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EVALUATION OF MAGNESIUM FLUXES IN RAT ERYTHROCYTES USING A STABLE ISOTOPE OF MAGNESIUM

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1. ABSTRACT

The mechanisms that maintain intracellular Mg concentration at physiologic levels are not fully understood. In this work, we described for the first time, a new method using ^{25}Mg stable isotopes, that allows simultaneous determination of Mg^{2+} efflux and Mg^{2+} influx in non-loaded cells at physiological levels of extracellular Mg. Erythrocytes from rats were suspended as a 10% suspension in NaCl medium or choline medium. The erythrocyte suspension was incubated at 37°C, and aliquots of the cell suspension were centrifuged at the beginning of the incubation and after 60 and 120 min. The quantification of ^{24}Mg, ^{25}Mg and ^{26}Mg in supernatants and in erythrocytes were determined by ICP/MS. Simultaneous Mg^{2+} efflux and Mg^{2+} influx were calculated from the intra-extracellular distribution of the three isotopes. By this new approach we characterized Mg^{2+} influx and Mg^{2+} efflux at 0.4 mM extracellular Mg in both NaCl and choline Cl medium. Mg^{2+} efflux and Mg^{2+} influx were largely inhibited by amiloride in NaCl medium and by cinchonine in choline Cl medium. Apparent velocity and LineWeaver-Burk kinetics showed that Mg^{2+} influx is different from Mg^{2+} efflux suggesting the involvement of two distinct transport mechanisms. Moreover, modifying extracellular Mg concentrations, to mimic hypo- or hyper-magnesaemia, we showed that Mg^{2+} efflux and Mg^{2+} influx increased with extracellular Mg up to 0.8 mM, the physiologic concentration of total extracellular Mg. Our data demonstrate that Mg^{2+} fluxes are directly related to the levels of extracellular Mg and that in physiological conditions, Na-dependent and Na-independent Mg^{2+} efflux counterbalance Mg influx to maintain constant intracellular Mg level.

2. INTRODUCTION

Magnesium (Mg) is the second most abundant cation in the intracellular fluid; it plays an essential role in many cells functions, including Ca^{2+} metabolism, 2nd messenger systems and enzymatic reactions involving ATP (1). In cells, the majority of Mg, present in the millimolar range, is bound to various structures like nucleus, intracellular organelles, membranes. Ionized free Mg^{2+} accounts for 1 to 5% of total cellular Mg. Interestingly, both intracellular free and bound Mg are maintained at a relatively constant level, even if extracellular Mg level varies (2); the underlying transport mechanisms remaining largely speculative.

Mg^{2+} fluxes across plasma membranes have been studied mainly in Mg-loaded cells, or in large cells which allowed microelectrode insertion or patch clamp techniques (3, 4). The different cell models and experimental approaches used evidenced several Mg^{2+} transport mechanisms. Erythrocytes have been largely used for efflux studies, as a significant net Mg^{2+} efflux was demonstrated from cells loaded with Mg (5). The most relevant, best characterized Mg^{2+} efflux occurs in exchange with extracellular Na (3). This cation exchanger, even though not characterized at the molecular level, consists of a plasma membrane protein, likely similar to other exchangers such as Na^+/H^+ antiporter, carrying out the electroneutral exchange of intracellular Mg^{2+} with extracellular Na^+. Na^+/Mg^{2+} exchange is probably driven by the transmembrane Na gradient, as indicated by the requirement for both cell ATP and Na-K ATPase activity

(5, 6). In addition, some authors have also proposed that ATP may also be necessary for the PKA-dependent phosphorylation of the Na^+/Mg^{2+} antiporter, resulting in the modulation of the transporter affinity for intracellular Mg^{2+} (5, 7). Besides Na^+/Mg^+ exchange, other ion exchange have been described, e.g. Ca^+/Mg^{2+} exchange, likely occurring in intracellular membranes or in discrete region of polarized cells, and Na^+-independent Mg^{2+} exchange pathways as that observed in sucrose or in choline chloride medium (8-10). Recently, Ebel and Gunther characterised the Na^+-dependent and Na^+-independent Mg^{2+} efflux in non- Mg-loaded erythrocytes and described the participation of a choline/ Mg^{2+} exchanger in Mg^{2+} efflux (9, 11).

