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DIFFERENCES IN THE UPTAKE OF CADMIUM AND MERCURY BY RAT HEPATOCYTE PRIMARY CULTURES

ROLE OF A SULFHYDRYL CARRIER*

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Abstract—Studies measuring the uptake of cadmium or mercury in isolated hepatocytes demonstrated that hepatocytes accumulated more cadmium than mercury in serum-containing medium, serum-free medium, or balanced salt solution. The preferential hepatocellular accumulation of cadmium, independent of medium composition, suggested that the uptake mechanism for cadmium and mercury might be different in hepatocytes. Pretreatment of hepatocytes with 50 μM *N*-ethylmaleimide decreased cadmium uptake by 23% while having no effect on the uptake of mercury uptake. Cadmium uptake in the presence of parachloromercuribenzenesulfonate or mercury was inhibited in a concentration-dependent manner. The uptake of cadmium was maximally inhibited (80%) with 75 μM parachloromercuribenzenesulfonate or 20 μM mercury respectively. Cadmium had no effect on mercury. Hepatocytes treated with parachloromercuribenzenesulfonate or mercury accumulated cadmium at a rate closely resembling the rate of mercury uptake in untreated hepatocytes. These results suggested that an SH-containing carrier may be operative in the uptake of cadmium by hepatocytes. Mercury can interact with this carrier to inhibit cadmium uptake; however, this carrier does not appear to facilitate mercury uptake.

The liver plays a key role in the disposition of cadmium and zinc [1, 2]. This is due not only to the inducibility of a metal-binding protein known as metallothionein [3] but also to the initial rapid uptake of these metals into the liver [4–8].

Reports from several groups suggest the existence of carrier-mediated transport, in addition to passive diffusion, as a mechanism for the rapid uptake of cadmium and zinc by the isolated perfused liver [6, 9, 10], isolated hepatocytes [11–14], and various other mammalian cell lines [15–17]. Failla *et al.* [13] and Failla and Cousins [14] also suggested that the uptake of both cadmium and zinc occurs via an SH-dependent carrier mechanism in isolated hepatocytes. These hypotheses are supported by the findings that: (a) at high cadmium concentrations (where passive diffusion would play a large role in the uptake process) cadmium uptake rate constants are lower than at low cadmium concentrations (where a facilitated mechanism would contribute more to uptake) [6]; (b) cadmium and zinc demonstrate competitive uptake kinetics [10–12, 14–16]; and (c) SH blockers are able to reduce significantly the uptake of both cadmium and zinc while metabolic inhibitors have little or no effect on metal uptake [13, 14, 17].

In contrast to cadmium, mercury is not accumulated significantly by the liver [18–22]. Although cadmium and mercury are chemically similar, recent findings indicate that they have different dispositions in hepatocyte primary cultures [23]. Differences in the uptake of these metals may contribute to the overall dispositional difference observed for cadmium and mercury in hepatocytes. No studies have been reported in which mercury uptake has been examined in hepatocyte primary cultures. The experiments reported in this paper were carried out to investigate the hepatocellular uptake of cadmium and mercury and to study the involvement of an SH carrier in the uptake process.

METHODS

Chemicals. Calcium-free Hanks' balanced salt solution (HBSS)‖, Dulbecco's modified Eagle medium (DMEM), and heat-inactivated calf serum were purchased from GIBCO (Grand Island, NY). HEPES buffer, parachloromercuribenzenesulfonate (PCMBS), and *N*-ethylmaleimide (NEM) were purchased from the Sigma Chemical Co. (St. Louis, MO). $HgCl_2$ was purchased from Fisher Scientific (Fairlawn, NJ). $CdCl_2$ was purchased from Alfa

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‖ Abbreviations: HBSS, Hanks' balances salt solution; DMEM, Dulbecco's modified Eagle medium; HEPES, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid; NEM, *N*-ethylmaleimide; PCMBS, parachloromercuribenzenesulfonate; and SH, sulfhydryl.

