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Effect of dexamethasone on zinc transport in rat hepatocytes in primary culture

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Abstract

Incubation of hepatocytes in medium supplemented with 1 μM dexamethasone and 15 or 30 μM $ZnCl_2$ for 24 h resulted in increased intracellular Zn and metallothionein levels. There was a positive correlation between the Zn and metallothionein concentrations. Initial rates of ^{65}Zn influx from balanced salt solutions containing albumin and $ZnCl_2$ to give known free Zn^{2+} concentrations were measured over 10 min. At 1.6 nM free Zn^{2+} the influx rate was the same in control and dexamethasone-treated cultures, but at 18 nM free Zn^{2+} there was an increased rate of influx after dexamethasone treatment. Measurements of ^{65}Zn efflux were quite hard to interpret, because of the existence of at least two intracellular Zn pools, a fast-exchanging and a slow-exchanging pool. The amount of Zn in the fast-exchanging pool appeared to increase as the external free Zn^{2+} concentration was increased, while the amount in the slow-exchanging pool increased on pre-treatment with Zn and dexamethasone. The fractional efflux of ^{65}Zn from the fast-exchanging pool appeared to increase with the pool size, implying that the rate of Zn efflux increases faster than the concentration of Zn^{2+} in that pool.

Key words: Hepatocyte; Zinc; Metallothionein; Glucocorticoid

1. Introduction

Synthesis of metallothionein is induced in hepatocytes in response to increased extracellular zinc concentrations and to a range of hormones and interleukins [1–4]. Hepatic zinc concentration rises and the plasma concentration falls as a result. A redistribution of total body zinc to the liver in vivo could be mediated solely by an increased metallothionein concentration leading to a net gain of cellular Zn. Increased intracellular binding of Zn to metallothionein would decrease Zn efflux from hepatocytes, and cell Zn content would increase without any change in Zn influx. Observations in vitro [5] are not in agreement with this prediction. It was found that Zn influx was increased in cultures pre-incubated with the synthetic glucocorticoid dexamethasone compared to controls, while ^{65}Zn efflux was similar in control cultures and cultures treated with dexamethasone. This suggests a regulatory role for Zn transport across the hepatocyte membrane.

Tracer studies have shown that zinc in hepatocytes has a short half-life [5,6]. At least two kinetic pools have been identified: radiolabelled ^{65}Zn rapidly reaches equilibrium with a fast-exchanging pool, which can in turn exchange with more tightly bound intracellular zinc [5]. Pattison and Cousins [5] showed that hepatocytes cultured with dexamethasone have more Zn in the slower-exchanging pool. ^{65}Zn efflux measured after short-pulse labelling will mostly be from the rapidly-exchanging pool, which may not reflect changes affecting the slower pool. We investigated this possibility by measuring ^{65}Zn efflux after varying loading times, with cells that had been pre-incubated under a variety of conditions designed to achieve different cellular Zn and metallothionein concentrations.

We also re-examined the regulation of ^{65}Zn uptake, by measuring the initial rate of ^{65}Zn influx in parallel. We have recently shown that ^{65}Zn uptake into cultured rat hepatocytes occurs via a saturable mechanism at low free Zn^{2+} concentrations ($K_m = 1.3 \cdot 10^{-8}$ M), and there is also a linear component at high Zn^{2+} concentrations [7]. For the experiments described here we measure initial rates of ^{65}Zn uptake using one Zn^{2+}

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concentration just above the K_m ($1.8 \cdot 10^{-8}$ M Zn^{2+}) and a lower concentration ($1.6 \cdot 10^{-9}$ M Zn^{2+}), closer to the estimated $2 \cdot 10^{-10}$ M Zn^{2+} measured in horse plasma [8].

2. Methods

2.1. Cell culture

Isolated hepatocytes from male Wistar rats were prepared by the method of Seglen [9] and cultured on collagen-coated plates (6 cm diameter, $5 \cdot 10^4$ cells cm^{-2}) in Williams medium-E supplemented with 5% foetal bovine serum, 0.2 μM bovine insulin, 1 mM glutamine and 40 $\mu g\,ml^{-1}$ gentamycin for 48 h before measuring ^{65}Zn fluxes [7]. The culture conditions were varied, as given in the legend to Table 2, in order to alter cellular Zn and metallothionein content. Under control conditions, the cells gain Zn during pre-incubation [7,10]. This was prevented by adding 2% (w/v) bovine serum albumin, which acts by chelating some of the Zn present in the culture medium. It has been recommended that the cells are allowed to recover overnight in culture before applying hormonal stimuli because receptors may be damaged by the isolation procedures [11] so 1 μM dexamethasone, when present, was added from a 10 mM stock solution in ethanol after 20 h in culture.