Concerning Mg^{2+} influx, it is well accepted that the entry of Mg^+ into the cell is basically driven by electrochemical gradient, via channels and carriers (12), but the mechanisms of Mg^{2+} influx lack of detailed characterization. It has been described that Na^+/Mg^{2+} antiport can function in the opposite direction when the Na gradient is reverted, suggesting that both Mg^{2+} influx and efflux can be driven by the same transport mechanisms (13, 14). It was also demonstrated that Mn^{2+} can be transported in exchange for Mg^{2+}, indicating the possible existence of a Mg^{2+}/Mg^{2+} reversible exchange (15, 16). Recently a candidate for the regulation of Mg^{2+} influx has been described and characterized at a molecular and functional level (17). TRPM7 channel is regulated by intracellular free Mg^{2+} and Mg-ATP and regulates Mg^{2+} influx into the cell (18). TRPM7 channel possess a PKA-dependent kinase domain at the cytoplasmic C-terminus that regulates channel opening and closing (19). For several aspects therefore the regulation of TRPM7 greatly resembles that of putative Na^+/Mg^{2+} antiport.

In this work, we described a new method using a stable isotope of Mg, which allows for the first time, the simultaneous determination of Mg^{2+} influx and efflux, avoiding Mg-loading of erythrocytes and in presence of physiological levels of extracellular Mg. This method is based on replacing extracellular ^{24}Mg with ^{25}Mg. This allows efflux to be determined as a function of ^{24}Mg appearance in the extracellular fluid and influx to be determined as a function of ^{25}Mg presence in the erythrocytes. We believe that this method will allow to better characterize Mg^{2+} transport at the cellular level.

3. MATERIALS AND METHODS

3.1. Reagents and equipment

Enriched Mg (96.7% ^{25}Mg, 2.2% ^{24}Mg and 1.1% ^{26}Mg) as oxide was obtained from Chemagas (Paris, France). Suprapure HNO_3, suprapure H_2O_2, suprapure HCl, lanthanum oxide and standard solutions of Mg (1g/L) were obtained from Merck (Darmstadt, Germany). Standard solution of Be (1g/L) was purchased from Spex Certiprep Instruments SA (Longjumeau, France). All other chemicals were of the highest quality available, and demineralized water was used throughout. The Mg isotope ratio measurements were performed using an ICP/MS instrument (quadrupole Elan 6100 DRC system, PERKIN ELMER, Courtaboeuf, France), fitted with a Meinhard-type nebulizer and cyclonic spray chamber.

3.2. Preparation of stable isotope solution

200 mg of enriched Mg (or 328 mg of MgO) were moistened with 2 mL of demineralized water, and then 2 mL of 12 moles/L HCl (suprapure) was added to transform the oxide into the soluble chloride of Mg. The solution was then neutralised with 2 ml of NaOH 1 mole/L and 25 ml of $NaHCO_3$ 1.67 moles/L. The solution was then brought to 100 ml with physiological solution (NaCl 0.9%). The pH and the osmolarity of the obtained solution were respectively 7.0 and 320 mOs. The concentration of Mg in this solution was 2 mg/mL.

3.3. Preparation of the washing solution and incubation media

The washing solution and the incubation media (NaCl or choline Cl) were prepared as described by Ebel and Gunther (9). Washing solution: KCL 140 mmol/l, Hepes 30 mmol/l pH 7.4, sucrose 50 mmol/l, glucose 5 mmol/l; NaCl medium: NaCl 150 mmol/l, Hepes 10 mmol/l pH 7.4, glucose 5 mmol/l; Choline Cl medium: choline Cl 150 mmol/l, Hepes 10 mmol/l pH 7.4, glucose 5 mmol/l. ^{25}Mg was add in the incubation medium to obtain a concentration of 0, 0.05, 0.1, 0.2, 0.4, 0.8 and 1.6 mmol/L, according to the experiment.

3.4. Preparation of erythrocytes

Blood (8ml) was obtained from anaesthetised (40mg/kg pentobarbital-sodium i.p.) male Wistar rats, weighing 350-400 g (from the Experimental Compared Nutrition Unit of INRA de Theix), by punction in the abdominal artery with a heparinized syringe, and was centrifuged at 1000 g for 10 min at 20°C. The plasma and buffy coat were aspirated and discarded. The erythrocytes were washed twice at 20°C in 10 ml of the washing solution.

