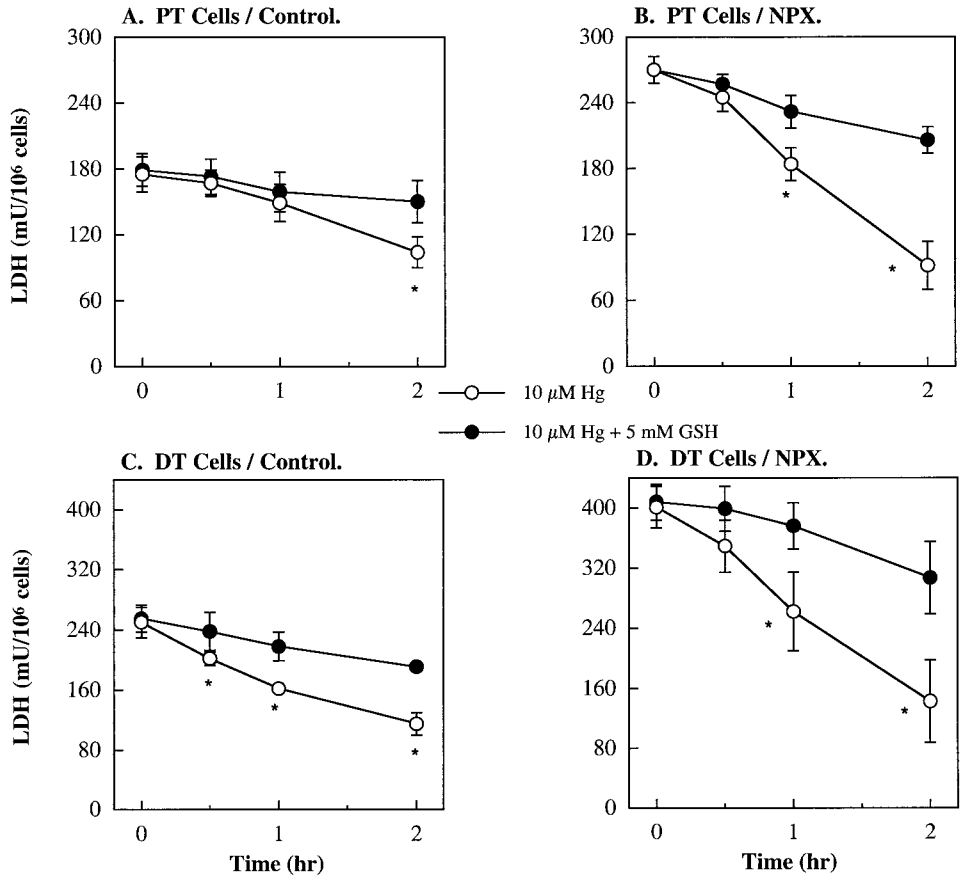

Fig. 3. Effect of the simultaneous addition of 5 mM GSH on 10 μM Hg^{2+}-induced decreases in LDH activity. Suspensions of PT (A and B) or DT (C and D) cells (2×10^6 cells/ml) isolated from control (A and C) or NPX (B and D) rats were incubated with either 10 μM Hg^{2+} or 10 μM Hg^{2+} plus 5 mM GSH for up to 2 h. After 1- or 2-h incubations, aliquots were removed, and LDH activity in the presence of Triton X-100 was measured. Results are the mean ± S.E. values of measurements from separate cell preparations from three control and four NPX rats. *, significantly different ($P < .05$) from sample incubated with Hg^{2+} alone at the same time point.

