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Species-specific Mn^{2+}/Mg^{2+} antiport from Mg^{2+}-loaded erythrocytes

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Mg^{2+}-loaded rat erythrocytes performed Mn^{2+}/Mg^{2+} antiport, which was nonspecifically stimulated by anions and cations. Mn^{2+}/Mg^{2+} antiport was shown to operate via the Na^+/Mg^{2+} antiporter because extracellular Na^+ and Mn^{2+} inhibited the intracellular uptake of each other's ions competitively. Furthermore, Mn^{2+}/Mg^{2+} antiport and Na^+/Mg^{2+} antiport were identically inhibited by various amiloride derivatives. Na^+/Mg^{2+} antiport of chicken and human erythrocytes cannot perform Mn^{2+}/Mg^{2+} antiport although chicken erythrocytes took up more Mn^{2+} than rat erythrocytes.

Mn^{2+} uptake; Mg^{2+} efflux; (Rat erythrocyte)

1. INTRODUCTION

In a preceding paper, Feray and Garay [1] described net Mg^{2+} efflux from Mg^{2+}-loaded rat erythrocytes in exchange for extracellular Mn^{2+}. This net Mg^{2+} efflux was probably performed by the Na^+/Mg^{2+} antiporter in the absence of extracellular Na^+.

Net Mg^{2+} efflux from erythrocytes can be performed by various Mg^{2+} transporting systems [2,3]: (i) by Na^+-dependent net Mg^{2+} efflux operating via Na^+/Mg^{2+} antiport [4,5]; (ii) by Na^+-independent net Mg^{2+} efflux in combination with Cl^- efflux for charge compensation. This Mg^{2+} efflux takes place in sucrose medium and is inhibited by $[Cl^-]_o$ and SITS [2,3]; (iii) in choline-Cl or KCl medium another net Mg^{2+} efflux occurs which is independent of $[Na^+]_o$ and is not inhibited by $[Cl^-]_o$ or SITS [2,3]. Therefore, we investigated whether other Mg^{2+} efflux systems can exchange extracellular Mn^{2+} for intracellular Mg^{2+} from Mg^{2+}-loaded erythrocytes.

2. MATERIALS AND METHODS

Blood was taken by heart puncture from anaesthetized rats (50 mg/kg Nembutal s.c.) and by venous puncture from chicken or human (J.V.) by means of a heparinized syringe and centrifuged at $1000 \times g$ for 10 min. The plasma and buffy coat were aspirated and the red cells were washed twice with 150 mM KCl.

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Abbreviations: $[Na^+]_o$, $[Cl^-]_o$, $[Mn^{2+}]_o$, extracellular concentration of Na^+, Cl^-, or Mn^{2+}; TCA, trichloroacetic acid; SITS, 4-acetamido-4′-isothiocyanatostilbene-2,2′-disulfonic acid

The cells were loaded with Mg^{2+} by incubating a 10% cell suspension for 30 min at 37°C in KCl medium (in mM: 140 KCl, 50 sucrose, 5 glucose, 30 Hepes-Tris, pH 7.4) with the addition of 12 mM $MgCl_2$ and 6 μM A23187 (dissolved in dimethyl sulfoxide). For removal of the ionophore, the cells were incubated 4 times in KCl-$MgCl_2$ medium plus 1% bovine serum albumin for 10 min at 37°C. The KCl-$MgCl_2$ medium was removed by washing the cells twice with sucrose, KCl, NaCl or choline-Cl medium. The sucrose medium contained (in mM): 350 sucrose, 5 glucose, 30 Hepes-Tris, pH 7.4. NaCl or choline-Cl medium was prepared by substitution of KCl in KCl medium by 140 mM NaCl or 140 mM choline-Cl. Under these conditions of Mg^{2+}-loading intracellular Mg^{2+} content of rat, chicken and human erythrocytes amounted to 20, 17.5, and 20 mmol/l cells.

Mg^{2+} efflux was measured by reincubation of a 10% cell suspension at 37°C in sucrose, KCl, choline-Cl or NaCl medium, as indicated.

In some efflux experiments, the cations or anions were substituted by positively or negatively charged ampholine. Ampholine (1809006), pH 2.5-4, or ampholine (1809046), pH 9-11 (Pharmacia, Bromma, Sweden), were neutralized to pH 7.4 with 1 N KOH or 1 N NaOH or 1 N HCl. Aliquots of the neutralized ampholine were added to the media instead of KCl or NaCl to yield 145 mM K^+, Na^+ or Cl^-.