3.5. Mg efflux and Mg influx

The erythrocytes were suspended as a 10% suspension in one of the incubation media and were incubated at 37°C. 1.5-ml aliquots of the cell suspension were centrifuged (5000g, 5 min, 20°C) at the beginning of the incubation (0) and after 60 and 120 min. The supernatant was separated, and the erythrocytes were washed twice with the washing solution and hemolised (1/11). ^{24}Mg, ^{25}Mg and ^{26}Mg contents of supernatants and erythrocytes were determined by ICP/MS (Elan 6100 DRC, PERKIN ELMER, Courtaboeuf, France) after appropriate dilution with 0.14 M-HNO_3 using Be as internal standard, and external calibration of the ICP-MS (20).

The mass spectrometer settings and plasma conditions were as follows: RF Power 1050 W, Auxiliary Ar flow rate 1.2 l/min, Plasma Ar flow rate 15 l/min. The nebulizer Ar flow rate was optimized daily using a solution of 1 µg In/l. Data acquisition conditions were as follows: Sweeps/reading 40, Readings/replicate 1, Number of replicates 3, Dwell time 100 ms, Scanning mode: peak hopping.

Mg^{2+} efflux and influx were calculated according to the following equations:

Mg^{2+} efflux in µmol/L cells = $[^{24}Mg]_T - [^{24}Mg]_{T0}$ in supernatant x erythrocyte dilution in the cell suspension

Mg^{2+} influx in µmol/L cells = $[^{25}Mg$ in cells - ^{26}Mg in cells$]_T$ x 0.1 - $[^{25}Mg$ in cells - ^{26}Mg in cells$]_{T0}$ x 0.1, where 0.1 is the natural abundance of ^{25}Mg.

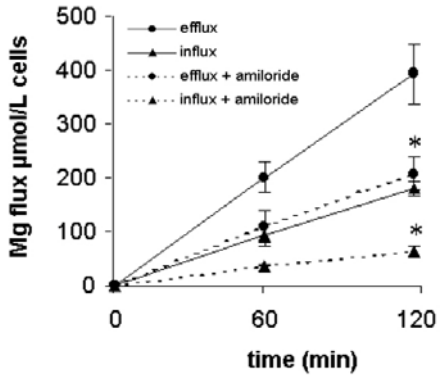

Figure 1. Mg efflux and Mg influx from rat erythrocytes incubated in NaCl medium in the presence of 0.4 mM extracellular ^{25}Mg. Amiloride, a specific inhibitor of Na transport, was used at a concentration of 1 mM. Mean value ± SD, n=6, *p<0.001 in Mg flux without amiloride vs Mg flux with amiloride.

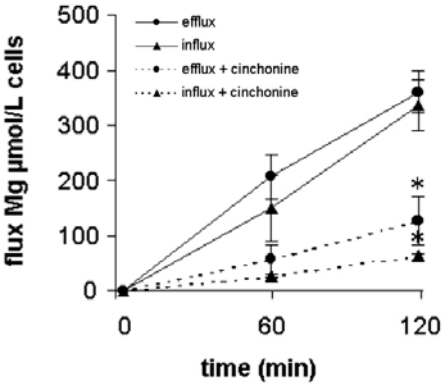

Figure 2. Mg efflux and Mg influx from rat erythrocytes in choline Cl medium in the presence of 0.4 mM ^{25}Mg. Cinchonine, a specific choline transport inhibitor, was used at a concentration of 2.4 mM. Mean value ± SD, n=6, *p<0.001 in Mg flux without cinchonine vs Mg flux with cinchonine.

Given the calibration process, the ICP/MS machine gives the quantitative results of each measured isotope as if the natural abundance for each isotope was 100%. So, it was necessary to subtract ^{26}Mg amount from ^{25}Mg amount in the erythrocyte. Indeed, erythrocyte ^{26}Mg represents the natural erythrocyte Mg but erythrocyte ^{25}Mg represents both the natural erythrocyte Mg and ^{25}Mg coming from the incubation medium.

To exclude Mg^{2+} efflux due to cell damage erythrocytes hemolysis was systematically measured by Hb determination in the supernatants (cyanmethemoglobin asssay, at 546 nm). An hemolysis in supernatants lower than 1.5% of total haemolysis, which did not increase significantly during the experiment, was considered in the range of normality and was therefore not taken into account.

Amiloride (1mM final concentration) in NaCl medium and cinchonine (2.4 mM final concentration) in choline medium were utilized as specific inhibitors of respectively, Na-dependent and choline-depedent Mg^{2+} efflux. The concentration of amiloride and cinchonine were chosen on dose response curve and checking that it did not induce any haemolysis.

3.6. Statistical analysis

Results were expressed as means ± SD. The statistical significance of differences between means was assessed using Student's t-test. The limit of statistical significance was set at $P<0.05$. Statistical analyses were performed using the GraphPad program (V3.00, GraphPad Software, San Diego, CA).

