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Characterization of Na^+-dependent Mg^{2+} efflux from Mg^{2+}-loaded rat erythrocytes

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Na^+-dependent Mg^{2+} efflux from Mg^{2+}-loaded rat erythrocytes was determined from the increase of extracellular Mg^{2+} concentration or decrease of intracellular Mg^{2+} content, as measured by means of atomic absorption spectrophotometry. Mg^{2+} efflux was specifically combined with the uptake of Na^+ at a stoichiometric ratio of $2Na^+ : 1Mg^{2+}$, indicating electroneutral Na^+/Mg^{2+} antiport. Na^+/Mg^{2+} antiport depended on intracellular ATP and was inhibited by amiloride and quinidine, but was insensitive to strophanthin. Net Mg^{2+} efflux was only occurring at increased concentration of intracellular Mg^{2+} ($[Mg^{2+}]_i$), and stopped when the physiological Mg^{2+} content was reached. Intracellular Mg^{2+} acted cooperatively with a Hill coefficient of 2.4, which may indicate gating of Na^+/Mg^{2+} antiport at increased $[Mg^{2+}]_i$. At increased intracellular Na^+ concentration, Na^+ competed with intracellular Mg^{2+} for Mg^{2+} efflux and Na^+ could leave the rat erythrocyte via this transport system. Na^+/Mg^{2+} antiport was working asymmetrically with respect to extra- and intracellular Na^+ and Mg^{2+}, and did not perform net Mg^{2+} uptake.

Introduction

In preceding experiments, we investigated net Mg^{2+} efflux from Mg^{2+}-loaded human, chicken and rat erythrocytes [1]. In all types of erythrocytes, there was an Na^+-dependent and Na^+-independent Mg^{2+} efflux. However, both kinds of Mg^{2+} efflux were differently expressed in these types of erythrocytes. In rat erythrocytes, Na^+-dependent net Mg^{2+} efflux exhibited the highest rate amounting to 8.9 mmol/l cells per 30 min. Thus, Na^+-dependent net Mg^{2+} efflux from rat erythrocytes was 5.5-times higher than from chicken erythrocytes and 55-times higher than from human erythrocytes. The high rate of Na^+-dependent net Mg^{2+} efflux offers the possibility to investigate more exactly the properties of Na^+-dependent net Mg^{2+} efflux from rat than human erythrocytes.

Abbreviations: TCA, trichloroacetic acid; PCMBS, *p*-chloromercuribenzenesulfonate; $[Na^+]_i$, $[Na^+]_o$, intracellular, extracellular Na^+ concentration; $[Mg^{2+}]_i$, $[Mg^{2+}]_o$, intracellular, extracellular Mg^{2+} concentration; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

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Materials and Methods

Cell-loading

Blood was taken from anesthetized rats (50 mg/kg Nembutal sc.) by heart puncture with 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.

The cells were loaded with Mg^{2+} by incubating a 10% cell suspension for 30 min at 37 °C in KCl medium (140 mM KCl, 50 mM sucrose, 5 mM glucose, 30 mM Hepes/Tris, pH 7.4) with the addition of 12 mM $MgCl_2$ and 6 μM A23187 dissolved in dimethyl sulfoxide.

For Na^+-loading of the cells, the cells were incubated with 30 μg/ml nystatin in the presence of 10 mM (Na^+-unloaded) or 150 mM Na^+ (Na^+-loaded) in the loading medium (substitution of KCl in the medium by NaCl, as indicated) for 20 min at 37 °C, followed by incubation at 0 °C for 20 min. For removal of A23187 or nystatin, the cells were incubated four times in loading media plus 1% bovine serum albumin for 10 min at 37 °C. The loading media were removed by washing the cells twice with NaCl medium (substitution of KCl in KCl medium by 140 mM NaCl).

For measuring $^{22}Na^+$ efflux, after removal of A23187 or nystatin, the cells were loaded with $^{22}Na^+$ by incubation with 1 μCi $^{22}NaCl$/ml loading medium (see Table I) at 37 °C for 30 min ($^{22}NaCl$, specific activity 100–

1000 mCi/mg Na^+, Amersham). Extracellular $^{22}Na^+$ was removed by washing the cells in NaCl medium.

Mg^{2+} efflux

Mg^{2+} efflux was measured by reincubating a 10% suspension of Mg^{2+}-loaded cells at 37 °C in Mg^{2+}-free NaCl medium (140 mM NaCl, 50 mM sucrose, 5 mM glucose, 30 mM Hepes/Tris, pH 7.4). At the beginning of reincubation and after different times, as indicated, 0.5-ml aliquots of the cell suspension were centrifuged for 1 min at $10000 \times g$. 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, SP9). In some experiments Mg^{2+} efflux was determined by the decrease of cellular Mg^{2+} content (see below).

