Characterization of Na^+-independent Mg^{2+} efflux from erythrocytes

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Na^+-independent Mg^{2+} efflux from Mg^{2+}-loaded human, rat and chicken erythrocytes was reduced by extracellular Cl^-. Na^+-independent Mg^{2+} efflux at low extracellular Cl^- concentration (sucrose medium) was inhibited by SITS and was nearly insensitive to SITS in 150 mM choline Cl medium. The inhibition of Mg^{2+} efflux by extracellular Cl^- and DIDS could be overcome by the lipophilic permeant tetraphenylphosphonium cation. Na^+-independent Mg^{2+} efflux from human and rat erythrocytes in sucrose and choline Cl medium was inhibited by cAMP and by amiloride and amiloride analogues. The results indicate that Na^+-independent Mg^{2+} efflux in high Cl^- medium is performed by a similar or the same Mg^{2+} efflux system, operating in sucrose medium in which the efflux of Mg^{2+} is accompanied by the efflux of Cl^- for charge compensation.

Magnesium; Chloride; Efflux; Amiloride; cAMP; Erythrocyte

1. INTRODUCTION

Mg^{2+} efflux from Mg^{2+}-loaded erythrocytes can be performed by Na^+-dependent Mg^{2+} efflux via Na^+/Mg^{2+} antiport [1–4] and by Na^+-independent Mg^{2+} efflux, in which Mg^{2+} efflux is accompanied by the efflux of Cl^- for charge compensation. The latter process can be inhibited by SITS, DIDS and Cl_o^- [5,6].

A residual efflux of Mg^{2+} exists in high Cl^- medium [5,6]. This residual Mg^{2+} efflux, which is independent of Cl_o^- and SITS, may be explained by artificial or undefined leak. In this paper, data are presented from which it can be assumed that this Cl^- and SITS-insensitive, Na^+-independent Mg^{2+} efflux is very similar, if not identical, to the Na^+-independent Mg^{2+} efflux in sucrose medium [5].

2. MATERIALS AND METHODS

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

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 four 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 or choline Cl medium. The sucrose medium contained (in mM): 350 sucrose, 5 glucose, 30 Hepes-Tris, pH 7.4. Choline Cl medium was prepared by substitution of KCl in KCl medium by 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 determined by reincubation of a 10% cell suspension at 37°C in sucrose or choline Cl medium, as indicated. At the beginning of reincubation and after 30 min, 0.5 ml aliquots of the cell suspension were centrifuged for 1 min at 10000 × *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). The rate of Mg^{2+} efflux was calculated from the increase of extracellular Mg^{2+} concentration.

An aliquot of the supernatant was taken for the determination of hemoglobin by means of the cyanmethemoglobin method.

For measuring intracellular Mg^{2+} content, the sedimented cells were washed twice with 150 mM KCl and hemolyzed 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+} content of the TCA extract was measured by atomic absorption spectrophotometry after dilution with 10% TCA/0.175% $LaCl_3$.

Cellular Mg^{2+} content was taken to correct Mg^{2+} efflux for hemolysis.

3. RESULTS AND DISCUSSION

In human erythrocytes, Na^+-independent Mg^{2+} efflux was inhibited by Cl_o^- up to 50 mM and thereafter remained nearly constant up to 150 mM. The remaining Mg^{2+} efflux was only slightly inhibited by SITS [5].

To clarify whether Na^+-independent Mg^{2+} efflux has the same properties in erythrocytes of other species, we tested Cl_o^- dependency of rat and chicken erythrocytes.

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Abbreviations: Cl_o^-, extracellular Cl^-; TCA, trichloroacetic acid; TPP^+, tetraphenylphosphonium; SITS, 4-acetamino-4′-isothiocyanatostilbene-2,2′-disulfonate; DIDS, 4,4′-diisothiocyanatostilbene-2,2′-disulfonate; dbcAMP, dibutyryl cAMP; IC_{50}, inhibitor concentration at 50% inhibition of Mg^{2+} efflux

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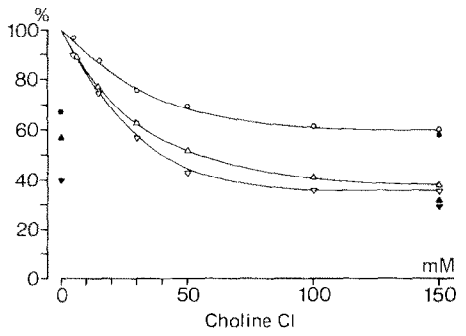


Fig. 1. Inhibition of Na^+-independent net Mg^{2+} efflux from chicken (○), rat (△) and human (▽) erythrocytes by choline Cl. Sucrose in sucrose medium was isoosmotically substituted by choline Cl. Filled symbols, Mg^{2+} efflux in the presence of 30 μM SITS. 100% values of net Mg^{2+} efflux in sucrose medium amounted to 0.4; 1.6 and 0.9 mmol/l cells × 30 min for chicken, rat and human erythrocytes. Mean of 3 experiments.

