Measurements of Free Mg^{2+} Content by Potenciometry in Human Red Blood Cells

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Zusammenfassung

E ist eine Methode zur Feststellung von intrazellulärem ionischem Magnesium entwickelt worden, und zwar durch potentiometrische Ablesung einer teilweise selektriven Elektrode für Magnesiumionen.
Diese Methode ist für die Feststellung von Erythrozyten angewandt worden, wobei 0.38 mM ± 0.02 erzielt wurden (Durchschnitt ± S.D. von 12 Feststellungen). Eine systematische Studie über den Einfluß der vorbereitenden Handhabung zeigte außerdem, daß die Sonofizierung das schnellste und genaueste Mittel war.

Summary

A new method for ionic magnesium determination has been devised. It is based on the potenciometric reading by an electrode partially selective to magnesium ions.
The method has been applied to the determination of ionic magnesium in erythrocytes, obtaining 0.38 mM ± 0.02 (mean ± S.D. of 12 determinations). Moreover, a systematic study of the influence of the preparatory manipulation on the determinations showed that the minimal sonication was the fastest and more accurate method.

Résumé

Une méthode a été mise au point pour la détermination du magnésium ionique intracellulaire, moyennant la lecture potentiométrique d'une électrode partiellement sélective pour les ions magnésium.
Cette méthode a été appliquée à la détermination des érythrocytes, avec obtention de 0.38 mM ± 0.02 (moyenne ± S.D. de 12 déterminations). En outre, une étude systématique de l'influence de la manipulation préparatoire a démontré que la sonication a été le moyen le plus rapide et le plus précis.

Introduction

Intracellular magnesium regulates more than 300 enzymes, especially those related with energetic metabolism. It is a cofactor of several membrane ATPases and plays an important role in the membrane permeability and electrolyte transport [1]. Moreover, small changes in free Mg^{2+} concentration can strongly modify cell activity [2].
Ultrafiltrable concentration of divalent cations in plasma is easily determinable by dialysis or ultrafiltration techniques [3]. However, the techniques used to determine the ionic forms are less developed. Whereas, there is a specific and selective electrode for calcium, magnesium has not this tool [4]. Its determination on human red cells has been tried by nuclear magnetic resonance [5], with a metallochromic indicator dye [6, 7] or null point plasma membrane permeabilization with the ionophore A23187 [8], being all of them very complex and long.
The method chosen to determine the erythrocytic ionic magnesium (EMg^{++}) is based on the potenciometric reading by an electrode partially selective to the ionic Mg. The selectivity of the electrode to Mg^{++} versus other cations present in the erythrocytes [9], is Na^{+} (1/100), K^{+} (1/150), and Ca^{++} (1/0.8); other divalent cations like iron, zinc and copper can not interfere because their concentrations are too small and appear to be bound to proteins; cesium was found to be the less interfering cation.
In this paper we tried three hemolysis methods to take out the intraerythrocytic fluid: the hypoosmotic breaking, the freezing and the ultrasonic splashing.

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Methods

Thirty milliliters of blood from 12 healthy donors was collected in heparinized tubes, the separation of erythrocytes was immediatly made by centrifugation at 1500 g for 10 minutes. The plasma and the buffy coat were aspirated. Three quarters of the erythrocytes were washed once with a solution of CICs 150 mM, MOPS-TRIS 5 mM pH 7.4 at 37° C and 30 µM EDTA, the rest was washed with the same solution added on 200 µM EGTA and again everything twice with CsCI 150 mM. The last centrifugation was made at 6000 g for 15 minutes, an after which, the samples were divided into four aliquots, one was frozen (one hour at –20° C), an other was splashed in cold with a sonifier cell disruptor W 185 (Heat Systems-Ultrasonics Inc. Plainview, L.I., N.Y.) the third one was hemolized to

several dilutions 1/3, 1/5, 1/8 y 1/10 with bidestiled water, and the one washed with EGTA was also splashed.
The determinations of Na^+ and K^+ were performed with the Electrolyte 2 (Beckman Instruments Inc. Palo Alto California), the ionic calcium and the pH were determined with a Nova 8 (Nova Massachusetts) calibrated with calcium (1-0.01 mM) solutions in KCl 100 mM.
The determination of the ionic magnesium was made in a set-up that accommodate a divalent cation electrode (ORION 93-32), a single junction reference electrode with a filling solution of KCI 4M saturated with Ag, a pH electrode and a temperature sounding. This set-up allows keeping the temperature stabilized at 37° C, and getting readings from 0.5 ml of the sample in anaerobic conditions. The sensing module membrane is gradually coated by proteins, so it needs carefully cleaning, being its life reduced to about two to four weeks.
The ionic magnesium was calculated with a computer programme. We obtained the ionic magnesium concentration from the differences between Mg activities of the sample and a solution prepared with identical Na^+, K^+, Ca^{++} in each sample. All the results were referred to erythrocytic water at 37° C.

Results and Discussion

The dilution results of erythrocytic Na^+ and K^+ have a good linear relation with their dilutions, but the Ca^{++} and Mg^{++} results have parabolic relations which make difficult the calculation of the concentration before diluting them.
The breaking method results of the calcium, sodium, potassium and pH are in tab. 1. The Na^+ and K^+ results agree with the literature values, assuming that they are given in mmol/l of proteins free water. The pH differences between the frozen and splashing techniques are not significant.

Tab. 1: Effect of hemolysis methods on erythrocytic cationic determinations. Mean and standard deviation of ionic cations mM/L H_2O at 37° (Calcium, Sodium, Potassium and Magnesium) and pH of the erythrocytic fluid obtained by dilution (1/5), freezing, sonication and sonication with EGTA (200 μM in the first washing).

	Calcium	Sodium	Potassium	Magnesium	pH
Diluted 1/5	0.03 ± 0.01	3.1 ± 0.19	18.7 ± 0.98	0.20 ± 0.09	7.17 ± 0.04
Frozen	0.17 ± 0.01	13.0 ± 1.17	93.8 ± 3.88	0.49 ± 0.17	7.22 ± 0.03
Sonicated	0.05 ± 0.01	12.7 ± 1.01	95.7 ± 4.81	0.39 ± 0.03	7.20 ± 0.02
Son. + EGTA	< 0.01	12.7 ± 1.10	94.7 ± 4.81	0.38 ± 0.02	7.20 ± 0.02

With calcium, the results are higher than in the literature [10] due to the lack of membrane calcium washing. We avoid washing in order to be able to determine the concentration of Ca^{++} with the Nova 8 range. Moreover the results are quite different depending on the membrane breaking method, this may be due to the alteration of the membrane proteins that bind calcium. It is important to select the method that releases a minimum of calcium because this alteration may affect in the same way the magnesium release.
The magnesium electrode readings are very stable and repeatable with a coefficient of variation of 7 %. Our EMg^{++} values with 40 ± 4 mmHg of PO_2 agree with those given by Flatman [8]. The results of magnesium are indirectly affected by the washing temperature. The membrane permeability to the magnesium is very small [8] but the decrease of temperature inhibits the calcium and sodium pumping, allowing their passive influx and increasing their erythrocytic concentration. This diminishes the accuracy of the magnesium reading by this method.
Assuming a similar behavior for magnesium than for calcium, we suggest that the minimal sonication is the best broken method.
An advantage of this technique over the null point plasma membrane permeabilization, consists on the possibility of obtaining magnesium activity results without the interference of its association with Cl^- and $CO3H^-$.
The accuracy of this technique may be improved with new electrodes [11] more selective to Mg^{++}.

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