ELSEVIER

Biochimica et Biophysica Acta 1234 (1995) 105–110

BBA Biochimica et Biophysica Acta

Reversibility of Na^+/Mg^{2+} antiport in rat erythrocytes

Theodor Günther *, Jürgen Vormann

Institute of Molecular Biology and Biochemistry, Free University of Berlin, Arnimallee 22, D-14195 Berlin, Germany

Received 22 June 1994; accepted 11 November 1994

Abstract

Rat erythrocytes loaded with Mg^{2+} plus Na^+ performed Mg^{2+} uptake under an intracellular/extracellular Na^+ gradient. Mg^{2+} uptake was coupled to Na^+ release at a stoichiometric ratio of 1 Mg^{2+}/2 Na^+. Mg^{2+} uptake was inhibited by amiloride, imipramine and quinidine. Mn^{2+} was taken up by the same transporter as Mg^{2+}. Similar results had been found for net Mg^{2+} efflux via Na^+/Mg^{2+} antiport in such rat erythrocytes. Hence, it can be concluded that Na^+/Mg^{2+} antiport in Mg^{2+}-loaded rat erythrocytes operates reversibly according to the direction of the Na^+ gradient which is a contributing driving force. Net Mg^{2+} influx was dependent on ATP which increased the affinity of intracellular Mg^{2+} by activating Na^+/Mg^{2+} antiport. Mg^{2+} uptake was increased by phorbol ester and inhibited by staurosporine, indicating that ATP may function via protein phosphorylation by protein kinase C.

Keywords: Sodium ion; Magnesium ion; Antiport; Amiloride; Protein kinase C; (Rat erythrocyte)

1. Introduction

Erythrocytes perform Na^+/Mg^{2+} antiport, which is active in Mg^{2+}-loaded cells, and results in net Mg^{2+} efflux [1,2]. Na^+/Mg^{2+} antiport in rat erythrocytes was previously thought to be irreversible, operating only as Mg^{2+} efflux coupled to Na^+ influx [1]. In those earlier experiments, rat erythrocytes were loaded with Na^+ and reincubated in Na^+-free, Mg^{2+}-containing medium. However, Na^+/Mg^{2+} antiport takes place only in Mg^{2+}-loaded cells [1–3], indicating that Na^+/Mg^{2+} antiport must be activated by increased intracellular Mg^{2+} concentration. Therefore, we investigated whether Na^+/Mg^{2+} antiport can be reversed in Na^+-loaded rat erythrocytes following additional Mg^{2+} loading.

2. Materials and methods

2.1. Cell loading

Blood was taken from anaesthetized rats (50 mg/kg Nembutal i.p.) 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 in 150 mM NaCl. Each experiment was done with erythrocytes from a single rat.

The cells were loaded with Na^+ and Mg^{2+} by incubating a 10% cell suspension for 20 min at 37°C and for additional 20 min at 0°C in a high [NaCl] medium (150 mM NaCl, 5 mM glucose, 30 mM Hepes/Tris, pH 7.4) with the addition of 0.5–3.0 mM $MgCl_2$, 6 μM A23187 (Boehringer-Mannheim) and 30 μg/ml nystatin (Sigma), both dissolved in dimethyl sulfoxide. For removal of A23187 and nystatin, the cells were incubated four times in NaCl medium with the same Mg^{2+} concentration as in the loading medium plus 1% bovine serum albumin (Serva) for 10 min at 37°C. The NaCl medium was removed by washing the cells twice with cold choline Cl medium (150 mM choline Cl, 5 mM glucose, 30 mM Hepes/Tris, pH 7.4))

2.2. Mg^{2+} transport

Mg^{2+} transport was measured by reincubating a 10% suspension of $Na^+ + Mg^{2+}$-loaded cells at 37°C in media containing 5 mM glucose, 30 mM Hepes/Tris, pH 7.4, with various Na^+ and Mg^{2+} content, as indicated. Na^+ was isoosmotically substituted with choline Cl. At the beginning of reincubation and after different time points, as indicated, 0.5 ml aliquots of the cell suspensions were

Abbreviations: $[Mg^{2+}]_o$, $[Mg^{2+}]_i$, extracellular, intracellular Mg^{2+} concentration; $[Na^+]_o$, $[Na^+]_i$, extracellular, intracellular Na^+ concentration; pH_i, intracellular pH; [ATP], cellular ATP concentration; PMA, phorbol myristate acetate; db-cAMP, dibutyryl cAMP; TCA, trichloroacetic acid.

* Corresponding author. Fax: +49 30 8384802.