Fig. 1 shows that Na^+-independent Mg^{2+} efflux from all types of erythrocytes expressed an analogous inhibition by Cl_o^-.

Mg^{2+} efflux in sucrose was inhibited by SITS, whereas Mg^{2+} efflux at 150 mM choline Cl was almost SITS-insensitive (Fig. 1). Thus, Cl_o^- and SITS dependency of Mg^{2+} efflux showed similar properties as KCl efflux from human erythrocytes [7,8]. In human erythrocytes, KCl efflux at high Cl_o^- and low Cl_o^- concentration was limited by the activity of capnophorin [7,8].

In analogy to KCl efflux, Na^+-independent Mg^{2+} efflux in high Cl_o^- medium may also be performed by an efflux system.

In erythrocytes, Cl^--dependent K^+ efflux was affected by cAMP, depending on the osmolarity [9].

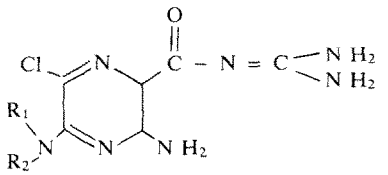
Table I

Inhibition of Na^+-independent net Mg^{2+} efflux from Mg^{2+}-loaded human and rat erythrocytes in sucrose and choline Cl medium by amiloride and amiloride derivatives

Derivative		Human erythrocytes		Rat erythrocytes	
R_1[a]	R_2	Sucrose	Choline Cl	Sucrose	Choline Cl
H	H (amiloride)	2.5	1	3	1
CH_3	$(CH_3)_2$-CH	0.1	0.06	0.2	0.06
H	$(CH_3)_3$-C	0.1	0.05	0.2	0.05
CH_3	HO-⟨⟩	0.1	0.06	0.2	0.06

Inhibitor concentrations (in mM) at 50% inhibition (IC_{50}) are listed. Mean of 3 experiments.

[a] Amiloride was substituted as indicated:



Therefore, we tested whether Na^+-independent Mg^{2+} efflux from erythrocytes is influenced in a similar way. As shown in Table I, Na^+-independent Mg^{2+} efflux from human and rat erythrocytes in choline Cl and sucrose medium was inhibited by dbcAMP.

From this result it can be concluded that Mg^{2+} efflux in choline Cl is performed by an efflux mechanism, which is affected by cAMP.

As another evidence for the existence of an Na^+-independent Mg^{2+} efflux system in choline Cl medium, we tested the inhibition of Mg^{2+} efflux by amiloride and amiloride derivatives.

Mg^{2+} efflux from chicken erythrocytes in sucrose and choline Cl medium was nearly resistant to amilorides (data not shown).

The concentrations at 50% inhibition of Mg^{2+} efflux by amiloride and various amiloride analogues are listed in Table II. Na^+-independent Mg^{2+} efflux from human and rat erythrocytes was more strongly inhibited by the amiloride derivatives than by amiloride. With respect to experimental error caused by the low rate of

Table II

Effect of dibutyryl cAMP on Na^+-independent Mg^{2+} efflux from Mg^{2+}-loaded chicken, rat and human erythrocytes

Medium		Chicken ery.	Rat ery.	Human ery.
Choline Cl (mM)	Sucrose (mM)			
100	–	80[a]	60	55
100	150	82	64	57
–	200	86	76	67
–	350	88	81	77

[a] Mg^{2+} efflux in the absence of dibutyryl cAMP was taken as 100%.

After Mg^{2+} loading, the cells were preincubated with 1 mM dibutyryl cAMP in different media for 10 min as indicated. Thereafter and 30 min later, 0.5 ml cell suspensions were taken and centrifuged at 10000 × g for 1 min. Mg^{2-} efflux was determined from the increase of Mg^{2+} concentrations in the supernatants. Mean of 4 experiments.

