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An Na^+-stimulated Mg^{2+}-transport system in human red blood cells

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The initial rate of net Mg^{2+} efflux was measured in human red blood cells by atomic absorption. In fresh erythrocytes incubated in Na^+,K^+-Ringer's medium this rate was 7.3 ± 2.8 μmol/l cells per h (mean ± S.D. of 14 subjects) with an energy of activation of 13200 cal/mol. Cells with total Mg^{2+} contents ($[Mg]_i$) ranging from 1.8 to 24 mmol/l cells were prepared by using a modified *p*-chloromercuribenzenesulphonate method. Mg^{2+} efflux was strongly stimulated by increases in $[Mg]_i$ and in external Na^+ concentrations ($[Na]_o$). A kinetic analysis of Mg^{2+} efflux as a function of $[Mg]_i$ and $[Na]_o$ revealed the existence of two components: (i) an Na^+-stimulated Mg^{2+} efflux, which exhibited a Michaelian-like dependence on free internal Mg^{2+} content (apparent dissociation constant = 2.6 ± 1.4 mmol/l cells; mean ± S.D. of six subjects) and on external Na^+ concentration (apparent dissociation constant = 20.5 ± 1.9 mM; mean ± S.D. of four subjects) and a variable maximal rate ranging from 35 to 370 μmol/l cells per h, and (ii) an Na^+-independent Mg^{2+} efflux, which showed a linear dependence on internal Mg^{2+} content with a rate constant of $(6.6 \pm 0.7) \cdot 10^{-3}$ h^{-1}. Fluxes catalyzed by the Na^+-stimulated Mg^{2+} carrier were partially dependent on the ATP content of the cells and completely inhibited by quinidine (IC_{50} = 50 μM) and by Mn^{2+} (IC_{50} = 0.5–1.0 mM).

Introduction

Studies of Mg^{2+} fluxes are scarce due to the relative unavailability and very short half-life of ^{28}Mg, the only radioactive isotope of Mg^{2+} (for review see Ref. 1). Nevertheless, the existence of specific membrane transport systems for this cation has been clearly demonstrated. Erdos and Maguire [2] characterized a transport system in murine S49 lymphoma cells which is able to catalyze $^{28}Mg^{2+}$ and not $^{45}Ca^{2+}$ influx. This system is inhibited by β-adrenergic agonists and prostaglandin E_1. Baker and Crawford found a transport system in squid giant axon which catalyzes a $^{28}Mg^{2+}$ efflux in the presence of ATP and external Na^+ (Ref. 3; see also Refs. 4 and 5). In chicken erythrocytes, Günther et al. [6] observed that external Na^+ stimulates an energy-dependent net Mg^{2+} efflux. In sheep, cows and other animals, net gastrointestinal absorption of Mg^{2+}, which can be measured by atomic absorption, is active and linked to Na^+ transport [7]. Finally, Mg^{2+} efflux from heart [8] and liver [9] mitochondria is respiration-dependent.

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Abbreviations: $[Mg]_i$, total internal Mg^{2+} content; $[fMg]_i$, free internal Mg^{2+} content; $[bMg]_i = Mg^{2+}$ content bound to internal buffers; $[Na]_o$, external Na^+ concentration; V_1, Mg^{2+} leak; *k*, rate constant of Mg^{2+} leak; $V_{Na}(Mg)$, Na^+-stimulated Mg^{2+} efflux; V_{max}, $V_{Na}(Mg)$ at saturating $[Mg]_i$ and $[Na]_o$; K_{Mg}, apparent dissociation constant for internal Mg^{2+}; K_{Na}, apparent dissociation constant for external Na^+; Mops, 4-morpholinepropanesulphonic acid; DIDS, 4,4′-diisothiocyano-2,2′-disulphonic acid stilbene; PCMBS, *p*-chloromercuribenzenesulphonate.

The human red cell membrane exhibits a very low permeability to $^{28}Mg^{2+}$ [10,11]. This was a further reason for the relative absence of information concerning Mg^{2+} movements in these cells.

In recent years we have developed atomic absorption and flame photometry methods for the precise determination of Na^+ and K^+ transport in human erythrocytes [12,13]. We therefore tried to adapt these methods in order to accurately determine the kinetic properties of the initial rate of net Mg^{2+} efflux in these cells. This allowed the discovery of a transport system able to catalyze (Na^+- and ATP-dependent) outward Mg^{2+} movements across the human red cell membrane.

Methods

Preparation of red cells. 30–50 ml of venous blood were collected in heparinized tubes and centrifuged at $1750 \times g$ for 10 min at 4°C. The plasma and buffy coat were aspirated and the red cell pellet was washed four times with cold 150 mM NaCl (or 150 mM KCl) and used immediately.

Mg^{2+}-loading procedure. In order to study the effect of internal Mg^{2+} content on Mg^{2+} efflux, five to seven aliquots of cells containing different Mg^{2+} contents were prepared by using a modified PCMBS method.

Washed red cells were resuspended to a hematocrit of 6–8% in the different cold loading media. Five to seven different loading media were prepared by mixing varying amounts of 110 mM $MgCl_2$ and Na^+/K^+ medium. The Na^+/K^+ medium contained 15 mM NaCl and 135 mM KCl. PCMBS was added to both media up to a final concentration of 50 μM. The osmolality of the loading media was adjusted to 295 ± 5 mosmol/kg.

