Concentration of intracellular free Mg^{2+} and Mg^{2+} efflux from magnesium-deficient erythrocytes

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Zusammenfassung

Die Konzentration des intrazellulären freien Mg^{2+} ($[Mg^{2+}]_i$) in Erythrozyten wurde mit Hilfe der Null-Punkt-Methode gemessen. $[Mg^{2+}]_i$ in den Erythrozyten war von 0.38 mmol/l bei Kontrollratten auf 0.22 mmol/l bei Mg-Mangelratten abgesunken.
Alle Fraktionen des Netto-Mg^{2+}-Effluxes aus Mg^{2+}-beladenen Erythrozyten waren bei den Mg-Mangelratten um ein Drittel angestiegen. Da der Mg^{2+} Efflnx und die intra-extrazelluläre Mg^{2+}-Verteilung bei den Erythrozyten im Blutkreislauf sich in einem steady-state befinden, könnte die verringerte $[Mg^{2+}]_i$ bei den Mg-Mangel-Erythrozyten durch eine erhöhte Affinität des Na^+/Mg^{2+} Antiporters für intrazelluläres Mg^{2+} und einen weitergehenden Mg^{2+} Efflux zustandegekommen sein.

Summary

The concentration of intracellular free magnesium ($[Mg^{2+}]_i$) in erythrocytes was measured by the null-point method. $[Mg^{2+}]_i$ was reduced from 0.38 mmol/l in controls to 0.22 mmol/l in the erythrocytes from Mg-deficient rats. All fractions of net Mg^{2+} efflux from Mg^{2+}-loaded erythrocytes were increased by one third in the cells from Mg-deficient rats. Since Mg^{2+} efflux and intra/extracellular Mg^{2+} distribution of erythrocytes in circulation must be in a steady-state, the reduced $[Mg^{2+}]_i$ in Mg-deficient erythrocytes may be caused by an increased affinity of the Na^+/Mg^{2+} antiporter for intracellular Mg^{2+}.

Résumé

La concentration intra-érythrocytaire de magnésium libre ($[Mg^{2+}]_i$) a été mesurée par la méthode du point-nul. La $[Mg^{2+}]_i$ qui était réduite de 0.38 mmol/l chez les rats témoins, n'était que de 0,22 mmol/l chez les rats carencés en magnésium.
Toutes les fractions de l'efflux net de Mg^{2+} à partir d'érythrocytes chargés en Mg^{2+} étaient augmentées de un tiers chez les rats présentant une carence en magnésium. Etant donné qu'il y a un équilibre entre l'efflux de Mg^{2+} et la distribution intra-/extra- érythrocytaire dans la circulation sanguine, la diminution de la $[Mg^{2+}]_i$ dans les érythrocytes carencés en magnésium pourrait s'expliquer par une augmentation de l'affinité de l'antiporteur Na^+/Mg^{2+} pour le Mg^{2+} intracellulaire et de l'efflux de Mg^{2+}.

Introduction

The concentration of intracellular free Mg^{2+} ($[Mg^{2+}]_i$) in erythrocytes was frequently measured. $[Mg^{2+}]_i$ of erythrocytes was found to be reduced in patients with essential hypertension [12] and in spontaneously hypertensive rats [10] when measured by ^{31}P-NMR. However, other investigators, using the same method, found no reduction of $[Mg^{2+}]_i$ in erythrocytes of hypertensive subjects. For discussion see [13, 17].
There were also controversial results for $[Mg^{2+}]_i$ of stored erythrocytes. When measuring by the ^{31}P-NMR method a decrease of $[Mg^{2+}]_i$ was found [1], whereas an increase of $[Mg^{2+}]_i$ was found when using the null-point method [2].