Na^+ efflux

Na^+ efflux was measured by the increase of $^{22}Na^+$ activity in the medium and by the decrease of $^{22}Na^+$ in the cells. $^{22}Na^+$ radioactivity was measured in the cells and in 200-µl aliquots of the supernatants in a γ-spectrometer (Berthold BF 5300). $^{22}Na^+$ efflux was calculated from the increase of the extracellular $^{22}Na^+$ activity by means of the specific activity of intracellular Na^+. An aliquot of the supernatant was taken for determination of hemoglobin by means of the cyanmethemoglobin method [2].

Cellular Mg^{2+} and Na^+ content

For measuring cellular Mg^{2+} or Na^+ contents, the cells were washed twice with 150 mM KCl and hemolysed 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 was measured by atomic absorption spectrophotometry after dilution with 10% TCA/0.175% $LaCl_3$. Na^+ content was measured by flame photometry (KLiNa-Flame, Beckman). Cellular Mg^{2+} content was also taken to correct Mg^{2+} efflux for hemolysis.

ATP content

ATP content of Mg^{2+}-loaded erythrocytes was determined enzymatically in an optical test by means of phosphoglycerate phosphokinase and glyceraldehydephosphate dehydrogenase according to the instructions of the manufacturer (Sigma, procedure No. 336-UV).

Preparation of membranes

Preparation of erythrocyte membranes was performed according to Hanahan and Ekholm [3]. Briefly, 0.4 mg/ml saponin was added to a 10% erythrocyte suspension in 150 mM NaCl, 3 mM L-histidine (pH 7.5) at 4 °C, then placed at room temperature for 15 min. Thereafter the sample was cooled and 1 vol. of the hemolysate was washed four times at 4 °C with 6 vol. of 150 mM NaCl, 3 mM L-histidine (pH 7.5).

ATPase assay

ATPase activity was measured at 37 °C according to Foder and Scharff [4]. The assay medium contained 80 mM NaCl, 80 mM L-histidine (pH 7.2), 1 mM ATP, 1 or 11 mM $MgCl_2$, 60 mM KCl, 1 mM EGTA, 1 mM G-strophanthin, 0.5 mM quinidine or 0.5 mM imipramine, as indicated in Table II, 2.3 mM phospho*enol*-pyruvate, 0.45 mM NADH, 90 µg/ml pyruvate kinase/lactate dehydrogenase (3 : 1, Boehringer), and approximately 0.5 mg membrane protein/ml. Protein was determined according to Lowry et al. [5].

Results and Discussion

Role of Na^+ in Mg^{2+} efflux

The high rate of Na^+-dependent Mg^{2+} efflux from rat erythrocytes offers the opportunity to measure the stoichiometric relationship between Mg^{2+} efflux and Na^+ uptake. Since quinidine is a strong inhibitor of Na^+-dependent Mg^{2+} efflux [6,7], the reduction of intracellular Mg^{2+} and the increase of intracellular Na^+ were measured in the absence and presence of 0.5 mM quinidine.

Additional tests were run with 1 mM strophanthin. From Fig. 1 it can be seen that the molar ratio of strophanthin-insensitive, quinidine-sensitive Na^+ uptake/Mg^{2+} efflux amounted to 2, indicating electroneutral Na^+/Mg^{2+} antiport as was found for Na^+/Mg^{2+} antiport in chicken erythrocytes [2]. This conclusion relies on there being no significant other Na^+ influx than the Mg^{2+}-dependent one under these experimental conditions. A ratio of 3 Na^+ : 1 Mg^{2+} was reported for Na^+/Mg^{2+} antiport of human erythrocytes [8].

The effect of $[Na^+]_0$ in the activation of Na^+/Mg^{2+} antiport was specific. LiCl (Fig. 2), choline Cl [7] and KCl (not shown) could not substitute for NaCl. The same Na^+ specificity was already found for the Na^+/Mg^{2+} antiport from chicken erythrocytes [2]. From the values of Fig. 2 the kinetic properties of $[Na^+]_0$ in net Mg^{2+} efflux can be derived. Fig. 3 shows that $[Na^+]_0$ operates in net Mg^{2+} efflux by Michaelis-Menten kinetics, K_m amounting to 62 mM. For comparison, in chicken erythrocytes, the corresponding K_m amounted to 25 mM [9]. Thus, both Na^+/Mg^{2+} exchangers did not express cooperativity with respect to $[Na^+]_0$, as may be expected for a coupled $2Na^+/1Mg^{2+}$ antiport. Further experiments are needed for clarification.