4. RESULTS

4.1. Mg^{2+} fluxes from erythrocytes incubated in NaCl medium and choline Cl medium with 0.4 mmol/L ^{25}Mg

Mg^{2+} influx and efflux was measured in isolated erythrocytes incubated in NaCl medium for 120 min at 37°C in the presence of 0.4 mmol/L ^{25}Mg. As can be seen in Figure 1, the rate of Mg^{2+} influx was about half the rate of Mg^{2+} efflux (apparent velocity 1.5 and 3.2 µmol/L/min, respectively). Amiloride (1 mM), a specific inhibitor of Na^+ transport, inhibited 2/3 of Mg^{2+} influx and ½ of Mg^{2+} efflux.

Figure 2 shows Mg^{2+} efflux and influx from rat erythrocytes incubated in the same conditions but with Na replaced with choline Cl. Noteworthy, the rate of Mg^{2+} influx was similar to the rate of Mg^{2+} efflux (apparent velocity 2.8 and 3.0 µmol/L/min, respectively) which was quantitatively comparable to that observed in Na-containing medium (see Figure 1). Cinchonine (2.4 mM), a selective inhibitor of the choline transporter (11) was able to inhibit a large amount of both Mg^{2+} efflux and influx.

4.2. Mg^{2+} fluxes from erythrocytes incubated in the presence of different concentration of ^{25}Mg (from 0.05 to 1.6 mmol/L)

Figures 3 reports data on the effects of extracellular Mg concentration on the rate of net Mg^{2+} efflux and influx in NaCl medium. Net Mg efflux results from subtracting the Mg^{2+} efflux at nominally 0 mM extracellular Mg. Both net Mg^{2+} efflux and influx increased as extracellular Mg concentration increased reaching a plateau at 0.8 mM extracellular Mg, and were of similar extent. Plotting the data of Mg^{2+} influx as a function of extracellular Mg according to LineWeaver-Burk equation, the curve displayed Michaelis-Menten kinetic (Figure 3, lower panel) with Km and Vmax reported in Table 1. Concerning the kinetic of net Mg^{2+} efflux, the curve also displayed LineWeaver-Burk kinetic (Figure 3, lower panel and Table 1). Also in choline Cl media, (Figure 4) both net Mg^{2+} efflux and influx increased when extracellular Mg level increased up to the concentration of 0.8 mM then reaching a plateau, but Mg efflux was lower than Mg influx. Figure 4 lower panels reports LineWeaver-Burk equation of Mg^{2+} influx and efflux and Table 1 the corresponding values of Km and Vmax.

5. DISCUSSION

Mg^{2+} transport within cells has been investigated over the last 20 years, the elective cells being erythrocytes. Data showed that Mg^{2+} efflux occurred mainly by a Na^+/Mg^{2+} antiport that was inhibited by amiloride and quinidine (21-24), a small portion of Mg^{2+} efflux was Na-independent, several other ions being proposed to be exchanged

