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# MERCURY-INDUCED $H_2O_2$ PRODUCTION AND LIPID PEROXIDATION *IN VITRO* IN RAT KIDNEY MITOCHONDRIA

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**Abstract**—Mercuric ion (Hg(II)) causes oxidative tissue damage in kidney cortical cells. We studied the *in vitro* effects of Hg(II) on hydrogen peroxide ($H_2O_2$) production by rat kidney mitochondria, a principal intracellular target of Hg(II). In mitochondria supplemented with a respiratory chain substrate (succinate or malate/glutamate) and an electron transport inhibitor (antimycin A (AA) or rotenone), Hg(II) (30 nmol/mg protein) increased $H_2O_2$ formation approximately 4-fold at the uniquinone-cytochrome *b* region (AA-inhibited) and 2-fold at the NADH dehydrogenase region (rotenone-inhibited). Concomitantly, Hg(II) increased iron-dependent lipid peroxidation 3.5-fold at the NADH dehydrogenase region, but only by 25% at the ubiquinone-cytochrome *b* region. The mitochondrial concentration of reduced glutathione (GSH) decreased both with incubation time and Hg(II) concentration. Hg(II), at a concentration of 12 nmol/mg protein, caused almost complete depletion of measurable GSH in substrate-supplemented mitochondria after a 30-min incubation. In electron transport-inhibited mitochondria, Hg(II) caused greater depletion of GSH in rotenone-inhibited than in AA-inhibited mitochondria, consistent with the effects of Hg(II) on lipid peroxidation. These results suggest that Hg(II) at low concentrations depletes mitchondrial GSH and enhances $H_2O_2$ formation in kidney mitochondria under conditions of impaired respiratory chain electron transport. The increased $H_2O_2$ formation by Hg(II) may lead to oxidative tissue damage, such as lipid peroxidation, observed in mercury-induced nephrotoxicity.

Mercury(II) (Hg(II)) a classic nephrotoxicant, causes oxidative damage to renal tissue, principally in the region of the proximal tubule cells. Previous research on the mechanism of this effect has shown that intracellular mercury is associated with impairment of mitochondrial electron transport [1, 2], uncoupling of oxidative phosphorylation [1–4], and enzyme inhibition [4, 5]. Oxidative stress (e.g. thiol depletion and lipid peroxidation) is a likely ultimate cause of renal failure by Hg(II) [6–8], although the specific action(s) underlying Hg(II)-promoted oxidative tissue damage is largely unknown.

One possible mechanism of Hg(II)-induced oxidative stress is impairment of the structure and function of the mitochondrial inner membrane [9, 10], resulting in enhanced state 4 respiration. This condition is known to be associated with increased production of superoxide ($O_2^-$) and hydrogen peroxide ($H_2O_2$) by the mitochondrial electron transport chain [11]. $O_2^-$ and $H_2O_2$ are formed at the NADH dehydrogenase (complex I) and ubiquinone-cytochrome *b* (complex III) regions under state 4 conditions, or in the presence of electron transport inhibitors, such as rotenone and antimycin A (AA†) [12]. Because Hg(II) accumulates in kidney mitochondria [2, 3], we investigated the *in vitro* effect of $HgCl_2$ on $H_2O_2$ formation by the mitochondrial respiratory chain, and the consequences of this effect on iron-dependent lipid peroxidation.

We report a positive relationship of Hg(II)-induced $H_2O_2$ formation and mitochondrial lipid peroxidation at Hg(II) concentrations previously observed [2, 3] in renal mitochondria during exposure to mercury compounds.

## MATERIALS AND METHODS

*Materials.* Dichlorofluorescin diacetate was obtained from Molecular Probes Inc., Eugene, OR. Antimycin A, bovine liver catalase, buthionine sulfoximine, diethyl maleate, $HgCl_2$ (+99.999%), horseradish peroxidase (type VIA), rotenone and scopoletin were purchased from the Sigma Chemical Co., St. Louis, MO. Monobromobimane was obtained from Calbiochem, La Jolla, CA. Other chemicals were reagent grade and were purchased from standard commercial sources. All solutions were prepared in doubly deionized water. Male Sprague–Dawley rats (200–250 g) were obtained from Tyler Laboratories, Bellevue, WA.