Products (Beverly, MA). $^{109}CdCl_2$ (378 mCi/mg) and $^{203}HgCl_2$ (2.56 mCi/mg) were purchased from the New England Nuclear Corp. (Boston, MA).

Hepatocyte primary cultures. Primary cultures of hepatocytes were prepared as described previously [23]. Cells were plated at a density of 2×10^6 cells per 60 × 15 mm petri dish and incubated in DMEM with 10% calf serum overnight before uptake experiments were performed.

Measurement of metal uptake. Hepatocytes were exposed to a given concentration of cadmium or mercury in medium (with or without serum) or HBSS (pH 7.3) labeled with either 0.03 μCi/ml $^{109}Cd^{2+}$ or 0.06 μCi/ml $^{203}Hg^{2+}$. After incubation, the metal-containing medium was decanted, and the cells were washed four times with HBSS buffered with HEPES (pH 7.65). The cells were harvested in 2 ml of distilled water and sonicated at 50 W for 10 sec (model W 185 Sonifier, Heat Systems Ultrasonics, Plainview, NY); radioactivity was determined in a Packard Auto Gamma Scintillation Counter. An aliquot of the resulting homogenate was taken for protein determination by the method of Lowry *et al.* [24]. In experiments measuring the effect of SH blockers on metal uptake, cells were preincubated with either NEM, PCMBS or mercury in serum-free medium for 30 min prior to the addition of metal. Cells preincubated with NEM were washed four times with HEPES-buffered HBSS, and the uptake of cadmium or mercury was measured in serum-containing medium without NEM. In cells preincubated with PCMBS of mercury, the uptake of cadmium was then measured in the presence of PCMBS or mercury in serum-free medium. These experiments were conducted in serum-free medium to eliminate potential confounding interactions between endogenous SH-containing serum components and the SH blockers on metal uptake. After incubation with metal, all cells were harvested and uptake was determined as described. All experiments were conducted at 37° in 5% CO_2, 95% air.

RESULTS

Uptake of cadmium and mercury by hepatocytes. The uptake of cadmium and mercury by hepatocytes during the course of a 60-min incubation was measured in serum-free medium containing either 3 μM cadmium or 3 μM mercury (Fig. 1). A rapid uptake of mercury occurred during the first 5 min of metal incubation after which a slower second phase of uptake was observed. The uptake rate for cadmium in serum-free medium was rapid during the entire incubation period. At the end of the 60-min incubation period, hepatocytes had accumulated 4.3 times more cadmium than mercury.

To exclude any possible interaction of the metals with the medium as being the basis for the observed differences in uptake [25], the uptake of cadmium and mercury was also examined in HBSS. A comparison of the amount of metal accumulated by hepatocytes during a 30-min incubation with 3 μM cadmium or mercury in serum-free medium, HBSS (pH 7.3) and serum-containing medium is shown in Table 1. No significant difference was observed when comparing the uptake of each respective metal in serum-free medium versus its uptake in HBSS. The uptake of both metals was significantly lower in serum-containing medium when compared with uptake in either serum-free medium or HBSS.

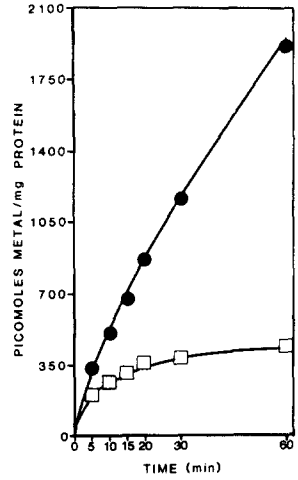

Fig. 1. Time course of cadmium and mercury uptake in serum-free medium. Hepatocytes were exposed to 3 μM cadmium or mercury in serum-free medium labeled with $^{109}Cd^{2+}$ or $^{203}Hg^{2+}$ for up to 60 min and then washed four times to remove metal-containing medium. The amount of cadmium (●) or mercury (□) associated with the hepatocytes was determined and standardized to hepatocyte protein. Each point represents the mean of at least four separate determinations; standard errors are smaller than the symbols.