2.2. Measurement of ^{65}Zn fluxes

All fluxes were measured after 20–48 h in culture. ^{65}Zn uptake rates were measured in a balanced salt solution (BSS) containing 140 mM NaCl, 15 mM Hepes (pH adjusted to 7.4 with about 8 mM KOH), 5 mM glucose, 1 mM $MgCl_2$ and 1.8 mM $CaCl_2$, as previously described [7]. ^{65}Zn incorporation (specific radioactivity 0.3–4.0 kBq/nmol) was measured in duplicate at 0, 5 and 10 min and, although Zn uptake against time is slightly curvilinear, the curvature is only slight at these short times (data not shown) and rates were calculated by linear regression analysis.

The procedure was adapted to measure ^{65}Zn efflux. Hepatocytes were washed twice in BSS and then loaded with ^{65}Zn by incubation at 37°C in BSS supplemented with different free Zn^{2+} concentrations in the range $1.6 \cdot 10^{-9}$ to $5 \cdot 10^{-6}$ M, as shown in Table 1. Cell-surface radioactivity was removed by four rapid washes in ice-cold BSS, the first two washes containing added 2 mM calcium ethylene diaminetetraacetic acid (Ca-EDTA) in order to remove extracellular ^{65}Zn [5,7]. Plates were returned to the 37°C bath and 2 ml pre-warmed BSS containing 2 mM Ca-EDTA added. This medium contained 1.8 mM Ca in excess of the Ca-EDTA, because the cells begin to show signs of rounding up after 30–60 min if Ca is omitted. Samples were usually taken immediately after adding the efflux medium and 2 and 5 min later. The supernatants were aspirated with a Pasteur pipette and the cells were solubilised in 0.2 M NaOH. Cell and supernatant fractions were counted for ^{65}Zn content. Under some conditions (e.g., after short pre-loading) there was a substantial fraction of ^{65}Zn in the medium in the earliest sample and plots of the log (fraction intracellular ^{65}Zn) against time were not linear (Fig. 1). Efflux data have therefore been presented as the fraction of ^{65}Zn in the supernatant after 2 min so that the rapid initial efflux is not disregarded but accurate timing of the efflux period was achieved.

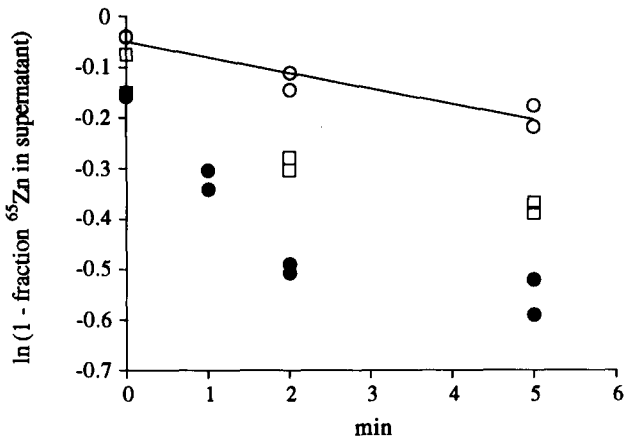

Fig. 1. Typical time course of ^{65}Zn efflux from one hepatocyte preparation. Efflux into BSS with Ca-EDTA was measured after 10 min loading (●) or 2 h loading (○) with 1.6 nM Zn^{2+} and 2 h loading with 18.2 nM Zn^{2+} (□). Each point is from a single plate. A straight line was fitted by the method of least squares to points (○). Intracellular ^{65}Zn concentrations at the start of efflux were 0.38 ± 0.04 (●), 0.15 ± 0.01 (○) and 1.27 ± 0.06 (□) nmol(mg protein)$^{-1}$.