*Methods.* For mitochondrial preparations, three rats were fasted overnight, anesthetized with $CO_2$, and killed by exsanguination. The kidneys were removed rapidly and the cortex was separated, minced, and homogenized in ice-cold Tris (10 mM), mannitol (225 mM), sucrose (75 mM), EDTA (3 mM), bovine serum albumin (BSA, 0.5 mg/mL), pH 7.5. Free fatty acids were removed from BSA

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† Abbreviations: AA, antimycin A; BSA, bovine serum albumin; BSO, buthionine sulfoximine; DEM, diethyl maleate; GSH, reduced glutathione; MDA, malondialdehyde; SMP, sub-mitochondrial particles; and TBARS, thiobarbituric acid reactive substances.

Table 1. Effect of $HgCl_2$ and order of addition of inhibitor, substrate and $HgCl_2$ on $H_2O_2$ formation by kidney mitochondria

| Order of additions | $H_2O_2$ formation (nmol $H_2O_2$/mg protein/min) |
|---|---|
| (AA) + succinate | 0.21 ± 0.01 |
| (AA) + succinate + $HgCl_2$ | 0.34 ± 0.01*† |
| (AA + $HgCl_2$) + succinate | 0.89 ± 0.08* |
| ($HgCl_2$) + succinate + AA | 0.89 ± 0.01* |
| (Rotenone) + malate/glutamate | 0.12 ± 0.02 |
| (Rotenone) + malate/glutamate + $HgCl_2$ | 0.19 ± 0.01* |
| (Rotenone + $HgCl_2$) + malate/glutamate | 0.18 ± 0.01* |
| ($HgCl_2$) + malate/glutamate + rotenone | 0.16 ± 0.02* |

Mitochondria (0.33 mg/mL) were incubated in the presence of electron transport inhibitor (1 nmol AA/mg or 3 nmol rotenone/mg protein), substrate (6 mM succinate or 2.5 mM/2.5 mM malate/glutamate) and 12 nmol $HgCl_2$/mg protein as indicated in the table. Substances within parentheses were preincubated with mitochondria for 1 min before other additions were made. $H_2O_2$ formation was measured by scopoletin oxidation as described in Materials and Methods. Values are means ± SD of triplicate determinations.

* Statistically different from rates without Hg(II) present, $P < 0.05$.

† Statistically different from when $HgCl_2$ was added before substrate to the reaction mixture, $P < 0.05$.

as described by Chen [13]. The mitochondrial fraction was prepared by differential centrifugation according to Johnson and Lardy [14]. The mitochondria were finally suspended in Tris (30 mM), 3-(*N*-morpholino)propanesulfonic acid (MOPS) (30 mM), mannitol (225 mM), sucrose (75 mM), $MgCl_2$ (5 mM), pH 7.5 (buffer A), except for mitochondria used to assess lipid peroxidation, which were suspended in Tris (30 mM), KCl (155 mM), pH 7.5. GSH-depleted mitochondria were prepared by injecting rats with buthionine sulfoximine (BSO, 0.45 mmol/kg, i.p.) and diethyl maleate (DEM, 3.4 mmol/kg, i.p.) [14] 2.5 hr before sacrifice, as described by Bagget and Berndt [15]. Protein concentrations were determined according to Smith *et al.* [16], using BSA as a standard. Mitochondrial respiratory chain substrates (6 mM succinate or 2.5 mM malate/2.5 mM glutamate), electron transport inhibitors (1 nmol antimycin A (AA)/mg protein, dissolved in ethanol, or 3 nmol rotenone/mg protein, dissolved in dimethyl sulfoxide), and $HgCl_2$ (dissolved in doubly deionized water) were used in assays at the indicated concentrations.

*$H_2O_2$ assay.* Mitochondria (0.33 mg/mL), horseradish peroxidase (30 μM) and scopoletin (2 μM) were incubated at 30° in a total volume of 3 mL buffer A. Substrate, inhibitor and $HgCl_2$ were added where indicated. $H_2O_2$ formation was followed continuously spectrofluorometrically by the horseradish peroxidase-dependent oxidation of scopoletin (ex. 365 nm, em. 450 nm) [11]. The rates of $H_2O_2$ formation were calculated based on a standard curve obtained from the oxidation of scopoletin by horseradish peroxidase and known amounts of $H_2O_2$.

*Oxygen consumption.* Oxygen consumption of mitochondria (2 mg protein/3 mL buffer A) was measured with a Clark oxygen electrode at 30°. The incubation mixture contained substrate, inhibitor and $HgCl_2$ when indicated. The $O_2$ concentration was assumed to be 200 nmol $O_2$/mL.