The loading solutions were devoid of phosphate or Tris buffers because after the recovery step (i) cells loaded for more than 5 h in cold phosphate buffers exhibited an extremely variable Mg^{2+} release, one or two orders of magnitude higher than normal; an effect which may result from adsorption or precipitation of magnesium phosphate at the membrane surface, and (ii) cation leak was increased in cells loaded with Tris. The pH of the loading solutions was about 5.5. Therefore, in all the experiments we added a control sample of fresh cells suspended in a (PCMBS-free) medium containing 15 mM NaCl and 135 mM KCl and 2.5 mM of sodium phosphate (pH 7.2 at 4°C) (see below). In addition, it is important to note that the red cells' buffering capacity increased the pH of the cell suspensions up to about 6.9.

The red cell suspensions were incubated in the loading solutions for 20 h at 4°C. At the end of the treatment with PCMBS, the cells were spun down at $1750 \times g$ for 5 min at 4°C and the supernatants were discarded.

The red cells were resuspended at a hematocrit of 20% in a 'recovering medium' containing (mM): 135 KCl, 15 NaCl, 5.5 potassium phosphate buffer (pH 7.4 at 37°C), 0.5 EGTA, 4 cysteine, 10 glucose, 3 inosine and 2 adenine. The final pH was adjusted to 7.4 at 37°C with Trizma base. The cell suspensions were incubated at 37°C for 1 h.

Mg^{2+}-loaded erythrocytes were washed five times with cold 150 mM NaCl (or 150 mM KCl) and resuspended in efflux medium at a hematocrit of 20–25%. For most of the experiments, three different efflux media were prepared: (i) Na^+,K^+-Ringer's medium containing (mM): 138 NaCl, 5 KCl, 10 glucose and 10 Mops-Tris buffer (pH 7.4 at 37°C) (Mg^{2+} efflux was not modified by replacing Mops-Tris with 2.5 mM sodium phosphate buffer, pH 7.4 at 37°C), (ii) Na^+ or K^+ media (see variation of the external cation composition). A portion of each cell suspension was set aside to measure hematocrit, hemoglobin by spectrophotometry and internal content of Mg^{2+} by atomic absorption (in an IL 457 Atomic Absorption Spectrophotometer). Internal contents of Na^+ and K^+ were measured by flame photometry (in an Eppendorf flame photometer) according to a previously published procedure [12,13].

Mg^{2+} fluxes in control cells (incubated in PCMBS-free media at pH 7.2) were similar to those in PCMBS-treated cells of similar Mg^{2+} content. This showed that after the recovery step, Mg^2 permeability was restored to normal values (see also Refs. 6 and 12).

Total internal Mg^{2+} content of the cells ranged from 1.8 to 24 mmol/l cells. Hemoglobin content per liter of cells (an indirect indicator of cell volume) was slightly increased in cells with high Mg^{2+} contents, indicating a moderate cell shrin-

kage. We therefore performed control experiments by adding different amounts of hypertonic Na^+ media to the PCMBS-loading solutions. The final Mg^{2+} fluxes were not significantly changed by cell shrinkage during the loading period (see also Results: the effect of osmolality).

Measurement of Mg^{2+} efflux. 0.5 ml of each cell suspension (Mg^{2+}-loaded or fresh erythrocytes) were added to twelve tubes containing 2 ml of efflux medium (the final hematocrit was approx. 4–5%). A duplicate of tubes was studied before and after incubation at 37°C for 1, 2, 3, 4 and 5 h. Incubation was stopped by sudden cooling to 4°C for 1 min. The tubes were then centrifuged at $1750 \times g$ for 4 min at 4°C. The supernatants were transferred into tubes for Mg^{2+} analysis (in an IL 457 Atomic Absorption Spectrophotometer) and for measurement of hemoglobin absorbance (at 540.5 nm).

Mg^{2+} efflux (V) was calculated from the slope (s) relating the external Mg^{2+} concentration with time (Fig. 1) by using the following equation:

$$V = \frac{s(100\% - \text{final hematocrit})}{\text{final hematocrit}} \quad (1)$$

The measurement of hemoglobin absorbance in the supernatants allowed us to calculate the real values of Mg^{2+} efflux by subtracting Mg^{2+} release due to a slight and variable red cell lysis from the measured Mg^{2+} efflux (see Fig. 1).

Variation of osmolality and pH. In order to study the influence of the osmolality, the Na^+/K^+-Ringer's medium was diluted with distilled water up to half of the original concentration. Different amounts of sucrose when then added and the final osmolalities were measured with a Fiske Osmometer. Fluxes were performed in these particular media using the above protocol.

The effect of external pH was studied by using the above protocol with the following modifications: (i) the pH values of the efflux media were modified by changing the relative concentrations of Tris and Mops, (ii) 10 μM DIDS was added to each efflux media in order to minimize variations in Cl^- distribution, cell volume, membrane potential and other parameters through changes in the ionic state of hemoglobin [14], and (iii) washed red cells were resuspended in each of the efflux media and incubated at 37°C for only 1 h.

Variation of the external cation composition. In order to study the effect of external Li^+, Na^+, K^+, Rb^+ and Cs^+, the red cells were washed with cold isotonic solutions of these cations and resuspended in efflux media containing: 150 mM XCl, 10 mM Mops-Tris (pH 7.4 at 37°C) and 10 mM glucose, where $X = Li^+$, Na^+, K^+, Rb^+ or Cs^+. All the other cations were added in powder or from freshly prepared concentrated stock solutions in water to the efflux media (Na^+,K^+-Ringer's, Na^+ or K^+ media) at different concentrations. In all cases the final osmolality was adjusted to 295 ± 5 mosmol/kg. Mg^{2+} efflux was measured according to the above protocol.