2.3. Determination of cell integrity

The integrity of the cells was routinely tested by Trypan blue exclusion. Cells were only plated if the dye exclusion was greater than 85% and the adherent cells in culture all excluded Trypan blue. In addition lactate dehydrogenase (LDH) release was measured using the method of Vassault [12]. Plates were pre-incubated for 2 h in BSS containing either 1.3 nM Zn^{2+} or 5 μM $ZnCl_2$ and washed four times in cold medium to simulate the conditions used for efflux measurements. Fresh medium at 37°C was added and LDH measured in the cell and supernatant fractions after 5 min incubation at 37°C. LDH release was usually less than 2% and never more than 4%, in plates from five different hepatocyte preparations. This was negligible compared to ^{65}Zn efflux so no correction was made.

2.4. Analytical methods

Total Zn content was measured by atomic absorption spectrophotometry (AAS) as described by Kalfakakou and Simons [13]. Analysis of triplicate plates gave a coefficient of variation of 1–2%. Protein was measured by the method of Lowry et al. [14] and metallothionein content measured by the ^{109}Cd binding method of Eaton and Toal [15].

2.5. Sources of materials

Calf-skin collagen, insulin and BSA were purchased from the Sigma, Williams-E medium and foetal calf serum from Gibco, $^{65}ZnCl_2$ (specific activity 55 MBq mg^{-1}) from DuPont, Stevenage, Herts and $^{109}CdCl_2$ (specific activity 32 GBq mg^{-1}) from Amersham International, Aylesbury, UK.

3. Results

As expected, the rate of ^{65}Zn efflux was affected by the time of the incubation used to load the cells and the concentration of zinc in the loading solution. ^{65}Zn efflux was particularly rapid after short pre-loading times.

3.1. The effect of ^{65}Zn concentration on efflux

In one set of experiments, cells were loaded for 2 h with the five different extracellular zinc concentrations shown in Table 1. Much of the ^{65}Zn that entered the cells replaced unlabelled Zn. For example, at the highest Zn^{2+} loading concentration, the cellular Zn content increased from 2.49 ± 0.25 to 3.30 ± 0.08 nmol (mg protein)$^{-1}$ (means ± S.E.), while the ^{65}Zn content increased by 2.20 ± 0.21 nmol (mg protein)$^{-1}$. In these experiments fractional ^{65}Zn efflux increased with increasing intracellular ^{65}Zn content (Fig. 2).

In contrast, there was an inverse relationship between ^{65}Zn concentration and fractional efflux when cells were loaded with ^{65}Zn for different times. Intracellular ^{65}Zn increased with time (Fig. 3), while the fraction of ^{65}Zn exiting in 2 min decreased as the loading time and intracellular ^{65}Zn concentration increased (Fig. 4). Thus, any measurement of ^{65}Zn efflux is likely to depend not only on how rapidly ^{65}Zn can cross the membrane but also on its equilibrium with intracellular Zn pools.

Table 1
Composition of media used to load cells with ^{65}Zn

Concentration of BSA (gl^{-1})	Concentration of $ZnCl_2$ (M)	Calculated free [Zn^{2+}] (M)
10	$1.0 \cdot 10^{-5}$	$1.62 \cdot 10^{-9}$
10	$8.0 \cdot 10^{-5}$	$1.82 \cdot 10^{-8}$
10	$1.2 \cdot 10^{-4}$	$3.56 \cdot 10^{-8}$
0	$2.0 \cdot 10^{-6}$	$2.0 \cdot 10^{-6}$
0	$5.0 \cdot 10^{-6}$	$5.0 \cdot 10^{-6}$

BSA, $ZnCl_2$ and $^{65}ZnCl_2$ were added to BSS to give the final concentrations shown and the free Zn^{2+} calculated as described previously [7].

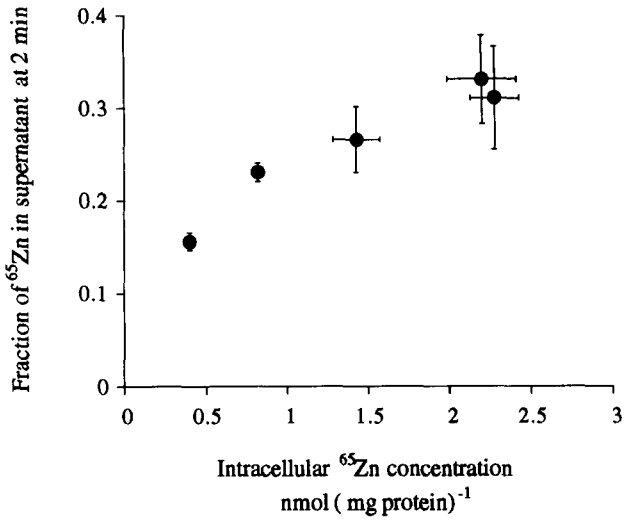

Fig. 2. Effect of intracellular ^{65}Zn concentration on efflux after 2 h pre-loading. Hepatocytes were loaded with ^{65}Zn in BSS containing five different Zn^{2+} concentrations. Data are presented as mean ± S.E. of (from left to right) 14, 3, 4, 7 and 3 preparations.