*Lipid peroxidation assay.* Mitochondria (0.33 mg/mL) were incubated for 30 min in Tris (30 mM), KCl (155 mM), pH 7.5, at 30° with substrate, inhibitor and $HgCl_2$ included where indicated. Butylated hydroxyanisole (1 mM) was added and thiobarbituric acid reactive substances (TBARS) were measured spectrophotometrically at 532 nm, according to Buege and Aust [17]. TBARS were converted to malondialdehyde (MDA) concentrations using $E = 1.56 \times 10^5\ M^{-1}\ cm^{-1}$ for the MDA–TBA chromophore.

*Glutathione assay.* Mitochondria (2 mg/mL) were incubated for 30 min with substrate, electron transport inhibitor, Fe(III):ADP (20:100 μM) and, where indicated, $HgCl_2$, in a total volume of 1 mL buffer A at 30°. Following incubation, mitochondria were immediately layered onto dibutylphthalate and centrifuged at 15,000 *g* for 3 min into 5-sulfosalicylic acid (1 M), as described by Meredith and Reed [18]. This process allowed separation of mitochondria from the incubation buffer and the immediate release of the intramitochondrial contents into the acid, avoiding breakdown of GSH by γ-glutamyl transpeptidase. Glutathione (GSH) was derivatized with monobromobimane and analyzed by reverse phase HPLC as described by Anderson [19], except for the use of tetra-butylammonium phosphate (1 mM, pH 3.0) in methanol as the mobile phase.

*Statistical analyses.* Statistical analyses were conducted using the two-tailed Student's *t*-test, at $P < 0.05$ level of significance. In Figs. 1–4, where mean values are used, the average variability between duplicate samples was less than 10%.

## RESULTS

The effect of $HgCl_2$ on $H_2O_2$ production by rat

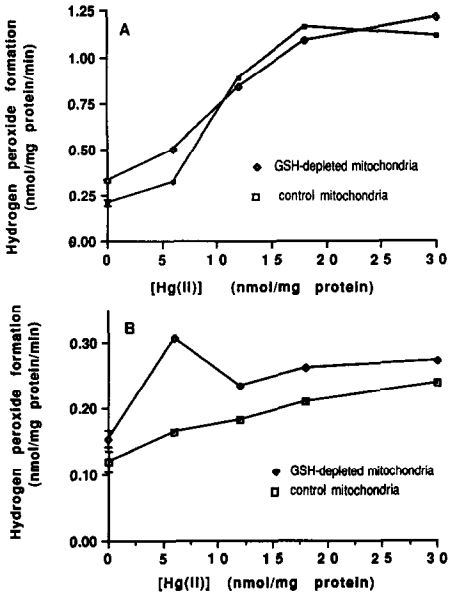


Fig. 1. Effects of $HgCl_2$ on $H_2O_2$ formation from control and GSH-depleted mitochondria. $HgCl_2$ and antimycin A (Fig. 1A, the ubiquinone-cytochrome *b* region) or rotenone (Fig. 1B, the NADH dehydrogenase region) were preincubated with control or GSH-depleted mitochondria (0.33 mg/mL) for 1 min. Then succinate (6 mM) or malate/glutamate (2.5 mM/2.5 mM) was added, and $H_2O_2$ formation was measured by scopoletin oxidation as described in Materials and Methods. GSH-depleted kidney mitochondria (0.39 ± 0.02 nmol GSH/mg protein, approximately 15% of control values) were prepared from rats injected with DEM-BSO 2.5 hr before they were killed. The figures show one representative experiment of two with mean values of duplicate or triplicate (at 0 nmol Hg(II)/mg protein) samples.