Variation of the anion composition. In experiments where Cl^- was replaced by NO_3^-, erythrocytes were washed with 150 mM $NaNO_3$ (or 150 mM KNO_3) and Cl^- in the efflux media was replaced by equivalent amounts of NO_3^-.

ATP depletion. Cells were depleted of ATP using a method previously described by Lew [15]. Briefly, before the flux experiment the erythrocytes were incubated for 30 min at 37°C in a medium containing: 135 mM KCl, 15 mM NaCl, 5 mM iodoacetamide, 5 mM inosine and 10 mM Mops-Tris (pH 7.4 at 37°C). The cells were then incubated in an efflux medium without glucose and Mg^{2+} efflux was measured as before.

Results

Mg^{2+} efflux in fresh human red cells

Mg^{2+} concentrations and hemoglobin absorbances were measured in the efflux medium ((Mg^{2+}-free) Na^+,K^+-Ringer's) of fresh erythrocytes. Fig. 1 shows that both parameters increased linearly with time for at least 5 h. Red cell lysis was about 0.035% per hour (see figure legend). External Mg^{2+} concentrations were therefore corrected for hemolysis (filled circles). Mg^{2+} efflux, calculated by regression analysis from the slope of this function ($r = 0.989$), was 7.6 μmol/l cells per h.

Taking into account the above considerations we measured Mg^{2+} efflux in fresh erythrocytes from 14 subjects and obtained a mean value of 7.3 ± 2.8 μmol/l cells per h (mean $\pm$ S.D.). No correlation was found with the sex or age of the studied subjects (data not shown).

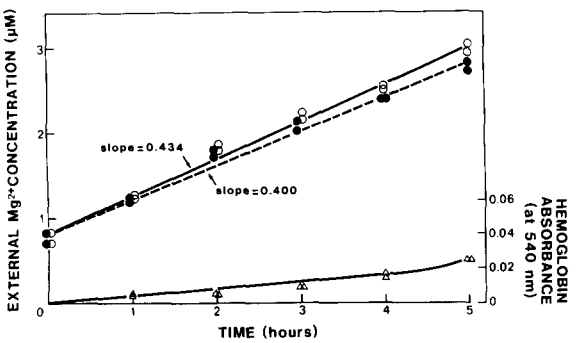

Fig. 1. Mg^{2+} efflux from fresh human red cells in Na^+,K^+-Ringer's medium (cells from a 35-yr-old healthy male). Measured values of external Mg^{2+} concentrations are indicated in open circles and hemoglobin absorbances in triangles. Both parameters increased linearly with time for at least 5 h. The slope of Mg^{2+} release (obtained by regression analysis; filled line, $r = 0.989$) was 0.434 μmol/l cells per h. External Mg^{2+} concentrations were corrected for hemolysis and indicated in filled circles (erythrocyte Mg^{2+} content = 2.2 mmol/l cells, final hematocrit = 0.052%, hemoglobin absorbance per liter of cells = 284 and rate of hemolysis = 0.035% per h). The slope of Mg^{2+} release corrected by red cell lysis (dotted line) was 0.400 μmol/l cells per h (note that hemolysis contributes with about 8% of total Mg^{2+} release). Mg^{2+} efflux (calculated by using Eqn. 1), was 7.6 μmol/l cells per h.

The effect of temperature, osmolality and pH

Fig. 2 shows an Arrhenius plot of Mg^{2+} efflux as a function of the temperature of incubation, it can be seen that this flux is strongly dependent on temperature (energy of activation = 13200 cal/mol).

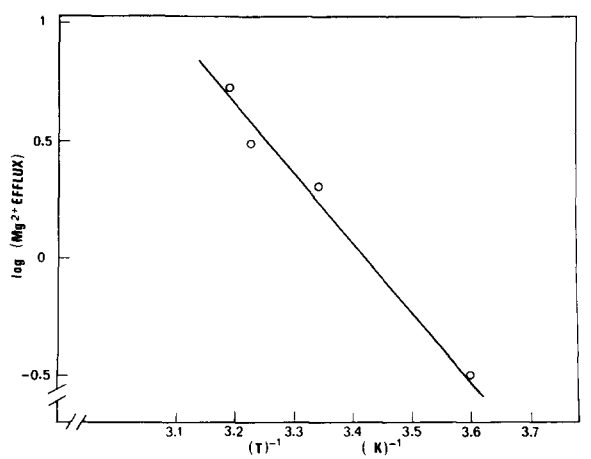

Fig. 2. Arrhenius plot of the effect of temperature on Mg^{2+} efflux in fresh human red cells. The energy of activation (calculated from the slope) was 13200 cal/mol.

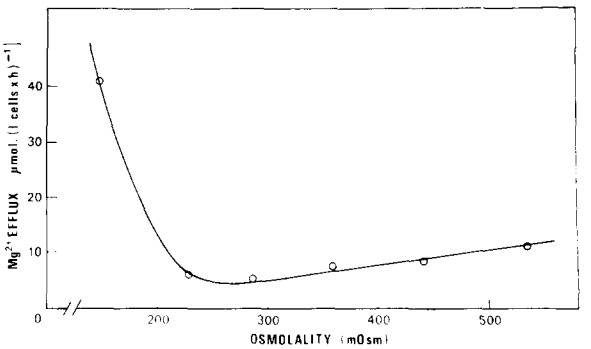

Fig. 3. Mg^{2+} efflux in fresh human red cells as a function of the external osmolality. A high flux-stimulation was observed in hypotonic medium (cell swelling). Hypertonicity (cell shrinking) slightly stimulated Mg^{2+} efflux. Similar results were obtained in two other experiments with fresh cells and in two experiments with Mg^{+2}-loaded cells.