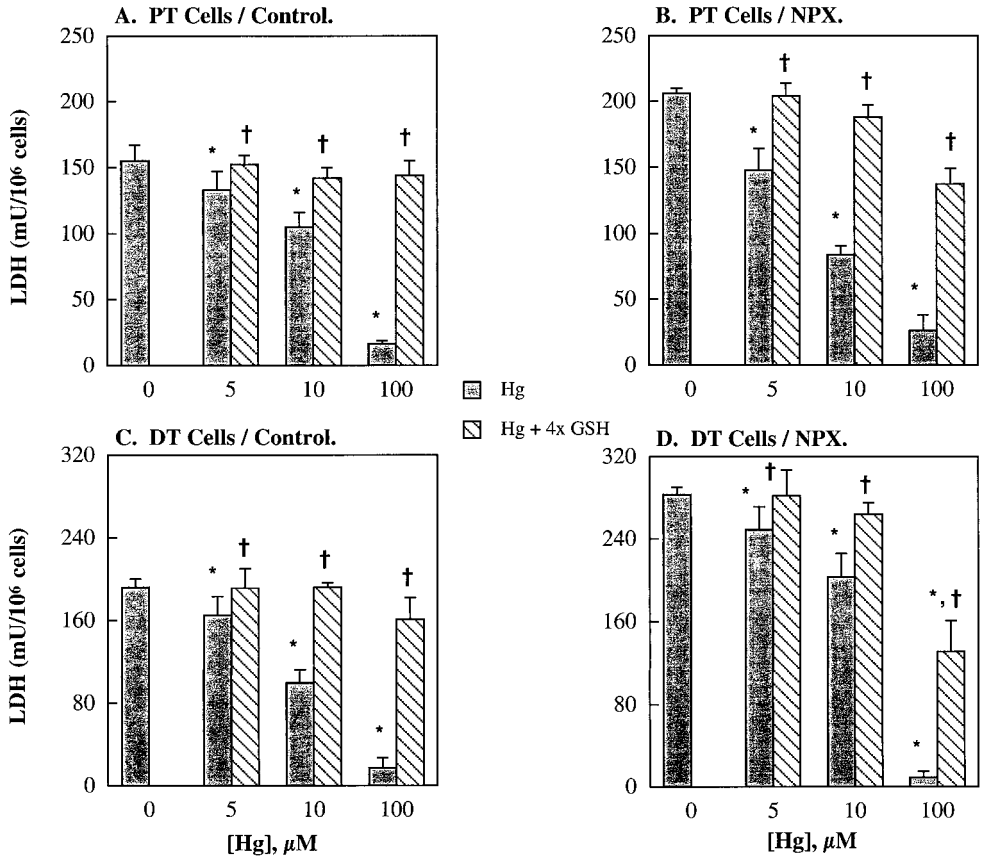

Fig. 4. Effect of the simultaneous addition of a 4-fold molar excess of GSH on Hg^{2+}-induced decreases in LDH activity. Suspensions of PT (A and B) or DT (C and D) cells (2×10^6 cells/ml) isolated from control (A and C) or NPX (B and D) rats were incubated with 0, 5, 10, or 100 μM Hg^{2+} in the absence or presence of a 4-fold molar excess of GSH for up to 2 h. After 1- or 2-h incubation, aliquots were removed, and LDH activity in the presence of Triton X-100 was measured. Data are shown for 2-h incubations, and results are the mean ± S.E. values of measurements from separate cell preparations from four control and five NPX rats. *, significantly different ($P < .05$) from sample incubated with buffer. †, significantly different ($P < .05$) from corresponding sample incubated without GSH.

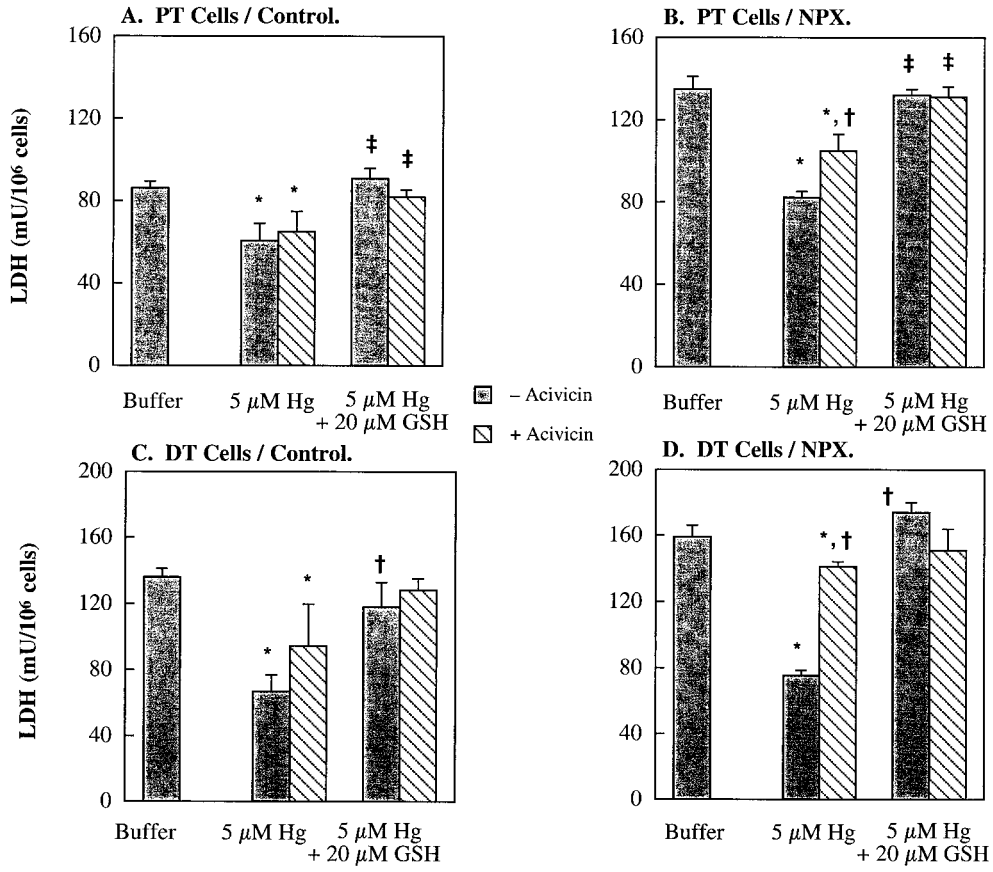

Fig. 5. Effect of acivicin on GSH-dependent protection from Hg^{2+}-induced decreases in LDH activity. Suspensions of PT (A and B) or DT (C and D) cells (2×10^6 cells/ml) isolated from control (A and C) or NPX (B and D) rats were preincubated for 15 min with either buffer or 0.25 mM acivicin and were incubated with 5 μM Hg^{2+} in the absence or presence of 20 μM GSH for up to 2 h. After 1- or 2-h incubations, aliquots were removed, and LDH activity in the presence of Triton X-100 was measured. Data are shown for 2-h incubations, and results are the mean ± S.E. values of measurements from separate cell preparations from four control and four NPX rats. *, significantly different ($P < .05$) from sample preincubated and incubated with buffer. †, significantly different ($P < .05$) from corresponding sample not preincubated with acivicin. ‡, significantly different ($P < .05$) from corresponding sample incubated without GSH.