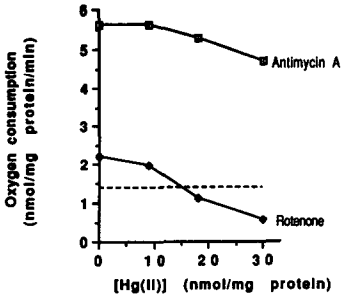


Fig. 2. Effects of $HgCl_2$ on $O_2$ consumption in electron transport-inhibited mitochondria. Mitochondria (2 mg/3 mL buffer A) were incubated at 30° in the presence of $KH_2PO_4$ (5 mM). Succinate (6 mM)-AA (1 nmol/mg protein), malate/glutamate (2.5 mM/2.5 mM)-rotenone (3 nmol/mg protein) and $HgCl_2$ were added as indicated in the figure and $O_2$ consumption was measured with a Clark oxygen electrode. The figure hows one representative experiment of two with mean values of duplicate samples. The dashed line indicates the KCN-insensitive oxygen consumption.

kidney mitochondrial preparations in the presence of electron transport inhibitors is shown in Table 1. $H_2O_2$ formation in mitochondria supplemented with either succinate and AA or malate/glutamate and rotenone was 0.21 ± 0.01 and 0.12 ± 0.02 nmol $H_2O_2$/mg protein/min (mean ± SD), respectively. The addition of 12 nmol $HgCl_2$/mg protein caused a 4.2-fold and 1.9-fold increase in $H_2O_2$ formation at the ubiquinone-cytochrome *b* (AA-inhibited) and NADH dehydrogenase (rotenone-inhibited) regions, respectively. Maximal $H_2O_2$ formation was observed in mitochondria when $HgCl_2$ was added prior to succinate, whereas when malate-glutamate was used as respiratory chain substrate, $HgCl_2$ increased the $H_2O_2$ formation to a similar extent, irrespective of when added.

Panels A and B of Fig. 1 show the effects of GSH depletion on Hg(II)-promoted $H_2O_2$ formation by renal mitochondria. In the absence of Hg(II), greater initial rates of $H_2O_2$ formation were observed from both the ubiquinone-cytochrome *b* (0.33 ± 0.001 nmol/mg protein/min, +65%, $P < 0.001$) and NADH dehydrogenase regions (0.16 ± 0.01 nmol/mg protein/min, +33%, $P < 0.05$, mean ± SD) in mitochondria depleted of GSH compared to control mitochondria. Increased $H_2O_2$ formation in GSH-depleted mitochondria compared to control mitochondria was also observed at low but not at higher $HgCl_2$ concentrations.

As an alternative method for measuring $H_2O_2$, the $H_2O_2$-dependent intramitochondrial oxidation of 2,7-dichlorofluorescin diacetate [20] was assayed after a 30-min incubation with mitochondria. This method also showed that Hg(II) at concentrations up to 12 nmol/mg protein enhanced $H_2O_2$ formation in mitochondria supplemented with succinate and AA (data not shown). However, the sensitivity of this assay was not sufficient to detect $H_2O_2$ formation at the NADH dehydrogenase region.

To study the effects of Hg(II) on the rate of electron flow through the inhibited electron transport chain, the oxygen consumption of electron transport-inhibited mitochondria was examined. Figure 2 shows that Hg(II) decreased the oxygen consumption by both AA- and rotenone-inhibited mitochondria at concentrations up to 30 nmol Hg(II)/mg protein. The dashed line indicates the amount of $O_2$ consumption that was not inhibited by 1 mM KCN for either AA- or rotenone-inhibited mitochondria.

The increased mitochondrial $H_2O_2$ formation by Hg(II) was associated with increased rates of mitochondrial lipid peroxidation. Because $H_2O_2$-dependent lipid peroxidation requires a transition metal, TBARS formation occurred only in the presence of added iron (Fe(III):ADP). As shown in Table 2, low levels of TBARS formation were detected when only Fe(III):ADP and succinate or malate/glutamate were present in the mitochondrial reaction mixture. TBARS formation was increased by addition of AA or rotenone to substrate-supplemented mitochondria. Addition of 18 nmol $HgCl_2$/mg protein, the concentration that maximally increased $H_2O_2$ production under the present conditions, further increased the TBARS formation to 18 nmol MDA/mg protein/30 min at the NADH dehydrogenase region and to 11 nmol MDA/mg

Table 2. Effects of $HgCl_2$ and electron transport inhibitors on mitochondrial lipid peroxidation

| Additions | TBARS formation (nmol MDA/mg protein/30 min) |
|---|---|
| Succinate | 4.0 ± 0.7 (5) |
| Succinate + AA | 9.5 ± 1.1* (5) |
| Succinate + AA + $HgCl_2$ | 11.0 ± 1.1* (5) |
| Malate/glutamate | 3.8 ± 0.8 (4) |
| Malate/glutamate + rotenone | 4.9 ± 1.0 (4) |
| Malate/glutamate + rotenone + $HgCl_2$ | 18.4 ± 1.8† (4) |