Fig. 3 shows the effect of the osmolality. Flux stimulation was obtained by increasing and particularly by decreasing the osmolality of the incubation media.

Mg^{2+} efflux was slightly affected by pH. Indeed, only 20–30% of flux stimulation was brought about by a pH unit of acidification or alkalinization of the incubation media (data not shown).

The effect of the internal cation composition

Internal Mg^{2+} content. Mg^{2+} efflux was measured in cells with total Mg^{2+} contents ($[Mg]_i$) ranging from 1.8 to 24 mmol/l cells. Fig. 4 shows results obtained with erythrocytes from seven different blood donors. In all the cases, Mg^{2+} efflux was a sigmoidal and saturable function of $[Mg]_i$. The maximal rate was extremely variable among the seven individuals, ranging from 40 to 500 μmol/l cells per h (Fig. 4). Conversely, the EC_{50} of flux stimulation was 4–5 mmol/l cells for most subjects (Fig. 4). In most of the following experiments we used Mg^{2+}-loaded erythrocytes because the magnitude of the fluxes (and the experimental precision) was much higher than in fresh erythrocytes.

Internal Na^+ and K^+ contents. Mg^{2+} efflux was measured in cells with high Na^+ (and low K^+) or with high K^+ (and low Na^+) contents. Table I shows mean values of 11 experiments. It can be seen that Mg^{2+} efflux was almost the same in the two kinds of cells.

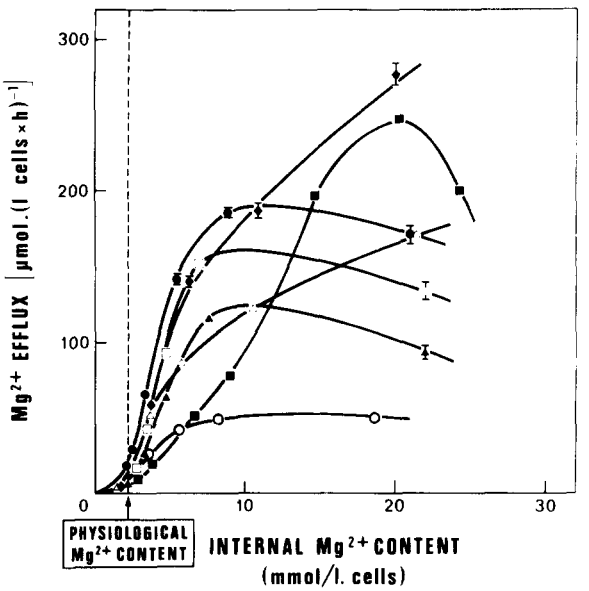

Fig. 4. Stimulation of Mg^{2+} efflux by increases in total erythrocyte Mg^{2+} contents. Small increases in physiological Mg^{2+} contents induced sharp flux-stimulations. The maximal rate of Mg^{2+} efflux exhibited high inter-individual variation.

The effect of the external cation composition

Several cations were added to the efflux medium up to concentrations just below the limit of toxicity (increase in passive cation permeability, hemolysis or agglutination) or solubility. Some divalent or trivalent cations (including Ca^{2+} at 10 mM, Sr^{2+} and Ba^{2+} at 1 mM, Be^{2+} at 0.05 mM and La^{3+} at 0.01 mM) slightly inhibited Mg^{2+} efflux (between 5 and 15%; data not shown). This inhibition was particularly significant with Mn^{2+}. Fig. 5 shows a dose-response curve where Mn^{2+} was able to inhibit 80% of Mg^{2+} efflux with an IC_{50} between 0.5 and 1 mM.

TABLE I

THE EFFECT OF INTERNAL CATION COMPOSITION ON Mg^{2+} EFFLUX FROM HUMAN RED CELLS

Values in this table are given as means ± S.D. of 11 experiments.

Cells	Internal cation content (mmol/l cells)			Mg^{2+} efflux (μmol/l cells per h)
	Na^+	K^+	Mg^{2+}	
High K^+	0.8 ± 0.2	106 ± 31	6.5 ± 2.7	71 ± 33
High Na^+	103 ± 13	1.6 ± 0.4	7.0 ± 3.5	70 ± 22 (n.s.)

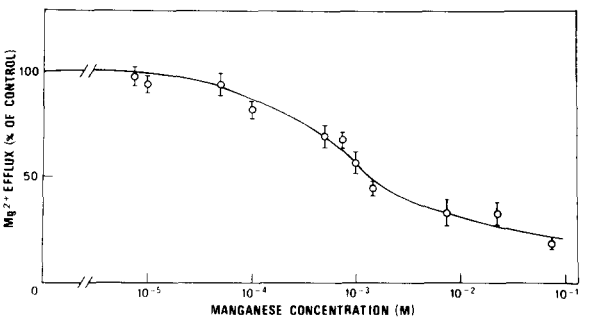

Fig. 5. Inhibition of Mg^{2+} efflux in human red cells by external Mn^{2+}. This cation was able to inhibit 50–80% of Mg^{2+} efflux with an IC_{50} between 0.5 and 1 mM. Values are given as means ± range of two experiments. The erythrocytes contained 18.0 ± 1.5 mmol of Mg^{2+} per liter of cells.