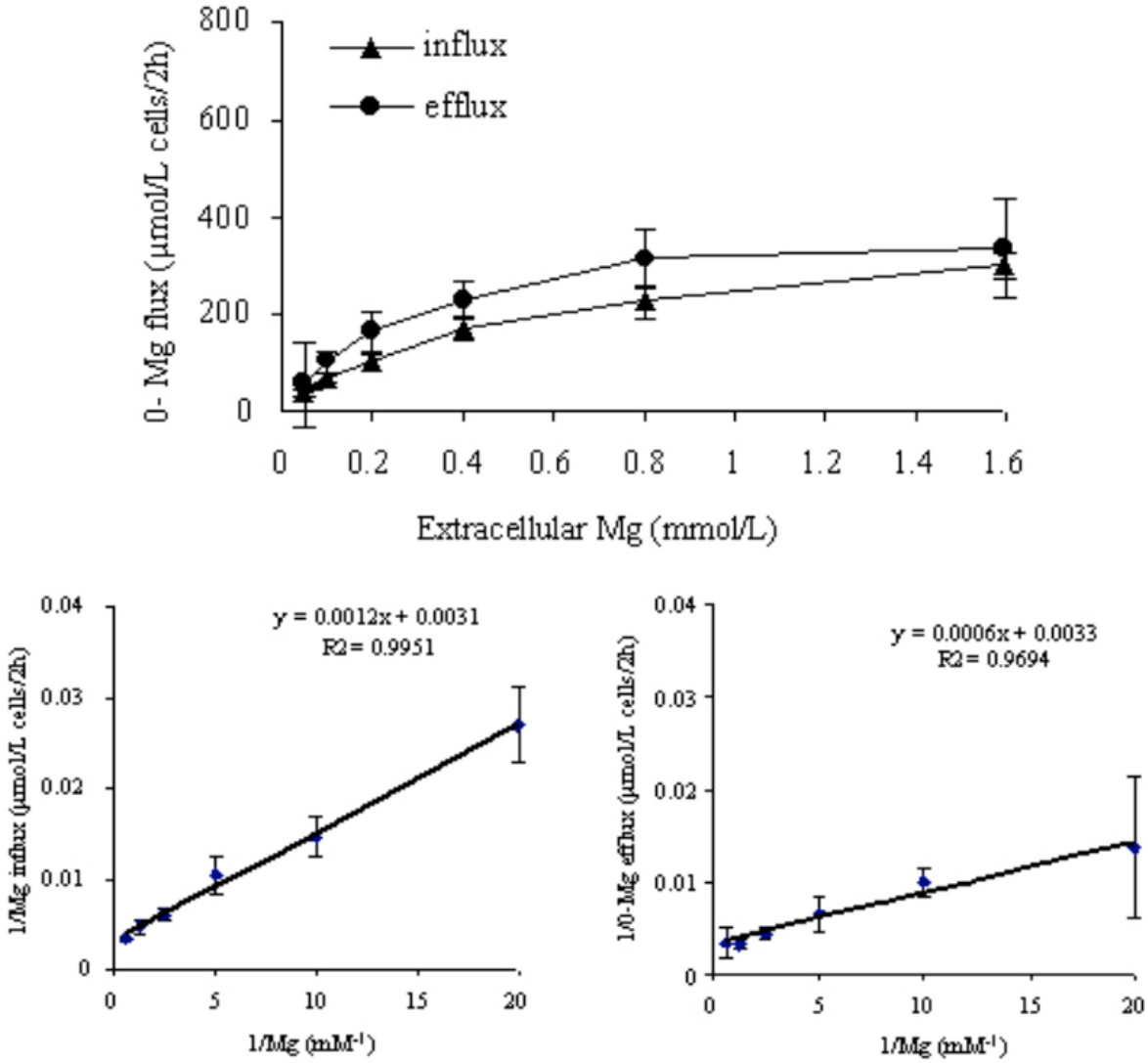

Figure 3. Effect of extracellular Mg concentration on net Mg^{2+} efflux and Mg^{2+} influx of rat erythrocytes in NaCl medium. Upper panel: Mg^{2+} influx and net efflux at increasing extracellular concentration of Mg. Mean value $\pm$ SD, n=6. Lower panels: LineWeaver Burk plots of Mg^{2+} influx and net efflux from rat erythrocytes in NaCl medium relative to extracellular Mg concentration. Note that for Mg^{2+} efflux the value at 0 mM extracellular Mg was subtracted from all data.

with Mg. Since these studies were conducted in Mg-loaded erythrocytes or in cells incubated in a Mg-free medium, they were limited in the way that they do not permit to evaluate Mg^{2+} efflux in the presence of physiological extracellular Mg levels nor to investigate the influence of modifications of extracellular Mg levels on Mg^{2+} fluxes. Moreover, they do not allow the study of Mg^{2+} influx which was studied, up to the '80 utilizing the radioactive isotope ^{28}Mg, with serious limitations due to the very short half life (21.3h), its scarce availability and high cost (25). The recent important improvements in analytical techniques of stable isotopes rendered possible the use of ^{25}Mg and ^{26}Mg. We thus developed a new approach, based on the use of stable isotope enriched media and ICP/MS, that makes possible the contemporaneous study of Mg^{2+} influx and Mg^{2+} efflux in physiologic conditions. The concentration of 0.4 mM of ^{25}Mg in extracellular media was chosen as plasma free Mg^{2+} is around 0.4 mmol/L. Lower and higher extracellular Mg levels, reflecting severe or mild Mg deficiency and Mg overload, were also studied.

