Reversible inactivation of Na^+/Mg^{2+} antiport in rat erythrocytes by glucose starvation

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Zusammenfassung

Wenn Rattenerythrozyten bei 37°C für 12 Stunden bei physiologischer (5 mM) Glucosekonzentration aufbewahrt wurden, war der Na^+/Mg^{2+}-Antiport um 90% reduziert, wohingegen der ATP- und Na^+-unabhängige Mg^{2+}-Efflux nicht beeinflußt wurde. Dieser Effekt wurde durch den Verbrauch der extrazellulären Glucose hervorgerufen. Durch hohe (20 mM) Glucosekonzentration im Inkubationsmedium ließ sich der Abfall der Na^+/Mg^{2+}-Antiportkapazität vermeiden. Die Inaktivierung des Na^+/Mg^{2+}-Antiportes war nach längerer Inkubation (12 h) der Glucoseverarmten Zellen in Medium mit Glucose reversibel. Kurzzeitige Reinkubation in Glucose-haltigem Medium für 1,5 h führte zu einer Erholung des intrazellulären ATP-Gehaltes aber nicht zu einem normalen Na^+/Mg^{2+}-Antiport.

Summary

When rat erythrocytes were stored for 12 h at 37°C at physiological (5 mM) glucose concentration, Na^+/Mg^{2+} antiport was reduced by 90%, whereas ATP- and Na^+-independent Mg^{2+} efflux was unaffected. The effect was due to an exhaustion of the available glucose in the medium. The reduction of Na^+/Mg^{2+} antiport by storage was prevented by high glucose concentration (20 mM) in the incubation medium. The inactivation of Na^+/Mg^{2+} antiport could be reversed by long-time (12 h) reincubation of the glucose-starved cells in media with glucose. Short-term reincubation in glucose-containing media for 1.5 h restored cellular ATP content but not Na^+/Mg^{2+} antiport capacity.

Introduction

The concentration of intracellular free Mg^{2+} ($[Mg^{2+}]_i$) in oxygenated erythrocytes amounts to 0.2 mM [1, 2] which is below the thermodynamic equilibrium. The low level of $[Mg^{2+}]_i$ is established by almost missing net Mg^{2+} influx under physiological conditions and by net Mg^{2+} efflux, which becomes activated when $[Mg^{2+}]_i$ is increased. The mechanism of Mg^{2+} efflux has been investigated in Mg^{2+}-loaded erythrocytes; two transport systems were characterized:

1. Na^+/Mg^{2+} antiport,
2. Na^+-independent MG^{2+} efflux [3].

Na^+/Mg^{2+} antiport depends on ATP, which may act by phosphorylation via protein kinase (PK) C in erythrocytes [4] and PKA in lymphocytes [5] and is inhibited by amiloride, imipramine and quinidine [3].

Na^+-independent Mg^{2+} efflux which is coupled to Cl^- efflux for charge compensation and inhibited by DIDS [3] is independent of ATP [6] and inhibited by amiloride [6].

In addition, in rat and chicken erythrocytes Na^+-independent Mg^{2+} efflux is inhibited by plasma [7]. The reduced Mg^{2+} efflux in plasma is due to inhibition by albumin [7] and by glucose [8, 9]. The effect of glucose was not specific; a smaller inhibition of Mg^{2+} efflux was also found with D-galactose and D-fructose, whereas pentoses were ineffective [9]. The mechanism is not known as yet. On the other hand, Na^+-dependent Mg^{2+} efflux was higher in glucose-containing medium compared to glucose-free medium [10]. During our experiments on Mg^{2+} efflux we observed an inactivation of net Mg^{2+} efflux when erythrocytes were stored at 37°C for prolonged time periods.

Therefore, in the present paper we report on experiments to explain the mechanism of inactivation.

Materials and Methods

The experiments were done with rat erythrocytes since these cells express a high rate of net Mg^{2+} efflux. Blood was taken from anaesthetized adult rats (50 mg/kg Nembutal i.p.) by heart puncture with a heparinized syringe. Blood was centrifuged at 1000 g for 10 min, plasma and buffy coat were aspirated, the cells washed in 150 mM NaCl and resuspended in Na^+ medium to the original cell concentration of approximately 50%.

Preincubation

Preincubation was performed with erythrocytes suspended in Na^+ medium, with blood directly taken or with erythrocytes resuspended in heat-activated rat plasma (30 min at 60°C) at the original hematokrit. The cells were preincubated for 12 h in a water bath at 37°C with slight shaking to prevent sedimentation. Glucose concentration of blood amounted to 5 mM. Glucose concentration of erythrocyte suspensions in Na^+ medium amounted to 0–20 mM as indicated. Na^+ medium contained (in mM) : 140 NaCl, 5 KCl, 1 $MgCl_2$, 1 phosphate, 1.2 $CaCl_2$, 50 saccharose, 0–20 glucose, 30 Hepes-Tris, pH 7.4. Each experiment was done with erythrocytes from a single rat. In some experiments air was substituted by 100% N_2 or O_2 as indicated.

