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Asymmetry of the magnesium sodium exchange across the human red cell membrane

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(1) Net Mg^{2+} inflow into human red cells through the Mg^{2+}–Na^+ exchange system is slower, for a given driving force defined by the ionic gradients and E_m, than outflow for a similar and opposite force. This is not incompatible with an asymmetric, equilibrating exchange carrier. (2) However, the finding that near zero force the rate of outflow does not tend towards zero implies an active component, i.e., direct input of metabolic energy in addition to the energy provided via the Na^+-concentration gradient by the Na^+/K^+-pump.

In the last eight years it has become clear that red blood cells from many species (birds [1,2], rats [3], ferrets [4,5] and humans [6–8]) display an outward trans-membrane transport of magnesium which depends on external sodium and requires the presence of ATP inside the cells [9]. This resembles the Mg^{2+} transport system in the giant axon of the squid [10,11] and is considered to be a Mg^{2+}/Na^+ antiport, activated by phosphorylation via a protein kinase. Curiously, the system cannot easily be induced to reverse the direction of the ionic movement. Recently, Flatman [5] has presented convincing evidence for reversal of the transport in ferret red cells. However, these differ from human erythrocytes in being high-Na cells provided with a rapid Ca^{2+}/Na^+ exchange [12,13]. Failure to invert the transport direction was noticed in human cells by Lüdi et al. [7] and in rat cells by Günther et al. [3]. In these experiments $[Mg_i^{2+}]$ was low [7] or not reported [3]. Mg^{2+} is quite likely to be necessary for a conditioning protein phosphorylation which in turn presumably is essential regardless of the direction of transport.

An attempt was made, therefore, to amend the procedure. Human red cells were treated with *p*-chloromercuribenzene sulfonic acid (PCMBS) in order to load them with or deplete them of Na^+ and simultaneously increase their Mg^{2+} content. Subsequent incubation with cysteine restored the high cation impermeability (e.g., Na_i^+ fell from 110.5 mM to 105 mM during 4 h in a K-medium with ouabain at 37°C). Free $[Mg_i^{2+}]$ was 2.74 mM and the media contained 1.64 mM, thus $\bar{E}_{Mg}$ was −6.9 mV (see legend to Fig. 1). Since even at high $[Na_i^+]$ and low $[Na_o^+]$ the membrane potential (E_m) should not deviate from −10 mV the electrochemical gradient for Mg^{2+} was slightly inward. Cells with ~100 mM Na_i^+ (Na-cells) were incubated in a medium of 2 mM Na_o^+ (Fig. 1, line *c*) and compared to cells with 6.4 mM Na_i^+ in a medium of ~100 mM Na_o^+ (K-cells, also treated with PCMBS; Fig. 1, line *b*). A third batch of cells was kept at nearly zero Na^+-gradient (Fig. 1, line *d*) with ~100 mM Na^+ on each side of the membrane. Each experiment was accompanied by a control of K-cells (3.5 mM Na_i^+) in a low Na^+-medium (0.8 mM). In human cells K_{Na} is 16–20 mM on both sides, K_{Mg} 1.3–2.6 mM on the inside [6,7] while in bird cells K_{Mg} is 0.2 mM on the outside [15]. Thus high $[Na^+]$ was well above K_{Na} and $[Mg_i^{2+}]$ was near or above K_{Mg}, while the same may be presumed for $[Mg_o^{2+}]$. From Fig. 1 it may be seen that the Mg^{2+}-movement was in the outward direction in all the arrangements and that the rate decreased during the 4 h of the experiment. It seems possible that the initial rate exceeded the later rate owing to some Mg^{2+} emerging from exhaustible sources such as the membrane surface or a fraction of damaged cells. Therefore, only the late part of the experiment (2–4 h), when the control no longer showed much Mg^{2+} release (10 μmol/(l cells) per h), is taken into account.