Mitochondria were incubated for 30 min in the presence of Fe(III):ADP (20:100 μM). Succinate (6 mM), AA (1 nmol/mg), malate/glutamate (2.5 mM/2.5 mM), rotenone (3 nmol/mg) and $HgCl_2$ (18 nmol/mg protein) were added as indicated in the table. TBARS were assayed at 532 nm ($E = 1.56 \times 10^5\ M^{-1}\ cm^{-1}$) as described in Materials and Methods. Values are means ± SD of the number of determinations (different mitochondrial preparations) shown within parentheses.

* Statistically different from rates in the absence of AA, $P < 0.05$.

† Statistically different from rates in the absence of $HgCl_2$, $P < 0.05$.

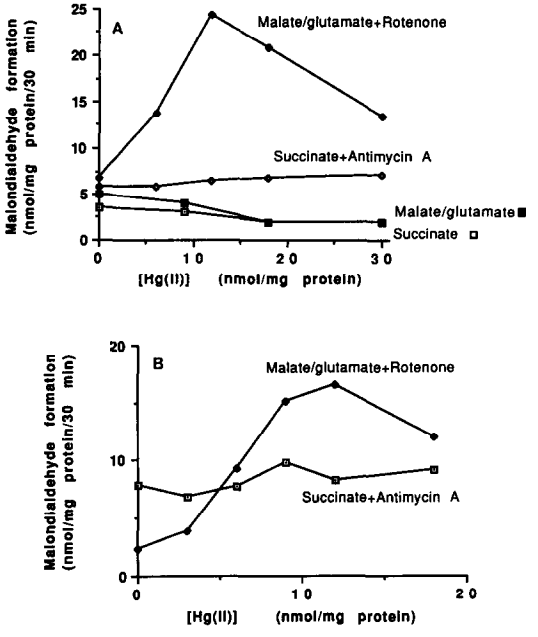


Fig. 3. Effects of $HgCl_2$ on lipid peroxidation in control and GSH-depleted mitochondria. Control (A) or GSH-depleted (B) mitochondria were incubated for 30 min in the presence of Fe(III):ADP (20:100 μM). Succinate (6 mM), succinate-AA (1 nmol/mg), malate/glutamate (2.5 mM/2.5 mM), malate/glutamate-rotenone (3 nmol/mg) and $HgCl_2$ were added as indicated in the panels. GSH-depleted kidney mitochondria (approximately 15% of control values) were prepared from rats 2.5 hr after injection of BSO-DEM, as described in Materials and Methods. TBARS were assayed at 532 nm ($E = 1.56 \times 10^5\ M^{-1}\ cm^{-1}$) as described in Materials and Methods. The figure shows one representative experiment of three with mean values of duplicate samples.

protein/30 min at the ubiquinone-cytochrome *b* region.

The concentration-dependent effects of $HgCl_2$ on lipid peroxidation are shown in Fig. 3A. In the absence of electron-transport inhibitor, $HgCl_2$ caused a slight decrease in TBARS formation at concentrations up to 30 nmol/mg protein regardless of substrate used. In contrast, formation of TBARS was slightly increased in AA-inhibited mitochondria and maximally increased by 4-fold in rotenone-inhibited mitochondria at 12 nmol Hg(II)/mg protein. Similar results were obtained when citrate was used as iron chelator instead of ADP (data not shown). Figure 3B shows the effect of $HgCl_2$ on TBARS formation in GSH-depleted mitochondria ([GSH] approximately 15% of control values). Comparison of panels A (control mitochondria) and B of Fig. 3 indicates that GSH depletion does not alter significantly the effects of Hg(II) on mitochondrial TBARS formation.

Studies were conducted to determine the effects of electron transport inhibitors and Hg(II) on mitochondrial GSH content. The GSH concentration in freshly prepared, substrate-supplemented mitochondria was 1.7 nmol GSH/mg protein. Mitochondrial GSH decreased during a 30-min incubation period to approximately 0.9 nmol GSH/mg protein. As seen in Fig. 4 (at 0 nmol Hg(II)/mg protein), the presence of rotenone in the reaction mixture during a 30-min incubation seemed to slightly prevent GSH depletion, since the GSH concentration decreased to only 1.4 nmol/mg protein, as opposed to 0.9 nmol/mg protein when rotenone was absent. In contrast, the presence of AA in the reaction mixture slightly increased the loss of GSH, which decreased to 0.5 nmol/mg protein during a 30-min incubation.

