Unchanged Mg^{2+} Metabolism of Erythrocytes from Patients with Tetanic Syndrome and Hypertension

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Zusammenfassung

In den Erythrozyten von 15 Patienten mit tetanischem Syndrom, die eine essentielle Hypertonie entwickelt hatten, waren der Na^+-abhängige und Na^+-unabhängige Mg^{2+}-Efflux sowie die Konzentration des intrazellulären freien Mg^{2+} unverändert im Vergleich zu gesunden Kontrollpersonen oder unbehandelten Patienten mit tetanischem Syndrom oder essentieller Hypertonie.
Wenn eine Änderung des Mg^{2+}-Stoffwechsels an der Pathogenese des tetanischen Syndroms oder der essentiellen Hypertonie beteiligt ist, müssen andere Zellen als Erythrozyten betroffen sein.

Summary

In erythrocytes from 15 patients who were suffering from tetanic syndrome and had developed essential hypertension, Na^+-dependent and Na^+-independent Mg^{2+} efflux and concentration of intracellular free Mg^{2+} were not different from healthy controls or untreated patients suffering either from tetanic syndrome or essential hypertension.
Therefore, when an alteration in Mg^{2+} metabolism is involved in the pathogenesis of tetanic syndrome or essential hypertension it must concern cells other than erythrocytes.

Résumé

Chez 15 patients atteints de spasmophilie et présentant une hypertension artérielle essentielle, l'efflux de Mg^{++} Na^+-dépendant et Na^+-indépendant et la concentration intracellulaire de Mg^{++} libre dans les érythrocytes ne présentaient pas de différences par rapport à des sujets témoins en bonne santé ou à des patients non traités également atteints de spasmophilie ou d'hypertension artérielle essentielle.
Ces résultats indiquent que les cellules impliquées ne sont pas les érythrocytes lorsqu'une altération du métabolisme du Mg^{++} intervient dans l'apparition d'une spasmophilie ou d'une hypertension essentielle.

Introduction

The relationship between Mg and hypertension is controversial. In patients suffering from hypertension who had been exposed to long-term treatment with diuretics, oral Mg supplementation was reported to have a beneficial effect [4, 13, 19]. However, other investigators found that oral Mg treatment had no effect on blood pressure [3, 7]. For review see [20]. In patients with essential hypertension an inverse relationship between blood pressure and intracellular free Mg^{2+} concentration ($[Mg^{2+}]_i$) of erythrocytes was measured by means of ^{31}P-NMR [14, 15]. After the discovery of an Mg^{2+} efflux system in erythrocytes [5, 11], it was reported [12] that in patients with essential hypertension, the Mg^{2+} efflux system may have an increased activity in relation to blood pressure. This can explain the reduced concentration of intracellular free Mg^{2+} ($[Mg^{2+}]_i$) found in erythrocytes from patients with essential hypertension. However, in similar ^{31}P-NMR measurements, $[Mg^{2+}]_i$ in the erythrocytes of hypertensive patients was found not to be reduced [21]. Also, with an A23187-method no reduction of $[Mg^{2+}]_i$ was measured in erythrocytes of hypertensive patients [18].
For discussion of the controversial results on $[Mg^{2+}]_i$ in erythrocytes of patients with essential hypertension see Ref. [16, 22].
Also, the reduction of $[Mg^{2+}]_i$ in cold-stored erythrocytes, as measured by ^{31}P-NMR [1], could not be reproduced when $[Mg^{2+}]_i$ was measured in erythrocytes by means of the null-point titration with A 23187 [2].
Since we have characterized the Mg^{2+} efflux system from human erythrocytes in greater detail [9], we reinvestigated Mg^{2+} efflux and $[Mg^{2+}]_i$ in erythrocytes from hypertensive patients.
In order to find an alteration in Mg^{2+} metabolism, if at all, we used erythrocytes from patients who had been suffering from chronic tetanic syndrome, requiring Mg supplementation for many years and who had developed hypertension.

