Direct Effect of Inorganic Mercury on Citrate Uptake by Isolated Rat Renal Brush Border Membrane Vesicles

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Abstract: Occupational exposure to mercury has long been associated with renal proximal injury and an increased incidence of proteinuria, as has such exposure to cadmium. Renal citrate excretion is very important with respect to acid-base balance since the metabolism of citrate generates three bicarbonate ions. In this study, we exposed isolated rat renal brush border membrane vesicles (BBMV) to mercury (Hg\(2^{+}\)) and examined their citrate uptake characteristics. BBMV were prepared by the divalent cation precipitation method. Citrate uptake was measured by the Millipore rapid membrane filtration method. The preincubation of BBMV with 0.5 and 2 mM HgCl\(_2\) for 1 min significantly inhibited citrate uptake compared with that of BBMV without Hg preincubation. The analysis of the time course of citrate uptake during a 30-min preincubation of BBMV with 0.1 mM Hg\(^{2+}\) also revealed a significant reduction in the uptake compared with that of the control BBMV without preincubation. These findings indicate that the preincubation of BBMV with mercury results in a time- and concentration-dependent inhibition of citrate uptake.

Key words: Mercury, Citrate, Brush border membrane vesicles (BBMV)

Introduction

The kidney is the organ most critically affected by the ingestion of inorganic bivalent mercury salts\(^{1}\). An increased prevalence of proteinuria was found in workers exposed to mercury compared with a control group, and a significant correlation between urinary mercury excretion and protein excretion has been demonstrated\(^{1}\). Cadmium also induces renal proximal injury\(^{2}\) and inhibits Na-amino acids\(^{3,4}\) and citrate\(^{5}\) cotransport systems in the rat renal brush border membrane. Membrane-impermeable chelating agents removed only about 60–65% of the bound cadmium from intact vesicles preincubated with Cd, and almost all Cd was removed by chelators when membrane permeability was increased by 1-butanol\(^{6}\). Mercury inhibits rat renal Na\(^{+}\)-Pi cotransporter\(^{7}\), but the mechanism by which mercury affects the transport system in the renal brush border membrane has not been clarified.

On the other hand, urinary citrate excretion is important with respect to acid-base balance, since the metabolism of citrate generates HCO\(_3^{−}\) ions. The excretion of citrate is equivalent to the loss of three HCO\(_3^{−}\) ions\(^{8}\). Citrate is freely filtered at the glomerulus, and its reabsorption in the proximal tubule is the major determinant of rate of the renal excretion\(^{9}\).

In the present study, we exposed rat renal brush border membrane vesicles (BBMV) to mercury and investigated the direct effect of the mercury exposure on the citrate uptake of the BBMV. The results presented here suggest that mercury
inhibits the Na-citrate co-transport system in renal brush border membranes.

**Materials and Methods**

**Materials**

\(^{14}\)C-citrate (spec. act. 50 mCi/m mol) was obtained from the New England Nuclear Corp. (Boston, MA). All other chemicals were reagent grade and purchased from commercial sources.

**Animals**

The animals used for these experiments were male Wistar rats (CLEA, Japan, Inc.) weighing 285–365 g (n=4). They were maintained in a temperature- and photoperiod-controlled animal house with *ad libitum* access to a standard diet and tap water.

**Brush border membrane preparation**

Each membrane specimen was prepared from the cortex of both kidneys of a single rat by a modification of the MgCl\(_2\)-precipitation technique\(^{12}\) and used within 4 h after preparation. During preparation, all materials were maintained on ice or at 4°C. Briefly the rats were anesthetized with an injection of chloral hydrate (360 mg/kg body wt) intraperitoneally. The renal cortices were removed and placed in an isolation buffer consisting of 50 mM mannitol and 2 mM Tris-(hydroxymethyl)-aminomethane (Tris)/HC1 (pH 7.0). They were homogenized in a glass Teflon homogenizer (Iuchi Co. Ltd., Japan) and a polytron homogenizer. A concentration of MgCl\(_2\) (1M) was added to the resultant homogenate to give a final concentration of 10 mM. This mixture was stirred for 20 min in a cold room and then centrifuged for 15 min at 3,000 g. The supernatant was collected and centrifuged for 20 min at 43,000 g. The pellet containing the BBMV was resuspended in isolation buffer and centrifuged for 15 min at 3,000 g. The supernatant was centrifuged for 20 min at 43,000 g. The final pellet containing the purified BBMV was resuspended in 260 mM mannitol and 20 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES)/Tris (pH 7.0).

**Marker enzyme assays**

The purity of membrane preparations was assessed from the enrichment of alkaline phosphatase (Alp)\(^{13}\) and leucine aminopeptidase (LAP)\(^{14}\) in the final preparation as compared with that of the original homogenate.

**Protein determination**

Protein determination was conducted by the method of Lowry *et al.*\(^{15}\) with crystalline bovine serum albumin as the standard.