SSDI 0005-2736(94)00267-3

centrifuged for 0.5 min at 10000 × *g*. For Mg^{2+} determination, 100 μl supernatant was diluted with 1 ml 5% TCA/0.175% $LaCl_3$ and Mg^{2+} was measured by atomic absorption spectrophotometry (Philips, SP9). Mg^{2+} influx or Mg^{2+} efflux were calculated from the alterations in extracellular Mg^{2+} concentration and were related to the cell volume measured by haematocrit.

2.3. Na^+ efflux

Na^+ efflux was determined from the same supernatants by flame photometry (KLiNa, Beckman). For correction of Mg^{2+}-independent Na^+ efflux, controls were run with Mg^{2+}-free incubation medium.

2.4. Cellular Mg^{2+}, Ca^{2+}, Mn^{2+} and Na^+ content

For measuring cellular Mg^{2+}, Ca^{2+}, Mn^{2+} and Na^+ contents, the cells were washed twice with 150 mM choline Cl and deproteinized with 5% TCA/0.175% $LaCl_3$. Mg^{2+}, Ca^{2+} and Mn^{2+} contents of the supernatants were measured by atomic absorption spectrophotometry, Na^+ content by flame photometry.

2.5. Cellular ATP content

ATP content of normal and ATP-depleted erythrocytes was determined enzymatically in TCA extracts by the luciferase method, according to the instructions of the manufacturer (Boehringer-Mannheim, No. 567 736).

Cells were ATP-depleted by addition of 5 mM 2-deoxyglucose instead of glucose and 1 mM iodoacetate during washing and incubation of the cells. Control experiments without iodoacetate revealed a smaller decrease of ATP but no effect of iodoacetate on Mg^{2+} transport.

3. Results

3.1. Induction of Mg^{2+} uptake

When $Na^+ + Mg^{2+}$-loaded rat erythrocytes were incubated in a high [NaCl] medium, net Mg^{2+} efflux occurred, which decreased with increasing $[Mg^{2+}]_o$ (Fig. 1), as found in previous experiments [1]. However, when the $Na^+ + Mg^{2+}$-loaded cells were incubated in choline Cl medium, containing 0. 5 mM Na^+, the cells took up Mg^{2+} (Fig. 1). In cells, that were not loaded with Mg^{2+}, there was no Mg^{2+} uptake (data not shown). These results show that Mg^{2+} uptake can be induced in Mg^{2+}-loaded rat erythrocytes when the Na^+ gradient was in the direction $high_i/low_o$.

3.2. Effect of $[Mg^{2+}]_o$ and $[Mg^{2+}]_i$ on Mg^{2+} uptake

The rate of Mg^{2+} uptake was dependent on $[Mg^{2+}]_o$, obeying Michaelis-Menten kinetics with a K_m of 0.3 mM

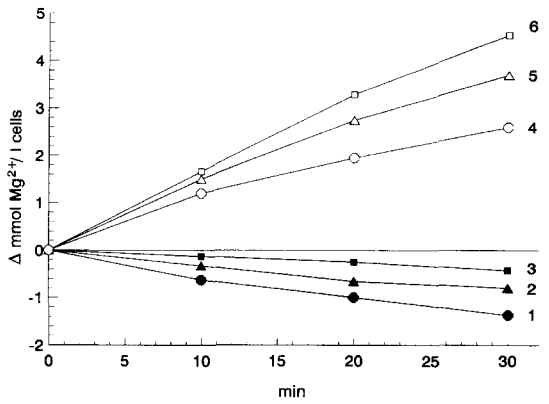

Fig. 1. Net Mg^{2+} efflux (below zero line) and net Mg^{2+} influx (above zero line) in $Na^+ + Mg^{2+}$-loaded rat erythrocytes. The cells were loaded with 6 μM A23187 and 30 μg/ml nystatin in the presence of 3 mM $MgCl_2$ and 150 mM NaCl ($[Na^+]_i = 73 \pm 2$ mM). Mg^{2+} transport was measured in NaCl medium with 150 mM NaCl and 0.5 mM $MgCl_2$ (1), 1.5 mM $MgCl_2$ (2), and 3.0 mM $MgCl_2$ (3), resulting in Mg^{2+} efflux, or in choline Cl medium with 0.5 mM NaCl and 0.5 mM $MgCl_2$ (4), 1.5 mM $MgCl_2$ (5), and 3 mM $MgCl_2$ (6), resulting in Mg^{2+} influx. Mean of two experiments was plotted.