At the beginning of reincubation and after 30 min, 0.5 ml aliquots of the cell suspension were centrifuged for 1 min at $10\,000 \times g$. For Mg^{2+} determination, 100 μl supernatant was diluted with 1 ml 10% TCA/0.175% $LaCl_3$ and Mg^{2+} was measured by atomic absorption spectrophotometry (Philips, SP 9). An aliquot of the supernatant was taken for the determination of hemoglobin by means of the cyanmethemoglobin method. For measuring Mn^{2+}-induced Mg^{2+} efflux, $MnCl_2$ was added to the reincubation media, as indicated.

For measuring intracellular Mg^{2+}, Mn^{2+} and Na^+ content, the sedimented cells were washed twice with 150 mM KCl and hemolized by adding 750 μl H_2O. 50 μl of the hemolysate were taken for determination of hemoglobin, the rest was deproteinized by addition of 50 μl 75% TCA and centrifuged.

Mg^{2+} and Mn^{2+} content was measured by atomic absorption spectrophotometry after dilution with 10% TCA/0.175% $LaCl_3$. Na^+ content was measured after addition of LiCl by flame photometry (KliNa-Flame, Beckman). Cellular Mg^{2+} content was taken to correct Mg^{2+} efflux for hemolysis. Cellular Mn^{2+} and Na^+ content was taken to determine Mn^{2+} influx or Na^+ influx.

Amiloride and its four analogs (see legend fig.3) were synthesized for this study by methods described earlier [6].

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Table 1

Effects of extracellular $MnCl_2$ on net Mg^{2+} efflux from rat erythrocytes incubated in sucrose, NaCl or choline-Cl medium

Medium	$MnCl_2$ (mM)	Mg^{2+} efflux (mmol Mg^{2+}/l cells × 30 min)
Sucrose	0	1.74
	1.0	2.38
KCl	0	0.70
	1.0	3.41
Choline-Cl	0	0.71
	1.0	3.36
NaCl	0	9.25
	1.0	8.19

Mean of two experiments

Table 2

Effects of various anions and cations on Mn^{2+}-dependent Mg^{2+} efflux from rat erythrocytes

Medium	$MnCl_2$ (mM)	Mg^{2+} efflux (mmol Mg^{2+}/l cells × 30 min)
Choline-Cl	0.0	0.92
	0.5	4.35
KCl	0.0	1.00
	0.5	4.84
KI	0.0	1.05
	0.5	4.52
KNO_3	0.0	1.07
	0.5	4.99

Mean of two experiments

3. RESULTS AND DISCUSSION

As shown in table 1, Mn^{2+}-induced Mg^{2+} efflux was low in sucrose medium but occurred to a high degree in KCl or choline-Cl medium.

These results indicate that Mn^{2+}-dependent net Mg^{2+} efflux was activated in salt-containing media. Mn^{2+}-induced Mg^{2+} efflux at 0 mM KCl amounted to 0.8 mmol Mg^{2+}/l cells × 30 min and increased linearly with KCl concentration to 4.2 mmol/l cell × 30 min at 150 mM KCl (fig.1).

Since Mn^{2+}-induced Mg^{2+} efflux was the same in KCl and choline-Cl medium (table 1), this transport system is not specifically dependent on cations. Therefore, we tested anion specificity. As can be seen from table 2, Mn^{2+}-induced net Mg^{2+} efflux was also not specifically dependent on anions. The role of either cations or anions was tested with ampholines (table 3).

In the presence of 145 mM Cl^-, compensated by polycationic ampholine, and in the presence of 145 mM K^+, compensated by polyanionic ampholine, Mn^{2+} did not significantly induce net Mg^{2+} efflux. From this result it can be concluded that small cations and small anions must be present simultaneously in the medium to permit Mn^{2+}-induced Mg^{2+} efflux.

Na^+/Mg^{2+} antiport was also active in a medium with 150 mM Na^+ compensated by polyanionic ampholine (table 3) and thus not dependent on the presence of small anions in the medium. The meaning of this result is not clear.

As indicated in table 1, Mg^{2+} efflux in NaCl medium was reduced by $[Mg^{2+}]_o$. Therefore, this relationship between $[Na^+]_o$, Mg^{2+} efflux and Mn^{2+} influx was investigated in greater detail.