**Uptake studies**

As with most organic solutes reabsorbed in the proximal tubule, citrate reabsorption was found to be sensitive to ouabain\(^{16}\). The uptake of citrate by the BBMV was determined by the Millipore rapid membrane filtration technique\(^{17-20}\). For the experiment in which BBMV were preincubated with HgCl\(_2\), membrane vesicles (10 µl) were preincubated at 30°C with 10 µl of preincubation solution consisting of 260 mM mannitol and 20 mM HEPES/Tris (pH 7.0) with or without HgCl\(_2\), for an appropriate interval. Citrate uptake was initiated by the addition of 40 µl of uptake solution consisting of 100 µM \(^{14}\)C-citrate, 130 mM NaCl and 20 mM HEPES/Tris (pH 7.0). After thirty seconds, the uptake was terminated by the addition of 4 ml of ice-cold solution consisting of 130 mM NaCl and 20 mM HEPES/Tris (pH 7.0). The solution was then rapidly filtered through a Millipore filter (DAWP02500: pore size 0.65 µm) and washed twice with 4 ml of ice-cold stop solution under vacuum suction. The background uptake was similarly determined by adding 4 ml of ice-cold stop solution to membrane vesicles (10 µl) before adding 40 µl of uptake solution at 4°C. The radioactivity associated with the filters was measured with a liquid scintillation counter (LSC-3500, Aloka). All uptake measurements were performed at 30°C in triplicate, and the uptake was calculated on the basis of the specific activity measured in each experiment. The non-specific binding value was subtracted from the experimental value. The vesicular uptake is expressed as picomoles \(^{14}\)C-citrate per mg protein and presented as means ± S.E. Student’s t-test was used to analyze differences among the groups. Significance was accepted at P<0.05.

**Results**

The purity of the BBMV preparations was assayed by determining the BBM-specific marker enzymes Alp and LAP. The specific activities of the two enzymes in the BBM fraction were enriched to a 10-fold higher level than those in the original homogenate.

**Effect of mercury concentration on citrate uptake by BBMV**

The effect of preincubation of BBMV with 0–2 mM HgCl\(_2\) for 1 min at 30°C is illustrated in Figure 1. The preincubation...
with 0.5 and 2 mM Hg significantly inhibited the citrate uptake compared with that without preincubation; the preincubation with Hg resulted in a concentration-dependent inhibition of citrate uptake by the BBMV.

Effect of mercury on citrate uptake by BBMV with respect to preincubation time

Figure 2 illustrates the time course of the inhibition of citrate uptake after preincubation with 0.1 mM HgCl₂. Following preincubation for 1 min, the citrate uptake by BBMV was decreased, but not significantly. Following 10 and 30-min preincubations, the citrate uptake was significantly decreased compared with that of the control without preincubation. These results indicate that Hg preincubation resulted in time- and concentration-dependent inhibition of citrate uptake by BBMV.

Discussion

Occupational exposure to inorganic mercury has been investigated in chloralkali plants, mercury mines, thermometer factories, refineries, and dental clinics. High mercury levels have been reported in all of these occupational exposure situations, although the levels vary according to work environment conditions. For the general population, the main sources of mercury exposure are dental amalgam restoration and thimerosal preservation in vaccines. The kidney is the organ most affected by the ingestion of inorganic bivalent mercury salts. Oliguria, anuria, and death from renal failure resulting from acute tubular necrosis has occurred not infrequently in the past following the ingestion of mercuric chloride either accidentally or with suicidal intent, and such conditions have also followed the therapeutic administration of mercurials. Organic mercurials have until recent years been used extensively in medical practice as effective diuretics in the management of congestive cardiac failure.

We previously reported that the preincubation of rat BBMV with cadmium results in time- and concentration-dependent inhibition of citrate uptake. In the present study, we found that mercury inhibited the citrate uptake by BBMV in the same manner. Although the preincubation of BBMV with 0.5 mM CdCl₂ for 1 min did not significantly inhibit citrate uptake, the preincubation with 0.5 mM HgCl₂ for 1 min significantly inhibited citrate uptake. Compared with our cadmium data, the present results indicate that mercury may be slightly more toxic than cadmium to renal brush border membranes.

Inhalation exposure to mercury may be followed by
dyspnea, interstitial pneumonia and renal tubular dysfunction\(^1\), which might induce respiratory and metabolic acidosis. Since the excretion of citrate is equivalent to the loss of three $\text{HCO}_3^-$ ions, the inhibition of citrate reabsorption contributes to the maintenance of metabolic acidosis during mercury intoxication.

The volume of BBMV in Cd-intoxicated rats induced by daily subcutaneous injections of CdCl\(_2\) at a dose of 2 mg Cd/kg body wt per day for 17 to 20 days was significantly reduced (50%) compared with that of control rats. Percentages of equilibrium value were also significantly inhibited in Cd-intoxicated rats\(^2\)\(^3\)\(^4\). Cadmium has a long biological half-life (about 30 years in humans)\(^2\)\(^4\)\(^5\). The half-time in the kidneys for inorganic mercury in the studies by Hursh et al.\(^2\)\(^5\),\(^2\)\(^6\) was 64 days, about the same as that for the body as a whole. Therefore, in vivo experiments in mercury-intoxicated rats are needed to further clarify the mechanism of renal disturbance and acid-base balance in chronic mercury intoxication.

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