To characterize the driving force of Na^+/Mg^{2+} antiport, we measured Mg^{2+} efflux in the presence of 0.5 mM PCMBS, which rapidly increased the intracellular

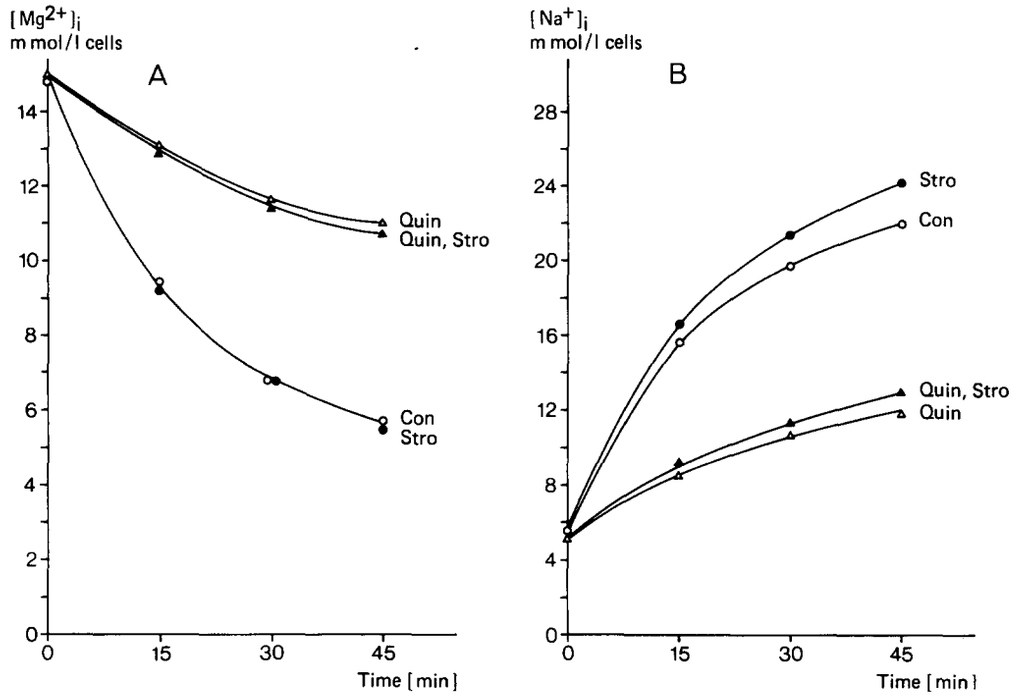

Fig. 1. Mg^{2+} efflux (A) and simultaneous Na^+ uptake (B) from Mg^{2+}-loaded rat erythrocytes. Con, control; Stro, 1 mM strophanthin; Quin, 0.5 mM quinidine were added during measurement of Mg^{2+} efflux and Na^+ uptake. Mean of three experiments.

Na^+ concentration and thus reduced the extracellular/intracellular Na^+ gradient. Under these conditions, Mg^{2+} efflux was inhibited (Fig. 4). From the measured $[Na^+]_i$, the $[Na^+]_0/[Na^+]_i$ gradients were calculated ($[Na^+]_0 = 140$ mM) and from the time course of intracellular Mg^{2+} content, the rate of Mg^{2+} efflux was calculated. Both parameters were plotted in Fig. 5. From Fig. 5 it may be concluded that the Na^+ gradient may be a driving force for Mg^{2+} efflux.

To investigate the role of $[Na^+]_i$ in Na^+/Mg^{2+} antiport, we measured $^{22}Na^+$ efflux from rat erythrocytes either unloaded or loaded with Na^+, or with Mg^{2+} or with Na^+ plus Mg^{2+}. As shown in Table I, in unloaded cells, there was a strophanthin-insensitive $^{22}Na^+$ efflux at constant $[Na^+]_i$ of 5 mmol/l cells, indicating $^{22}Na^+/Na^+$ exchange, which was inhibited by amiloride and quinidine. The rate of strophantin-insensitive $^{22}Na^+/Na^+$ exchange was increased in Na^+-loaded cells ($[Na^+]_i = 60$ mmol/l cells) in the absence of net Mg^{2+} efflux. In Mg^{2+}-loaded (Na^+-unloaded) cells, which quickly exchanged intracellular Mg^{2+} for extracellular Na^+, $[Na^+]_i$ increased rapidly (see also Fig. 1B). Probably by the increased $[Na^+]_i$, $^{22}Na^+/Na^+$ exchange was also increased. In ($Na^+ + Mg^{2+}$)-loaded cells, the rate of net Mg^{2+} efflux (Na^+/Mg^{2+} antiport) was reduced. Simultaneously, the efflux of $^{22}Na^+$ was increased. The increase in $^{22}Na^+$ efflux by additional Mg^{2+}-loading amounted to 7.2 mmol/l cells per 15 min