Pre-incubation with zinc and dexamethasone caused a significant increase in cellular zinc and metallothionein, whilst pre-incubation with albumin prevented

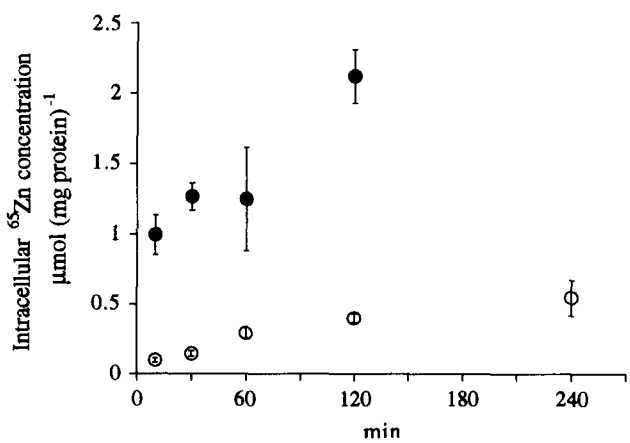

Fig. 3. ^{65}Zn concentration after different influx times. Hepatocytes were incubated in BSS with either 5 µM ^{65}Zn (●) or 1.62 nM Zn^{2+} (○). Data (mean ± S.E.) are calculated from duplicate measurements of 4, 3, 10, 7 (●), 10, 3, 3, 14 and 3 (○) hepatocyte preparations, reading from left to right.

Table 2
Cellular zinc and metallothionein concentrations after different culture conditions

Culture conditions	BSA	Control	1 μM dex 15 μM Zn	1 μM dex 30 μM Zn
Estimated Zn^{2+} in medium (M)	$2.5 \cdot 10^{-10}$	$7.4 \cdot 10^{-10}$	$2.4 \cdot 10^{-9}$	$1.7 \cdot 10^{-8}$
Total Zn in cells (nmol (mg protein)$^{-1}$)	1.70 ± 0.10 (4)	2.20 ± 0.18 (10)	3.80 ± 0.87 (10)	5.63 ± 0.72 (10)
Cell metallothionein (μg (mg protein)$^{-1}$)	0.35 ± 0.05 (4)	0.20 ± 0.05 (9)	1.35 ± 0.41 (7)	1.75 ± 0.08 (4)

Cells were cultured in Williams Medium E supplemented with 5% fetal calf serum for 48 h before ^{65}Zn fluxes were measured. This medium contains 5 μM Zn and, except in the control condition, was modified as follows: (BSA), 20 g/l fatty acid-free bovine serum albumin present for 48 h; (1 μM dex 15 μM Zn), medium supplemented with 1 μM dexamethasone and 15 μM $ZnCl_2$ from 24–48 h; (1 μM dex 30 μM Zn), medium supplemented with 1 μM dexamethasone from 24–48 h. The free Zn^{2+} concentrations were estimated from the concentrations of Zn, albumin and amino acids present in the supplemented media [22,23]. The cells were analysed for Zn and metallothionein after 48 h, data given as mean ± S.E. (number of preparations). Zinc content in all four groups was significantly different ($P < 0.01$). Metallothionein concentrations were significantly different ($P < 0.05$) comparing the BSA with control or 1 μM dex 15 μM Zn with 1 μM dex 30 μM Zn. Dexamethasone treatment led to a highly significant increase in metallothionein ($P < 0.001$) compared with control or BSA treatment. The Zn concentration of freshly isolated cells was 1.72 ± 0.16 nmol (mg protein)$^{-1}$ ($n = 5$).

the increase in hepatocyte zinc content seen under control conditions (Table 2). The mean metallothionein concentration was higher after BSA addition compared to controls, and therefore suggests that intracellular free Zn^{2+} is rediced after culture with BSA compared to the control condition. Zinc and metallothionein levels were correlated in these cells ($P < 0.01$).