(not shown). Mg^{2+} uptake was also increased with Mg^{2+} loading (Fig. 2). When the values of Fig. 2, measured at $[Na^+]_o = 0.5$ mM, were analyzed according to Hill as a function of $[Mg^{2+}]_o$ during Mg^{2+} loading, a Hill coefficient of 2.2 ± 0.2 was obtained (Fig. 3, curve A).

3.3. Effect of the Na^+ gradient on Mg^{2+} transport

To analyze further the kinetics of net Mg^{2+} influx, we investigated net Mg^{2+} transport of Na^+-loaded cells ($[Na^+]_i = 73 \pm 2$ mM, mean ± S.E., $n = 6$) which were simultaneously Mg^{2+}-loaded at $[Mg^{2+}]_o = 0.5$–3.0 mM by replotting the values of Fig. 2 as a function of $[Na^+]_i/[Na^+]_o$ (Fig. 4). Cellular Mg^{2+} content amounted to 2.0 mmol/l cells at $[Mg^{2+}]_o = 0.5$ mM up to 4.9

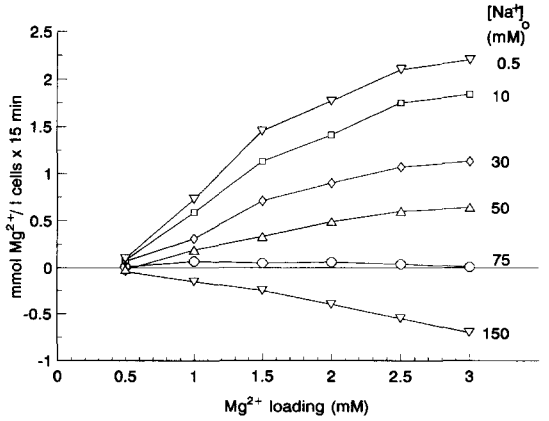

Fig. 2. Net Mg^{2+} efflux (below zero line) and net Mg^{2+} influx (above zero line) in $Na^+ + Mg^{2+}$-loaded rat erythrocytes as a function of Mg^{2+} loading. The cells were loaded in the presence of 0.5–3.0 mM $MgCl_2$ and 150 mM NaCl. Reincubation was performed in media with 1 mM $MgCl_2$ and 0.5, 10, 30, 50, 75, and 150 mM NaCl as indicated. NaCl was isoosmotically substituted by choline Cl. Mean of two experiments.

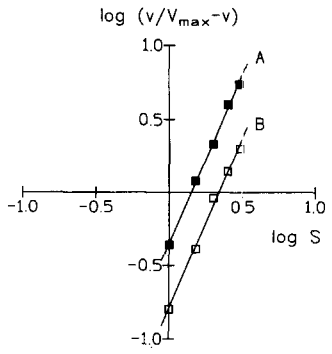

Fig. 3. Hill plot (log $(v/V_{max}-v)=n$ log $S-$ log K') of Mg^{2+} uptake by $Na^+ + Mg^{2+}$-loaded rat erythrocytes. log $(v/V_{max}-v)$ was plotted as a function of $[Mg^{2+}]_o$ in the loading medium. (A) Rat erythrocytes with normal ATP content. Values measured at 0.5 mM NaCl and 1 mM $MgCl_2$ were taken from Fig. 2. (B) ATP-depleted rat erythrocytes. Analogous values were taken from Fig. 6.

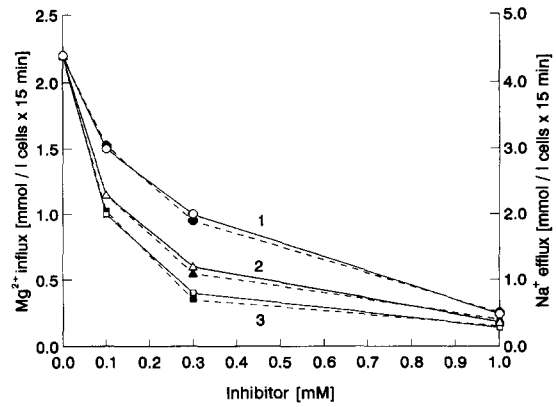

Fig. 5. Inhibition of net Mg^{2+} influx and net Na^+ efflux of $Na^+ + Mg^{2+}$-loaded rat erythrocytes by amiloride (1), imipramine (2) and quinidine (3). The cells were loaded in the presence of 3 mM $MgCl_2$ and 150 mM NaCl. Reincubation in choline Cl medium with 0.5 mM NaCl and 1 mM $MgCl_2$. Na^+ efflux was corrected for passive (Mg^{2+}-independent) Na^+ leak by subtraction of Na^+ leak in Mg^{2+}-free reincubation medium. Open symbols (solid line), net Mg^{2+} influx; filled symbols (dashed line), net Na^+ efflux. Mean of two experiments.

