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Metal Transport in Cells: Cadmium Uptake by Rat Hepatocytes and Renal Cortical Epithelial Cells

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The toxic metals appear to use the transport pathways that exist for biologically essential metals. In this regard interactions between the toxic and essential metals are possible. This report summarizes recent findings on the transport of cadmium in rat hepatocytes and renal cortical epithelial cells in the presence or absence of certain essential metals. The transport of cadmium in hepatocytes does not require energy and, therefore, is not an active process. It occurs primarily (80%) by temperature-sensitive processes, i.e., ion channels and carriers, that involve interaction with sulfhydryl groups. These processes apparently exist for the transport of essential metals like copper, zinc and calcium. The remaining 20% of the cadmium in hepatocytes is transported via a temperature-insensitive process, possibly by diffusion. In comparison with the hepatocytes, a smaller fraction (30%) of the cadmium transport through the basolateral membrane and none from the apical membrane of the renal cortical epithelial cells is temperature-sensitive. Total accumulation through the basolateral membrane is about twice that through the apical membrane. A majority of the cadmium transport in the renal cells is by diffusion. As in hepatocytes, copper, zinc and mercury antagonize cadmium transport through the apical membranes of the renal cells. The relative antagonism by copper is the same (25%); however, the antagonism by zinc (16%) and mercury (10%) is 4- to 6-fold lower than in hepatocytes. It appears that the relative contribution of various transport pathways available for cadmium uptake is different in each cell type and apparently depends on the morphological and functional differences between the cell membranes. — Environ Health Perspect 103(Suppl 1):73-75 (1995)

Key words: metal transport, cadmium transport, metal interactions, hepatocytes, kidney cells, renal tubular epithelial cells, cadmium, zinc, copper, mercury, calcium channels

Introduction

Transport of metals in cells can be visualized as a two-step process that involves binding to anionic sites on membrane phospholipids and proteins, including the plasma membrane receptors, and internalization (1). The metals may enter the cell by simple diffusion through nonspecific ion pores or through specific ion-channels like those for Ca^{++} , Na^+ , and K^+ . The internalization step may involve membrane carriers. A metal may also be internalized in a protein-bound form through receptor-mediated endocytosis, e.g., iron bound to ferritin (2).

Well-defined homeostatic uptake and efflux pathways exist for biologically essential metals. The toxic metals apparently compete with the essential metals to use the same pathways, thus disrupting the intracellular balance of the essential metals and resulting in toxicological consequences. Recent findings from our laboratory on the transport of cadmium and its

interactions with other metals are summarized in this report.

Materials and Methods

Two types of cells were studied: primary cultures of rat hepatocytes and renal cortical epithelial cells. Details of the cell isolation, culture, and the experimental procedures are provided in earlier publications (3-5). Briefly, the hepatocytes were isolated by a collagenase perfusion method (6) from Sprague-Dawley rats. The cells were cultured in six-well plastic plates for 24 hr in Waymouth's MB752/1 medium containing 2% bovine serum albumin. The renal cortical epithelial cells were isolated by collagenase digestion of rat renal cortex. These cells were cultured in Dulbecco's modified Eagle's medium and Ham's nutrient mixture F-12 containing 10 to 15% fetal bovine serum for up to 5 days, at which time the cultures reached confluence. The uptake of cadmium was examined after replacement of the culture medium to Hank's balanced salt solution that contained no serum proteins. The transport of cadmium was studied in hepatocytes after incubation with ^{109}Cd -labeled $3 \mu\text{M CdCl}_2$ and in renal cells after incubation with ^{109}Cd -labeled $1 \mu\text{M CdCl}_2$ for up to 30 min. The cells were washed with ice-cold phosphate-buffered saline containing 2 mM EGTA to remove the free and loosely bound cadmium. The cadmium remaining in the cells after washing was

quantitated and the amount of cadmium taken up normalized by the protein content. The data plotted in the figures are means \pm SE of six wells per data point.

Results and Discussion

Cadmium Uptake by Rat Hepatocytes

The uptake of cadmium in hepatocytes at 37°C was rapid during the first few minutes and continued at a slower rate for the duration of the observation period (Figure 1). The uptake of cadmium at 4°C , however, was considerably lower, and the accumula-

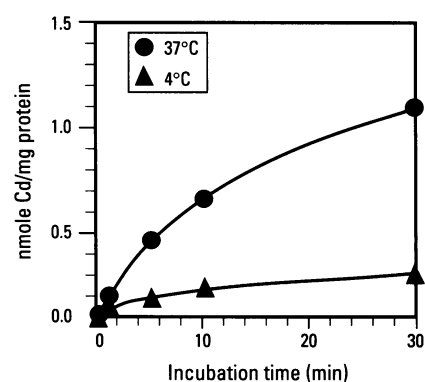


Figure 1. Effect of temperature on cadmium uptake in rat hepatocytes. The cells were incubated in HBSS for 30 min at either 4 or 37°C prior to incubation with $3 \mu\text{M CdCl}_2$ at the appropriate temperature. The standard errors are smaller than the symbols. Adapted from Blazka and Shaikh (4).