We showed that, in the presence of physiologic extracellular Mg, it is possible to detect a Na-dependent Mg^{2+} efflux which was amiloride sensitive and a Na-independent Mg^{2+} efflux, confirming previous observations (9). Our data, allowing the contemporaneous measurement of Mg^{2+}influx and efflux in physiologic conditions, show that in Na medium net Mg^{2+} efflux is similar to Mg^{2+} influx. In contrast, the rate of net Mg^{2+} efflux was lower to Mg^{2+} influx when NaCl medium was substituted with choline Cl. Our results confirm the existence of a Na-dependent and a Na-independent Mg^{2+} efflux, and show that

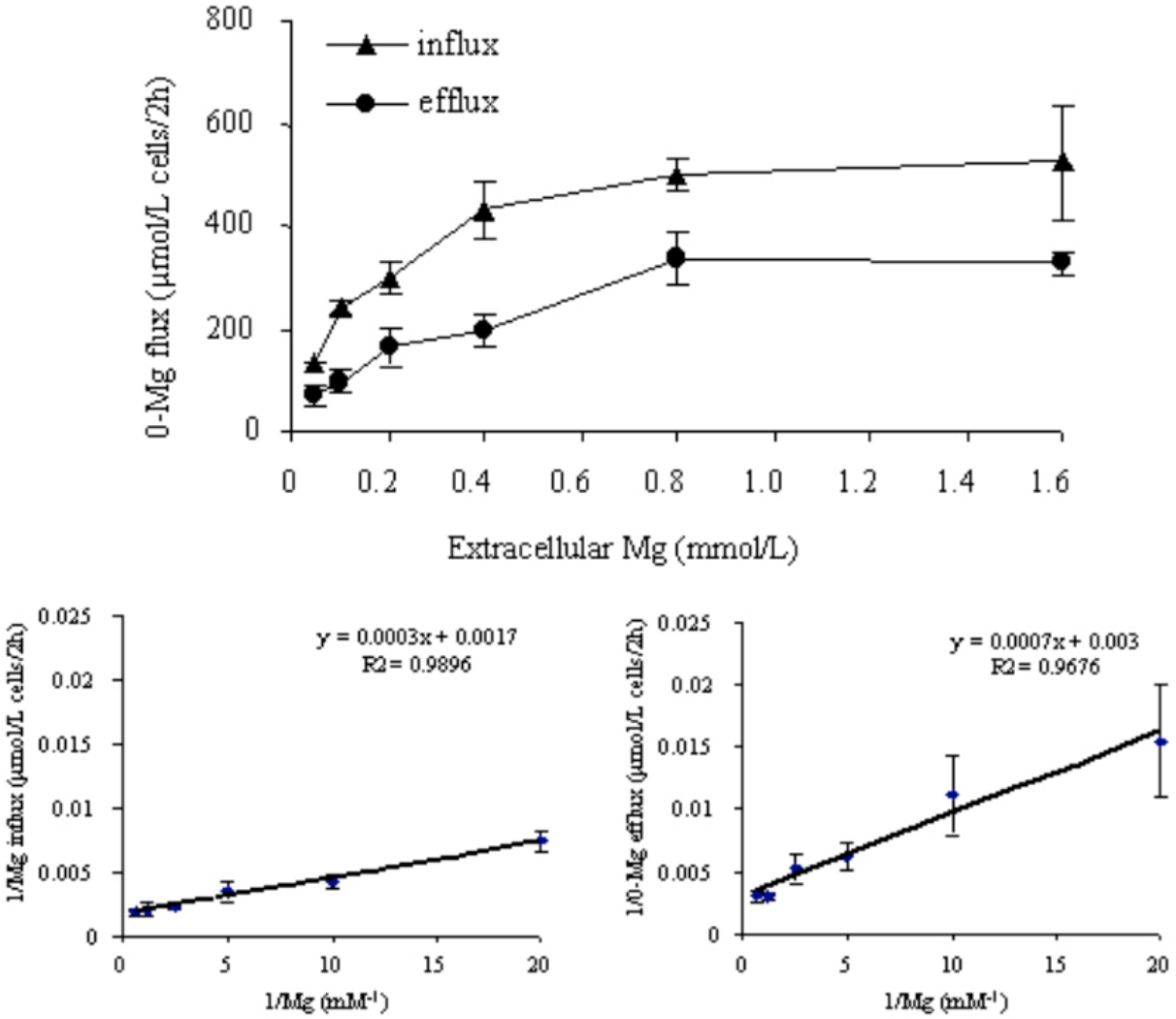

Figure 4. Effect of extracellular Mg concentration on net Mg^{2+} efflux and Mg^{2+} influx of rat erythrocytes in choline Cl medium. Upper panel: Mg influx and net efflux at increasing extracellular Mg. Mean value ± SD, n=6. Lowe panels: LineWeaver Burk plot of Mg^{2+} influx and net efflux from rat erythrocytes in choline Cl medium in relation to extracellular Mg concentration. Note that for Mg^{2+} efflux the value at 0 mM extracellular Mg was subtracted from all data.

in physiological conditions, Na-dependent and Na-independent Mg^{2+} efflux counterbalance Mg influx to maintain intracellular Mg level constant.