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Patients

The experiments were performed with erythrocytes from 15 patients (1 male, 14 females) suffering from tetanic syndrome who had developed essential hypertension. Their age amounted to 50.1 ± 2.2 years (Mean ± SEM). The tetanic syndrome was diagnosed at the age of 34.1 ± 2.9 years. The maximal systolic blood pressure amounted to 193 ± 9 Torr and the maximal diastolic blood pressure to 120 ± 5 Torr (class I according to WHO criteria).
However, blood pressure in these

patients often showed drastic changes. 9 of these 15 patients were frequently suffering from transistoric ischemic attacks (TIAs). TIAs occurred on average 4.5 years after the tetanic syndrome had been diagnosed. Before Mg treatment, 6 of the patients showed hypomagnesemia, 3 hypocalcemia and 1 hypokalemia. The patients were treated with oral doses of Mg (500–700 mg/d). Some patients were additionally treated with Ca antagonists, dihydralazine and β blockers.

To test whether Mg^{2+} supplementation and the other medication had any influence on these patients, blood from 6 untreated patients (2 males, 4 females, 47.6 ± 2.1 years old) suffering from essential hypertension (class I according to WHO criteria) and blood from 8 untreated normotensive patients (1 male, 7 females, 44.1 ± 3.3 years old) with tetanic syndrome was taken. As controls, blood from 5 healthy normotensive persons (1 male, 4 females) of similar age was used.

Methods

Blood was taken 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. 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, 12 $MgCl_2$, 50 sucrose, 5 glucose, 30 HEPES/Tris, pH 7.4) with the addition of 6 µmol/l A23187 dissolved in dimethyl sulfoxide (DMSO). For removal of the ionophore the cells were incubated four times in KCl medium plus 1 % bovine serum albumin for 10 min at 37 °C. The KCl medium was removed by washing the cells twice with 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 % LaCl3 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 [11].

For measuring cellular Mg content, the cells were washed twice with 150 mmol/l KCl and hemolysed by adding 750 µl H2O. 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 % LaCl3. Cellular Mg content of Mg^{2+}-loaded erythrocytes was taken to correct Mg^{2+} efflux for hemolysis. Mg and Ca concentration in serum were measured by AAS after dilution with 10 % TCA/0.175 % LaCl3.

Determination of intracellular free Mg^{2+} concentration was performed by the null-point method [2, 17]. 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 MgCl2). Half of the tests were run with 10 µmol/l A23187. After 15 min incubation, aliquots were centrifuged at 13,000 g for 1 min. To 100 µl supernatant 2 ml 10 % TCA/0.175 % LaCl3 was added and Mg content was measured by AAS. A23187 caused a rapid equilibration between extra- and intracellular Mg^{2+} concentration. By interpolation the intracellular 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.

Results

As shown in Tab. 1, serum Mg and Ca concentrations, Mg content and $[Mg^{2+}]_i$ of erythrocytes were the same in controls and in patients with tetanic syndrome and additional hypertension.

When during the equilibration of free Mg^{2+} by means of the neutral A23187-Mg^{2+} complex, $[Mg^{2+}]_i$ may be enhanced due to a Donnan effect, the values for $[Mg^{2+}]_i$ in control and patient erythrocytes must be increased. Since the extracellular/intracellular Cl^- concentration gradient in the erythrocytes of hypertensives seems to be somewhat higher than in controls [18, 23], the resulting $[Mg^{2+}]_i$ in our hypertensive patients would be somewhat higher than in the controls. In any case, there would be no reduction of $[Mg^{2+}]_i$ in patients' erythrocytes. In patients, not treated with Mg, suffering from tetanic syndrome or essential hypertension there were somewhat lower serum Mg and Ca concentrations than in Mg-supplemented patients or healthy persons.

Net Mg^{2+} efflux from Mg^{2+}-loaded erythrocytes can be performed by Na^+-independent Mg^{2+} efflux (in sucrose), which is inhibited by SITS and Cl^-, and by Na^+-dependent Mg^{2+} efflux (NaCl minus choline Cl) (10). As shown in Tab.1, all fractions of Mg^{2+} efflux from the erythrocytes of all groups were identical.