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tion over 30 min was only 20% of that at 37°C. After 1 min incubation at 37°C about 13% of the accumulated Cd was associated with cell membranes. This fraction remained essentially unchanged even after 30 min.

From the above results it can be deduced that cadmium uptake is biphasic and temperature-sensitive. Furthermore, that membrane-associated component accounts for a relatively small fraction of total cadmium accumulation. This implies that cadmium is efficiently transported across the hepatocyte plasma membrane, which is in accord with the *in vivo* observation of rapid cadmium accumulation in the liver after iv administration in rats (7). Marked inhibition of cadmium uptake (55 and 65%, respectively) by sulfhydryl blockers, PCMBS and NEM (4), indicates that binding of cadmium to plasma membrane sulfhydryl groups is an essential step in transport of about two-thirds of the cadmium in these cells.

A part of the temperature-sensitive component of cadmium transport was determined to occur through receptor-operated Ca^{++} channels. Of the four Ca^{++} channel blockers tested, verapamil was the most effective and inhibited about one-third of the cadmium uptake through this route (Figure 2). The hydrated ionic radii of cadmium and calcium are very similar, thus, cadmium can easily pass through the Ca^{++} channels. Furthermore, the binding affinity of cadmium to the calcium-binding sites is greater, which facilitates the displacement of calcium by cadmium (8–10).

Not only copper and zinc but also mercury antagonized cadmium accumulation in hepatocytes (Figure 3). The antagonism was very pronounced in the presence of 10 times molar excess of zinc or mercury (62 and 61%, respectively). In comparison, the antagonism by copper was only 22%. Zinc, copper, mercury, and cadmium compete for binding to sulfhydryl ligands that also include transport carriers (11–13). Of the two essential metals, zinc was the most potent antagonist of cadmium accumulation. The inability of copper to antagonize cadmium accumulation to the same extent as zinc goes along with the notion that there are different sulfhydryl-containing membrane carriers or ion channels for copper (14).

The competitive nature of the antagonism was confirmed by the Lineweaver-Burk plots of the initial rates of cadmium uptake in the absence or presence of 30 μ M copper, zinc, or mercury. Neither of these metals had any significant effect on the

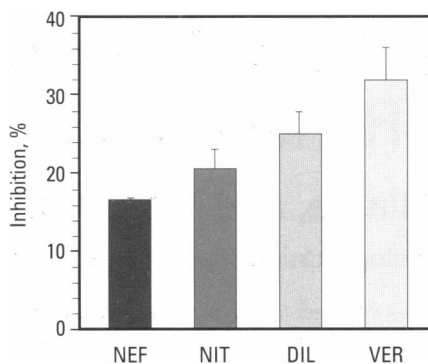


Figure 2. Effect of cadmium channel blockers on cadmium uptake in rat hepatocytes. The cells were preincubated for 30 min with 100 μ M nifedipine (NEF), nitrendipine (NIT), 250 μ M diltiazem (DIL), or verapamil (VER), followed by coincubation with 3 μ M cadmium for 30 min. Adapted from Blazka and Shaikh (3).

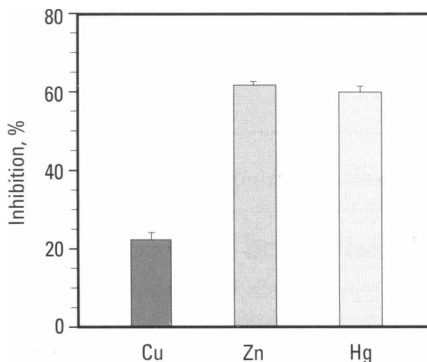


Figure 3. Antagonism of cadmium uptake by copper, zinc, and mercury in rat hepatocytes. The cells were coincubated with 3 μ M $CdCl_2$ and 30 μ M $CuCl_2$, $ZnCl_2$, or $HgCl_2$ for 30 min. Adapted from Blazka and Shaikh (4).

V_{max} but increased the K_m , which is typical of the competitive inhibition kinetics (4). The competitive inhibition of cadmium accumulation by copper and zinc was limited to the inhibition of its uptake and was not accompanied by acceleration of its efflux from the cells.

Cadmium Uptake by Renal Cortical Epithelial Cells

As in hepatocytes, cadmium uptake in the renal cells at 37°C was also rapid initially and then continued at a slower rate (Figure 4). However, total accumulation after 30 min was about 19% of that in the hepatocytes. Taking into account the difference in cadmium concentration in the medium (1 vs. 3 μ M), the accumulation in the renal cells was only about a sixth of that in hepatocytes. The temperature effect in the renal cells was dependent on the cell density

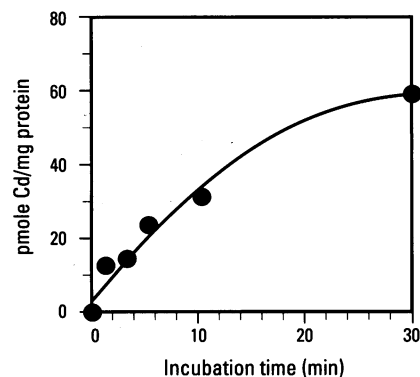


Figure 4. Time-course of cadmium uptake by rat renal cortical epithelial cells. The cells were preincubated for 30 min in HBSS at 37°C prior to incubation with 1 μ M $CdCl_2$ at the appropriate temperature. The SE was smaller than the size of the symbol.