3.3. Zn uptake

Initial rates of Zn uptake were measured at two free Zn^{2+} concentrations. At 1.6 nM Zn^{2+} the rate of ^{65}Zn influx was similar in the two conditions tested but at 18.2 nM Zn^{2+} the influx rate was higher ($P < 0.01$) in the Zn and dexamethasone pre-treated cultures (Fig. 5). Cells were also loaded with ^{65}Zn for 10 min at these two Zn^{2+} concentrations for ^{65}Zn efflux measurements. The amount of ^{65}Zn loaded in 10 min corresponded to the influx measurements in Fig. 5, i.e., at 1.6 nM Zn^{2+} amounts were similar in control and dexamethasone-treated cultures, while at 18.2 nM Zn^{2+}, more ^{65}Zn was loaded in dexamethasone-treated cultures than in controls (Table 3). This difference was significant ($P = 0.03$ using Student's t-test) when paired samples from the same hepatocyte preparations were compared.

3.4. Zn efflux

When efflux was measured after 10 min loading with ^{65}Zn there was no significant effect of the pre-treat-

Table 3
^{65}Zn content after loading with isotope

Pre-culture conditions	BSA	Control	1 μM dex 15 μM Zn	1 μM dex 30 μM Zn
^{65}Zn content after 10 min with 1.6 nM Zn^{2+} (nmol (mg protein)$^{-1}$)	not measured	0.10 ± 0.03 (3)	0.10 ± 0.025 (3)	not measured
^{65}Zn content after 2 h with 1.6 nM Zn^{2+} (nmol (mg protein)$^{-1}$)	0.26 ± 0.01 (5)	0.27 ± 0.01 (5)	0.39 ± 0.02 (5)	0.38 ± 0.02 (4)
^{65}Zn content after 10 min with 18.2 nM Zn^{2+} (nmol (mg protein)$^{-1}$)	0.27 ± 0.03 (4)	0.34 ± 0.03 (4)	0.48 ± 0.09 (4)	0.46 ± 0.05 (4)
^{65}Zn content after 2 h with 18.2 nM Zn^{2+} (nmol (mg protein)$^{-1}$)	0.96 ± 0.05 (5)	0.96 ± 0.04 (7)	1.50 ± 0.10 (7)	1.60 ± 0.14 (5)

Results are given as means ± S.E., number of preparations in parenthesis. After 10 min loading there was no difference between the control condition and dexamethasone treatment when 1.6 nM Zn^{2+} was used, but a significant difference after loading with 18.2 nM Zn^{2+} ($P < 0.03$ using a paired t-test to compare either control or BSA treatment with the dexamethasone groups). After 2 h loading there was a highly significant difference ($P < 0.01$) between cultures that were treated with Zn and dexamethasone and those that were not.

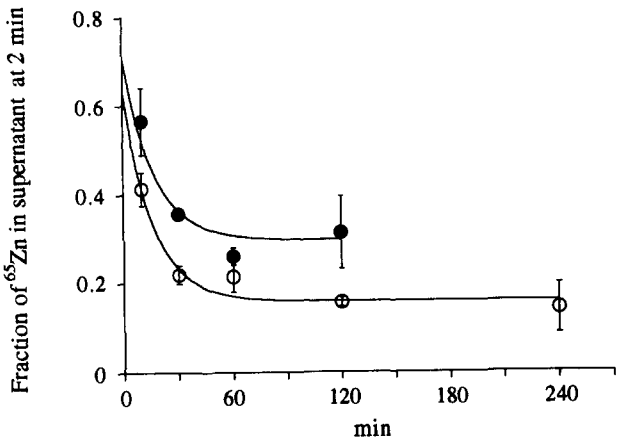

Fig. 4. ^{65}Zn efflux after different influx times. Hepatocytes were incubated in BSS with either 5 μM ^{65}Zn (•) or 1.62 nM Zn^{2+} (○). Data are mean ± S.E. calculated from the same plates as presented in Fig. 3. Lines are the best fit to the equation $y = a + b\exp(-cx)$. For data at 5 μM ^{65}Zn (•): $a = 0.297 \pm 0.035$, $b = 0.408 \pm 0.101$, $c = 0.060 \pm 0.026$, $n = 24$, $r^2 = 0.721$ and for data at 1.62 nM Zn^{2+} (○): $a = 0.158 \pm 0.027$, $b = 0.470 \pm 0.132$, $c = 0.062 \pm 0.027$, $n = 33$, $r^2 = 0.834$. The y intercepts are 0.704 ± 0.106 and 0.628 ± 0.135, respectively.