Effect of SH blockers on the uptake of cadmium and mercury. Pretreatment of hepatocytes with 50 μM NEM resulted in a 23% decrease in the uptake of 3 μM cadmium at 30 min (Table 2). No significant difference in the uptake of 3 μM mercury was observed after this treatment. Hepatocytes exposed to higher concentrations of NEM showed signs of toxicity as determined by trypan blue uptake, cellular rounding, and detachment from dishes.

Table 1. Comparison of cadmium and mercury uptake in serum-containing medium, serum-free medium, and buffered HBSS*

	Metal uptake (pmoles/mg protein)	
	Cadmium	Mercury
Serum-containing medium	393 ± 12	259 ± 8
Serum-free medium	1161 ± 18†	371 ± 11†
HBSS	1115 ± 29†	407 ± 14†

* Hepatocytes were exposed to 3 μM cadmium or mercury in either serum-containing medium, serum-free medium, or HBSS (containing either $^{109}Cd^{2+}$ or $^{203}Hg^{2+}$ label) for 30 min. The amount of radioactivity associated with the hepatocytes was then determined. Each value represents the mean ± S.E. of at least four separate determinations.

† Significantly different from serum-containing medium at $P < 0.05$.

Table 2. Effect of NEM pretreatment on cadmium and mercury uptake by hepatocytes*

	Metal uptake (pmoles/mg protein)	
	Cadmium	Mercury
Control	427 ± 6	324 ± 5
50 μM NEM	330 ± 4†	328 ± 6

* Hepatocytes were preincubated in 50 μM PCMBS for 30 min in serum-free medium and then washed four times with HEPES-buffered HBSS. The uptake of 3 μM cadmium or mercury during a 30-min incubation in serum-containing medium (labeled with either $^{109}Cd^{2+}$ or $^{203}Hg^{2+}$) was determined. Each value represents the mean ± S.E. of four separate determinations.

† Significantly different from control at $P < 0.05$.

Treatment of hepatocytes with PCMBS [26] inhibited cadmium uptake in a concentration-dependent manner (Fig. 2). Maximal inhibition of cadmium uptake to 20% of control was obtained at a concentration of 75 μM PCMBS. The IC_{50} for PCMBS inhibition of cadmium uptake was approximately 26 μM. A consistent, concentration-dependent inhibition of cadmium by PCMBS was observed at concentrations between 0.5 and 3 μM cadmium (Table 3). No consistent effect of PCMBS on mercury uptake was observed (data not shown).

Treatment of hepatocytes with mercury also inhibited cadmium uptake in a concentration-dependent manner (Fig. 3 and Table 4). A maximal inhibition of cadmium uptake to 20% of control was obtained at a concentration of 20 μM mercury. The IC_{50} for mercury inhibition of cadmium uptake was approximately 8 μM. Concentrations of cadmium between 0.5 and 3 μM had no effect on the uptake of 10 and 20 μM mercury (data not shown).

As shown above, PCMBS and mercury significantly decreased the net uptake of cadmium by hepatocytes. PCMBS and mercury also significantly decreased the rate of cadmium uptake in hepatocytes when compared with control. Treatment of hepatocytes with 50 μM PCMBS or 20 μM mercury produced a time course of uptake for cadmium that closely resembled that of an equimolar concentration of mercury (Fig. 4).

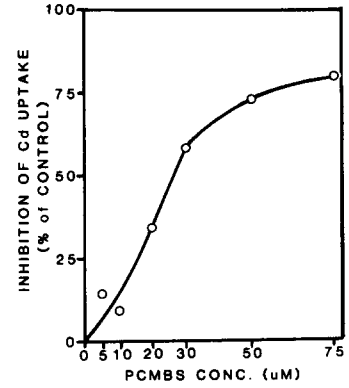

Fig. 2. Inhibition of cadmium uptake by PCMBS. Hepatocytes were preincubated with various concentrations of PCMBS in serum-free medium for 30 min. The uptake of 3 μM cadmium at 30 min was then determined in serum-free medium (labeled with $^{109}Cd^{2+}$) containing PCMBS and compared with the uptake of cadmium in control hepatocytes. Each point represents the mean calculated percent inhibition of four separate determinations.