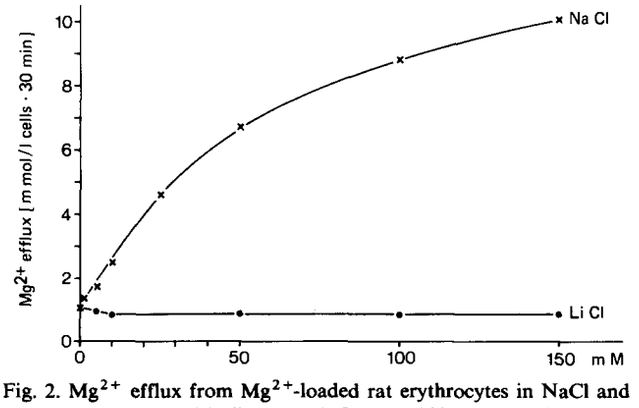

Fig. 2. Mg^{2+} efflux from Mg^{2+}-loaded rat erythrocytes in NaCl and LiCl media. Media with different NaCl and LiCl concentrations were used. NaCl and LiCl were isoosmotically substituted by sucrose.

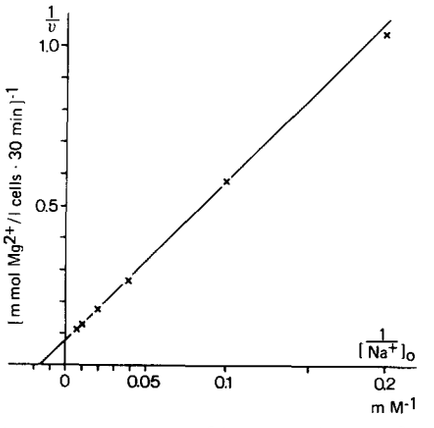

Fig. 3. Na^+ dependency of Mg^{2+} efflux from Mg^{2+}-loaded rat erythrocytes. Lineweaver-Burk plot of the values from Fig. 2.

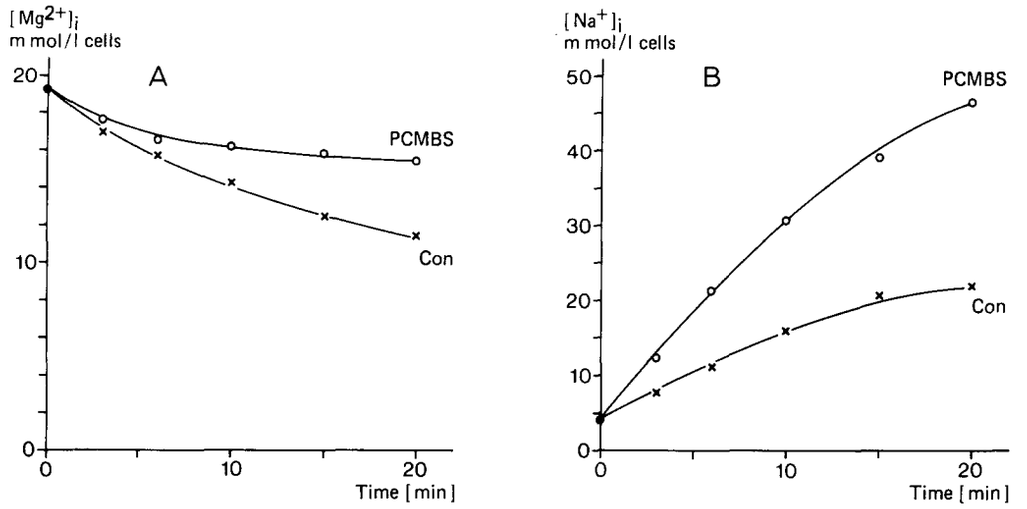

Fig. 4. Mg^{2+} efflux (A) and Na^+ uptake (B) of Mg^{2+}-loaded rat erythrocytes in the presence of 0.5 mM PCMBS.

(calculated from Table I), whereas the reduction in net Mg^{2+} efflux by additional Na^+-loading amounted only to 2.5 mmol/l cells per 15 min (calculated from Table I). This result might indicate that intracellular Na^+ can be exchanged for extracellular Na^+ via the Na^+/Mg^{2+} antiport system. Such a mechanism can explain the reduction of net Mg^{2+} efflux by increased $[Na^+]_i$. However, part of ^{22}Na efflux in $(Na^+ + Mg^{2+})$-loaded cells must be produced by a separate strophanthin-insensitive $^{22}Na^+/Na^+$ exchange system which is also operating in Mg^{2+}-unloaded cells.

