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ZINC UPTAKE BY ISOLATED RAT HEPATOCYTES *

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Summary

Isolated rat hepatocytes were used to investigate the uptake of zinc at early exposure times. Hepatocytes were incubated with ^{65}Zn (1—500 μM) and samples were withdrawn at times ranging from 25 s to 60 min. A biphasic pattern of uptake was observed with a rapid first phase of uptake followed by a slower second phase. The relationship between velocity of uptake and substrate concentration for the first phase was nonlinear, while that of the second phase was linear. The presence of 10 μM cadmium produced a decrease in the velocity of uptake of only the first phase. This suggests that the first phase is at least partly carrier mediated, while there is no indication of involvement of a carrier in the second phase. KCN (1 mM) and carbonyl cyanide *m*-chlorophenylhydrazone (2 μM), did not cause any change in the uptake of ^{65}Zn (1 μM), which suggests that there is no active component in the uptake of zinc.

Introduction

The nutritional importance of dietary zinc is widely appreciated [1]. For dietary zinc to exert its effects on cellular metabolism, it must, after ingestion, undergo absorption and transport from the gastrointestinal tract. These processes are therefore nutritionally important and have been reviewed by Evans [2] and Cousins [3]. Also important for expression of cellular effects is uptake of zinc into the cell itself [3].

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The advantages of cell cultures have been utilized to study uptake of zinc at the cellular level. Cox [4] and Cox and Ruckenstein [5] using HeLa S_3 cells, found that zinc uptake was reduced by incubation at low temperatures, by the presence of sulfhydryl blocking agents and cadmium and that glucocorticoid hormones could enhance zinc uptake. Another study showed that both RNA and protein synthesis are required for the hormone-induced increase in zinc uptake [6].

Schwarz and Matrone [7], who used the 3T3 line of mouse fibroblasts (clone 4A), found evidence of carrier mediation for zinc uptake. Their data also showed evidence of a rapid initial uptake with a later slower phase.

Using primary cultures of isolated adult rat hepatocytes, which do not suffer from a marked loss of organ specific function, Failla and Cousins [8,9] obtained results similar to those of the above studies. They were able to extend the studies to show that the accumulated zinc was bound to a low molecular weight protein.

The apparent rapid early uptake noted in these studies was not investigated in detail. The use of isolated hepatocyte suspensions and a technique for the rapid separation of hepatocytes from the incubation medium allows investigation of cellular uptake of various compounds over very short time intervals. The purpose of the present study, therefore, was to make use of the isolated hepatocytes and the rapid sampling procedure to examine in detail the cellular uptake process for zinc, especially at the early times.

Methods

Male Sprague-Dawley rats (230—320 g) from Bio-Lab (White Bear, MN) were used as liver donors. They were allowed free access to food (Purina lab chow) and water. Urethane (2.2 g/kg, intraperitoneally) was used as the anesthetic and surgery was performed at about 8:30 a.m. for each experiment.

Hepatocytes were isolated essentially by using the method of Berry and Friend [10] with some modifications as previously described [11].

Suspensions of hepatocytes (3.6 ml) were pre-incubated for 5 min at 37°C in a Dubnoff metabolic shaking bath (80 oscillations per min) before addition of $^{65}ZnCl_2$ (^{65}Zn, carrier-free, New England Nuclear, Boston, MA) dissolved in saline (150 μl) at final concentrations of 1—500 μM. Each incubation vessel contained a similar amount of radioactivity (100 nCi per ml suspension). Incubation was for a total of 60 min during which duplicate 200-μl aliquots were removed at 25, 50, 75 and 100 s and at 15, 30, 45 and 60 min and processed to determine uptake of ^{65}Zn by the isolated hepatocytes, as previously described for ^{109}Cd [11].

In some experiments the effects of metabolic inhibitors and $CdCl_2$ on cellular uptake of ^{65}Zn (1 μM) were examined. Either KCN (Mallinckrodt Inc., St. Louis, MO) or carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) (Calbiochem, San Diego, CA) at final concentrations of 1 mM and 2 μM, respectively, was added to the cell suspension just prior to pre-incubation, as was $CdCl_2$ (2 or 10 μM). In these experiments duplicate incubation vessels were used. Furthermore, duplicate aliquots (150 μl) were withdrawn from each flask for determination of cellular uptake of ^{65}Zn at each time point.