DISCUSSION

The data presented in this paper demonstrate that cadmium uptake is greater than mercury uptake in hepatocyte primary cultures. Whether these uptake differences are due to differences in transmembrane transport, metal efflux rates or differences in membrane adsorption characteristics cannot be ascertained from this study. It has been shown previously that, at equimolar metal concentrations in serum-containing medium, there is less diffusible (non-protein bound) mercury than cadmium [23]. While this may play a role in the differential uptake of these metals in serum-containing medium, measurements of cadmium and mercury uptake in serum-free medium and in balanced salt solution revealed that cadmium was still preferentially accumulated over mercury. This suggests that the uptake mechanism for these two metals in hepatocytes may be different.

Other investigators have proposed the existence of an SH-containing, facilitated carrier mechanism working in conjunction with passive diffusion in the uptake of cadmium and zinc in several systems [6, 9–17] and have reported reductions in cadmium

Table 3. Effect of PCMBS on cadmium uptake*

	Cadmium uptake (pmoles/mg protein)			
PCMBS (μM)	Cadmium (μM) 0.5	1	2	3
0	261 ± 10	494 ± 13	881 ± 16	1161 ± 18
30	134 ± 7 (49)	258 ± 5 (48)	448 ± 11 (49)	485 ± 39 (58)
50	79 ± 8 (70)	130 ± 9 (74)	231 ± 10 (74)	315 ± 12 (73)

* Hepatocytes were preincubated in serum-free medium containing either 30 or 50 μM PCMBS for 30 min; the uptake at 30 min of 0.5, 1, 2 and 3 μM cadmium was determined in this medium. Each value represents the mean ± S.E. of at least four separate determinations. Numbers in parentheses represent the percent inhibition of cadmium uptake with respect to control.

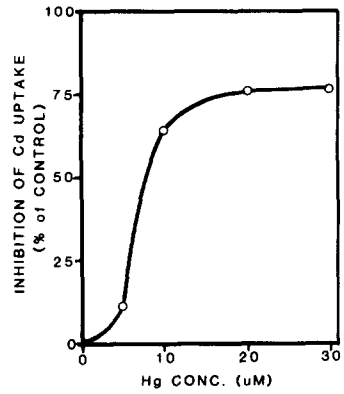

Fig. 3. Inhibition of cadmium uptake by mercury. Hepatocytes were preincubated for 30 min with various concentrations of mercury in serum-free medium. The uptake of 3 μM cadmium at 30 min was determined in serum-free medium (labeled with $^{109}Cd^{2+}$) containing mercury and compared with the uptake of cadmium in control hepatocytes. Each point represents the mean calculated percent inhibition of four separate determinations.

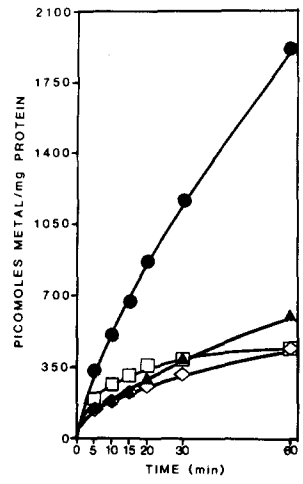

Fig. 4. Comparison of cadmium uptake in hepatocytes pretreated with SH blockers with the uptake of mercury in untreated hepatocytes. The uptake of 3 μM cadmium (●) or mercury (□) was determined in serum-free medium during the course of a 60-min incubation. The time course of uptake of 3 μM cadmium in hepatocytes treated with 50 μM PCMBS (◇) or 20 μM mercury (▲) was determined as described in Methods. All points represent the mean of at least four separate determinations; standard errors were smaller than the symbols.