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During this period the 'normal' arrangement of Na_i^+ and Na_o^+ produced the expected Mg^{2+} outward movement at a rate of 51 μmol/(l cells) per h. In contrast, the inverse Na^+ arrangement did not lead to a measurable Mg^{2+} translocation across the membrane, although from a symmetrical Mg^{2+}/Na^+ exchange system an uptake of more than 50 μmol/(l cells) per h is expected. There is compelling reason for this expectation in the fact that the driving force in the inverted arrangement is opposite and larger than in the normal arrangement for all the possible transport stoichiometries (n). This is easily seen if the distance from equilibrium is calculated for both instances. A simple way to do this is solving the equilibrium equation

$$\frac{Mg_i^{2+}}{Mg_o^{2+}} = \left(\frac{Na_i^+}{Na_o^+}\right)^n \exp\left[(n-2)\cdot \bar{E}_{ex}\cdot \frac{F}{RT}\right]$$

for $\bar{E}_{ex}$, the equilibrium potential of the exchanger, at each value of n (the number of Na^+ ions transported per one Mg^{2+} ion) with the four given concentrations. $\bar{E}_{ex}$ is the membrane potential at which there is not net ionic movement by exchange. $\bar{E}_{ex}$ must not be confused with $\bar{E}_{Mg}$ mentioned above, at which there is no Mg^{2+} movement through diffusional pathways. Subtracting $\bar{E}_{ex}$ from the actually prevailing membrane potential (E_m) of -10 mV gives the distance from equilibrium in volts (for $n=2$ the ion movement is electrically neutral; thus $(Na_i^+/Na_o^+)^2 = (r_{Na})$ is converted to the electrical potential $E_{Na} = (RT/F)\ \ln(r_{Na})$ and this is subtracted from 13.8 mV ($2\ \bar{E}_{Mg}$)). It is seen from Table I that for the cells with the inverted Na^+-gradient the driving force on Mg^{2+} for all stoichiometries is inward and larger than in those with normally oriented Na^+-gradient, yet no inward Mg^{2+} flow is detectable. This is not due to the ionic concentrations being far below half-saturation (see above). In view of the considerable scatter of the data the deviation from the slightly outward flow in the control may or may not signal some inward flow. However, an inward flow of ~ 50 μmol/(l cells) per h might easily be demonstrated statistically. It seems indisputable, therefore, that compared with the normal situation the reversal of the Na^+ gradient results in a much slower net flow of cations through the exchanger. At the very small outward driving force for high Na_i^+ and Na_o^+ (line d in Fig. 1) the outflow of Mg^{2+} was 31 μmol/(l cells) per h, more than 1/2 of what is indicated by line b.

What does the asymmetry mean?

(1) It is unlikely that the PCMBS treatment wrecks the system in such a selective way.

(2) High internal Na^+ does not poison the system because its inhibitory effect on Mg^{2+}-outflow is competitively overcome by Na_o^+ [7].

(3) Asymmetric equilibrating carriers are well known; they behave like valves and do not conflict with the second law of thermodynamics [14]. However, if the net flow at, e.g., infinite cis concentration from inside is faster than from outside the intrinsic, and thus also the apparent, K_{diss} must be commensurately larger on the inside. Otherwise zero rate at equal concentrations (and no electric potential) will not obtain (which indeed is the obvious thermodynamic constraint). In the present experiments the ionic concentrations were not saturating but near or above $K_{0.5}$. There is evidence that the affinities for Na^+ are similar on both sides of the membrane [7,8], however, for human cells the external $K_{0.5}$ for Mg^{2+} is unknown, whereas Günther and Vormann showed for bird cells that it is, in the absence of Na_o^+, 0.2 mM [15], while the internal $K_{0.5}$ for Mg^{2+} is similar as in human cells (3.5 mM [1]). This then would agree with unequal rates of flow in opposite direction.