Figure 4 also shows the effects of $HgCl_2$ on the mitochondrial GSH concentration after a 30-min incubation period. In substrate-supplemented mitochondria, $HgCl_2$ (12 nmol/mg protein) decreased the GSH concentration to approximately 10% of control levels. When electron transport inhibitors were also present, however, Hg(II) decreased the GSH concentration in rotenone-inhibited, but not in AA-inhibited, mitochondria.

## DISCUSSION

Numerous studies have suggested that oxidative

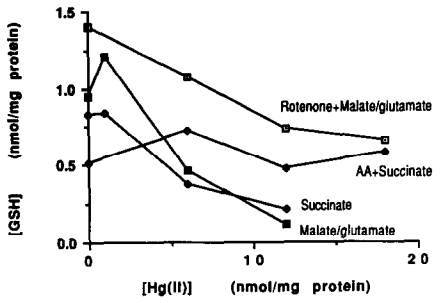


Fig. 4. Effects of $HgCl_2$ on mitochondrial GSH concentration. Mitochondria (2 mg/mL) were incubated for 30 min in the presence of Fe(III):ADP (20:100 $\mu$M). Succinate (6 mM), malate/glutamate (2.5 mM/2.5 mM), succinate-AA (1 nmol/mg protein), malate/glutamate-rotenone (3 nmol/mg protein) and $HgCl_2$ were included as indicated in the figure. The GSH concentration was determined as described in Materials and Methods. The GSH concentration at the start of the incubation was $1.75 \pm 0.11$ nmol GSH/mg mitochondrial protein. The figure shows one representative experiment of two with mean values of duplicate samples.

tissue damage is a principal underlying action of mercury-induced nephrotoxicity [6–8, 21, 22]. The present study provides evidence that this effect involves Hg(II)-dependent increases in mitochondrial $H_2O_2$ formation and lipid peroxidation. $HgCl_2$ maximally increased the $H_2O_2$ formation by approximately 2-fold at the NADH dehydrogenase region and 5-fold at the ubiquinone-cytochrome *b* region of the mitochondrial respiratory chain when rotenone or AA, respectively, were employed as the electron transport inhibitors. Preliminary studies also indicated increased $H_2O_2$ production in kidney mitochondria isolated from rats treated *in vivo* with $HgCl_2$ (unpublished observations).

The precise mechanism(s) whereby $HgCl_2$ interacts with the electron transport chain to increase $H_2O_2$ formation is not presently clear. One explanation of this effect is that mercury disrupts the structural integrity of the mitochondrial inner membrane resulting in altered ion permeability and membrane potential [3, 23], with secondary effects on the rate of $H_2O_2$ formation [11]. This possibility is supported by previous studies from this laboratory which demonstrate ultrastructural changes in the mitochondrial membrane as a primary event in mercury-induced loss of respiratory control and other biochemical functions [10]. Additionally, indications from the present study imply direct effects of Hg(II) on specific componenets of the respiratory chain. First, in AA-inhibited mitochondria, Hg(II) decreased only the $CN^-$-inhibitable $O_2$ consumption, whereas in rotenone-inhibited mitochondria, Hg(II) inhibited both $CN^-$-insensitive and -sensitive $O_2$ consumption. Second, the order of addition of $HgCl_2$ and substrate had differential effects on the NADH dehydrogenase and ubiquinone-cytochrome *b* regions; thus, $H_2O_2$ formation from the ubiquinone-cytochrome *b* region was greatest when the components of the respiratory chain were in a predominantly oxidized state (no substrate present) at the time of $HgCl_2$ addition, whereas the NADH dehydrogenase region was insensitive to the order of addition.