Discussion

The patients suffered from tetanic syndrome and essential hypertension. It has not been defined as yet whether both diseases are related in these patients. There is the possibility that both diseases are not related. Hypertension may occur randomly and independent in patients with tetanic syndrome as in other persons. The other possibility is that hypertension is a complication occuring late in some of the patients with tetanic syndrome.

In the patients with chronic tetanic syndrome suffering from hyperten-

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Tab. 1: Serum Ca and Mg concentrations, erythrocyte Mg content, $[Mg^{2+}]_i$ as well as Mg^{2+} efflux from erythrocytes of patients with tetanic syndrome and hypertension.

	Controls (5)[a]	Tet. Syn + Hypert. (treated) (15)	Tet. Syn (untreated) (8)	Hypert. (untreated) (6)
Serum Ca (mmol/l)	2.45 ± 0.07	2.43 ± 0,.03	2.21 ± 0.05	2.21 ± 0.02
Serum Mg (mmol/l)	0.81 ± 0.02	0.80 ± 0.03	0.72 ± 0.02	0.74 ± 0.02
$[Mg^{2+}]_{tot}$ (Ery) (mmol/l cells)	2.43 ± 0.08	2.50 ± 0.04	–	–
$[Mg^{2+}]_i$ (Ery) (mmol/l cells)	0.21 ± 0.01	0.23 ± 0.01	–	–
Mg^{2+} Efflux (mmol/l cells· 30 min)				
Sucrose medium	0.86 ± 0.03	0.87 ± 0.04	0.93 ± 0.06	1.00 ± 0.03
Sucrose + SITS[b]	0.20 ± 0.02	0.22 ± 0.03	0.20 ± 0.02	0.22 ± 0.01
NaCl medium	0.40 ± 0.04	0.42 ± 0.04	0.36 ± 0.03	0.35 ± 0.04
Choline Cl medium	0.11 ± 0.03	0.16 ± 0.02	0.12 ± 0.01	0.14 ± 0.01

[a] Mean ± SEM, number of patients in parenthesis.
Tet. Syn., tetanic syndrome; Hypert., hypertension.
[b] SITS, 4-acetamido-4'-isothiocyanatostilbene-2,2'-disulfonie acide.

sion, we found no alterations in total and free Mg^{2+} concentration of erythrocytes and Mg^{2+}efflux from erythrocytes.
From these results it can be concluded that

1. Mg^{2+} metabolism in erythrocytes is not changed in patients with tetanic syndrome plus hypertension, although oral Mg supplementation has a beneficial effect in normalizing the serum Mg concentration which is reduced to borderline values in some patients with tetanic syndrome.

2. The therapeutic treatment of these patients may have normalized Mg^{2+} efflux from the erythrocytes. This possibility is not likely because the erythrocytes were washed a few times in albumin-containing medium and were loaded with Mg^{2+}. A normalisation of Mg^{2+} efflux by treatment of the patients would have been overcome by the experimental procedure. Any alterations in Na^+-dependent and Na^+-independent Mg^{2+} efflux would have been measurable under these conditions. In agreement with this conclusion, in untreated patients suffering either from tetanic syndrome or hypertension alterations in Mg^{2+} efflux were not detected.

3. If treatment of the patients with tetanic syndrome plus hypertension had normalized the Mg^{2+} metabolism of the erythrocytes, an alteration of Mg^{2+} metabolism cannot play a significant role in the pathogenesis of this disease, because these patients are still sick, although the Mg^{2+} metabolism in their erythrocytes was normal. Also, the suggestion that a different dietary status may cause a different $[Mg^{2+}]_i$ [16] shows that $[Mg^{2+}]_i$ in erythrocytes cannot be a significant parameter in essential hypertension. When an alteration of Mg^{2+} metabolism is involved in the pathogenesis of the tetanic syndrome or essential hypertension, it must concern cells other than erythrocytes which is in agreement with preceding results [8].

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