Mn^{2+} was taken up in Mg^{2+}-unloaded cells, incubated in sucrose, NaCl or KCl medium (table 4). However, in Mg^{2+}-loaded cells, Mn^{2+} uptake was 3 times higher in sucrose and 9 times higher in NaCl and KCl medium than in unloaded cells (table 4). Mn^{2+} up-

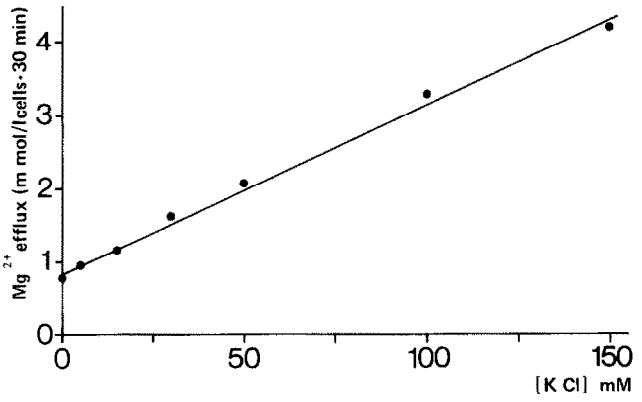

Fig.1. Stimulation of Mn^{2+}-induced Mg^{2+} efflux from Mg^{2+}-loaded rat erythrocytes by KCl. Sucrose of sucrose medium was isoosmotically substituted by KCl. Difference of Mg^{2+} efflux between tests without and with the addition of 0.5 mM $MnCl_2$ at various KCl concentrations was plotted.

Table 3

Effects of cations and anions on Mn^{2+}-induced Mg^{2+} efflux from Mg^{2+}-loaded rat erythrocytes

Incubation		$MnCl_2$ (mM)	Mg^{2+} efflux (mmol Mg^{2+}/l cells × 30 min)	
Cation	Anion			
A^{+a}	Cl^-	0.0	1.16	
A^{+a}	Cl^-	0.5	1.44	0.28[c]
K^+	A^{-b}	0.0	2.90	
K^+	A^{-b}	0.5	2.93	0.03[c]
K^+	Cl^-	0.0	0.70	
K^+	Cl^-	0.5	2.50	1.80[c]
$Choline^+$	Cl^-	0.0	0.66	
$Choline^+$	Cl^-	0.5	2.38	1.72[c]
Na^+	Cl^-	0.0	11.66	
Na^+	A^{-b}	0.0	9.71	

Mean of two experiments

[a] A^+, ampholine pH 9–11, neutralized with 1 N HCl to pH 7.4, Cl^- concentration in the medium amounted to 145 mM

[b] A^-, ampholine pH 2.5–4, neutralized with 1 N KOH or 1 N NaOH to pH 7.4, Na^+ and K^+ concentration in the medium amounted to 145 mM

[c] Mn^{2+}-induced Mg^{2+} efflux

Table 4

Mg^{2+} efflux and Mn^{2+}-influx of unloaded and Mg^{2+}-loaded rat erythrocytes

Medium	$MnCl_2$ (mM)	Mg^{2+}-loaded (mmol/l cells × 30 min		Mg^{2+}-unloaded[a]
		Mg^{2+} efflux	Mn^{2+} influx	Mn^{2+} influx
Sucrose	0	1.84	0.00	0.00
	1	2.58	0.80	0.29
KCl	0	0.92	0.00	0.00
	1	3.54	2.07	0.29
NaCl	0	10.45	0.00	0.00
	1	9.41	1.29	0.19

Mean of two experiments

[a] In Mg^{2+}-unloaded cells no significant Mg^{2+} efflux was measured

take in Mg^{2+}-unloaded cells was not correlated to efflux of Mg^{2+}. In Mg^{2+}-loaded cells incubated in sucrose medium Mn^{2+}-induced Mg^{2+} efflux was approximately the same as Mn^{2+} uptake. However, in KCl medium Mn^{2+}-induced Mg^{2+} efflux exceeded Mn^{2+} uptake. These results indicate that there are two mechanisms for Mn^{2+} uptake. (i) Mn^{2+} uptake into unloaded cells, which is not related to Mg^{2+} efflux. Its mechanism has not yet been defined. (ii) Mn^{2+} uptake into Mg^{2+}-loaded cells is accompanied by an additional efflux of Mg^{2+}. From the quantitative relationship between the Mg^{2+} and Mn^{2+} fluxes it can be assumed that there was an Mn^{2+}/Mg^{2+} antiport in sucrose medium. However, in KCl medium, Mg^{2+} efflux exceeded Mn^{2+} uptake. Therefore, also in KCl medium a major part of Mg^{2+} efflux may occur in exchange for extracellular Mn^{2+}, as found by Feray and Garay [1]. However, Mn^{2+} may cause an additional efflux of Mg^{2+} in KCl medium.