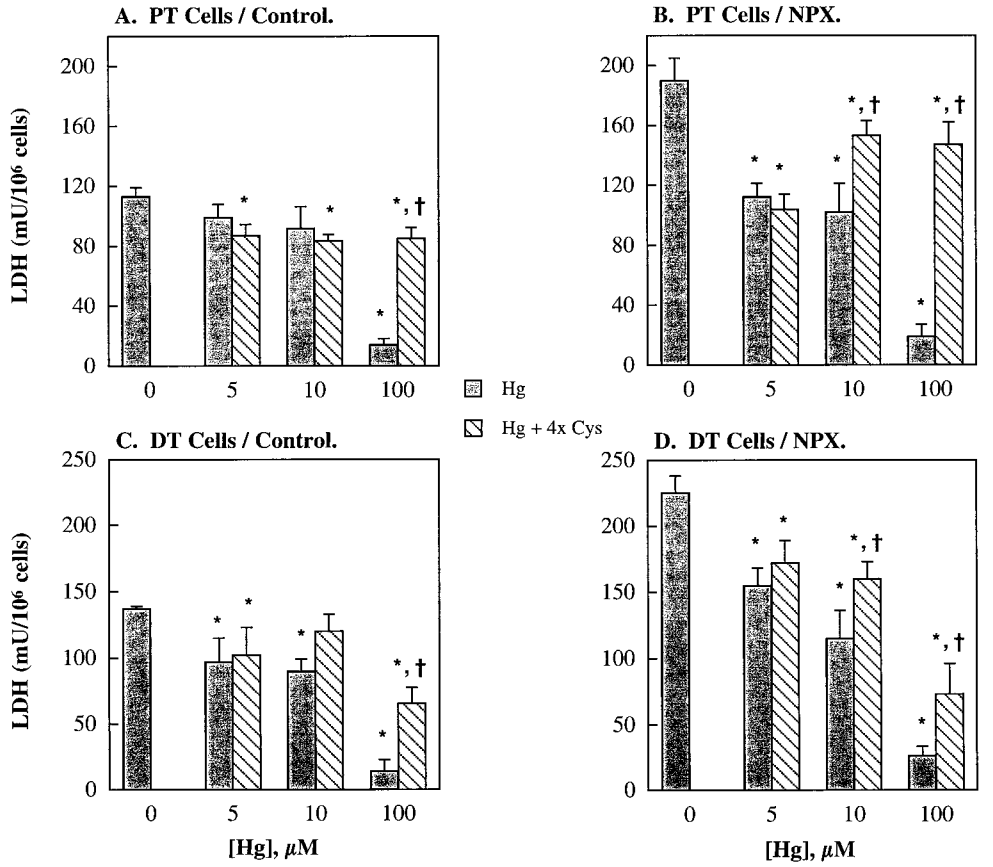

Fig. 6. Effect of the simultaneous addition of a 4-fold molar excess of Cys on Hg^{2+}-induced decreases in LDH activity. Suspensions of PT (A and B) or DT (C and D) cells (2×10^6 cells/ml) isolated from control (A and C) or NPX (B and D) rats were incubated with 0, 5, 10, or 100 μM Hg^{2+} in the absence or presence of a 4-fold molar excess of Cys for up to 2 h. After 1- or 2-h incubations, aliquots were removed, and LDH activity in the presence of Triton X-100 was measured. Data are shown for 2-h incubations, and results are the mean ± S.E. values of measurements from separate cell preparations from four control and five NPX rats. *, significantly different ($P < .05$) from sample incubated with buffer. †, significantly different ($P < .05$) from corresponding sample incubated without Cys.