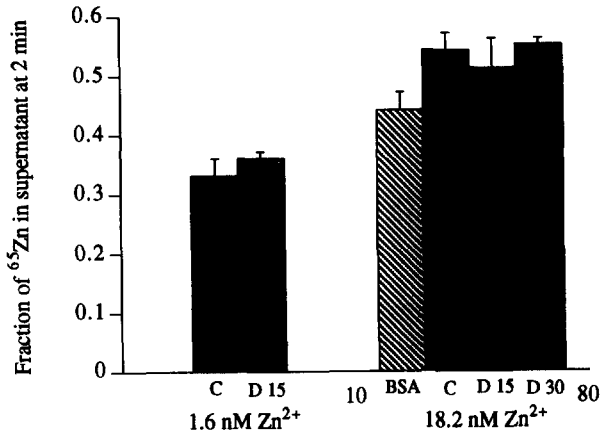

Fig. 6. Effect of pre-culture conditions on ^{65}Zn efflux after 10 min loading. Efflux was measured after 10 min loading with 1.6 nM Zn^{2+} after culture under control conditions (C) or with 1 μM dexamethasone and 15 μM Zn (D 15) and after 10 min loading with 18.2 nM Zn^{2+} following pre-culture under all four conditions tested (see Table 2). Each experiment was measured in triplicate using three hepatocyte preparations at 1.6 nM $^{65}Zn^{2+}$ and 5 at 18.2 nM $^{65}Zn^{2+}$. Data are mean ± S.E.

ments which alter intracellular Zn and metallothionein levels (Fig. 6). After 2 h loading with ^{65}Zn, cultures with increased Zn and metallothionein concentrations also accumulated significantly more ^{65}Zn (Table 3). ^{65}Zn efflux, when expressed as fractional loss in 2 min, was significantly reduced ($P < 0.01$) in these dexamethasone pre-treated cells, compared with controls (Fig. 7). However, comparing the cells pre-treated with dexamethasone and either 15 or 30 μM Zn, their metallothionein and total Zn concentrations were markedly different (Table 1), but their intracellular ^{65}Zn concentration after 2 h loading with ^{65}Zn (Table 3), and fractional efflux of ^{65}Zn (Fig. 7) were very similar ($P > 0.3$).

4. Discussion

4.1. Efflux rates

Efflux of ^{65}Zn from cultured rat hepatocytes was extremely rapid. 10%–60% of the isotope was found in the medium after only 2 min (Figs. 2 and 4). This

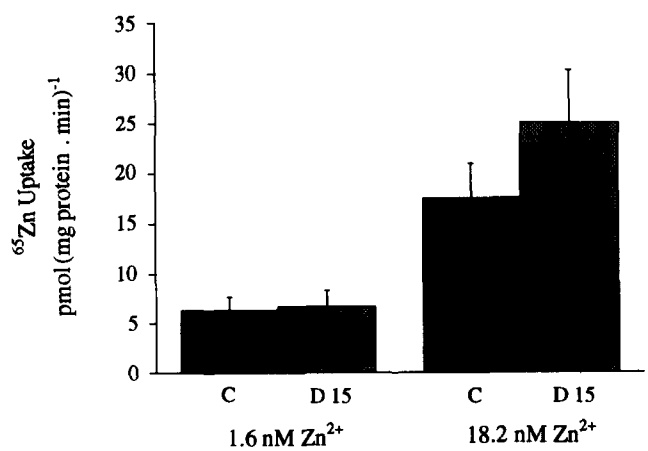

Fig. 5. Initial rates of ^{65}Zn influx into hepatocytes after 48 h culture. ^{65}Zn Influx rates from BSS with either 1.6 nM free Zn^{2+} or 18.2 nM Zn^{2+} were calculated from duplicate measurements at three times in control plates (C) and from cultures supplemented for 24 h with 1 μM dexamethasone and 15 μM $ZnCl_2$ (D 15). Data are mean ± S.E. from five separate hepatocyte preparations.