Mg^{2+}-loading

After preincubation the cells were centrifuged, washed and loaded with Mg^{2+} by incubating a 10% cell suspension for 20 min at 37°C in Na^+ medium with 5 mM glucose in the presence of 6 μM of the cation ionophore A23187 (Boehringer, Mannheim) and 12mM

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$MgCl_2$, in some experiments 3mM $MgCl_2$ was used as indicated (Tab. 1). A23187 was removed by incubating the cells 4 times in Na^+ medium with the same Mg^{2+} concentration as in the loading medium plus 1% bovine serum albumin (Serva) for 10min at 37°C.

Mg^{2+} efflux

Mg^{2+} efflux was measured by reincubating a 10% cell suspension at 37°C in Mg^{2+}-free Na^+ medium or choline medium in which NaCl of Na^+ medium was isoosmotically substituted by choline Cl. Glucose concentration amounted to 5 mM. At the beginning of reincubation and after 20 or 30 min, 0.5 ml aliquots of the cell suspension were centrifuged for 1 min at 10.000 g. 100 μl supernatant was diluted with 1ml 10% trichloroacetic acid/0.175% $LaCl_3$ and Mg^{2+} was measured by atomic absorption spectrophotometry (Philips SP9). Na^+/Mg^{2+} antiport was determined by subtraction of Mg^{2+} efflux in choline medium (Na^+-independent Mg^{2+} efflux) from total Mg^{2+} efflux.

Measurements

Glucose in media was measured enzymatically (D-Glucose-Test, Boehringer). ATP in erythrocytes was determined by an optical test (Sigma, Test Kit No: 336-UV)

Results and Discussion

In previous experiments on Mg^{2+} efflux from erythrocytes we found an inactivation of Na^+/Mg^{2+} antiport by oxygen-derived free radicals [11]. Since erythrocytes were stored under air, oxygen and reactive Fe from erythrocytes may have caused inactivation of net Mg^{2+} efflux by oxygen-derived free radicals. Therefore, we preincubated rat erythrocytes at 4°C and 37°C under N_2 and O_2 for 18 h. Thereafter, the erythrocytes were loaded with Mg^{2+} in the presence of 3 and 12 mM $MgCl_2$ for measurement of Mg^{2+} efflux. As shown in table 1, after preincubation at 4°C Na^+/Mg^{2+} antiport was unchanged amounting to 9.49 mmol/l cells x 20 min. Na^+/Mg^{2+} antiport from fresh rat erythrocytes loaded in the presence of 12 mM $MgCl_2$ amounted to 9.45 mmol/l cells x 20 min [12]. The lower rate of Na^+/Mg^{2+} antiport after loading in the presence of 3 mM $MgCl_2$ (Tab. 1) corresponds to previous results [4]. However, after preincubation at 37°C Na^+/Mg^{2+} antiport was drastically reduced by about 90%. Free radicals do not seem to be involved in the inactivation process as the same low rate of Na^+/Mg^{2+} antiport was found in N_2 and O_2 atmosphere. Cl^--dependent Mg^{2+} efflux was – not significantly – increased by preincubation at 37°C. At higher Mg^{2+}-loading due to an increased intracellular-extracellular Mg^{2+} gradient the rate of Cl^--dependent Mg^{2+} efflux was also elevated.

Tab. 1: Na^+/Mg^{2+} antiport and Na^+-independent net Mg^{2+} efflux from Mg^{2+}-loaded rat erythrocytes. Blood was stored at 4°C and 37°C for 18 h in O_2 and N_2. Thereafter, the cells were loaded with Mg^{2+} in the presence of 3 or 12 mM $MgCl_2$ as indicated.
Values in mmol/l cells x 20 min. Mean ± SEM, n number of experiments.

Atmosphere	°C	$MgCl_2$ (mM) during loading	n	Na^+/Mg^{2+} antiport	Na^+-independent Mg^{2+} efflux
N_2	4	3	4	2.52 ± 0.03	0.73 ± 0.10
	4	12	4	9.49 ± 0.50	2.66 ± 0.37
	37	3	5	0.28 ± 0.02	0.97 ± 0.22
	37	12	5	1.08 ± 0.23	2.96 ± 0.43
O_2	4	3	3	2.29 ± 0.05	0.76 ± 0.21
	4	12	3	8.72 ± 0.65	2.81 ± 0.34
	37	3	9	0.27 ± 0.04	0.85 ± 0.14
	37	12	9	0.94 ± 0.07	2.95 ± 0.30

Tab. 2: Effect of temperature during preincubation on Na^+/Mg^{2+} antiport and Na^+-independent Mg^{2+} efflux of rat erythrocytes (in mmol/l cells x 20 min). The cells were preincubated in plasma or Na^+ medium with 5 mM glucose for 12 h at 37, 20 or 4°C. Mean ± SEM of 3 experiments.