Consistent with an increased formation of $H_2O_2$ by Hg(II) in electron-transport inhibited mitochondria, an increased rate of TBARS formation was observed in the presence of $HgCl_2$. The effect of Hg(II) in promoting increased TBARS formation was greater when rotenone was used as an electron-transport inhibitor, despite a greater rate of $H_2O_2$ formation in AA-inhibited mitochondria. This apparent discrepancy with regard to sites of $H_2O_2$ formation and lipid peroxidation might be explained by the chain-breaking antioxidant activity of reduced coenzyme Q (ubiquinol) [24–26]. When AA is added to substrate-supplemented mitochondria, a greater steady-state concentration of reduced coenzyme Q (ubiquinol) predominates, and thus a greater antioxidant capacity towards lipid peroxidation prevails. Conversely, rotenone blocks the electron flow between NADH dehydrogenase and coenzyme Q. Thus, in the presence of rotenone, coenzyme Q is predominantly in the oxidized form (ubiquinone), which lacks chain-breaking antioxidant activity. Therefore, in rotenone-inhibited mitochondria, an increased formation of $H_2O_2$, although less than that observed in the presence of AA, could result in increased lipid peroxidation (i.e. TBARS formation) because of the relatively lower concentration of ubiquinol. It is important to note that no attempt at equalizing the rate of electron flow was made in the present studies. Hence, the two systems (AA- versus rotenone-inhibited mitochondria) should not be considered directly comparable.

Another consequence of mercury exposure which is relevant to its prooxidant potential is the depletion of antioxidants, particularly GSH. Because of the high affinity of Hg(II) for GSH via mercaptide formation, GSH depletion by Hg(II) might be expected to also affect lipid peroxidation. In this study, Hg(II) decreased the GSH concentration in rotenone-inhibited, but not in AA-inhibited mitochondria, consistent with a greater stimulatory effect of Hg(II) on lipid peroxidation in rotenone-inhibited than in AA-inhibited mitochondria. However, extensive depletion of mitochondrial GSH by Hg(II) was observed also in mitochondria supplemented with substrate alone. Hg(II)-induced depletion of GSH could render the mitochondria more susceptible to oxidative stress, contributing to the mercury-induced increase in lipid peroxidation observed. This effect, however, is probably minor in this study, because lipid peroxidation increased to a comparable extent in both control and GSH-depleted mitochondria following addition of Hg(II).

The lack of a measurable effect of GSH depletion on mercury-promoted oxidant formation implies that reduced coenzyme Q (ubiquinol), a natural component of the electron transport chain, may be a more important mitochondrial antioxidant than GSH in preventing Hg(II)-induced lipid peroxidation. Indications supporting this view include: (i) lipid peroxidation and GSH depletion were minor when mitochondrial coenzyme Q was reduced by succinate, and (ii) no apparent correlation of mitochondrial GSH concentration and extent of

mitochondrial lipid peroxidation was observed. Other studies have shown that coenzyme Q is the most important lipophilic antioxidant in low-density lipoproteins [27] and α-tocopherol-loaded liposomes [28] exposed to oxidative stress. Frei *et al.* [28] also showed that, unlike α-tocopherol, GSH does not interact with coenzyme Q.

*In vivo* in rat kidney, Hg(II) inhibits several of the enzymes involved in the protection of cells against oxidative stress, including glutathione disulfide (GSSG)-reductase and GSH-peroxidase, as well as other enzymes involved in renal GSH metabolism, such as γ-glutamylcysteine synthetase and γ-glutamyl transpeptidase [5, 7]. Further studies of the effects of Hg(II) on the antioxidant capacity of the complete mitochondrial GSH-system (GSSG-reductase, GSH-peroxidase, and NADH-regeneration) are required to clearly establish the role(s) of GSH and coenzyme Q in Hg(II)-induced mitochondrial lipid peroxidation.

The finding that Hg(II) is capable of promoting oxidant formation by renal mitochondria while compromising cellular antioxidant systems suggests that oxidative damage to cellular constituents may constitute a fundamental mechanism of mercury-induced toxicity to tissue cells. Mercury has been shown to produce oxidative damage to subcellular membranes [29, 30] and to damage DNA by an oxidative-type mechanism [31]. Mercury-promoted formation of reactive oxidant species by tissue mitochondria has also been linked to facilitated oxidation of reduced porphyrins [22]. Should further research demonstrate that mercury promotes production of reactive oxidant species in the intact cell, such findings would provide a mechanistic basis for the tissue-damaging properties observed both biochemically and ultrastructurally during exposure to mercury compounds [4, 10, 32].

In summary, Hg(II) enhances the formation of $H_2O_2$ in rat kidney mitochondria *in vitro* under conditions of impaired respiratory chain electron transport. Hg(II) also promotes enhanced iron-catalyzed mitochondrial lipid peroxidation. These effects may underlie oxidative tissue damage associated with mercury exposure.

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