In experiments with Na^+-loaded human erythrocytes, Lüdi and Schatzmann [10] also found inhibition of net Mg^{2+} efflux by increased $[Na^+]_i$. In their experiments, the inhibition was caused by reduction of V_{max} at unchanged K_m for $[Na^+]_i$ and $[Mg^{2+}]_i$, indicating no competition of intracellular Na^+ and Mg^{2+}. To clarify the interaction of Na^+ efflux and net Mg^{2+} efflux, we measured net Mg^{2+} efflux from rat erythrocytes loaded with different Na^+ and Mg^{2+} concentrations. As shown by Dixon plot in Fig. 6, intracellular Na^+ inhibited net Mg^{2+} efflux competitively, K_i amounted to 15 mmol/l cells. This result completes the $^{22}Na^+$ efflux experiment indicating that $^{22}Na^+$ efflux is partly performed by the Na^+/Mg^{2+} antiport. Thus, at increased $[Na^+]_i$ net Mg^{2+} efflux was reduced.

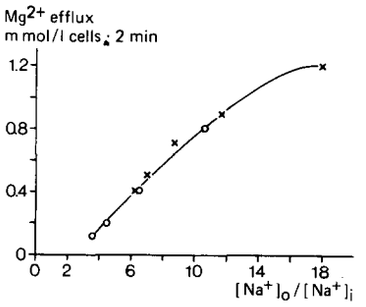

Fig. 5. Mg^{2+} efflux as a function of $[Na^+]_o/[Na^+]_i$ gradient. Values were taken from Fig. 4. $[Na^+]_o = 140$ mM. ×, values from control; ○, values from the experiment with PCMBS at various times.

Role of Mg^{2+} in Mg^{2+} efflux

To test whether the Mg^{2+} gradient is an additional driving force, Mg^{2+} efflux was measured at increasing $[Mg^{2+}]_o$ and thus at changed $[Mg^{2+}]_i/[Mg^{2+}]_o$ gradi-

TABLE I

$^{22}Na^+$ efflux and net Mg^{2+} efflux from rat erythrocytes

Loading [a]	Inhibitor [b]	Efflux (mmol/l cells per 15 min)	
		$^{22}Na^+$ efflux	Net Mg^{2+} efflux
Unloaded	–	1.54	0
	amiloride	0.56	0
	quinidine	0.77	0
Na^+-loaded	–	14.7	0
	amiloride	1.95	0
	quinidine	1.24	0
Mg^{2+}-loaded	–	7.0	4.0
	amiloride	1.66	1.6
	quinidine	1.03	1.0
$(Na^+ + Mg^{2+})$-loaded	–	21.9	1.5
	amiloride	6.79	0.7
	quinidine	5.96	0.6

[a] Unloaded cells, loading medium with 0.5 mM Mg^{2+} and 10 mM Na^+, Na^+-loaded cells, loading medium with 0.5 mM Mg^{2+} and 150 mM Na^+, Mg^{2+}-loaded cells, loading medium with 12 mM Mg^{2+} and 10 mM Na^+, $(Na^+ + Mg^{2+})$-loaded cells, loading medium with 12 mM Mg^{2+} and 150 mM Na^+. Mg^{2+}-loading was performed by means of A23187, Na^+-loading by means of nystatin, and $^{22}Na^+$-loading by incubation with $^{22}NaCl$. Mean of two experiments.

[b] Amiloride concentration, 1 mM; quinidine concentration, 0.5 mM. $^{22}Na^+$ efflux and net Mg^{2+} efflux were measured in the presence of 2 mM strophanthin.

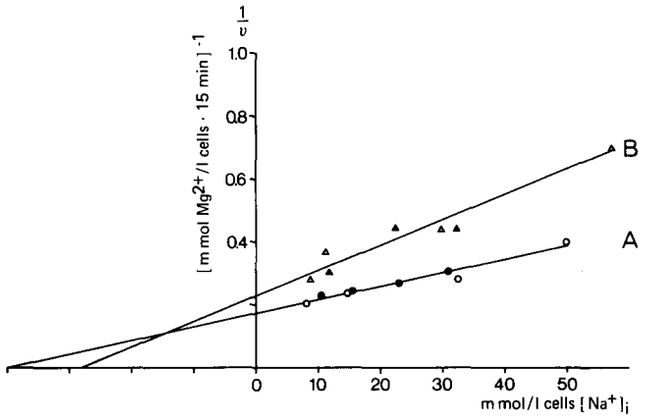

Fig. 6. Dixon plot of net Mg^{2+} efflux. Rat erythrocytes were Mg^{2+}-loaded with A23187 at $[Mg^{2+}]_0 = 12$ mM (A) and $[Mg^{2+}]_0 = 6$ mM (B) and additionally Na^+-loaded by means of nystatin at $[Na^+]_0$ of 10, 50, 100 and 150 mM. Two experiments were plotted with different symbols.