Of the monovalent cations tested (including Li^+, Na^+, K^+, Rb^+, Cs^+ and choline up to 150 mM and Tl^+ up to 0.1 mM), only Na^+ induced a significant change in Mg^{2+} efflux. Table II shows a mean value of Na^+-stimulated Mg^{2+} efflux ($V_{Na}(Mg)$) of 142 ± 35 μmol/l cells per h (mean ± S.D. of five subjects).

Interestingly, Mn^{2+} was able to inhibit Mg^{2+} efflux in Na^+ medium but not in K^+ medium (Table II). Indeed Table II shows that this cation at 1.5 mM concentration almost completely blocks $V_{Na}(Mg)$ (Na^+-stimulated Mg^{2+}-efflux).

Anion composition

The anion composition of the incubation media had little or no effect on Mg^{2+} efflux. Indeed, no effect was observed by replacing Cl^- with phos-

TABLE II

GENERAL PROPERTIES OF Na^+-STIMULATED Mg^{2+} EFFLUX IN HUMAN RED CELLS

Values in this table are given as means ± S.D. The number of experiments is indicated in brackets.

Condition	Mg^{2+} efflux (μmol/l cells per h)		
	K^+ medium	Na^+ medium	Na^+-stimulated
Control	151 ± 54 (5)	293 ± 89 (5)	142 ± 35 (5)
1.5 mM Mn^{2+}	141 ± 57 (4)	162 ± 61 (4)	21 ± 19 (4)
ATP-depleted cells	142 ± 21 (4)	182 ± 32 (4)	40 ± 21 (4)
NO_3^- medium	177 ± 20 (3)	311 ± 44 (3)	134 ± 29 (3)
0.5 mM quinidine	132 ± 48 (3)	130 ± 51 (3)	−2 ± 7 (3)

phate (up to 50 mM; data not shown) or with NO_3^- (Table II).

The effect of ATP

Total Mg^{2+} efflux was reduced by about 30–50% in ATP-depleted cells. Table II shows that this effect results from a 60–85% reduction of $V_{Na}(Mg)$ without any significant effect on Mg^{2+} efflux in K^+ medium.

The effect of drugs active on cation transport

Ouabain (0.1 mM), furosemide (1 mM), DIDS (10 μM) and bumetanide (20 μM) were not able to modify Mg^{2+} efflux (data not shown). Conversely, Mg^{2+} efflux was inhibited by cinchona alkaloids and phloretin (Fig. 6). The most powerful inhibitor, quinidine, was able to inhibit 40–80% of Mg^{2+} efflux with an $IC_{50} = 50$ μM (Fig. 6).

Table II shows that the effect of quinidine is exerted on the Na^+-stimulated fraction of Mg^{2+} efflux. Indeed, 0.5 mM of this drug was able to completely block $V_{Na}(Mg)$ (Table II).

A kinetic analysis of outward Mg^{2+} movements in human red cells

Fig. 7 shows Mg^{2+} efflux as a function of external Na^+ concentration $[Na]_o$. At each $[Na]_o$, $V_{Na}(Mg)$ was calculated by subtracting the Mg^{2+} efflux in K^+ medium from the total efflux. Fig. 7 (inset) shows a Hanes plot of the data. The obtained straight line strongly suggested that

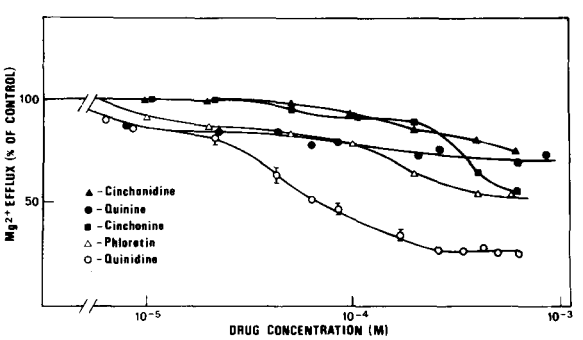

Fig. 6. Inhibition of Mg^{2+} efflux by cinchona alkaloids and phloretin. The most active agent, quinidine, was able to inhibit 40–80% of Mg^{2+} efflux with an IC_{50} of about 50 μM. The experiment represented in the figure was performed in Mg^{2+}-loaded cells. Values are given as means ± range. Similar results were obtained in two other experiments.

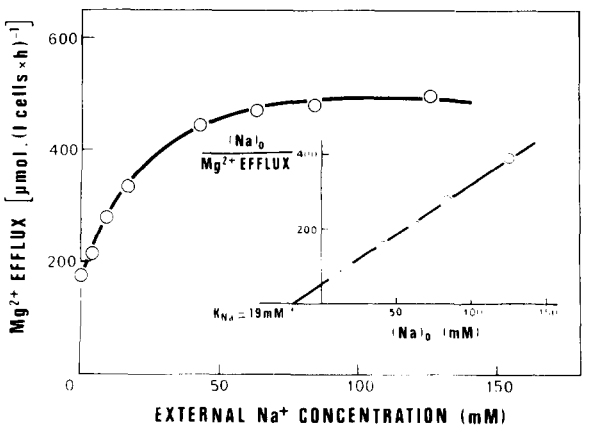

Fig. 7. Stimulation of Mg^{2+} efflux by the rise in external Na^+ concentration. Isotonicity was maintained by replacing NaCl with equivalent amounts of KCl. Inset: Hanes plot of the Na^+-stimulated Mg^{2+} efflux ($V_{Na}(Mg)$) as a function of $[Na]_o$. An apparent dissociation constant for external Na^+ of 19 mM was obtained from the intercept with the x-axis.