mmol/l cells at $[Mg^{2+}]_o = 3.0$ mM, as found in previous experiments [1]. After reincubation in medium with $[Na^+]_o = 150$ mM, giving $[Na^+]_i/[Na^+]_o = 0.49$, net Mg^{2+} efflux occurred. Its rate rose with increasing Mg^{2+}-loading as characterized in previous experiments [1]. In media with $[Na^+]_o < 75$ mM, giving $[Na^+]_i/[Na^+]_o > 1$, net Mg^{2+} efflux switched to net Mg^{2+} influx. Its rate increased with Mg^{2+} loading and with increasing $[Na^+]_i/[Na^+]_o$ (Fig. 4).

These results show: (1) The increased rate of Mg^{2+} uptake with Mg^{2+} loading (Figs. 2, 4), indicates that the increase in $[Mg^{2+}]_i$ activated the Mg^{2+} influx system. According to Fig. 3 this activation was brought about by a co-operative effect.

(2) The intracellular/extracellular Na^+ gradient contributes to the driving force of Mg^{2+} uptake. As shown in Fig. 4, at $[Na^+]_i/[Na^+]_o = 1$, there was no net Mg^{2+} transport. At $[Na^+]_i/[Na^+]_o > 1$, net Mg^{2+} influx occurred, whereas at $[Na^+]_i/[Na^+]_o < 1$ net Mg^{2+} efflux was observed. The curves of Mg^{2+} transport cross the zero line of net Mg^{2+} flux at $[Na^+]_i/[Na^+]_o = 1$. Within experimental error, the curves (Fig. 4) have the same slope below and above the crossing point. This result is evidence that the action of the Na^+ gradient is the same on each side of the membrane. Moreover, since the crossing point is at $[Na^+]_i/[Na^+]_o = 1$, net Mg^{2+} flux under the used conditions may only depend on the Na^+ gradient.

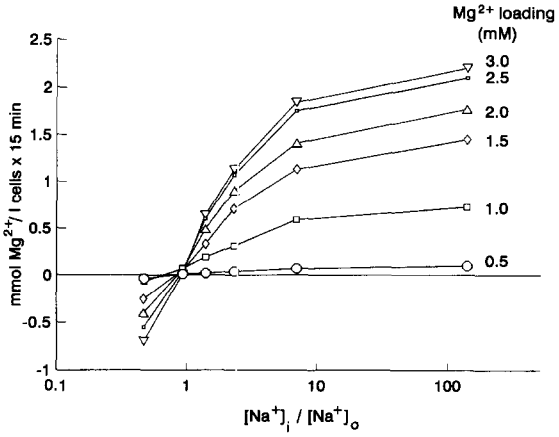

Fig. 4. Net Mg^{2+} efflux (below zero line) and net Mg^{2+} influx (above zero line) in $Na^+ + Mg^{2+}$-loaded rat erythrocytes as a function of the Na^+ gradient. Same experiments as in Fig. 2. The cells were differently loaded with Mg^{2+} as indicated. Mg^{2+} transport was plotted as a function of $[Na^+]_i/[Na^+]_o$ (log scale), $[Na^+]_i = 73 \pm 2$ mM.

When Mg^{2+}-loaded rat erythrocytes were loaded in the presence of 75 mM Na^+ instead of 150 mM resulting in $[Na^+]_i = 25$ mM and incubated at various $[Na^+]_o$ the rate of Mg^{2+} transport as a function of the Na^+ gradient was identical (data not shown). Again, this result shows that the Na^+ gradient contributes to the driving force for Mg^{2+} transport in Mg^{2+}-loaded rat erythrocytes.

3.4. Na^+ coupling of Mg^{2+} uptake

During Mg^{2+} uptake, Na^+ is released from $Na^+ + Mg^{2+}$-loaded rat erythrocytes. Mg^{2+} uptake and Na^+ release were inhibited by amiloride, imipramine and quinidine (Fig. 5). Each of these substances inhibited Mg^{2+} uptake and Na^+ release to the same extent, indicating that net Mg^{2+} influx is coupled to net Na^+ efflux. The stoichiometric ratio can be obtained by comparing the rate of Mg^{2+} uptake and Na^+ release. From the scales in Fig. 5 it can be seen that the stoichiometric ratio is 2 Na^+ for 1 Mg^{2+}. Hence, the stoichiometry is the same as was found for Na^+/Mg^{2+} antiport from Mg^{2+}-loaded rat erythrocytes [1].