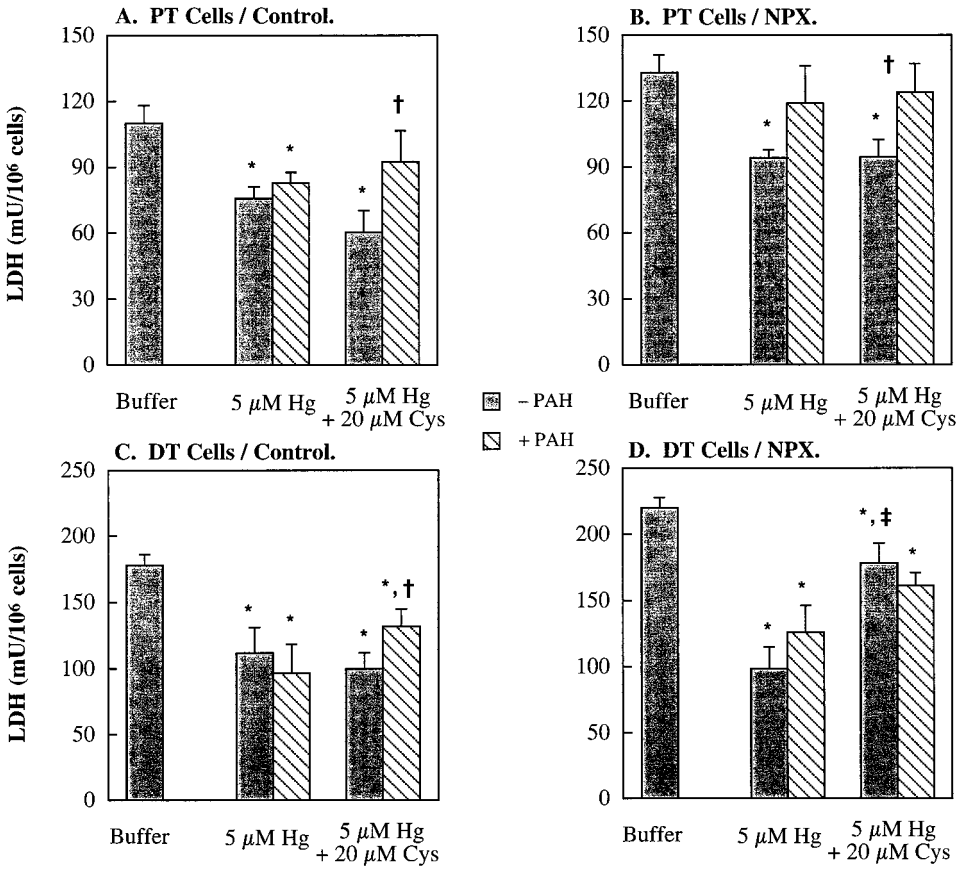

Fig. 7. Effect of PAH on Cys-dependent enhancement of Hg^{2+}-induced decreases in LDH activity. Suspensions of PT (A and B) or DT (C and D) cells (2×10^6 cells/ml) isolated from control (A and C) or NPX (B and D) rats were incubated with 5 μM Hg^{2+} in the absence or presence of 20 μM Cys and in the absence or presence of 1 mM PAH for up to 2 h. After 1- or 2-h incubation, aliquots were removed, and LDH activity in the presence of Triton X-100 was measured. Data are shown for 2-h incubations, and results are the mean ± S.E. values of measurements from separate cell preparations from four control and five NPX rats. *, significantly different ($P < .05$) from sample incubated with buffer. †, significantly different ($P < .05$) from corresponding sample without PAH. ‡, significantly different ($P < .05$) from corresponding sample incubated without Cys.

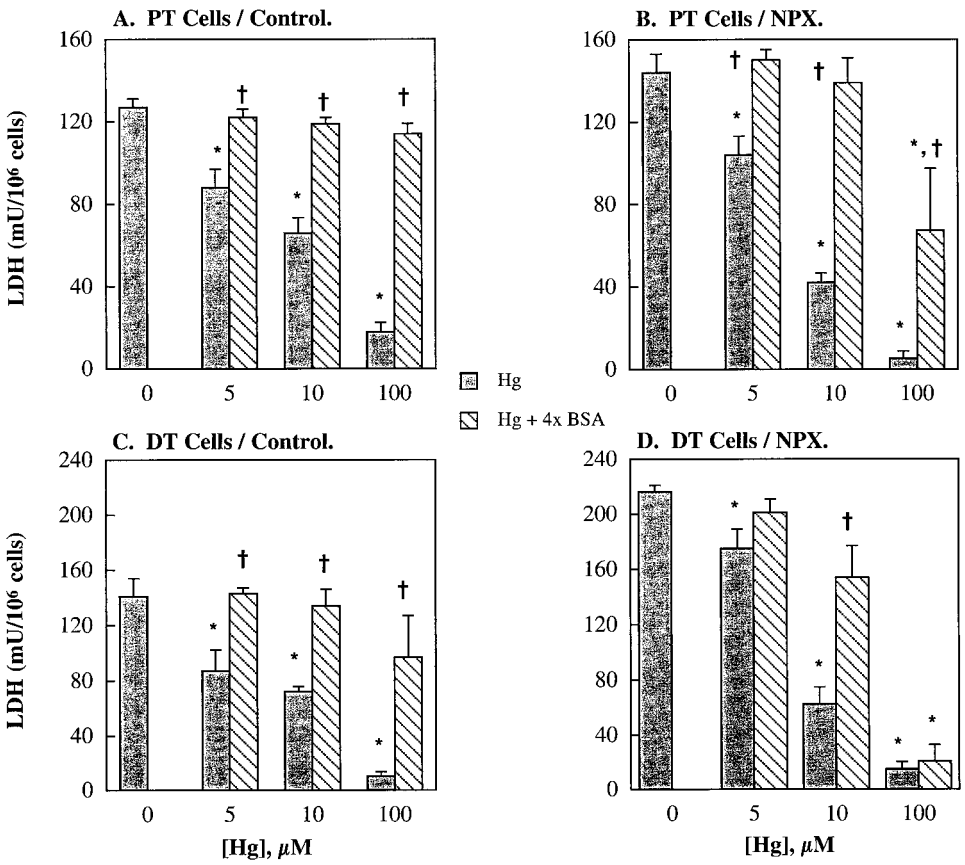

Fig. 8. Effect of the simultaneous addition of a 4-fold molar excess of BSA on Hg^{2+}-induced decreases in LDH activity. Suspensions of PT (A and B) or DT (C and D) cells (2×10^6 cells/ml) isolated from control (A and C) or NPX (B and D) rats were incubated with 0, 5, 10, or 100 μM Hg^{2+} in the absence or presence of a 4-fold molar excess of BSA for up to 2 h. After 1- or 2-h incubations, aliquots were removed, and LDH activity in the presence of Triton X-100 was measured. Data are shown for 2-h incubations, and results are the mean ± S.E. values of measurements from separate cell preparations from four control and four NPX rats. *, significantly different ($P < .05$) from sample incubated with buffer. †, significantly different ($P < .05$) from corresponding sample incubated without BSA.