Generally, five or six rats were used to supply the isolated hepatocyte preparations to obtain the data for this report. Each datum point represents a mean value with $n = 5$ or 6, with each n value being supplied by hepatocytes from a different rat. Statistical evaluation was by the paired t-test, with significance set at the 5% level.

Results

The uptake zinc (1, 5, 25, 100, 250 and 500 μM) by isolated rat hepatocytes over 60 min is shown in Fig. 1. The curves show a rapid early phase of uptake followed by a slower second phase of increasing accumulation with time. Uptake rates (v_0) over the first 100 s of incubation were calculated from the samples taken at 25, 50, 75 and 100 s. The v_0 values are plotted against their respective substrate concentration (S_0) in Fig. 2, which depicts a nonlinear

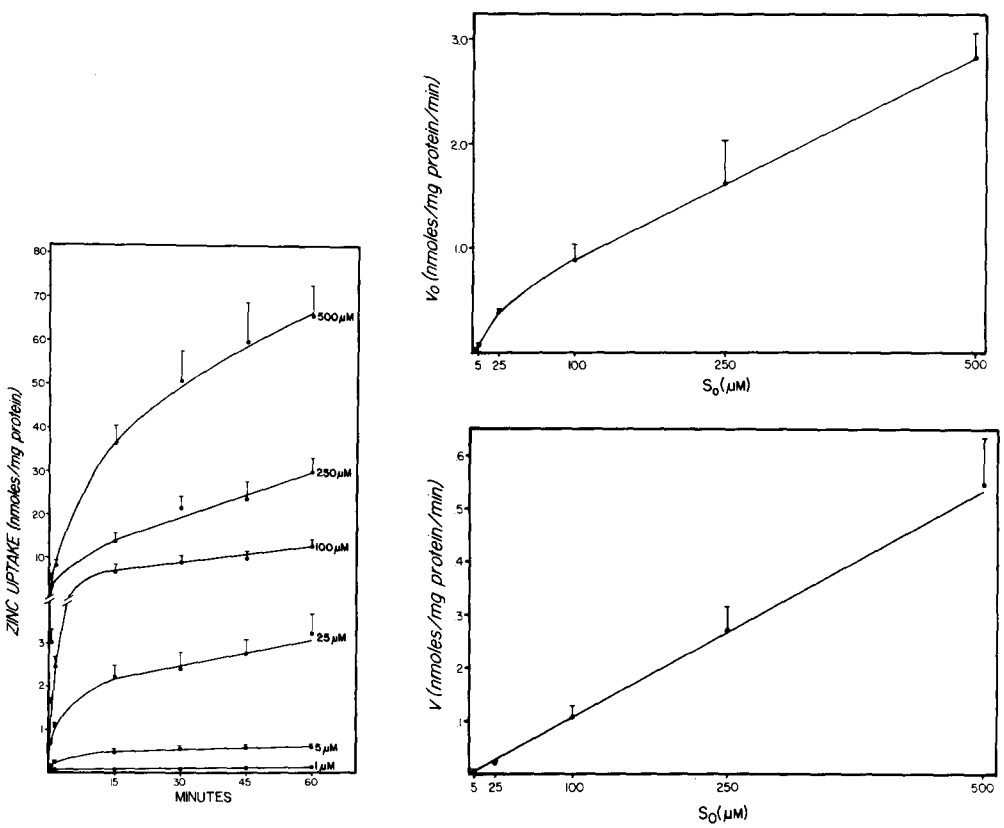

Fig. 1. Uptake of zinc (1—500 μM) by isolated hepatocytes. Each point represents the mean (±S.E.) from five or six separate hepatocyte preparations.

Fig. 2. Velocity of uptake of the first phase, v_0, calculated from 25—100 s of incubation. Each point represents the mean (±S.E.) from five or six separate hepatocyte preparations.

Fig. 3. Velocity of uptake, v, calculated from 15—60 min of incubation. Each point represents the mean (±S.E.) from five or six separate hepatocyte preparations.