From Fig. 5 the K_i values can be obtained. K_i for amiloride amounts to 0.3 mM, for imipramine to 0.15 mM and for quinidine to 0.1 mM. These values correspond to

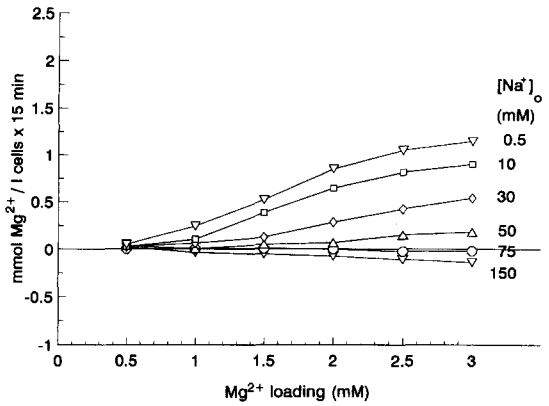

Fig. 6. Mg^{2+} transport in $Na^+ + Mg^{2+}$-loaded, ATP-depleted rat erythrocytes as a function of Mg^{2+} loading. The cells were loaded in the presence of 0.5–3.0 mM $MgCl_2$ and 150 mM NaCl, resulting in $[Na^+]_i = 72 \pm 2$ mM. Reincubation was performed in media with 0.5, 30, 50, 75 and 150 mM NaCl and 1 mM $MgCl_2$ with the addition of 5 mM 2-deoxyglucose instead of glucose and 1 mM iodoacetate for ATP depletion. Analogous experiments as in Fig. 4. Mean of two experiments.

the K_i values which were obtained for inhibition of net Mg^{2+} efflux via Na^+/Mg^{2+} antiport [4].

3.5. Role of ATP in Mg^{2+} uptake

Net Mg^{2+} efflux via Na^+/Mg^{2+} antiport in rat erythrocytes was dependent on ATP [1]. Its function has not been defined so far. The function of ATP in net Mg^{2+} influx was analyzed by repeating the experiment shown in Figs. 2 and 4 with ATP-depleted cells. Incubating $Na^+ + Mg^{2+}$-loaded cells with 5 mM 2-deoxyglucose plus 1 mM iodoacetate instead of glucose reduced ATP content by 85% (from 0.6 to 0.09 mmol/l cells). The results with ATP-depleted cells were similar to those obtained from control cells. Again, Mg^{2+} uptake was increased with Mg^{2+} loading of the cells and with the Na^+ gradient (Figs. 6 and 7). However, the rates of Mg^{2+} flux were

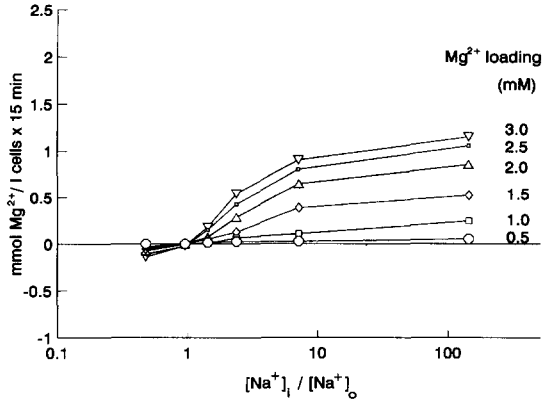

Fig. 7. Mg^{2+} transport in $Na^+ + Mg^{2+}$-loaded, ATP-depleted rat erythrocytes as a function of the Na^+ gradient (log scale). Same experiments as in Fig. 6. $[Na^+]_i = 72 \pm 2$ mM.

Table 1
Regulation of Mg^{2+} uptake by rat erythrocytes

Addition	M	Mg^{2+} uptake (mmol Mg^{2+} /l cells per 30 min)
Control	–	1.50 ± 0.09
PMA	10^{-6}	1.91 ± 0.11 [a]
Staurosporine	10^{-7}	0.94 ± 0.10 [b]
Okadaic acid	10^{-6}	1.72 ± 0.04 [a]
db-cAMP	10^{-4}	1.51 ± 0.08
PMA	10^{-6}	
+ staurosporine	10^{-7}	0.99 ± 0.04 [b]
PMA	10^{-6}	
+ okadaic acid	10^{-6}	1.99 ± 0.02 [b]