$V_{Na}(Mg)$ was a Michaelian-like function of $[Na]_o$:

$$V_{Na}(Mg) = \frac{V_{max}}{1 + (K_{Na}/[Na]_o)} \quad (2)$$

The apparent dissociation constant for external Na^+ (K_{Na}) was obtained from the intercept with the x-axis and the maximal rate (V_{max}) from the reciprocal of the slope (Fig. 7, inset). Table III shows mean values of these kinetic parameters. A constant value of $K_{Na} = 20.5 \pm 1.9$ mM (mean ± S.D.) was obtained in erythrocytes from four subjects (Table III). In contrast with this constancy of K_{Na}, V_{max} varied from 35 to 370 μmol/l cells per h.

We investigated the effect of external Na^+ on the stimulation of Mg^{2+} efflux by $[Mg]_i$. Fig. 8 shows a typical experiment where we measured Mg^{2+} efflux as a function of $[Mg]_i$ in Na^+ medium and in K^+ medium (or in Na^+ medium containing 0.5 mM quinidine).

Mg^{2+} transport depends on the free Mg^{2+} content of the cells ($[fMg]_i$), which is related to $[Mg]_i$ by:

$$[Mg]_i = [fMg]_i + [bMg_T]_i \quad (3)$$

where $[bMg_T]_i$ is the Mg^{2+} content bound to internal buffers.

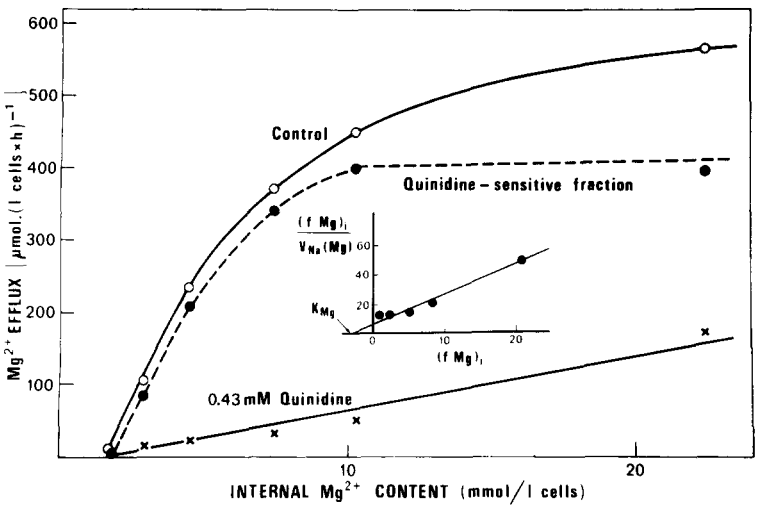

Fig. 8. Stimulation of Mg^{2+} efflux by increases in internal Mg^{2+} content in the presence and absence of 0.43 mM quinidine. It can be seen that Mg^{2+} efflux has two components: (i) a saturable, quinidine-sensitive Mg^{2+} efflux, and (ii) a linear quinidine-resistant Mg^{2+} efflux. Similar results were obtained in two other experiments and in three experiments where, instead of quinidine, Mg^{2+} efflux was measured in K^+ medium. Inset: Hanes plot of quinidine-sensitive Mg^{2+} efflux. Free Mg^{2+} content in the x-axis represents $[Mg]_i - C_{(0+1)}$ (see text).

A kinetic analysis of the interaction of Mg^{2+} transport with $[Mg]_i$ requires a detailed knowledge of $[bMg_T]_i$ as a function of $[fMg]_i$. Unfortunately this is a very complex function (see Ref. 19).

Flatman and Lew [19] found that $[bMg_T]_i$ comprises three intracellular buffer systems:

$$[bMg_T]_i = [bMg_0]_i + [bMg_1]_i + [bMg_2]_i \quad (4)$$

Two of them ($[bMg_0]$ and $[bMg_1]$) have low binding capacity (C_0 and C_1) and high affinity and a third one ($[bMg_2]$) has high capacity (C_2) and low affinity ($1/K_2$).

In the range of $[Mg]_i$ contents of our experiments (1.8–24 mmol/l cells), the numerical values obtained by Flatman and Lew [19] allow us to transform Eqn. 4 by assuming saturation for C_0 and C_1 and by setting $fC_2 \approx C_2$ into:

$$[bMg_T]_i \approx C_{(0+1)} + ([fMg]_i \times C_2)/K_2 \quad (5)$$

where $C_{(0+1)}$ is the binding capacity of $[bMg_0]$ and $[bMg_1]$ and fC_2 is unsaturated C_2.

Using Eqns. 3 and 5 we can easily obtain:

$$[fMg]_i(1 + (C_2/K_2)) \approx [Mg]_i - C_{(0+1)} \quad (6)$$

Eqn. 6 now allows a simple kinetic analysis of the data. Fig. 8 shows that the quinidine-resistant Mg^{2+} efflux was a linear function of $[Mg]_i$ with an intercept with the x-axis of about 2 mmol/l cells (similar results were obtained in K^+ medium). This is the kinetic behaviour predicted by Eqn. 6 for an Mg^{2+} leak (V_1) as a function of $[Mg]_i$:

$$V_1 = k[fMg]_i \approx k'[Mg]_i - k'C_{(0+1)} \quad (7)$$

where k is the rate constant of Mg^{2+} leak and k' is:

$$k' = \frac{k}{1 + (C_2/K_2)} \quad (8)$$

Eqn. 7 thus allowed us to obtain k' and $C_{(0+1)}$ from the slope and intercept with the x-axis of the quinidine resistant Mg^{2+} efflux as a function of $[Mg]_i$ (Fig. 8). Table III shows mean values of these kinetic parameters. Constant values of $k' = (6.6 \pm 0.7) \cdot 10^{-3}$ h^{-1} and of $C_{(0+1)} = 2.1 \pm 0.3$ mmol/l cells were obtained in erythrocytes from six different individuals (Table III).