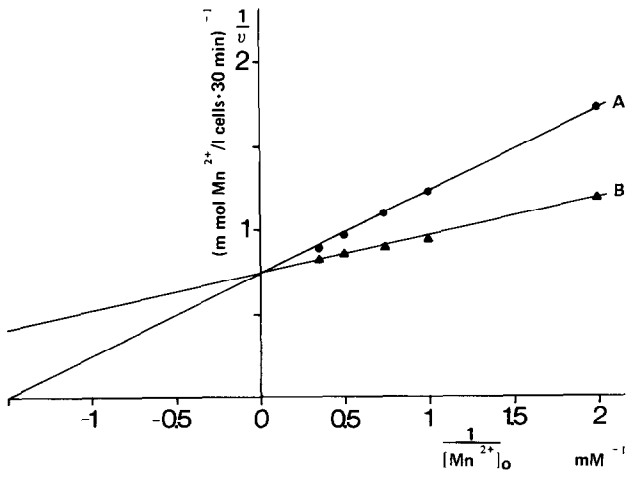

Fig.2. Lineweaver-Burk plot of Mn^{2+} influx into Mg^{2+}-loaded rat erythrocytes. (A) $[Na^+]_o$ = 150 mM; (B) $[Na^+]_o$ = 30 mM (NaCl of NaCl medium was isoosmotically substituted by KCl).

When Mn^{2+}/Mg^{2+} antiport is performed via the Na^+/Mg^{2+} antiport system, extracellular Na^+ should be a competitive inhibitor of net Mn^{2+} uptake. As shown in fig.2, extracellular Na^+ inhibited Mn^{2+} uptake competitively, and vice versa, Na^+ uptake into Mg^{2+}-loaded rat erythrocytes was competitively inhibited by $[Mn^{2+}]_o$ (not shown).

To elucidate the problem whether the Na^+/Mg^{2+} antiporter is functioning in Mn^{2+}/Mg^{2+} antiport, we tested inhibition of Mn^{2+}-induced Mg^{2+} efflux by various amiloride derivatives.

As shown in fig.3, Mn^{2+}-induced Mg^{2+} efflux was inhibited by various amiloride derivatives. The order of potency of inhibition was identical to the inhibition of

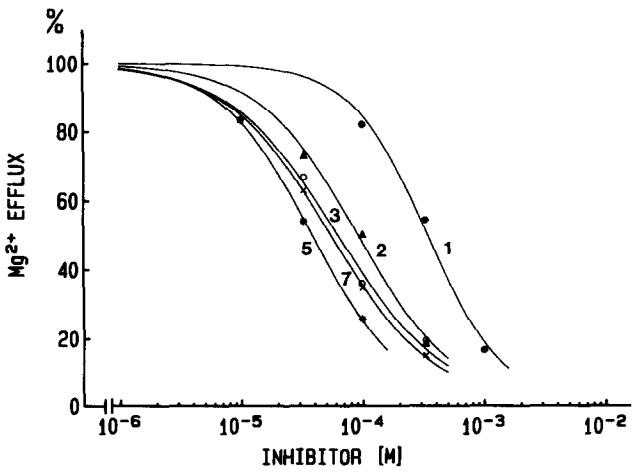

Fig.3. Inhibition of Mn^{2+}-induced Mg^{2+} efflux from Mg^{2+}-loaded rat erythrocytes by various amiloride derivatives. KCl medium with 1 mM $MnCl_2$ was taken. 100% amounted to 5.93 mmol Mg^{2+}/l cells × 30 min. (1) Amiloride; (2) 5-(*N*-ethyl-*N*-isopropyl)amiloride; (3) 5-(*N*-methyl-*N*-isobutyl)amiloride; (5) 5-(*N*-ethyl-*N*-4-chlorobenzyl) amiloride; (7) 5-(*N*-methyl-*N*-4-hydroxyphenyl)amiloride. The amiloride derivatives were dissolved in dimethyl sulfoxide (DMSO). The final DMSO concentration in the tests amounted to 1%. 100% values were run with 1% DMSO. Same numbering of amiloride derivatives as in [7].