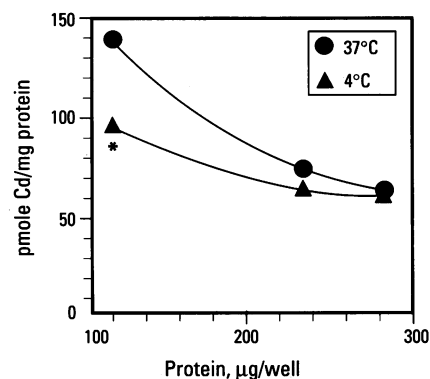


Figure 5. Effect of cell density on cadmium accumulation in renal cortical epithelial cells. The cells were cultured for 3, 4, or 5 days and the accumulation of 1 μ M cadmium was determined after 30 min incubation at 4 or 37°C. Total protein content was used as an index of relative cell density. *Significantly different from 37°C ($p < 0.05$). Adapted from Endo and Shaikh (5).

(Figure 5). At low cell density (111 μ g protein/well), lowering the temperature to 4°C reduced cadmium accumulation by about 30%. The temperature effect was completely abolished when the cell culture reached confluence (>280 μ g protein/well). Also, cadmium accumulation was lower at high density than at low density; at 37°C the confluent cultures accumulated only 45% of the amount taken by the nonconfluent. The explanation for these observations lies in the morphologic changes associated with confluence of the renal cortical epithelial cells. These cells develop brush borders on their apical surface, a recreation of the *in vivo* morphology, only upon forming a confluent monolayer. The two types of membranes, basolateral and apical, have very different physiologic functions and,

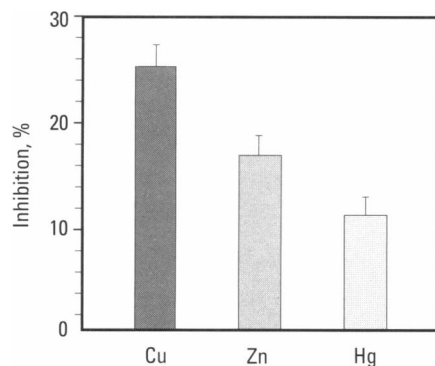


Figure 6. Antagonism of cadmium uptake by copper, zinc and mercury. The renal cortical epithelial cells were coincubated with 1 μ M cadmium and 30 μ M copper or zinc, or 1 μ M mercury for 30 min. Adapted from Endo and Shaikh (5).

therefore, exhibited very different transport characteristics.

Temperature dependence of a part of cadmium uptake in nonconfluent cultures indicates that from the basolateral membrane surface a fraction of the metal is

taken up through ion channels and/or membrane carriers that are not influenced by a change in membrane fluidity. In comparison, temperature insensitivity of cadmium transport in confluent cultures and its lack of requirement for metabolic energy (5) indicates an uptake process that is composed of simple diffusion.

In confluent cultures of renal cortical epithelial cells, the antagonism of cadmium uptake by 30 μ M copper (Figure 6) was the same as in hepatocytes (25% vs. 22%). The antagonism by 30 μ M zinc, however, was much smaller than in hepatocytes (16 vs. 62%), even though the molar ratio of zinc to cadmium in the medium was 3 times higher in the case of renal cells. Similarly, the antagonism by equimolar mercury was markedly lower than in hepatocytes (11 vs. 61%). Mercury concentrations greater than 1 μ M produced cytotoxicity in the renal cortical epithelial cells and enhanced rather than inhibited cadmium accumulation, possibly due to damage to the plasma membrane caused by this highly nephrotoxic metal.

As in hepatocytes, the antagonism by copper and zinc was competitive in nature. These metals antagonized cadmium accumulation by inhibiting its uptake and not by enhancing its efflux (5).

In conclusion, the toxic metal cadmium appears to use the transport pathways intended for the essential metals, zinc, copper, calcium, etc. Furthermore, these pathways are not available to the same extent in all cell types. Directionality of the cell surface, i.e., apical (brush border) versus basolateral side is also important, as it may offer different mechanisms for metal transport. Thus, it is important to study metal transport not only in the target cell types but also under conditions that allow the cells to mimic their *in vivo* morphology. Presence of other metals in the medium provides opportunities for metal interactions that could influence mutual transport, resulting in excesses and deficiencies of the toxic and essential metals in the cell, respectively. Such interactions have obvious toxicological consequences and, therefore, need to be further characterized.

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