Regarding $V_{Na}(Mg)$ (quinidine-sensitive Mg^{2+} efflux), this was obtained at each internal Mg^{2+} content, by subtracting the Mg^{2+} efflux in the presence of quinidine from that in its absence (Fig. 8, inset). This together with Eqn. 6 and the extrapolated values of $C_{(0+1)}$ allowed us to calculate $V_{Na}(Mg)$ as a function of $[Mg]_i$. Fig. 8 (inset) shows a Hanes plot of the data. The obtained linearity suggests that $V_{Na}(Mg)$ is a Michaelian-like function of free Mg^{2+} content.

$$V_{Na}(Mg) \approx \frac{V_{max}}{1 + (K'_{Mg}/([Mg]_i - C_{(0+1)}))} \quad (9)$$

where V_{max} is the maximal rate of $V_{Na}(Mg)$, K_{Mg}

TABLE III

A KINETIC MODEL FOR OUTWARD Mg^{2+} MOVEMENTS ACROSS HUMAN RED CELL MEMBRANES
Values in this table are given as means ± S.D. The number of subjects studied is indicated in brackets.

Mg^{2+} leak		Na^+-stimulated Mg^{2+} carrier		
k' ($10^3 \times h^{-1}$)	$C_{(0+1)}$ (mmol/l cells)	K_{Na} (mM)	K'_{Mg} (mmol/l cells)	V_{max} [a] (μmol/l cells per h)
6.6 ± 0.7 (6)	2.1 ± 0.3 (6)	20.5 ± 1.9 (4)	2.6 ± 1.4 (6)	143 ± 113 (10)

[a] In four subjects this was obtained from the dependence of $V_{Na}(Mg)$ on $[Na]_o$ and in six other subjects it was obtained from the dependence of $V_{Na}(Mg)$ on $[Mg]_i$.

is the apparent dissociation constant for internal Mg^{2+} and K'_{Mg} is:

$$K'_{Mg} = K_{Mg}(1 + (C_2/K_2)) \quad (10)$$

Table III shows mean values of these kinetic parameters (calculated from the Hanes plot). A constant value of $K'_{Mg} = 2.6 \pm 1.4$ mmol/l cells and a variable $V_{max} = 143 \pm 113$ μmol/l cells per h were obtained in erythrocytes from different blood donors (Table III).

Discussion

The human red cell membrane has been widely used for studying the molecular mechanism of ion transport. Several transport systems for Na^+, K^+, Ca^{2+}, Cl^- and HCO_3^- have been characterized [12,16–18]. In contrast, due to methodological reasons, Mg^{2+} transport has remained almost unexplored. We show here that the initial rate of net Mg^{2+} efflux can be accurately measured in human red cells by atomic absorption. We thus used this method in order to investigate the kinetic properties of outward Mg^{2+} movements across the red cell membrane.

The main result of our study is the finding that human erythrocytes contain a transport system which catalyzes outward Mg^{2+} movements in the presence of external Na^+. These fluxes are partially dependent on the ATP content of the cells and completely inhibited by quinidine and Mn^{2+}. The remaining Na^+-independent Mg^{2+} efflux exhibits properties compatible with an Mg^{2+} leak, i.e.: (i) linear dependence on free internal Mg^{2+} content and (ii) independence of the (cis and trans) concentration of other cations and of ATP. However, the magnitude of this Mg^{2+} leak (calculated from Eqn. 8 and Ref. 19) is about $45 \cdot 10^{-3}$ h^{-1}, a value higher than the membrane leaks for Na^+ and K^+ [12] or Ca^{2+} [18]. This does not result from the PCMBS treatment because a similar value can be calculated for fresh erythrocytes in K^+ medium. It may indicate that a fraction of Na^+-independent Mg^{2+} efflux is mediated by some other low-affinity Mg^{2+}-transport system.

It is hard to ascribe Na^+-stimulated Mg^{2+} fluxes to any of the ion-transport systems already described in human red cells. Indeed, it is well established that the Ca^{2+} pump cannot move Mg^{2+} out of the cells [16]. In addition, the lack of effect of ouabain, bumetanide and DIDS strongly suggests that the Mg^{2+} carrier is different from the Na^+ pump, the Na^+,K^+-cotransport system or the anion carrier. Curiously, it shares a common sensitivity to quinine and quinidine with the Ca^{2+}-dependent K^+ channel [18]. However, it is hard to believe that a channel which can discriminate between K^+ and Na^+, which is opened by a rise in cytosolic free Ca^{2+} content and which catalyzes extremely rapid fluxes of K^+, can catalyze slow Mg^{2+} effluxes in low Ca^{2+} erythrocytes. Finally, we do not believe that Mg^{2+} movements are catalyzed by a $Na^+ : Ca^{2+}$ exchange because this system does not appear to exist in human red cells [18].