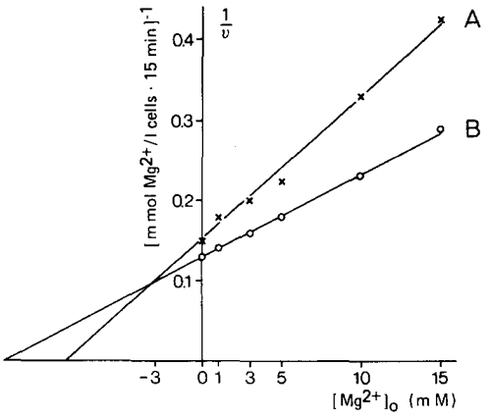

Fig. 8. Dixon plot of net Mg^{2+} efflux from Mg^{2+}-loaded rat erythrocytes. Values were taken from Fig. 7.

ent. Figs. 7 and 8 show that net Mg^{2+} efflux was increased at higher $[Mg^{2+}]_i$. This result may indicate that net Mg^{2+} efflux is also driven by the Mg^{2+} gradient. From Fig. 8, which was derived from Fig. 7, it can be seen that extracellular Mg^{2+} inhibited net Mg^{2+} efflux competitively, the K_i value amounted to 3 mM. Competitive inhibition of net Mg^{2+} efflux by $[Mg^{2+}]_0$ can be explained by simultaneous uptake of extracellular Mg^{2+}, its rate being increased with increasing $[Mg^{2+}]_0$. Thus, a smaller rate of net Mg^{2+} efflux is obtained when extracellular Mg^{2+} is simultaneously taken up. Uptake of extracellular Mg^{2+} (in competition with extracellular Na^+) during Na^+/Mg^{2+} antiport was demonstrated with $^{28}Mg^{2+}$ [11].

In an analogous experiment, the Na^+/Mg^{2+} antiport system of rat erythrocytes took up extracellular Mn^{2+}, which may function as an analogue for Mg^{2+} [12].

Role of ATP in Mg^{2+} efflux

As a next question, we investigated whether net Mg^{2+} efflux is driven by ATP. To test ATP dependence, rat erythrocytes were ATP-depleted by preincubation with 2-deoxyglucose instead of glucose at 37 °C for various times up to 5 h and thereafter loaded with Mg^{2+}. Table II shows that Na^+-dependent net Mg^{2+} efflux was dependent on intracellular ATP. ATP dependence of Na^+-dependent Mg^{2+} efflux obeyed Michaelis-Menten kinetics, the K_m value for ATP amounted to 0.1 mmol/l cells (not shown). A similar K_m value was determined for Na^+/Mg^{2+} antiport of human erythrocytes [13].

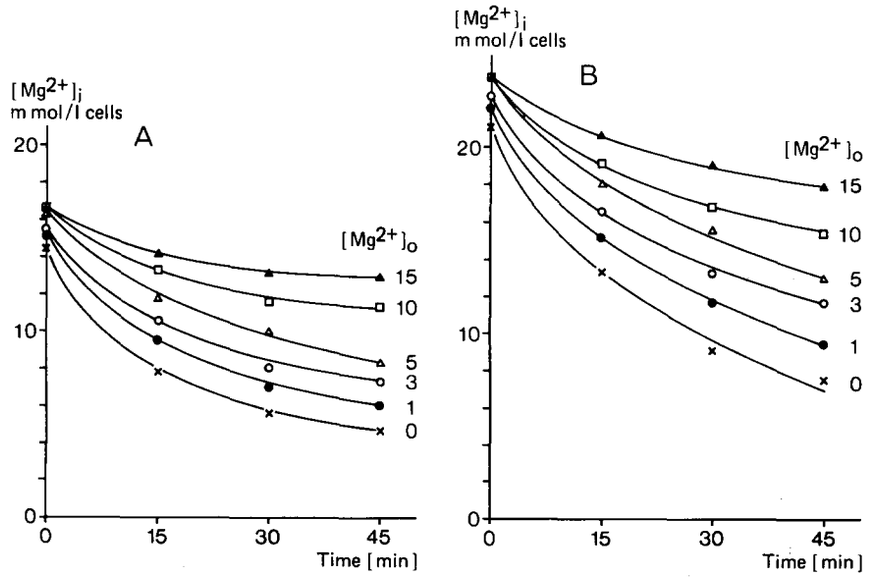

Fig. 7. Inhibition of net Mg^{2+} efflux from Mg^{2+}-loaded rat erythrocytes by $[Mg^{2+}]_0$. $[Mg^{2+}]_0$ in the reincubation medium amounted to 0 up to 15 mM as indicated. Rat erythrocytes were loaded with Mg^{2+} in the presence of 6 mM Mg^{2+} (A) and 12 mM Mg^{2+} (B).