control and NPX rats exhibited a similar pattern as PT cells, except that at 100 μM Hg^{2+}, BSA had no effect on the extent of cellular injury (Fig. 8, C and D).

Modulation of Hg^{2+}-Induced Cellular Injury by DMPS. To study the effect of the dithiol chelator DMPS on Hg^{2+}-induced cellular injury, cells were first preincubated with 5 mM DMPS and then incubated with 5 or 10 μM Hg^{2+} (Fig. 9). DMPS preloading provided complete protection from Hg^{2+}-induced LDH inactivation in PT cells from either control or NPX rats but provided only partial protection in DT cells.

Simultaneous incubation of PT cells from either control or NPX rats with Hg^{2+} and a 4-fold molar excess of DMPS resulted in complete protection at 5 and 10 μM Hg^{2+} but only partial protection at 100 μM Hg^{2+} (Fig. 10, A and B). DMPS provided only modest protection in DT cells and, like BSA, had no effect on the cytotoxicity of 100 μM Hg^{2+} in DT cells from NPX rats (Fig. 10, C and D).

Discussion

The present study sought to define the susceptibility of PT cells from control and NPX rats to Hg^{2+}, to determine whether DT cells are also susceptible to Hg^{2+}, and to determine the influence of thiols on Hg^{2+}-induced cellular injury. The use of suspensions of freshly isolated PT cells as an in vitro model allowed manipulation of incubation conditions in a controlled environment. Earlier studies showed that isolated PT cells from NPX rats maintained their hypertrophic state, increased cellular contents of protein and GSH, and increased enzymatic activities that are observed in vivo (Lash and Zalups, 1992, 1994). These cells are thus a suitable model in which to explore the mechanisms by which compensatory renal cellular hypertrophy alters Hg^{2+}-induced cellular injury.

Relative Susceptibility of PT and DT Cells. Although the PT region of the nephron is the major site that accumulates Hg^{2+} and exhibits pathological changes after exposure to Hg^{2+} in vivo (Zalups, 1991a,b), freshly isolated DT cells from control rats exhibited modestly greater susceptibility to Hg^{2+} than PT cells from control rats. These results suggest that the distal nephron can be intoxicated if it is exposed to sufficient amounts of Hg^{2+}, which may occur if the transport activities in the proximal nephron that enable cellular accumulation of Hg^{2+} are defective. This in turn may occur under conditions such as energy depletion that lead to proximal tubular dysfunction. Hg^{2+} may then be delivered to more distal segments of the nephron, thereby leading to cellular injury in those epithelial cells.

In the absence of exogenous thiols, both PT and DT cells appeared to exhibit a threshold for Hg^{2+} between 5 and 10 μM, whereas 100 μM Hg^{2+} caused death of virtually all cells within 2 h of incubation. It is noteworthy, however, that there was some variability between experiments. For this reason, control incubations containing no Hg^{2+} were always performed and paired with incubations containing Hg^{2+}.

Effect of Compensatory Renal Growth. PT cells from NPX rats were indeed more sensitive to Hg^{2+} than were PT cells from control rats. In contrast, no significant effect of compensatory renal growth was observed in DT cells. This finding is consistent with the minimal changes in cellular biochemistry observed in DT cells from NPX rats (Lash and Zalups, 1994) and the heterogeneity with respect to hypertrophy occurring in distal segments of the nephron.