The cells were loaded in the presence of 150 mM NaCl and 1 mM $MgCl_2$. Mg^{2+} uptake was measured in choline Cl medium with 0.5 mM NaCl and 1 mM $MgCl_2$. Mean ± S.E. of four experiments. Significant differences to controls by unpaired Student's t-test. [a] $P < 0.05$, [b] $P < 0.01$.

lower than those in control cells (Figs. 2 and 4). In accordance with this result, in ATP-depleted cells, V_{max} of Mg^{2+} uptake was reduced by 33% (data not shown). When the rate of Mg^{2+} uptake in ATP-depleted cells, measured at $[Na^+]_o = 0.5$ mM (Fig. 6), was analyzed according to Hill as a function of $[Mg^{2+}]_o$ during Mg^{2+} loading, a Hill coefficient of 2 was obtained (Fig. 3, curve B). The same Hill coefficient was found in control cells (Fig. 3, curve A). However, log K' was increased in ATP-depleted cells by $\Delta \log K' = 0.48$ (factor of 3) (Fig. 3), indicating a lower affinity of $[Mg^{2+}]_i$. For further characterisation of the function of ATP, we tested the effect of protein phosphorylation by protein kinase A (activated by db-cAMP) and protein kinase C (activated by PMA) which are both inhibited by staurosporine [5]. Moreover, the involvement of phosphorylated protein was tested by addition of okadaic acid, which inhibits Ser/Thr-protein phosphatases 1 and 2A [6,7]. As shown in Table 1, Mg^{2+} uptake was stimulated by PMA and inhibited by staurosporine. Okadaic acid had a small stimulatory effect, and db-cAMP was ineffective, indicating stimulation by protein kinase C. Stimulation of Mg^{2+} uptake by PMA was less expressed in cells loaded in the presence of 3.0 mM $MgCl_2$ compared to 1.0 mM $MgCl_2$ (data not shown).

3.6. Cation specificity of Mg^{2+} uptake

In previous experiments with Mg^{2+}-loaded rat erythrocytes additional Ca^{2+} loading had no significant effect on Mg^{2+} efflux via Na^+/Mg^{2+} antiport [8]. However, in these experiments Ca^{2+} loading was limited due to an effective Ca^{2+} pump which prevented sufficient Ca^{2+} loading.

Reversibility of Na^+/Mg^{2+} antiport offers the possibility to test cation specificity by extracellular addition of divalent cations, which would be toxic when applied intracellularly. As shown in Fig. 8, Mg^{2+} uptake was inhibited by Ca^{2+}, Mn^{2+}, Ni^{2+}, and La^{3+}. At the used concentrations, Sr^{2+} did not affect Mg^{2+} uptake.

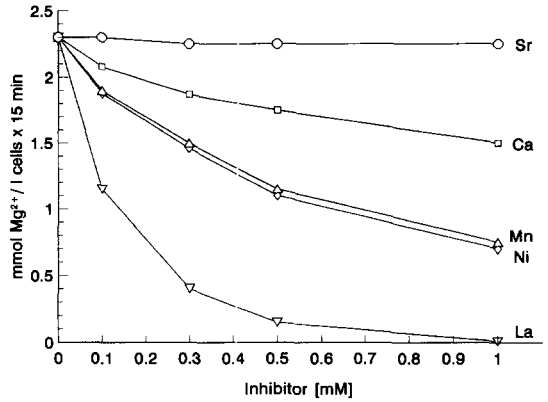

Fig. 8. Inhibition of Mg^{2+} uptake in $Na^+ + Mg^{2+}$-loaded rat erythrocytes by extracellular $SrCl_2$, $CaCl_2$, $MnCl_2$, $NiSO_4$ and $LaCl_3$. The cells were loaded in the presence of 3 mM $MgCl_2$ and 150 mM NaCl and reincubated in choline Cl medium with 0.5 mM NaCl and 1.3 mM $MgCl_2$. Mean of two experiments.

Using Dixon plots the inhibition was defined as being competitive (not shown). The K_i values amounted to 0.4 mM for Ca^{2+}, 0.02 mM for Mn^{2+} and Ni^{2+}, and to 0.005 mM for La^{3+}. Mn^{2+} was taken up by the cells (data not shown). Also, in preceding experiments Mn^{2+} was taken up by rat erythrocytes in exchange for Mg^{2+} via Na^+/Mg^{2+} antiport [9,10], indicating that Mn^{2+} can substitute for Mg^{2+} in Na^+/Mg^{2+} antiport. Ca^{2+} was not taken up by the cells (data not shown). Uptake of Sr^{2+}, Ni^{2+} and La^{3+} via Na^+/Mg^{2+} antiport was not investigated.