	37°C	20°C	4°C
Na^+/Mg^{2+} antiport	0.84 ± 0.15	7.00 ± 0.30	6.74 ± 0.07
Na^+-independent Mg^{2+2} efflux	0.40 ± 0.02	0.50 ± 0.08	0.45 ± 0.08

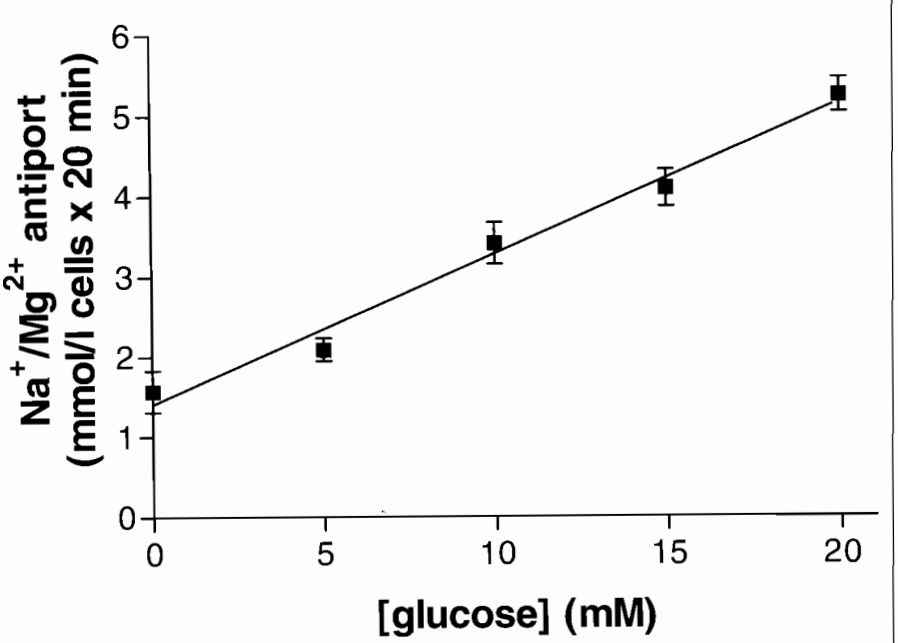

Fig. 1: Na^+/Mg^{2+} antiport in rat erythrocytes, preincubated in Na^+ medium with 0–20 mM glucose at 37°C for 12 h. Thereafter, the cells were loaded with Mg^{2+} in the presence of 6 μM A23187 and 12 mM $MgCl_2$. Na^+/Mg^{2+} antiport was measured in Mg^{2+}-free Na^+ medium with 5 mM glucose. Mean ± SEM of 7 experiments.

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In previous experiments we found inhibition of Na^+/Mg^{2+} antiport by serum albumin [7]. Therefore, in a second set of experiments we tested whether a serum factor might have inactivated Na^+/Mg^{2+} antiport at 37°C. However, preincubation of rat erythrocytes in serum or serum-free medium yielded the same inactivation of Na^+/Mg^{2+} antiport, excluding the action of a serum factor (data not shown).

Next, we tested the temperature depence of inactivation. Preincubation of erythrocytes at 4°C and 20°C yielded the same normal rate of Na^+/Mg^{2+} antiport after reincubation. However, preincubation at 37°C for 12 h caused drastic inhibition of Na^+/Mg^{2+} antiport, Na^+-independent Mg^{2+} efflux being unaffected (table 2).

Since Na^+-independent Mg^{2+} efflux is not energy-dependent, whereas Na^+/Mg^{2+} antiport needs ATP, we investigated the effect of energy metabolism on Na^+/Mg^{2+} antiport.

Preincubation of rat erythrocytes at 37°C for 12 h in medium with different glucose concentrations revealed a linear reduction of Na^+/Mg^{2+} antiport with decreasing glucose concentration (fig. 1).

The reduction of Na^+/Mg^{2+} antiport was reversible. When cells were preincubated at 37°C with 0 to 20 mM glucose for 12 h, the same reduction of Na^+/Mg^{2+} antiport was found as shown in fig. 1. When after the first 12 h preincubation period the cells were incubated for a second 12 h period at 37°C with 20 mM glucose, Na^+/Mg^{2+} antiport yielded the same high rate as was found in the cells incubated with 20 mM glucose from the beginning of incubation onwards (data not shown). From this experiment it can be concluded that it needs some time before Na^+/Mg^{2+} antiport is restored. In this experiment Na^+/Mg^{2+} antiport was restored within 12 h, whereas in the other experiments a reduced Na^+/Mg^{2+} antiport was measurable even though Mg^{2+}-loading and washing of the cells in the presence of 5 mM or 20 mM glucose took about 1.5 h after preincubation.