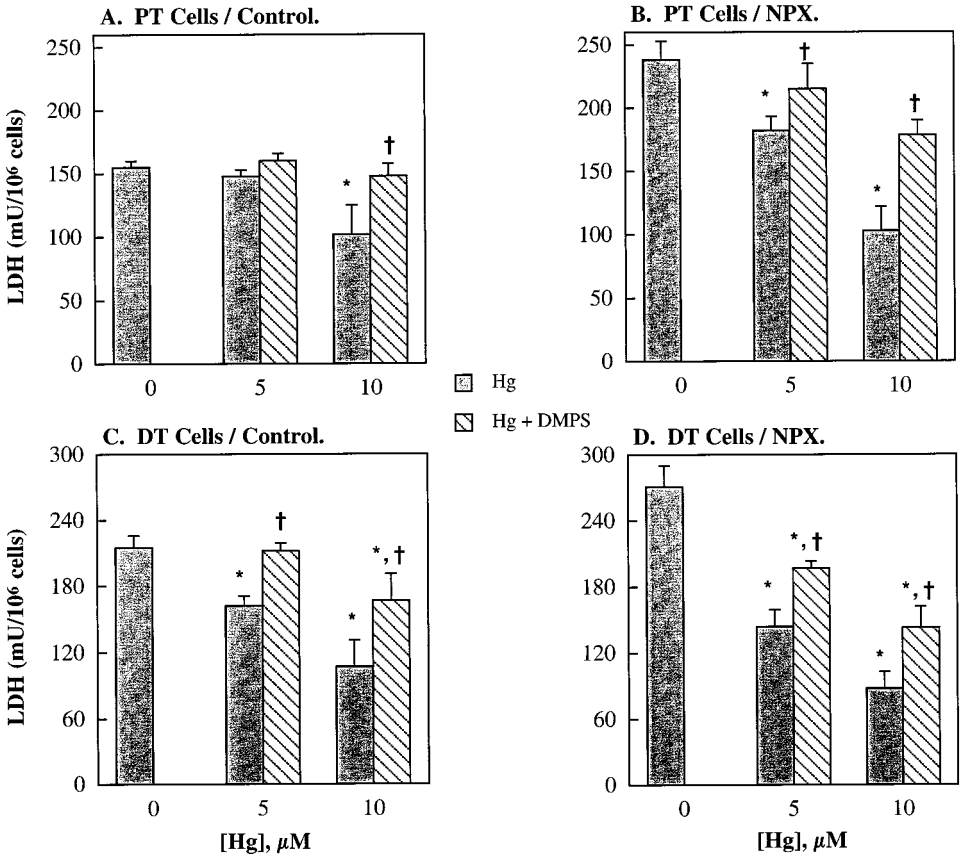

Fig. 9. Effect of preloading with 5 mM DMPS on Hg^{2+}-induced decreases in LDH activity. Suspensions of PT (A and B) or DT (C and D) cells (2×10^6 cells/ml) isolated from control (A and C) or NPX (B and D) rats were preincubated for 15 min with either buffer or 5 mM DMPS. Cells were then incubated with either 0, 5, or 10 μM Hg^{2+} for up to 2 h. After 1- or 2-h incubations, aliquots were removed, and LDH activity in the presence of Triton X-100 was measured. Data for 2-h incubations are shown, and results are the mean ± S.E. values of measurements from separate cell preparations from four control and four NPX rats. *, significantly different ($P < .05$) from sample incubated with buffer. †, significantly different ($P < .05$) from corresponding sample not preincubated with DMPS.

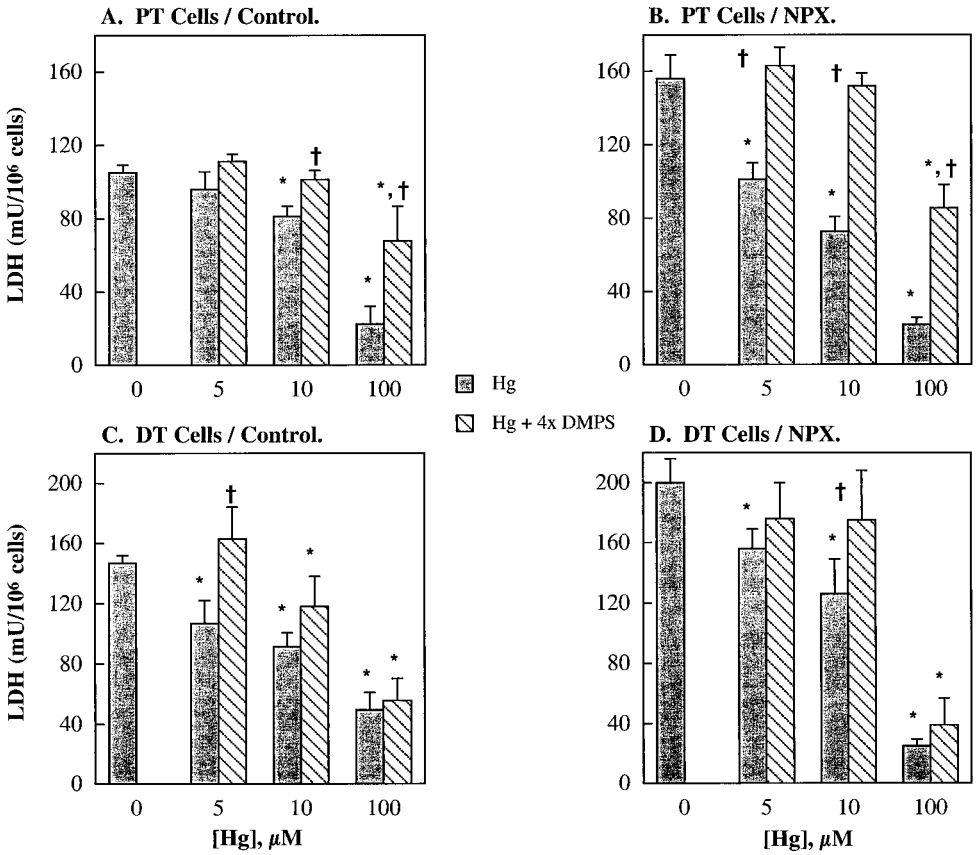

Fig. 10. Effect of the simultaneous addition of a 4-fold molar excess of DMPS on Hg^{2+}-induced decreases in LDH activity. Suspensions of PT (A and B) or DT (C and D) cells (2×10^6 cells/ml) isolated from control (A and C) or NPX (B and D) rats were incubated with 0, 5, 10, or 100 μM Hg^{2+} in the absence or presence of a 4-fold molar excess of DMPS for up to 2 h. After 1- or 2-h incubations, aliquots were removed, and LDH activity in the presence of Triton X-100 was measured. Data are shown for 2-h incubations, and results are the mean ± S.E. values of measurements from separate cell preparations from four control and four NPX rats. *, significantly different ($P < .05$) from sample incubated with buffer. †, significantly different ($P < .05$) from corresponding sample incubated without DMPS.