TABLE II

Mg^{2+} efflux and ATP contents of Mg^{2+}-loaded erythrocytes

The erythrocytes were ATP-depleted by incubation with 5 mM 2-deoxyglucose (2-DOG) for various times as well as during Mg^{2+}-loading and during reincubation for measurement of Mg^{2+} efflux (in NaCl medium). Control was incubated throughout with glucose. Mean of two experiments.

Preincubation with 2-DOG (h)	Mg^{2+} efflux (mmol/l cells per 30 min)	ATP content (mmol/l cells)
Control	5.93	0.45
0	4.71	0.18
2	4.20	0.09
3	3.79	0.06
5	2.97	0.04

For further clarifying the role of ATP in Na^+-dependent net Mg^{2+} efflux, we measured ATPase activity of rat ghosts and the effect of inhibitors which inhibited Na^+-dependent Mg^{2+} efflux [1,6,8,9]. As shown in Table III, ATPase activity was not inhibited at 0.5 mM quinidine or imipramine which inhibited net Mg^{2+} efflux by 50%.

The lacking inhibition of ATPase by quinidine and imipramine can be explained by the suggestion that both substances bind to the Na^+/Mg^{2+} antiporter without affecting a phosphorylation-dephosphorylation process. However, amiloride and one of its analogs which inhibited Na^+/Mg^{2+} antiport with different sensitivity [14] also inhibited strophanthin-insensitive ATPase by the same difference in sensitivity (Table III). From this result it can be concluded that ATP is operating in Na^+/Mg^{2+} antiport via phosphorylation.

Amiloride and its analog inhibited ATPase activity also at 1 mM Mg^{2+} and ATP. Under this condition the concentration of free Mg^{2+} is low by binding of Mg^{2+}

TABLE III

ATPase activity of rat erythrocyte membranes

Stroph, G-strophanthin; Quin, quinidine; Imi, imipramine; Ami, amiloride; E.C.Ami, 5-(*N*-ethyl-*N*-4-chlorobenzyl)amiloride.

Additions (mM)	ATPase activity [a]	
	1 mM Mg+ 1 mM ATP	11 mM Mg+ 1 mM ATP
–	3.02	2.95
Stroph. 1	1.49	1.47
Stroph. 1 + Quin 0.5	1.47	1.42
Stroph. 1 + Imi 0.5	1.48	1.40
Stroph. 1 + Ami 0.03	1.41	1.44
Stroph. 1 + Ami 0.10	1.20	1.23
Stroph. 1 + Ami 0.30	0.39	0.42
Stroph. 1 + E.C.Ami 0.01	1.45	1.40
Stroph. 1 + E.C.Ami 0.03	0.92	1.15
Stroph. 1 + E.C.Ami 0.10	0.30	0.36

[a] Values in μmol ADP/mg protein per h are the means of two experiments.

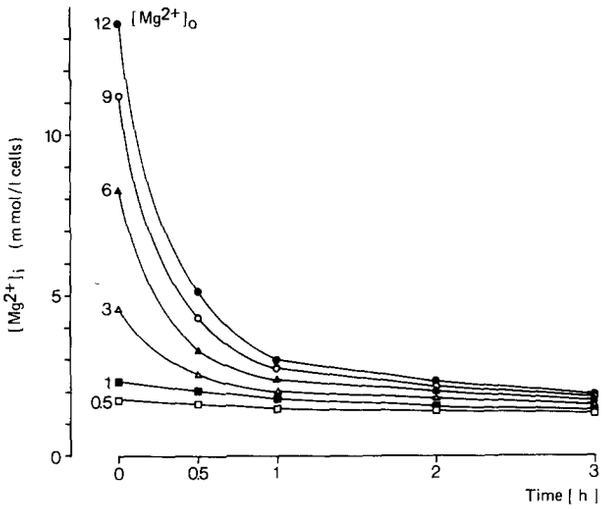

Fig. 9. Mg^{2+} efflux from rat erythrocytes loaded at different $[Mg^{2+}]_0$. The various $[Mg^{2+}]_0$ are indicated at the curves.

to ATP and net Mg^{2+} efflux is not operating at low $[Mg^{2+}]_i$ (Ref. 2 and Fig. 9). Therefore, amiloride can inhibit Na^+/Mg^{2+} antiport via inhibition of ATPase only at high $[Mg^{2+}]_i$, when Na^+/Mg^{2+} antiport is gated by increased $[Mg^{2+}]_i$ (see below).