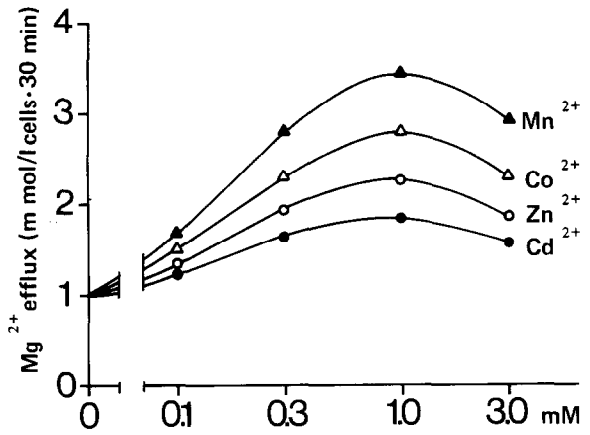

Fig.4. Stimulation of Mg^{2+} efflux by various divalent cations. Mg^{2+}-loaded rat erythrocytes were incubated in choline-Cl medium with the addition of $MnCl_2$, $CoCl_2$, $ZnCl_2$ and $CdCl_2$ as indicated.

Table 6

Effect of $[Mn^{2+}]_o$ on Mg^{2+} efflux and Mn^{2+} influx of Mg^{2+}-loaded human erythrocytes

Medium	$MnCl_2$ (mM)	Mg^{2+} efflux (mmol/l cells × 30 min)	Mn^{2+} influx (mmol/l cells × 30 min)
Sucrose	0	0.94	0.00
	1	0.57	0.16
	2	0.41	0.21
KCl	0	0.12	0.00
	1	0.07	0.45
	2	0.07	0.70
NaCl	0	0.28	0.00
	1	0.10	0.48
	2	0.08	0.68

Mean of two experiments

Na^+/Mg^{2+} antiport by the same amiloride derivatives [7]. Mn^{2+} uptake was simultaneously inhibited. The order of potency of inhibition of Mn^{2+} uptake by the amiloride derivatives was the same as the inhibition of Mg^{2+} efflux (data not shown), again indicating Mn^{2+}/Mg^{2+} antiport.

These results indicate that $[Mn^{2+}]_o$-induced Mg^{2+} efflux is performed via the Na^+Mg^{2+} antiporter. This mechanism is also operating in sucrose medium although to a lower degree because Mn^{2+}/Mg^{2+} antiport is nonspecifically stimulated by small anions and cations (fig.1).

This Mn^{2+}/Mg^{2+} exchange system in rat erythrocytes is not specific for Mn^{2+}. As shown in fig.4, Co^{2+}, Zn^{2+} and Cd^{2+} also induced net Mg^{2+} efflux from Mg^{2+}-loaded rat erythrocytes with decreasing effectivity in this order. This order corresponds to the increase of stability constants of these metal ions with various ligands (e.g. glycinate, oxalate, malonate [8]). Other divalent cations as Ba^{2+}, and Sr^{2+} were ineffective (data not shown).

Chicken erythrocytes possess an active Na^+/Mg^{2+} antiport [3,4,9] with similar properties as the Na^+/Mg^{2+} antiport of rat erythrocytes [5,7]. However, in chicken erythrocytes, extracellular Mn^{2+} did not induce (but inhibited) net Mg^{2+} efflux, although chicken erythrocytes took up more Mn^{2+} than rat erythrocytes (table 5).

Also in human erythrocytes Mn^{2+} did not induce Mg^{2+} efflux from Mg^{2+}-loaded cells but caused inhibition of Mg^{2+} efflux (table 6). Therefore, Na^+/Mg^{2+} antiport in Mg^{2+}-loaded chicken and human erythrocytes may be performed by proteins with different structures compared to rat erythrocytes.

Table 5

Effects of $[Mn^{2+}]_o$ on Mg^{2+} efflux and Mn^{2+} influx of unloaded and Mg^{2+}-loaded chicken erythrocytes

		Mg^{2+}-loaded (mmol/l cells × 30 min)		Mg^{2+}-unloaded[a]
Medium	$MnCl_2$ (mM)	Mg^{2+} efflux	Mn^{2+} influx	Mn^{2+} influx
Sucrose	0.0	1.79	0.00	0.00
	0.5	1.35	0.69	0.23
	1.0	1.19	1.14	0.31
	2.0	1.02	2.04	0.44
KCl	0.0	2.05	0.00	0.00
	0.5	1.37	3.60	0.31
	1.0	1.20	6.64	0.39
	2.0	0.91	10.34	0.52
NaCl	0.0	4.30	0.00	0.00
	0.5	3.01	3.46	0.16
	1.0	2.29	6.24	0.24
	2.0	1.49	10.56	0.36

Mean of two experiments

[a] In Mg^{2+}-unloaded chicken erythrocytes no significant Mg^{2+} efflux was measured

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