TABLE I

EFFECT OF KCN, CCCP AND $CdCl_2$ ON THE UPTAKE OF ZINC (1 μM)

	v	v_o
Control	15.3 ± 2.6 *	0.72 ± 0.10
KCN (1 mM)	15 ± 1.6	0.73 ± 0.12
CCCP (2 μM)	15 ± 0.9	0.70 ± 0.11
$CdCl_2$ (2 μM)	12.2 ± 1.6	0.65 ± 0.12
$CdCl_2$ (10 μM)	11 ± 1.7 **	0.63 ± 0.13

* Mean ± S.E. for five or six separate hepatocyte preparations.
** Statistically different from controls.

relationship between the two variables. This suggests involvement of a carrier in the uptake of zinc, at least in part.

The uptake rates for the second phase (v) calculated from the 15, 30, 45 and 60 min samples, however, showed a linear relationship with S_o (Fig. 3). This demonstrates a lack of saturability of the uptake process, suggesting that this second phase is not directly related to a carrier-mediated process. A comparison of the v_o values with the v values shows that v_o is at least 5-times greater than the corresponding v, which emphasizes the biphasic nature of the uptake process. The effects of the metabolic inhibitors and $CdCl_2$ on uptake of 1 μM ^{65}Zn on both v_o and v are shown in Table I. Only $CdCl_2$ at 10 μM reduced the v_o of ^{65}Zn, while none of the additions to the hepatocyte suspension caused any alteration in v. The reduction in v_o of ^{65}Zn in the presence of cadmium strengthens the conclusion that v_o is, at least partially, carrier mediated. The lack of effect of the metabolic inhibitors (Table I) indicates that neither phase of uptake is an active process.

Discussion

The results of this study with suspensions of freshly isolated hepatocytes have shown that uptake of zinc with time is a biphasic response. This is in agreement with the implications that can be drawn from the data of Schwarz and Matrone [7] and Failla and Cousins [8]. Furthermore, we have found that the first phase of uptake shows characteristics of mediated transport, but the second slower phase does not. In contrast to the findings of Failla and Cousins [8], we could not demonstrate any inhibition of uptake of Zn in the presence of the metabolic inhibitors, KCN and CCCP. Cox [4], who used cell cultures, reported that inhibitors of oxidative metabolism did not cause a decrease in the uptake of zinc. Only when inhibitors of glycolysis and oxidative metabolism were added together, was zinc uptake reduced. The authors had reservations as to the interpretation of these results, since the combination treatment caused changes in cellular characteristics.

The decrease in uptake rate for Zn induced by a 10-fold higher concentration of Cd than Zn in the medium was only minimal for v_o and non-existent for v. In fact, at 60 min of incubation there was no statistical difference in the amount of ^{65}Zn taken up in the absence or presence of Cd. This is in contrast

to the inhibition by Cd reported by other laboratories [4,8]. The reason for the observed difference may be related to protein in the external medium.

It has been suggested that zinc and cadmium share a common pathway for uptake by liver [12]. A comparison of the results of this present report on uptake of ^{65}Zn with a previous study from our laboratory for uptake of ^{109}Cd [1] shows similarities in the pattern of uptake. Firstly, both show a rapid first and slower second phase of uptake, neither of which is reduced by the metabolic inhibitors, KCN and CCCP. Secondly, both cadmium and zinc will inhibit only the early phase of uptake of the other when the competing metal is added to the medium in relative excess. The only observed difference is that the v_o vs. S_o curve for Cd is linear whereas that for Zn is not. However, since Zn inhibits v_o of Cd, then it is likely that this phase of uptake for Cd is at least partly carrier-mediated, which is consistent with the data for Zn uptake. Such interactions have toxicological relevance since zinc is known to protect against various manifestations of cadmium toxicity [13].

In conclusion, the data of this report show that the uptake of zinc by isolated rat hepatocytes is a biphasic process, and that only the first phase (v_o) shows characteristics of carrier-mediated transport. In contrast to other reports, metabolic inhibitors did not decrease the uptake of zinc. Cadmium, while inhibiting v_o to a small extent, did not have the marked inhibitory effects documented by others. Finally, this study allowed a comparison with a previous report for Cd uptake, showing consistency with the proposal that at least part of the uptake process for Cd and Zn is via a common pathway.

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