Asymmetry of net Mg^{2+} efflux

As shown in Fig. 7, net Mg^{2+} efflux can take place when $[Mg^{2+}]_0$ is higher during efflux than the Mg^{2+} concentration during the loading procedure. Probably, net Mg^{2+} efflux can operate uphill, as was also shown for Mg^{2+}-loaded human erythrocytes [10]. An uphill Mg^{2+} efflux may be driven by the Na^+ gradient and possibly by an ATP-dependent process.

Asymmetry of the Mg^{2+} transport system was tested by changing the concentration gradients. $[Na^+]_i$ was increased by means of PCMBS according to De Mendonca et al. [15], and it was tested wether Mg^{2+} was taken up during incubation of the Na^+-loaded erythrocytes in KCl medium with 12 mM $MgCl_2$. Under these experimental conditions, no significant uptake of Mg^{2+} could be measured (data not shown), indicating asymmetry of the Mg^{2+} efflux system as was already found for net Mg^{2+} efflux from human erythrocytes [10,13]. Thus, Na^+/Mg^{2+} antiport is not a simple exchange system like Na^+/H^+ or Na^+/Ca^{2+} exchange which can operate in both directions.

Gating of net Mg^{2+} efflux by $[Mg^{2+}]_i$

When Mg^{2+}-unloaded chicken erythrocytes were incubated, there was no significant Mg^{2+} efflux [2]. To investigate this property, rat erythrocytes were loaded with Mg^{2+} at various extracellular Mg^{2+} concentrations. Thus, different degrees of Mg^{2+}-loading were obtained. Fig. 9 shows Na^+-dependent net Mg^{2+} efflux from differently Mg^{2+}-loaded cells after reincubation in

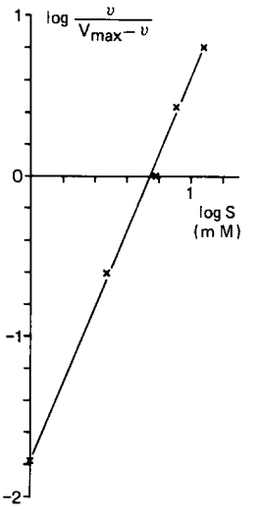

Fig. 10. Hill plot (log $v/(V_{max} - v) = n_H \cdot \log S$) of net Mg^{2+} efflux. For S, the concentrations $[Mg^{2+}]_0 = [Mg^{2+}]_i$ at equilibrium of Mg^{2+} distribution in the presence of A23187 were taken. v is the rate of Mg^{2+} efflux during the first 6 minutes of reincubation in NaCl medium. V_{max} was obtained by extrapolation.

NaCl medium. There was almost no net Mg^{2+} efflux after Mg^{2+}-loading at the low $[Mg^{2+}]_o$ (unloaded cells). Mg^{2+} efflux from Mg^{2+}-loaded cells stopped when the Mg^{2+} content of unloaded cells was reached. The same result was obtained with chicken erythrocytes, loaded with Mg^{2+} under similar conditions [2]. These results indicate that net Mg^{2+} efflux takes place only when $[Mg^{2+}]_i$ is increased.

When the rates of net Mg^{2+} efflux from this experiment were plotted according to Hill as the function log $v/(V_{max} - v) = n_H \cdot \log [Mg^{2+}]_i$, a Hill coefficient ($n_H$) of 2.4 was obtained (Fig. 10). The same cooperative effect of $[Mg^{2+}]_i$ on net Mg^{2+} efflux and the same value of $n_H = 2.4$ was obtained with human erythrocytes [10].

From this result it can be suggested that at least 3 Mg^{2+} are simultaneously needed for Na^+/Mg^{2+} antiport from rat erythrocytes. This might indicate that 1 Mg^{2+} is exchanged for 2 Na^+, the other 2 Mg^{2+} are simultaneously needed to activate the Na^+/Mg^{2+} antiport, e.g., by gating. This process (eventually in cooperation with ATP) may be responsible for the asymmetry of Na^+/Mg^{2+} antiport.

When $[Mg^{2+}]_i$ is reduced to the physiological value, Na^+/Mg^{2+} antiport is no longer gated, and thus net Mg^{2+} efflux stops when the physiological $[Mg^{2+}]_i$ is reached (Fig. 9). Thus, the increased $[Mg^{2+}]_i$ may have two functions: It may gate net Mg^{2+} efflux and it may work as an additional driving force.

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