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The above reviewed alterations in $[Mg^{2+}]_i$ [12] occurred, if at all, at constant total Mg content. Since intracellular Mg represents an Mg buffer with only 10 % of the total Mg being free Mg^{2+}, the reduction of $[Mg^{2+}]_i$ at constant total Mg is difficult to explain. To study the relationship between total and free Mg^{2+} concentration in erythrocytes, we used erythrocytes from Mg-deficient rats. In Mg deficiency the strongest decrease of intracellular Mg content was found in erythrocytes, amounting to 33 %, whereas in soleus muscle, cardiac muscle and lymphocytes the reduction of Mg content amounted only to 6, 10 and 9 % of the controls [15].
The small reduction of Mg content in muscle cells or lymphocytes may be related to a reduction of ribosomes which have a high Mg content. However, erythrocytes do not contain cell organelles. Therefore, the strong reduction of Mg content in Mg-deficient erythrocytes may have another mechanism.
In preceding experiments we have characterized Na^+-dependent and Na^+-independent Mg^{2+} efflux from erythrocytes [5, 7, 8, 9]. Therefore, we investigated the question whether these Mg^{2+} efflux systems and $[Mg^{2+}]_i$ are changed in Mg-deficient rat erythrocytes.

Materials and Methods

Female Wistar rats weighing 70 g were fed an Mg-deficient diet (Mg content: 3 mmol/kg, Ca content: 250 mmol/kg; Ssniff, Soest, FRG) and distilled water ad libitum. The composition of the diet was described in detail [6]. Control rats were fed the same diet enriched with $MgCl_2$ to 41

mmol/kg. The diets were fed for 4 to 5 weeks.

For preparation of reticulocytes female Wistar rats weighing 150 g received a daily subcutaneous injection of 10 mg/kg b. w. phenylhydrazine chloride dissolved in saline for 5 days. One day later blood was taken for determination of Mg^{2+} efflux. Blood was taken under Nembutal anesthesia (50 mg/kg s. c.) by heart puncture with a heparinized syringe and centrifuged at 1,000 g for 10 min. The plasma and buffy coat were aspirated and the red cells were washed twice with 150 mmol/l KCl.

Mg^{2+} efflux

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

For removal of the ionophore, the cells were incubated four times in KCl medium plus 12 mmol/l $MgCl_2$ and 1 % bovine serum albumin for 10 min at 37° C. Thereafter, the cells were washed twice in cold (4° C) sucrose medium (in mmol/l: 350 sucrose, 5 glucose, 30 Hepes/Tris, pH 7.4).

Mg^{2+} efflux was measured by reincubating a 10 % cell suspension at 37° C in Mg^{2+}-free medium. For reincubation, sucrose medium, NaCl or choline Cl medium (substitution of KCl in KCl medium by 140 mmol/l NaCl or 140 mmol/l choline Cl) were used. 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 g. For Mg determination, 100 µl supernatant was diluted with 1 ml 10 % TCA/ 0.175 % $LaCl_3$ and Mg was measured by atomic absorption spectrophotometry (AAS) (*Philips,* SP9). An aliquot of the supernatant was taken for determination of hemoglobin by means of the cyanmethemoglobin method [5].

For measuring cellular Mg content of Mg^{2+}-loaded and untreated erythrocytes, the cells were washed twice with 150 mmol/l 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 content was measured by AAS after dilution with 10 % TCA/0.175 % $LaCl_3$. Cellular Mg content of Mg^{2+}-loaded erythrocytes was taken to correct Mg^{2+} efflux for hemolysis.

Mg concentration in serum was measured by AAS after dilution with 10 % TCA/0.175 % $LaCl_3$.

Intracellular free Mg^{2+}

Determination of intracellular free Mg^{2+} concentration was performed by the null-point method [2, 4, 14]. The erythrocytes were washed 3 times with 150 mmol/l NaCl and incubated at 50 % hematocrit in media which contained (in mmol/l): 145 NaCl, 5 KCl, 5 glucose, 30 Hepes/Tris, pH 7.4, and 0, 0.1, 0.3, 0.5, or 0.7 $MgCl_2$. Half of the tests were run with 10 µmol/l A23187. After 15 min incubation, aliquots were centrifuged at 10 000 g for 1 min. To 100 µl supernatant 2 ml 10 % TCA/ 0.175 % $LaCl_3$ was added and Mg content was measured by AAS. A23187 caused a rapid equilibration between extra- and intracellular Mg^{2+} concentration. By means of interpolation, the extracellular Mg^{2+} concentration was determined at which no alteration in extracellular Mg^{2+} concentration occurred between the tests without and with A23187. Thus, membrane-binding of Mg^{2+} was eliminated. The concentration of intracellular free Mg^{2+} was calculated according to the formula [4]:

$[Mg^{2+}]_i = [Mg^{2+}]_o \cdot r^2$. ($r = [Cl^-]_o / [Cl^-]_i$)

The Cl^- distribution ratio (r) was determined in parallel tests by the addition of $^{36}Cl^-$ ($Na^{36}Cl$, specific activity: 110 MBq/g Cl, 3 mCi/g Cl^-, Amersham).