3.7. Cell type specificity of Mg^{2+} uptake

In analogous experiments human erythrocytes were loaded with Na^+ and Mg^{2+}. However, under identical conditions human erythrocytes did not perform Mg^{2+} uptake (data not shown). In similar experiments, also Schatzmann [11] could not obtain Mg^{2+} uptake in human erythrocytes, whereas in ferret erythrocytes Na^+/Mg^{2+} antiport could be reversed by reversing the Na^+ gradient [12,13]. These results show that in erythrocytes Na^+/Mg^{2+} antiporters are existing with different species-dependent properties.

4. Discussion

The results have shown that rat erythrocytes can be induced to take up extracellular Mg^{2+} in exchange for intracellular Na^+. Prerequisite is:

(1) The cells must be loaded with Mg^{2+},

(2) $[Na^+]_i/[Na^+]_o$ must be > 1.

In rat erythrocytes the Na^+ gradient contributes to the driving force for net Mg^{2+} transport. Additionally, 2 intracellular Na^+ are exchanged for 1 extracellular Mg^{2+} in Mg^{2+} uptake. The same stoichiometric ratio helds for net Mg^{2+} efflux via Na^+/Mg^{2+} antiport [1].

Net Mg^{2+} influx coupled to net Na^+ efflux was inhibited by about the same degree as net Mg^{2+} efflux via Na^+/Mg^{2+} antiport by amiloride, imipramine and quinidine. Moreover, Mn^{2+} inhibited Mg^{2+} uptake competitively and was taken up itself by Na^+/Mg^{2+} antiport [9,10]. These results suggest that Na^+/Mg^{2+} antiport in rat erythrocytes can operate reversibly.

A prerequisite for demonstrating reversible Na^+/Mg^{2+} antiport in rat erythrocytes was Mg^{2+} loading. Net Mg^{2+} efflux via Na^+/Mg^{2+} antiport was also only seen in Mg^{2+}-loaded cells [1]. In rat erythrocytes, net Mg^{2+} efflux as a function of $[Mg^{2+}]_i$ revealed a Hill coefficient of 2.44 [1]. This result was explained by the assumption that 1 intracellular Mg^{2+} is released in exchange for 2 extracellular Na^+ in Na^+/Mg^{2+} antiport and that 2 additional intracellular Mg^{2+} are operating co-operatively to activate the Na^+/Mg^{2+} antiporter. In the present experiments, the Hill coefficient for net Mg^{2+} influx amounted to 2.2, again indicating that 2 intracellular Mg^{2+} are needed in Mg^{2+} uptake via reversible Na^+/Mg^{2+} antiport. The increased Mg^{2+} uptake at increased Mg^{2+} loading (Fig. 2) fits into this scheme, indicating that more Na^+/Mg^{2+} antiporter molecules are activated by increasing $[Mg^{2+}]_i$. The Na^+/Mg^{2+} antiporter may have different Mg^{2+} sites: two intracellular Mg^{2+} binding sites for activating Na^+/Mg^{2+} antiport, an intracellular Mg^{2+} binding site for Mg^{2+} efflux and an extracellular Mg^{2+} binding site for Mg^{2+} influx. Whether the extracellular and intracellular Mg^{2+} transport site of the Na^+/Mg^{2+} antiporter in rat erythrocytes is operating with the same or with different affinity at both sides of the membrane cannot be decided because of the co-operative action of $[Mg^{2+}]_i$ in Mg^{2+} transport.

The role of ATP in net Mg^{2+} influx is to increase the affinity of the Na^+/Mg^{2+} antiporter for intracellular Mg^{2+}. Taking into account the co-operative function of $[Mg^{2+}]_i$ (Fig. 3) the possible action of ATP may be phosphorylation of the Na^+/Mg^{2+} antiporter by MgATP and increased binding of Mg^{2+} to a modifier site of the phosphorylated Na^+/Mg^{2+} antiporter. At lower cellular ATP, less Na^+/Mg^{2+} antiporters may be activated, resulting in lower V_{max} of Mg^{2+} uptake. This mechanism is in agreement with the stimulation of Mg^{2+} uptake by PMA and okadaic acid and its inhibition by staurosporine (Table 1), indicating that protein kinase C may phosphorylate the Na^+/Mg^{2+} antiporter or a protein, which is involved in Mg^{2+} uptake. This mechanism may represent regulation of Na^+/Mg^{2+} antiport. As shown by other experiments [7,14] PMA and okadaic acid enhance protein phosphorylation in erythrocytes. The results indicate that Na^+/Mg^{2+} antiport has very similar properties to Na^+/H^+ and Na^+/Ca^{2+} antiport. Na^+/H^+ is silent at physiological pH_i but is activated when the cytoplasm becomes acidic which is mediated by the allosteric effect of an intracellular modifier site. Besides acidification Na^+/H^+ antiport was activated by okadaic acid [6] and phorbol ester