(4) Plotting the rates of Mg^{2+} flow from the three arrangements (b, c, d) in Fig. 1 against calculated deviation from equilibrium for the passive exchanger (Fig. 2), however, suggests that there is net outward transport at zero force. The inset presents a similar experiment with eight data points taken from Ref. 7 which shows that the plot used results in an upward concave curve near zero driving force. Thus it is impossible that the lines in the main Fig. 2 pass through both

TABLE I

Distance of the Mg^{2+}/Na^+ exchanger from equilibrium expressed in mV as a measure for the driving force [a] acting on Mg^{2+}

$[Mg^{2+}]_i = 2.74$ mM (in cell-H_2O), $[Mg^{2+}]_o = 1.64$ mM; $E_m = -10$ mV. n = transport stoichiometry Na^+: Mg^{2+}.

	mV [b] (mean (±S.E.) [d])		
n:	1:1	2:1	3:1
Na-cells in K-medium [c]	−102 (±8)	−200 (±13.5)	−295 (±20)
Na-cells in Na-medium [c]	+0.45 (±2)	+7.3 (± 3.5)	+13.9 (± 5)
K-cells in Na-medium [c]	+77 (±4)	+160 (± 5)	+242 (± 8)

[a] Force on Mg^{2+} directed inwardly is taken as negative.

[b] Multiplying the values in mV by 0.09648 gives the electrochemical potential difference in kJ mol^{-1}.

[c] Na^+-concentrations as indicated in Fig. 1.

[d] The error is calculated from the S.E. of the ionic concentrations.

point *d* and the origin. Since the rate at point *d* is statistically different from the rate in the control this means that there is a (Na^+-dependent) Mg^{2+} outflow in the absence of a driving force exerted by the electrochemical potential of Na^+ or Mg^{2+}.

The tentative conclusion, therefore, seems that the unequal rate of Mg^{2+} transport in opposite directions by no means rules out that the system for Na^+-dependent Mg^{2+} translocation is a simple, passive exchange