Although preloading PT cells from control rats with 5 mM GSH enhanced Hg^{2+}-induced cellular injury, PT cells from NPX rats were afforded protection. The results obtained with PT cells from control rats are consistent with the function of a GSH transport system that serves to increase intracellular content of GSH (Visarius et al., 1996). One possible reason why PT cells from NPX rats were protected is that transport processes, possibly involving uptake and/or efflux of GSH itself or mercuric conjugates of various thiols, may be altered as a result of compensatory cellular hypertrophy, leading to a state where net cellular accumulation of Hg^{2+} was diminished.

Modulation by GSH. Mercuric conjugates with various extracellular thiols, rather than free Hg^{2+}, are likely the principal forms of Hg^{2+} that renal epithelial cells are exposed to in vivo. As discussed above, the relevance of incubating cells with free Hg^{2+} (in the absence of ligands) is that data from these experiments can be used as a reference point from which to assess the effects of various intracellular and extracellular thiols.

GSH has a complex role in the regulation of renal cellular disposition and cytotoxicity of Hg^{2+}. On the one hand, GSH can protect renal cells from Hg^{2+}-induced cellular injury by preventing it from binding to other essential, cellular thiols (Johnson, 1982; Houser et al., 1992; Lash and Zalups, 1992; Girardi and Elias, 1993). On the other hand, mercuric conjugates of GSH may be a physiological form by which Hg^{2+} is transported into the renal proximal tubule; this implies that the presence of extracellular GSH may function to enhance renal cellular accumulation of Hg^{2+} (Zalups and Lash, 1997b; Lash et al., 1998; Zalups, 1998). Indeed, the present results and the fact that other types of GSH conjugates are transported into renal PT cells (Lash and Jones, 1985) support these conclusions.

The effect of preloading cells with GSH on Hg^{2+}-induced cellular injury differed in PT and DT cells. As discussed above, PT cells from control rats whose intracellular GSH content was enhanced exhibited more toxicity. DT cells, however, were afforded protection under the same incubation conditions. The difference in effect is likely due to cellular heterogeneity along the nephron with respect to GSH-dependent metabolism and transport (Lash and Tokarz, 1989; Lash and Putt, 1999). Hence, the inability of DT cells to significantly accumulate GSH may account for the protective, rather than enhancing, effect of GSH preincubation.

Mercuric conjugates of GSH may be transported at both basolateral and brush-border membranes. However, it is likely that the corresponding Cys conjugates are the primary species that are transported across the brush-border membrane. In support of this are studies showing that inhibition of GGT activity by acivicin diminishes the renal cellular uptake and accumulation of Hg^{2+} (Berndt et al., 1985; Tanaka et al., 1990; Zalups, 1995). In the present study, however, acivicin had little or no effect on cellular injury induced by mercuric conjugates of GSH. This suggests that at least in this in vitro model, degradation of mercuric conjugates of GSH to the Cys conjugates is not an obligate step in the processes leading from cellular uptake and accumulation to injury because increased uptake of mercuric conjugates of GSH across the basolateral membrane may occur under these conditions.

Modulation by Cys. Data from the present study support the hypothesis that mercuric conjugates of Cys are important transport forms of Hg^{2+}. Exposure of PT cells from control