To investigate the role of glucose in the reduction of Na^+/Mg^{2+} antiport, we measured glucose concentration in the media after 12 h preincubation at 37°C. As shown in table 3, glucose concentration was drastically reduced during preincubation.

Rat erythrocytes are insulin-independent cells [13], glucose being transported into the cell via GLUT 1 [14]. At high glucose concentration more glucose is transported into the cells, followed by an increased rate of glycolysis. Consequently, the steady-state concentration of ATP may be increased. Measurement of ATP showed that preincubation of erythrocytes with low glucose at 37°C for 12 h yielded a similar decrease of ATP as Na^+/Mg^{2+} antiport activity (fig. 2).

The presence of 5 mM or 20 mM glucose during Mg^{2+}-loading and washing rapidly restored cellular ATP content (fig. 2), whereas restoration of Na^+/Mg^{2+} antiport required much more time.

These results show that exhaustion of glucose during preincubation leads to a reduced cellular ATP content. However, ATP depletion is not the only reason for the reduction of Na^+/Mg^{2+} antiport. ATP in rat erythrocytes was rapidly restored after reincubation, whereas the maximal capacity of Na^+/Mg^{2+} antiport was restored only after a longer time period. Therefore, ATP depletion may have caused secondary effects in rat erythrocytes at the cell membrane, e.g. by Mg^{2+}- and Ca^{2+}-activated enzymes, leading to reduced Na^+/Mg^{2+} antiport. Only after normalization of this unknown alteration, which takes more time than the replenishment of ATP, Na^+/Mg^{2+} antiport is normalized.

This mechanism may have practical importance in ischemia: when ATP declines, Mg^{2+} is released and cannot be transported out of the cells. Additionally, $[Ca^{2+}]_i$ is increased by

Tab. 3: Glucose concentration (in mM) in preincubation media with 0–20 mM glucose after 12 h preincubation at 37°C. Mean ± SEM of 6 experiments.

Glucose at start	0	5	10	15	20
Glucose after 12 h at 37°C	0	0.10 ± 0.04	0.94 ± 0.05	1.61 ± 0.16	3.85 ± 0.6

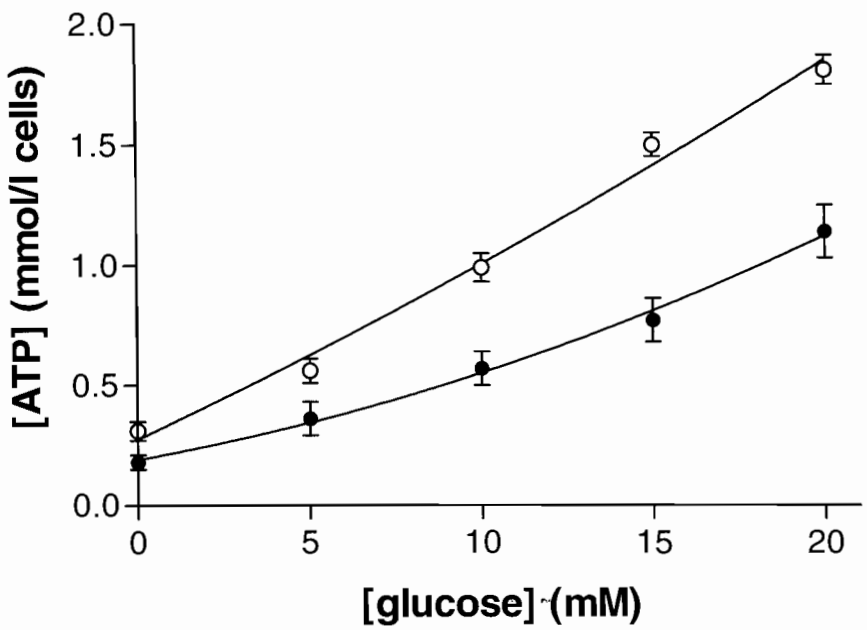

Fig. 2: ATP content of rat erythrocytes (in mmol/l cells)
●, ATP content directly after 12 h preincubation at 37°C in Na^+ medium with 0–20 mM glucose.
○, ATP content after Mg^{2+}-loading and washing procedure (lasting 1.5 h) in Na^+ medium with 5 mM glucose.
Mean of 4 experiments.

impaired Ca^{2+} pumping. The increased $[Mg^{2+}]_i$ and $[Ca^{2+}]_i$ may cause cell injury [15].

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