uptake by treating hepatocytes with SH-blocking agents (presumably by blocking the SH carrier) [13, 14, 17]. In the present studies, a 23% inhibition of cadmium uptake was achieved after pretreatment with 50 μM NEM which had no effect on mercury uptake. This suggests that, while an SH-dependent mechanism plays a role in the uptake of cadmium, this mechanism does not seem to play an important role in the uptake of mercury. Uptake of mercury in hepatocyte primary cultures may take place by passive diffusion.

It is well known that mercury can bind tightly to SH groups [27]. Our findings indicate that mercury can interact with the proposed SH carrier in such a manner as to inhibit cadmium uptake. Both the non-penetrating mercurial SH blocker PCMBS [26] and inorganic mercury inhibit cadmium uptake by hepatocytes in a concentration-dependent manner. The time course of uptake profile obtained for cadmium after treatment with these mercurials closely resembles that of mercury uptake itself. In these experiments the mercurial is probably blocking the SH carrier, making it unavailable for cadmium transport. Under such conditions passive diffusion may become the predominating factor in the uptake of cadmium, thus making cadmium uptake resemble the uptake of mercury. While both inorganic mercury and PCMBS maximally inhibit cadmium uptake by 80% in hepatocytes, inorganic mercury is approximately three times more potent in its inhibitory action. These compounds probably inhibit cadmium uptake by the same mechanism, and chemical differences (i.e. steric considerations) may account for the differences in inhibitory potency.

The proposed SH carrier may be unimportant in the uptake of mercury itself because either: (1) mercury binds to the carrier too tightly to effectively dissociate from it once in the intracellular compartment and/or (2) mercury promotes some conformational change in the carrier rendering it inoperative. In either case mercury would essentially block its own uptake.

The initial rapid uptake of mercury seen during the first 5 min of metal incubation probably represented a non-specific surface binding of mercury to the hepatocyte as well as its binding to the carrier. This was followed by a slower uptake phase, prob-

Table 4. Effect of mercury on cadmium uptake*

Mercury (μM)	Cadmium uptake (pmoles/mg protein)			
	Cadmium (μM)			
	0.5	1	2	3
0	261 ± 10	494 ± 13	881 ± 16	1161 ± 18
10	139 ± 2 (47)	203 ± 7 (59)	337 ± 29 (62)	386 ± 17 (67)
20	82 ± 1 (69)	144 ± 4 (71)	231 ± 5 (74)	244 ± 8 (79)

* Hepatocytes were preincubated in serum-free medium containing either 10 or 20 μM mercury for 30 min, the uptake at 30 min of 0.5, 1, 2 and 3 μM cadmium was determined in this medium (labeled with $^{109}Cd^{2+}$). Each value represents the mean ± S.E. of at least four separate determinations. Numbers in parentheses represent the percent inhibition of cadmium uptake with respect to control.

ably representing only the passive uptake of mercury. A similar explanation of the time course of cadmium uptake in PCMBS-treated cells would also apply.

Experiments attempting to examine the effect of PCMBS on mercury uptake did not produce consistent results. This is most probably due to the fact that the affinities of PCMBS and mercury for SH groups (and other amino acid functional groups) are similar and, therefore, they can easily exchange with each other at these sites. Hence, PCMBS is not effective in blocking SH groups in experiments utilizing inorganic mercury. This exchange is not a factor in experiments utilizing cadmium since the affinity of cadmium for SH groups is several orders of magnitude less than that of mercurials [27].

The results of this study suggest that an SH-containing carrier may facilitate the uptake of cadmium by hepatocytes. Mercury can interact with this carrier to block cadmium uptake; however, this carrier does not appear to play an important role in mercury uptake. This phenomena may be the basis for the preferential hepatic uptake of cadmium observed *in vitro* and *in vivo*.

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