The most interesting data of our work concerns Mg^{2+} influx. We demonstrated a significant time dependent Mg^{2+} influx in NaCl medium in the presence of physiologic concentration of extracellular Mg. Like efflux, also this influx was sensitive to amiloride. Since amiloride and derivatives operate primarily on Na^+ channels, it is possible that the inhibition occurs on both side of the transporter. Alternatively, it is conceivable that an increase of intracellular Mg due to inhibition of Mg^{2+} efflux by amiloride, determines an indirect inhibition of Mg^{2+} influx. The amiloride insensitive fluxes measured in Na medium also suggest the presence of a Na-independent Mg^{2+} fluxes which could be explained by a Mg^{2+}/Mg^{2+} exchange (15). This fact is consistent with the observation that Mg^{2+} influx was stimulated by increasing extracellular Mg. The concentration dependence of Mg^{2+} influx, as observed by plotting the data according to LineWeaver-Burk, demonstrated saturability of transport with increasing extracellular Mg. However, as Km was 0.4 mM in NaCl medium, which is the normal free Mg^{2+} in the blood, it can be suggested that Mg^{2+} influx may not be saturated under physiologic conditions.

Our data on the contemporaneous measurements of Mg^{2+} influx and efflux underline the differences in the kinetic parameters (see Table 1) suggesting that Mg transport is differently regulated, likely by intra- or extracellular cation concentrations (likely Na^+ or Mg^{2+}). Alternatively, Mg transport inside or outside the cell is driven by two different mechanisms. Based on the recent data on the role of TRPM7 channels in the regulation of Mg homeostasis (7, 10), our observations are compatible with

Table 1. LineWeaver Burk kinetics of Mg^{2+} influx and net efflux in erythrocytes incubated in NaCl- or choline-Cl medium

	NaCl-medium		choline-Cl medium	
	influx	**net efflux**	**influx**	**net efflux**
Km (mM)	0.398±0,108	0.213±0.153 [1]	0.179±0.056 [3]	0.218±0.072
Vmax (μmol/l cells/2h)	335±39	337±87	589±91 [4]	330±39 [2]

Influx vs efflux (same medium): [1] $p<0.05$; [2] $p<0.001$ NaCl vs choline (same flux): [3] $p<0.01$; [4] $p<0.001$

the hypothesis that Mg^{2+} influx might be driven by a TRPM7 cation channel, and Mg^{2+} efflux might be driven by the putative Na^{+}/Mg^{2+} antiport. The possibility to contemporaneously measure both fluxes in physiologic conditions opens exciting perspectives in the understanding the regulation of Mg^{2+} fluxes at the cellular level.

In conclusion, our new experimental approach of the contemporaneous evaluation of Mg^{2+} influx and Mg^{2+} efflux demonstrate that in rat erythrocytes, in the presence of physiologic concentration of extracellular Mg, both Na-dependent and Na-independent Mg^{2+} efflux and Mg^{2+} influx are present, confirming previous results obtained in the absence of extracellular Mg. The Na-independent transport show the characteristics of a Mg^{2+}/Mg^{2+} exchange. Data also show that these fluxes are proportional to extracellular Mg availability up to the physiologic concentration. Since Mg requirement is related to different cell functions, such as proliferation and differentiation, specific mechanisms should operate to modulate the above described Mg^{2+} fluxes in the case of physiopathologic changes of intracellular Mg requirement.

7. REFERENCES

1. Flatman, P. W: Magnesium transport across cell membranes. *J Membr Biol* 80, 1-14 (1984)

2. Wolf, F. I., S. Fasanella, B. Tedesco, A. Torsello, A. Sgambato, B. Faraglia, P. Palozza, A. Boninsegna and A. Cittadini: Regulation of magnesium content during proliferation of mammary epithelial cells (HC-11). *Front Biosci* 9, 2056-2062 (2004)

3. Gunther, T., J. Vormann and V. Hollriegl: Characterization of Na(+)-dependent Mg2+ efflux from Mg2(+)-loaded rat erythrocytes. *Biochim Biophys Acta* 1023, 455-461 (1990)