Na^+-stimulated Mg^{2+} efflux thus appears to be catalyzed by a new erythrocyte transport system. Interestingly, this system shares common properties with the Mg^{2+} carriers of squid axon and chicken erythrocytes, i.e., stimulation by external Na^+ and ATP or another metabolic factor [3–6] and with the Mg^{2+} carrier of lymphoma cells, i.e., inhibition by external Mn^{2+} [2].

A kinetic analysis of the interaction of the erythrocyte Mg^{2+} carrier with external Na^+ indicated that under physiological conditions this system operates under maximal Na^+-stimulation. Regarding the interaction with internal Mg^{2+}, the kinetic analysis was hampered by the fact that free Mg^{2+} content is a complex function of total internal Mg^{2+}. A simplified equation was obtained from the model of Flatman and Lew [19]. Interestingly, we obtained a binding-capacity for the high-affinity buffers ($C_{(0+1)} = 2.1 \pm 0.3$ mmol/l cells in Table III) which is slightly higher than that reported by these authors (1.2–1.3 mmol/l cells) [19]. This is due to the fact that the equations of Flatman and Lew predict a slight positive deviation with respect to the approximation of Eqn. 7. Indeed, a careful inspection of Fig. 8 (quinidine-resistant Mg^{2+} efflux) shows such a positive deviation.

Eqn. 10, Table III and the values of C_2 and K_2 reported by Flatman and Lew [19] allow an estimation of K_{Mg} of about 0.4 mmol/l cells, a value slightly higher than the physiological internal Mg^{2+} content. This agrees with the sharp stimulation of Mg^{2+} efflux by small increases in physiological Mg^{2+} contents and strongly suggests that this system may regulate erythrocyte free Mg^{2+} content. On the other hand, the free Mg^{2+} content of the cells is much below the value expected from chemical equilibrium [19]. We are thus tempted to speculate that the Na^+-stimulated Mg^{2+} carrier can move Mg^{2+} in an uphill direction against its electrochemical gradient. The energy may be supplied by ATP hydrolysis or by net inward Na^+ influx. Indeed, the trans-stimulation by external Na^+ suggests a possible exchange of internal Mg^{2+} for external Na^+. Unfortunately, the investigation of this point requires the difficult measurement of the effect of internal Mg^{2+} on $^{22}Na^+$ influx and the still more difficult measurement of net Mg^{2+} fluxes in Mg^{2+} media.

In conclusion, we report here that a significant fraction of outward Mg^{2+} movements in human red cells is catalyzed by an Na^+-stimulated Mg^{2+} carrier. These fluxes are partially dependent on the ATP content of the cells and completely inhibited by quinidine and Mn^{2+}. The Na^+-stimulated Mg^{2+} transport system may be responsible for the maintaining and regulation of a low free internal Mg^{2+} content. This is of critical importance for the activity of those enzymes and membrane transport systems where Mg^{2+} acts as a cofactor.

References

1 Flatman, P.W. (1984) J. Membrane Biol. 80, 1–14
2 Erdos, J.J. and Maguire, M.E. (1983) J. Physiol. 337, 351–371
3 Baker, P.F. and Crawford, A.C. (1972) J. Physiol. 337, 351–371
4 De Weer, P. (1976) J. Gen. Physiol. 68, 159–178
5 Mullins, L.J., Brinley, F.J., Spangler, S.G. and Abercrombie, R.F. (1977) J. Gen. Physiol. 69, 389–400
6 Günther, T., Vormann, J. and Förster, R. (1984) Biochem. Biophys. Res. Commun. 119, 124–131
7 Martens, H. (1983) Br. J. Nutr. 49, 153–158
8 Crompton, M., Capano, M. and Carafoli, E. (1976) Biochem. J. 154, 735–742
9 Silipandri, D., Toninello, A., Zoccarato, F. and Silipandri, N. (1977) Biochem. Biophys. Res. Commun. 78, 23–27
10 Rogers, T.A. (1961) J. Cell. Comp. Physiol. 57, 119–121
11 Ginsburg, S., Smith, J.G., Ginsburg, F.M., Reardon, J.Z. and Aikawa, J.K. (1962) Blood 20, 722–729
12 Garay, R.P., Adragna, N., Canessa, M. and Tosteson, D.C. (1981) J. Membrane Biol. 62, 160–174
13 Garay, R.P., Nazaret, C., Diez, J., Etienne, A., Bourgain, R., Braquet, P. and Esanu, A. (1984) Biochem. Pharmacol. 33, 2013–2020
14 Wieth, J.O. (1966) Acta Physiol. Scand. 68, 234
15 Lew, V. (1971) Biochim. Biophys. Acta 233, 827–830
16 Sarkadi, B. and Tosteson, D.C. (1979) in Membrane Transport in Biology. II. Transport across single biological membranes (Giebisch, G., Tosteson, D.C. and Ussing, H.H., eds.), pp. 117–160, Springer Verlag, Berlin
17 Gunn, R. (1979) in Membrane Transport in Biology. II. Transport across single biological membranes (Giebisch, G., Tosteson, D.C. and Ussing, H.H., eds.), pp. 59–80, Springer Verlag, Berlin
18 Lew, V.L. and Beaugé, L. (1979) in Membrane Transport in Biology. II. Transport across single biological membranes (Giebisch, G., Tosteson, D.C. and Ussing, H.M., eds.), pp. 81–115, Springer Verlag, Berlin
19 Flatman, P.W. and Lew, V.L. (1980) J. Physiol. (London) 305, 13–30