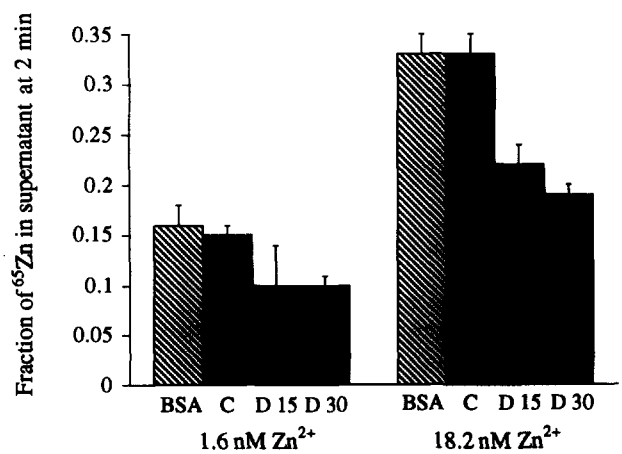

Fig. 7. Effect of pre-culture conditions on ^{65}Zn efflux after 2 h loading. Efflux was measured in triplicate after 2 h loading as described in Methods. Pre-culture conditions are as described for Fig. 6. Data are mean ± S.E. Number of preparation are as shown in Table 3.

Table 4
Estimation of labile pool size in control cells

Concentration of Zn^{2+} used for loading	1.6 nM	18.2 nM	5 μM
^{65}Zn content after 10 min (nmol(mg protein)$^{-1}$)	0.10 ±0.03	0.34±0.03	1.0 ±0.14
Fractional efflux after 10 min	0.41 ±0.04	0.54±0.04	0.57±0.07
Estimated ^{65}Zn content of labile pool (nmol(mg protein)$^{-1}$)	0.062±0.008	0.28±0.02	0.86±0.10

The ^{65}Zn content of the labile pool was calculated on the assumption that the fractional efflux (in 2 min) would have been 0.67 if all the ^{65}Zn had been in the labile pool (from Fig. 4, extrapolation to zero loading time corresponds to forcing all ^{65}Zn into the labile pool).

agrees with previous studies [5,10] and is consistent with the rapid turn over of hepatic Zn in vivo [6,16].

Schroeder and Cousins [10] found that adding Zn to their efflux medium increased its rate of efflux from hepatocytes. In order to prevent any effects of extracellular Zn, we added 2 mM Ca-EDTA to the BSS used for efflux measurements.

Increasing the time of loading with ^{65}Zn before measuring efflux resulted in the release of a smaller fraction into the medium than after shorter times (Fig. 4). This can best be explained by the equilibration of isotope with intracellular pools and highlights the difficulty of interpreting efflux data after hormonal treatment known to change pool sizes. When the intracellular ^{65}Zn content was increased by pulsing with higher free Zn^{2+} concentrations, rather than increasing the loading time, efflux was faster at higher concentrations (Fig. 2). From previous studies [5,7], one could reasonably suppose that in 10 min, ^{65}Zn entry into the labile pool will be proportional to the extracellular free Zn^{2+} concentration. As ^{65}Zn entry increases, the fraction of Zn that is ^{65}Zn in the labile pool will also increase.

The intracellular ^{65}Zn concentration and its fractional efflux are both increased by loading with higher extracellular Zn concentrations (Figs. 3, 4, Table 4). We attempted to estimate pool sizes in cells loaded with ^{65}Zn for 10 min, making the assumption that extrapolation of fractional efflux data to zero loading time (Fig. 4) would correspond to all the ^{65}Zn being in the labile pool. The analysis suggests that the labile Zn pool, as well as the fractional ^{65}Zn efflux increase more rapidly than the total cellular ^{65}Zn content, for different Zn^{2+} loading concentrations (Table 4). An increased fractional efflux as cellular ^{65}Zn content increases is also suggested by the measurements made after 2 h labelling (Fig. 2).

4.2. Induction of metallothionein

The metallothionein concentration in control cultures and after induction by Zn and dexamethasone is similar to previously published values [17,18]. Metallothionein has seven Zn-binding sites, and a molecular mass of approx. 7000 Da, so 1 μg is able to bind 1 nmol Zn [19]. Table 2 shows that the Zn concentration increases more than can be explained by additional metallothionein and therefore suggests that some of the additional Zn is bound to other molecules. This is in agreement with Schroeder and Cousins [18] who found approximately twice as much Zn in hepatocytes stimulated with dexamethasone than could be accounted for by the increase in metallothionein.