4. Rasgado-Flores, H. and H. Gonzalez-Serratos: Plasmalemmal transport of magnesium in excitable cells. *Front Biosci* 5, D866-879 (2000)

5. Vormann, J. and T. Gunther: Magnesium transport mechanisms. In: Magnesium and the cell. Ed: N. J. Birch (1993)

6. Wolf, F. I., A. Di Francesco, V. Covacci and A. Cittadini: Regulation of magnesium efflux from rat spleen lymphocytes. *Arch Biochem Biophys* 344, 397-403 (1997)

7. Romani, A. M. and A. Scarpa: Regulation of cellular magnesium. *Front Biosci* 5, D720-734 (2000)

8. Gunther, T. and J. Vormann: Characterization of Na(+)-independent Mg2+ efflux from erythrocytes. *FEBS Lett* 271, 149-151 (1990)

9. Ebel, H. and T. Gunther: Characterization of Mg(2+) efflux from rat erythrocytes non-loaded with Mg(2+). *Biochim Biophys Acta* 1421, 353-360 (1999)

10. Romani, A. M. and M. E. Maguire: Hormonal regulation of Mg2+ transport and homeostasis in eukaryotic cells. *Biometals* 15, 271-283 (2002)

11. Ebel, H., M. Hollstein and T. Gunther: Role of the choline exchanger in Na(+)-independent Mg(2+) efflux from rat erythrocytes. *Biochim Biophys Acta* 1559, 135-144 (2002)

12. Beyenbach, K. W: Transport of magnesium across biological membranes. *Magnes Trace Elem* 9, 233-254 (1990)

13. Flatman, P. W: Mechanisms of magnesium transport. *Annu Rev Physiol* 53, 259-271 (1991)

14. Gunther, T. and J. Vormann: Reversibility of Na+/Mg2+ antiport in rat erythrocytes. *Biochim Biophys Acta* 1234, 105-110 (1995)

15. Feray, J. C. and R. Garay: A one-to-one Mg2+:Mn2+ exchange in rat erythrocytes. *J Biol Chem* 262, 5763-5768 (1987)

16. Gunther, T., J. Vormann and E. J. Cragoe, Jr.: Species-specific Mn2+/Mg2+ antiport from Mg2(+)-loaded erythrocytes. *FEBS Lett* 261, 47-51 (1990)

17. Wolf, F. I: TRPM7: Channeling the Future of Cellular Magnesium Homeostasis? *Sci STKE* 2004, PE23 (2004)

18. Nadler, M. J., M. C. Hermosura, K. Inabe, A. L. Perraud, Q. Zhu, A. J. Stokes, T. Kurosaki, J. P. Kinet, R. Penner, A. M. Scharenberg and A. Fleig: LTRPC7 is a Mg.ATP-regulated divalent cation channel required for cell viability. *Nature* 411, 590-595 (2001)

19. Schmitz, C., A. L. Perraud, C. O. Johnson, K. Inabe, M. K. Smith, R. Penner, T. Kurosaki, A. Fleig and A. M. Scharenberg: Regulation of vertebrate cellular Mg2+ homeostasis by TRPM7. *Cell* 114, 191-200 (2003)

20. Coudray, C., D. Pepin, J. C. Tressol, J. Bellanger and Y. Rayssiguier: Study of magnesium bioavailability using stable isotopes and the inductively-coupled plasma mass spectrometry technique in the rat: single and double labelling approaches. *Br J Nutr* 77, 957-970 (1997)

21. Gunther, T., J. Vormann and R. Forster: Regulation of

intracellular magnesium by Mg2+ efflux. *Biochem Biophys Res Commun* 119, 124-131 (1984)

22. Gunther, T. and J. Vormann: Mg2+ efflux is accomplished by an amiloride-sensitive Na+/Mg2+ antiport. *Biochem Biophys Res Commun* 130, 540-545 (1985)

23. Gunther, T: Putative mechanism of Mg2+/Mg2+ exchange and Na+/Mg2+ antiport. *Mag-Bull* 18, 2-6 (1996)

24. Feray, J. C. and R. Garay: An Na+-stimulated Mg2+-transport system in human red blood cells. *Biochim Biophys Acta* 856, 76-84 (1986)

25. Flik, G., J. A. Van der Velden and Z. I. Kolar: Radiotracers probes in cellular magnesium transport studies. In: Magnesium and the cell. Ed: N. J. Birch (1993)

Key Words: Mg influx, Mg efflux, Na-Mg antiport, Mg-Mg exchange, Amiloride

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