For determination of trapped fluid (and trapped $^{36}Cl^-$) between the sedimented cells, ^{14}C inulin (inulin-[^{14}C]carboxylic acid, specific activity: 74–370 MBq/mmol, 2–10 mCi/mmol, Amersham) was added.

After centrifugation, aliquots of the supernatants and sediments were measured by liquid scintillation counting.

$[Cl^-]_i$ was calculated assuming that intracellular water amounts to 65 % of the cell volume [2, 4].

Results

Mg^{2+} efflux

As shown in Tab. 1, Na^+-independent Mg^{2+} efflux (in sucrose medium) and Na^+-dependent Mg^{2+} efflux (NaCl minus choline Cl medium) were about one third higher in erythrocytes from Mg-deficient rats than in those from controls. Mg^{2+} efflux was inhibited to the same percentage by 0.5 mmol/l quinidine or 30

Tab. 1: Net Mg^{2+} efflux from Mg^{2+}-loaded erythrocytes from 4 control and 8 Mg-deficient rats. Mean ± SEM. Significant difference to controls by unpaired Student's t-test.

Medium	Inhibitor	Control (mmol/l cells · 30 min)	Mg-deficient
Sucrose	–	1.94 ± 0.13	2.58 ± 0.18*
	Quin[a)]	1.51 ± 0.18	2.00 ± 0.10*
	SITS	1.31 ± 0.17	1.46 ± 0.18
NaCl	–	9.50 ± 0.33	12.12 ± 0.50**
	Quin	4.25 ± 0.52	5.14 ± 0.42
Choline Cl	–	0.98 ± 0.19	1.09 ± 0.13
	Quin	0.72 ± 0.18	1.17 ± 0.17

a) Quin, Quinidine (0.5 mmol/l SITS, 4-acetamido-4'-isothiocyanatostilbene-2,2'-disulfonic acid (30 µmol/l)

* ,$p < 0.05$; ** ,$p < 0.01$

µmol/l SITS in controls and Mg-deficient erythrocytes. Thus, net Mg^{2+} efflux from Mg-deficient erythrocytes showed the same properties as those from normal erythrocytes but all parameters of net Mg^{2+} efflux were increased by one third.
The simultaneously measured net K^- efflux was not significantly increased in Mg-deficient erythrocytes (data not shown), indicating the specificity of the effect.
Since in Mg deficiency hemolysis is increased resulting in a higher percentage of young erythrocytes [3], we tested Mg^{2+} efflux from rat reticulocytes, produced by injection of phenylhydrazine. This procedure caused an increase of reticulocytes up to 70 % of the red blood cells. However, all fractions of net Mg^{2+} efflux were unchanged in reticulocytes (data not shown).
Therefore, the increase in net Mg^{2+} efflux in Mg deficiency is not caused by the younger age of the erythrocytes. Moreover, this result shows that the Mg^{2+} efflux systems are not altered by maturation of erythrocytes.

Concentration of intracellular free Mg^{2+}

When the activity of Mg^{2-} efflux is increased, one may expect that $[Mg^{2+}]_i$ is reduced. Therefore, we measured $[Mg^{2+}]_i$ of Mg-deficient erythrocytes. By means of the null-point method we found (Tab. 2) that intracellular and extracellular Mg^{2+} was in equilibrium at $[Mg^{2+}]_o = 0.24$ mmol/l for normal erythrocytes and at $[Mg^{2+}]_o = 0.13$ mmol/l for Mg-deficient erythrocytes. Taking into account the $[Cl^-]_o / [Cl^-]_i$ distribution ratio, which was the same for both types of erythrocytes, $[Mg^{2+}]_i$ can be calculated to be 0.38 mmol/l for normal erythrocytes and 0.22 mmol/l for Mg-deficient erythrocytes. Thus, in control erythrocytes, $[Mg^{2+}]_i$ was the same as found by other authors who used the same method [2].