4.3. Zn efflux after dexamethasone treatment

Initially we assume that ^{65}Zn efflux over 2 min reflects the ^{65}Zn in an intracellular pool that can equilibrate rapidly with the exterior. The lack of effect of changing intracellular pools on fractional ^{65}Zn efflux after 10 min loading (Fig. 6) probably reflects a lag period while ^{65}Zn equilibrates with slower pools, and is in agreement with the results of Pattison and Cousins [5] after a 1 min ^{65}Zn loading pulse.

After 10 min loading at 18.2 nM Zn^{2+} the cells cultured with dexamethasone had accumulated more ^{65}Zn than the controls but the fractional efflux was the same as controls. This might be explained if the higher concentration of ^{65}Zn is accompanied by a higher unlabelled Zn concentration in the fast exchanging pool following dexamethasone treatment. A larger labile Zn pool in these cultures is also suggested by the relative increases in the Zn and metallothionein concentrations. However, the fractional efflux of ^{65}Zn after 10 min loading with 1.6 nM Zn^{2+} is the same in control and dexamethasone-pretreated cultures. In this case the lower fraction of ^{65}Zn in the rapidly exchanging pool following dexamethasone treatment might be matched by an increased rate of efflux at a higher Zn^{2+} concentration.

Fractional ^{65}Zn efflux after 2 h loading is reduced in dexamethasone-treated cultures compared with controls (Fig. 7), and there is more isotope in the cells (Table 3). Failla and Cousins [20] similarly found higher ^{65}Zn concentrations after 3 h incubation even though dexamethasone had been removed from the medium. This may not necessarily represent greater net accumulation of total Zn in the treated cultures. The Zn and dexamethasone treated cultures had higher total Zn concentrations before loading (Table 2), so it is likely that more of the ^{65}Zn enters the more stable pools during 2 h loading than in the control or BSA groups. The reduced fractional efflux after culture with Zn and

dexamethasone (Fig. 7) would be in agreement with this hypothesis.

Comparing cells cultured with dexamethasone and either 15 or 30 μM Zn, there was more Zn and metallothionein in the cells cultured at the higher Zn concentration, while the amount of ^{65}Zn entering the two cultures was the same. One would expect fractional efflux of ^{65}Zn from the 30 μM-Zn culture to be lower, but the fractional efflux measured after 2 min was no different (Fig. 7). The accessible pools of unlabelled Zn might be of similar magnitude after dexamethasone treatment with 15 and 30 μM Zn but this seems unlikely as the Zn and metallothionein concentrations have risen in parallel. It may be that the reduced fraction of ^{65}Zn in the labile pool at 30 μM Zn is balanced by an increased efflux rate corresponding to the increased pool size (Fig. 4).

4.4. Zn influx after dexamethasone treatment

A novel finding is that no difference could be detected in the rate of ^{65}Zn influx in control and dexamethasone-treated cultures when measured at a low free Zn^{2+} concentration (1.6 nM). Pattison and Cousins [5] measured Zn influx rates from 5 μM ^{65}Zn in Waymouth's medium containing 0.2% BSA after induction of metallothionein with dexamethasone (but without additional Zn). In their study pre-culture with dexamethasone increased the Zn influx rate to about twice the control level. We found a higher rate of influx in our treated cultures at the higher concentration of 18 nM Zn^{2+} but this concentration is about one hundred times that found in vivo and may have little physiological significance. An increase in Zn uptake rate might be explained by a reduction in intracellular Zn^{2+} concentration following metallothionein induction, but this would not explain why the effect only occurs at the higher extracellular Zn^{2+} concentration. Furthermore, the Zn/metallothionein ratios suggest that the free Zn^{2+} is higher following stimulation with dexamethasone. The increased Zn influx at 18 nM Zn^{2+} might be explained by an increase in the V_{max} for transport-mediated Zn uptake. From measurements at just two Zn^{2+} concentrations it is impossible to tell how much the K_m might have changed.

Transformed hepatoma cells (HTC) accumulate ^{65}Zn in response to dexamethasone and have faster initial rates of uptake after pre-treatment with dexamethasone, without induction of metallothionein [21]. This observation supports the hypothesis that Zn transport at the hepatocyte membrane may respond directly to dexamethasone treatment.

Acknowledgement

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