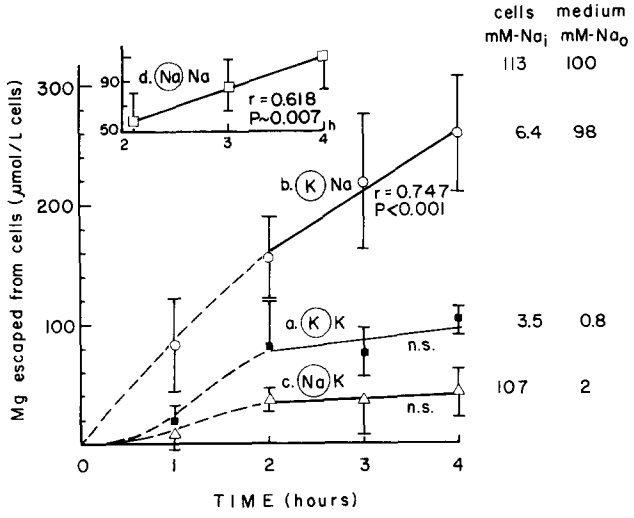

Fig. 1. Time course of Mg^{2+}-release from human red cells loaded to 8.95 mM Mg (giving 2.74 mM Mg^{2+} in the cell water) and the Na^+ concentrations (mean between 0 and 4 h in cell water) indicated. Na_i^+ and Na_o^+ are replaced by K^+. Circled K next to Na means K-cells in Na-medium, etc. Line (*a*) is a control at low Na^+ on both sides. Six experiments with cells from six donors, mean ± S.E. r = correlation coefficient if data are normalized by setting value at 4 h to 1; P = chance probability, n.s. = not statistically significant. Rate (slope of line) used in Fig. 2, from 2–4 h is (μmol/(l cells) per h) 10 in (*a*), 51 in (*b*), 3 in (*c*) and 31 in (*d*). Medium: (mM) NaCl + KCl 135, Tris-HCl 20, $MgCl_2$ 1.64 glucose 10 (in four experiments 0.17 mM ouabain was present in (*c*)), pH 7.4, 37°C, haematocrit 45.8 ± 0.3% (S.E.). Notice that when the leak in (*a*) has fallen to insignificant rate (after 2 h) outflow of Mg is rapid in (*b*), zero instead of negative in (*c*) and more than 1/2 of maximum in (*d*) (which overlies (*a*) and for clarity is moved up). Methods: For loading, intact cells (used within 4 days of collecting) were incubated at 5% haematocrit for 21 h at 4°C in 0.075 mM *p*-chloromercuribenzene sulfonic acid in NaCl or KCl 150 mM and $MgCl_2$ 60 mM. They were washed and recovered for 1 h at 37°C in (mM) cysteine 6 (to bind the mercurial), NaCl or KCl 140, $MgCl_2$ 1.5, Na-phosphate buffer 0.5 (pH 7.4), EGTA 0.1, glucose 10, inosine 3, adenine 2. Mg^{2+} was measured by atomic absorption flame photometry in dilutions of the medium and after trichloroacetic acid precipitation in cell dilutions. Samples and standards contained 62.5 mM $LaCl_3$. Changes in [Mg_o^{2+}] indicating flow rates, were carefully corrected for Mg^{2+}-loss due to haemolysis [7], which on average was 0.5% ± 0.03% (S.E.) in 2 h and similar for *a*, *b*, *c*, *d*. To assess free intracellular [Mg] magnesium-binding inside cells was measured according to Ref. 8 in four separate batches of cells not treated with PCMBS but incubated for 1 h at 37°C in 10 mM inosine to raise [ATP] as in the recovery procedure. Omitting PCMBS seemed admissible because it is unlikely that its presence alters concentrations of proteins, organic phosphates and the like; its membrane action is quite specific for small cations [16] and its action on enzymes is perfectly reversed by cysteine [17].

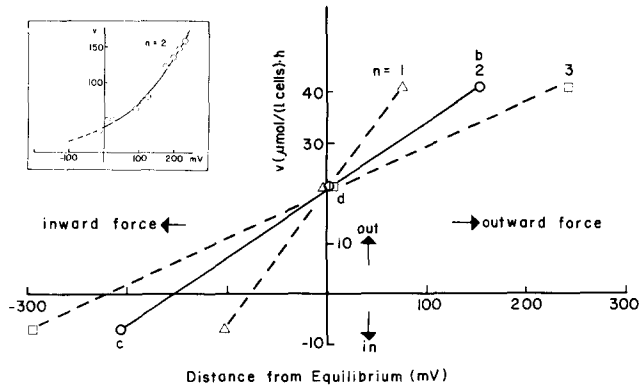

Fig. 2. Plot of rates from Fig. 1 versus driving force from Table I for three stoichiometries (*n*). Note that, even at larger force, inflow is slower than outflow and that there is net outflow at nearly zero driving force. Point *d* is statistically significantly different from zero (see *d* in Fig. 1) and (with $P \sim 0.024$) from control rate (*a* in Fig. 1) which, however, does not and should not differ significantly from zero. Nevertheless, Fig. 2 is corrected for this spurious outward rate in the control. The inset (same coordinates as main figure) shows a previous experiment (Fig. 5 from Ref. 7), unsuitable to test for large inflow because done at 0.02 mM Mg_o^{2+}, but demonstrating that near zero force the resulting curve is upward concave which precludes that in the main figure it passes through point *d and* the origin (the curvature is in the same sense also for $n = 1$ and $n = 3$).

carrier, but that the behaviour near electrochemical equilibrium warns against rash conclusions. It implies the possibility that some input of metabolic energy, assisting Na_o^+-driven Mg^{2+} outflow and opposing Na_i^+-dependent Mg^{2+} inflow, contributes to the asymmetry. If such were the case the system certainly still would differ from any ATP-driven P-type ion pump because it is not even partially inhibited by vanadate [8].

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