Discussion

A remarkable result was the reduction of $[Mg^{2+}]_i$ in Mg-deficient erythrocytes. In Mg-deficient erythrocytes the concentration of Mg-binding substances, such as ATP and 2,3 bisphosphoglycerate, is reduced [11]. However, a single reduction of Mg^{2+}-binding ligands would lead to an increase of $[Mg^{2+}]_i$, or $[Mg^{2+}]_i$ would remain constant because of intracellular buffering or because of Mg^{2+} efflux. Therefore, the decrease of $[Mg^{2+}]_i$ in Mg-deficient erythrocytes must have another reason.
At first glance, it may be assumed that the reduced $[Mg^{2+}]_i$ was caused by the increased rate of net Mg^{2+} efflux (Na^+/Mg^{2+} antiport) in Mg-deficient erythrocytes. However, this mechanism is not likely. Net Mg^{2+} efflux operates only in Mg^{2+}-loaded erythrocytes, when $[Mg^{2+}]_i$ is increased and Mg^{2+} efflux stops at physiological $[Mg^{2+}]_i$ [5]. Since the rate of net Mg^{2+} efflux from rat erythrocytes is high [9] and the life time of rat erythrocytes rather long, erythrocytes in vivo must be in a steady-state of extracellular – intracellular Mg^{2+} distribution. Under these conditions, net Mg^{2+} efflux is not operating.
The reduced $[Mg^{2+}]_i$ in Mg-deficient erythrocytes may be caused by an increased affinity of the Mg^{2+} efflux system for intracellular Mg^{2+} at the inner site of the membrane. Thus, the Mg^{2+} efflux system can transport Mg^{2+} out of the cell until a lower $[Mg^{2+}]_i$ is reached, compared to normal erythrocytes, and net Mg^{2+} efflux stops at this lower $[Mg^{2+}]_i$. Because of the long life time of erythrocytes, this state can be reached by an unchanged or by an increased activity of the net Mg^{2+} efflux system.
The increased affinity and activity of the net Mg^{2+} efflux system may be caused by an alteration of the erythrocyte membrane in Mg deficiency. In Mg deficiency an alteration of serum lipids and an alteration in the composition of lipids in the erythrocyte membrane occur [16]. Therefore, it may be suggested that conformation changes of the Mg^{2+} channel or Na^+/Mg^{2+} antiporter in the erythrocyte membrane, which may be involved in net Mg^{2+} efflux, are favored by the altered lipid composition and/or membrane fluidity of the cell membrane, resulting in a higher Mg^{2+} efflux.

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Tab. 2: Mg content of serum and erythrocytes and intraerythrocyte free Mg^{2+} concentration from control and Mg-deficient rats. Mean ± SEM of 12 control and 15 Mg-deficient rats. Significant difference to controls by unpaired Student's t-test.

	Control	Mg-deficient
$[Mg]_{serum}$ (mmol/l)	0.87 ± 0.03	0.18 ± 0.02***
$[Mg]_{tot}$ (mmol/l cells)	2.95 ± 0.22	1.73 ± 0.25**
$[Mg^{2+}]_o$ at equilibrium (mmol/l)	0.24 ± 0.01	0.13 ± 0.01***
r[a]	1.15	1.15
r[b]	1.26	1.31
$[Mg^{2+}]_i$ (mmol/l)[c]	0.32	0.17
	0.38	0.22

[a] determined in the presence of 10 µmol/l) A23187
[b] determined from tests without addition of A23187
[c] $[Mg^{2+}]_i = [Mg^{2+}]_o \cdot r^2$, $[Mg^{2+}]_i$ was calculated using $[Mg^{2+}]_o$ at equilibrium and the Cl^- distribution ratio (r) from [a] and [b]
** ,$p < 0.01$; *** ,$p < 0.001$

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