[15] via phosphorylation which increases the affinity of the Na^+/H^+ antiporter for H^+ at the internal regulatory site [6,15,16]. Similarly, also Na^+/Ca^{2+} antiport is activated by increased $[Ca^{2+}]_i$ and by phosphorylation [17–19]. Thus, Na^+/Mg^{2+} antiport of rat erythrocytes may have a similar structure to Na^+/H^+ and Na^+/Ca^{2+} antiporters including their isoforms [16,17,20,21]. The physiological function of Na^+/Mg^{2+} antiport in vivo may be the performance of net Mg^{2+} efflux. $[Mg^{2+}]_i$ is buffered [4] by reversible reaction with Mg^{2+}-binding ligands, particularly ATP. When $[Mg^{2+}]_i$ is increased by release from Mg^{2+}-binding ligands which can occur by ATP depletion or acidification [22], Mg^{2+} can be transported out of the cell to support constancy of $[Mg^{2+}]_i$ [22]. Mg^{2+} uptake via Na^+/Mg^{2+} antiport may play no role because in vivo the Na^+ gradient (e.g., in rat erythrocytes) does not become > 1.

References

[1] Günther, T., Vormann, J. and Höllriegl, V. (1990) Biochim. Biophys. Acta 1023, 455–461.
[2] Günther, T., Vormann, J. and Förster, R. (1984) Biochem. Biophys. Res. Commun. 119, 124–131.
[3] Günther, T. and Vormann, J. (1990) Magnesium Trace Elem. 9, 279–282.
[4] Günther, T. (1993) Min. Electr. Metab. 19, 259–265.
[5] Tamaoki, T., Nomoto, H., Takahashi, I., Kato, Y., Morimoto, M. and Tomita, F. (1986) Biochem. Biophys. Res. Commun. 135, 397–402.
[6] Bianchini, L., Woodside, M., Sardet, C., Pouyssegur, J., Takai, A. and Grinstein, S. (1991) J. Biol. Chem. 266, 15406–15413.
[7] Bordin, L., Clari, G., Bellato, M., Tessarin, C. and Moret, V. (1993) Biochem. Biophys. Res. Commun. 195, 723–729.
[8] Günther, T. and Vormann, J. (1989) FEBS Lett. 250, 633–637.
[9] Féray, J.C. and Garay, R. (1987) J. Biol. Chem. 262, 5763–5768.
[10] Günther, T., Vormann, J. and Cragoe, Jr. E.J. (1990) FEBS Lett. 261, 47–51.
[11] Schatzmann, H.J. (1993) Biochim. Biophys. Acta 1148, 15–18.
[12] Flatman, P.W. and Smith, L.M. (1990) J. Physiol. 431, 11–25.
[13] Flatman, P.W. and Smith, L.M. (1991) J. Physiol. 443, 217–230.
[14] Cohen, C.M. and Foley, S.F. (1986) J. Biol. Chem. 261, 7701–7709.
[15] Sardet, C., Counillon, L., Franchi, A. and Pouyssegur, J. (1990) Science 247, 723–726.
[16] Levine, S.A., Montrose, M.H., Tse, M. and Donowitz, M. (1993) J. Biol. Chem. 268, 25527–25535.
[17] Reeves, J.P. (1992) Arch. Biochem. Biophys. 292, 329–334.
[18] Caroni, P. and Carafoli, E. (1985) Eur. J. Biochem. 132, 451–460.
[19] DiPolo, R. and Beaugé, L. (1987) Biochim. Biophys. Acta 897, 347–354.
[20] Tse, C.M., Brant, S.R., Walker, M.S., Pouyssegur, J. and Donowitz, M. (1992) J. Biol. Chem. 267, 9340–9346.
[21] Nakasaki, Y., Iwamoto, T., Hanada, H., Imagawa, T. and Shigekawa, M. (1993) J. Biochem. 114, 528–534.
[22] Günther, T. and Vormann, J. (1994) Renal Physiol. Biochem. 17, 279–286.