Table III

Effect of tetraphenylphosphonium Cl (TPPCl) on Na^+-independent Mg^{2+} efflux from Mg^{2+}-loaded human and rat erythrocytes in sucrose and choline Cl medium

No.	Sucrose (mM)	Choline Cl (mM)	TPP Cl (mM)	DIDS (μM)	Human ery. (mmol Mg^{2+}/l cells × 30 min)	Rat ery. (mmol Mg^{2+}/l cells × 30 min)
1	330	10	–	–	0.74	1.00
2	330	10	–	40	0.21	0.50
3	330	–	10	–	0.65	1.02
4	330	–	10	40	0.61	1.09
5	50	150	–	–	0.33	0.58
6	50	150	–	40	0.29	0.53
7	50	140	10	–	0.72	1.02
8	50	140	10	40	0.59	0.80

Mean of 2 experiments.

Na^+-independent Mg^{2+} efflux, the ratio of IC_{50} for amiloride to IC_{50} for amiloride derivatives was the same in sucrose as in choline Cl medium. These ratios of IC_{50} were similar for human and rat erythrocytes.

Mg^{2+} efflux was somewhat more strongly inhibited by amiloride and amiloride derivatives in choline Cl than in sucrose medium. In agreement with this result, the renal Na^+/H^+ antiporter was also more strongly inhibited by amiloride in Cl^- than gluconate medium, because of a 2.2 times higher affinity of amiloride to the Na^+/H^+ antiporter in the presence of Cl^- [10].

The analogous inhibition of Mg^{2+} efflux in sucrose and choline Cl medium by amilorides is another evidence that both Na^+-independent Mg^{2+} efflux systems are very similar or identical.

Thus, it can be suggested that Na^+-independent Mg^{2+} efflux in choline Cl medium may also be accompanied by the efflux of intracellular Cl^- for charge compensation as shown for Na^+-independent Mg^{2+} efflux in sucrose [5].

The reduction of Na^+-independent Mg^{2+} efflux at high Cl_o^- concentration may be produced by the reduction of the Cl^- gradient or by changing the properties of capnophorin at high Cl_o^- concentration with respect to Cl^- conductivity and SITS sensitivity because at the same time, the net Cl^- efflux system becomes less sensitive to inhibition by SITS [7,8].

An experimental proof for the charge compensatory effect of Cl^- in Na^+-independent Mg^{2+} efflux offers the lipophilic permeant TPP^+ [11]. When Na^+-independent Mg^{2+} efflux in sucrose and choline Cl medium is accompanied by Cl^- efflux for charge compensation, it may be expected that the inhibition of Na^+-independent Mg^{2+} efflux by Cl_o^- and DIDS which attack at the Cl^- efflux system can be overcome by TPP^+. Indeed, the inhibition of Mg^{2+} efflux in sucrose by DIDS (Table III, No. 2) was overcome by TPP^+ (Table III, No. 4), and the inhibition of Na^+-independent Mg^{2+} efflux by high Cl_o^- (choline Cl medium) (No. 5) was also overcome by TPP^+ (No. 7).

Since the role of Cl^- efflux in Mg^{2+} efflux was substituted by TPP^+, it can be concluded that in Na^+-independent Mg^{2+} efflux, Mg^{2+} leaves the cell separately from Cl^- by a channel of its own, which may be the same at low or high Cl_o^-.

REFERENCES

[1] Günther, T., Vormann, J. and Förster, R. (1984) Biochem. Biophys. Res. Commun. 119, 124–131.
[2] Günther, T. and Vormann, J. (1985) Biochem. Biophys. Res. Commun. 130, 540–545.
[3] Günther, T. and Vormann, J. (1987) Biochem. Biophys. Res. Commun. 148, 1069–1074.
[4] Günther, T., Vormann, J. and Höllriegl, V. (1990) Biochim. Biophys. Acta 1023, 455–461.
[5] Günther, T. and Vormann, J. (1989) FEBS Lett. 247, 181–184.
[6] Günther, T. and Vormann, J. (1989) FEBS Lett. 350, 633–637.
[7] Fröhlich, O., Leibson, C. and Gunn, R.B. (1983) J. Gen. Physiol. 81, 127–152.
[8] Jones, G.S. and Knauf, P.A. (1985) J. Gen. Physiol. 86, 721–738.
[9] Kim, H.D., Sergeant, S., Forte, L.R., Sohn, D.H. and Im, J.H. (1989) Am. J. Physiol. 256, C722–C778.
[10] Warnock, D.G., Yang, W.-C., Huang, Z.-Q. and Cragoe, E.J. (1988) J. Biol. Chem. 263, 7216–7221.
[11] Lichtshtein, D., Kaback, H.R. and Blume, A.J. (1979) Proc. Natl. Acad. Sci. USA 76, 650–654.