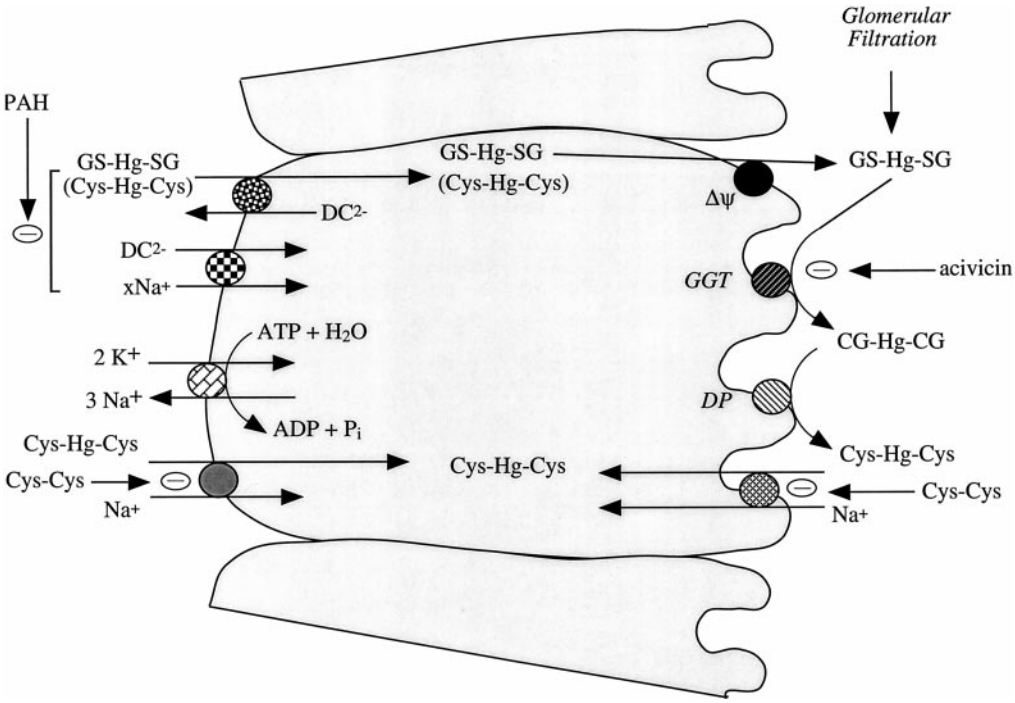

Fig. 11. Scheme of renal proximal tubular transport and metabolism of mercuric conjugates of thiols. Hg^{2+} is shown being presented to either the basolateral or brush-border membranes as complexes with two molecules of either GSH or Cys. Mercuric conjugates of GSH are presumably transported into the renal PT cell by a Na^+-dependent, PAH-sensitive process, possibly coupled to transport of dicarboxylates (DC^{2-}) and is transported into the lumen by a membrane potential-sensitive carrier, where it is degraded in successive reactions by GGT and dipeptidase activities to the cysteinylglycine (CG-Hg-CG) and, finally, the Cys conjugate. The resulting luminal as well as serosal mercuric conjugates of Cys are believed to be transported into the renal PT cell by carriers that may or may not cotransport Na^+ ions and are competitively inhibited by cystine.

rats to mercuric conjugates of Cys resulted in slight enhancement or little effect on cytotoxicity (depending on the concentration of Hg^{2+} used) compared with PT cells from control rats incubated with Hg^{2+} alone. This slight effect or lack of effect of Cys is very significant because other thiols (i.e., DMPS and BSA) provide almost complete protection from Hg^{2+}-induced injury (Lash and Zalups, 1992; present study).

Although exposure to mercuric conjugates of Cys caused LDH inactivation to increase slightly at 5 and 10 μM Hg^{2+} in PT cells from control rats, it was protective in PT cells from NPX rats and in DT cells from both control and NPX rats. These results, and the ability of PAH to protect normal PT cells (but not DT cells) from the toxic effects of Hg^{2+}, support a role for the PAH-sensitive organic anion transporter (which is absent in DT cells) in the uptake of mercuric conjugates of Cys in PT cells from control animals. The quantitative differences in response to mercuric conjugates of Cys in PT cells from control and NPX rats suggest that activity of this carrier relative to other transporters may be altered by compensatory renal cellular hypertrophy.

Modulation by BSA and DMPS. BSA and DMPS were by far the most effective, protective thiols. Incubation of PT cells from control rats with Hg^{2+} and either BSA or DMPS (presumably in the form of a mercuric conjugate) provided nearly complete protection from the toxic effects of Hg^{2+} at all three concentrations tested. In contrast, exposure to mercuric conjugates of BSA or DMPS provided partial protection to PT cells from NPX rats and DT cells from control rats at 100 μM Hg^{2+} and partial to no protection in DT cells from NPX rats with increasing Hg^{2+} concentrations. Compensatory cellular hypertrophy and cell type-dependent differences in the handling of BSA or mercuric conjugates of BSA may account for the various observed effects. Mercuric conjugates of BSA may accumulate in PT cells via a slow, low-capacity process involving endocytosis on the luminal membrane. Differences in the ability to transport DMPS or mercuric conjugates of DMPS or in the ability to reduce oxidized DMPS may play a role in the varied responses observed during coexposure to Hg^{2+} and DMPS. Results with DMPS are consistent with data showing that once Hg^{2+} binds to DMPS, the complex is not readily taken up by renal epithelial cells (Zalups et al., 1998). There also is no evidence that DMPS can be transported into epithelial cells from the distal nephron. Hence, the protection observed with DT cells that were preincubated with DMPS was likely due to extracellular chelation of Hg^{2+} rather than increases in intracellular content of DMPS.

Summary and Conclusions. The various pathways discussed above are summarized in Fig. 11, which illustrates the potential metabolic fates and action of several transporters in delivering Hg^{2+}, in the form of thiol conjugates, to the renal PT cell. One important caveat is that the isolated renal cell suspensions have lost the plasma membrane polarity that is characteristic of the intact renal tubular epithelium. Hence, transporters on both brush-border and basolateral plasma membrane surfaces have equal and simultaneous access to substrates. Consequently, inhibition of one transport mechanism may be more readily compensated by increased transport via another carrier. Accordingly, although all the data from various studies support the conclusion that mercuric conjugates of Cys and GSH are primary transport forms of Hg^{2+}, a role for other types of mercuric conjugates and other transport systems cannot be excluded.

In conclusion, the present study has demonstrated enhanced susceptibility of PT cells from NPX rats to Hg^{2+}; shown that DT cells, although they are not an in vivo target for Hg^{2+}, are very susceptible to Hg^{2+}-induced cellular injury in this in vitro model; and characterized differences in the effects of exogenous thiols on the cytotoxicity of Hg^{2+} in renal PT and DT cells and in cells from NPX and control rats.

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