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THE AFFINITY OF THE Ca^{2+} PUMP OF HUMAN ERYTHROCYTES FOR EXTERNAL Na^+ OR K^+

PEDRO J. ROMERO and ENEIDA ROMERO

Departamento de Biologia Celular, Facultad de Ciencias, UCV, Apartado 47114, Los Chaguaramos, Caracas (Venezuela)

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Ca^{2+} extrusion from human erythrocyte ghosts, resealed to Na^+ and K^+, was stimulated along a rectangular hyperbola, by raising the external Na^+ or K^+ concentration. The results suggest the presence on the Ca^{2+} pump of an alkaline cation-binding site, which possesses greater affinity for K^+ than for Na^+.

The Ca^{2+} pump of human erythrocytes is fundamental for maintaining the shape and integrity of the cell. Activity of this pump prevents both inhibition of the Na^+ pump and opening of the K^+ channel by internal Ca^{2+}.

Ca^{2+} pump modulation seems a complex function in human red cells, as may be inferred from the number of effectors which have been described occurring physiologically [1–4]. Among such modulators, Na^+ and K^+ are specially important [5–8].

Previous work on human red cells has provided some evidence that ($Ca^{2+}+Mg^{2+}$)-ATPase and Ca^{2+} extrusion are stimulated to a similar extent by each cation [9]. Moreover, the same activating effect of Na^+ and K^+ at low or high Ca^{2+} concentrations is obtained for both enzyme activity [5] and active transport [10,11]. However, a systematic study of the action of these ions on active Ca^{2+} efflux is lacking at present. This circumstance seems to arise from the difficulty of maintaining effective gradients of Na^+ and K^+ the membrane, while measuring Ca^{2+} extrusion.

In the present work, we have studied the stimulation by external Na^+ and K^+ of Ca^{2+} extrusion from human erythrocyte ghosts, which had been resealed to alkaline cations. Ghosts were prepared from fresh blood (mainly O(+) group), essentially as described earlier [11]. After restoring isotonicity with K^+, Na^+ or choline chloride, ghosts were resealed to alkaline cations in the presence of Na_2^+-ATP and an excess of Ca^{2+} over EGTA. The ghost-free Ca^{2+} was about 10–20 μM at the end of resealing. This concentration was sufficient to saturate the pump during subsequent incubation.

Ghosts were finally incubated for up to 6 min at 37°C and at a haematocrit of 5%, in a choline medium similar to that described in legend to Fig. 1. Where appropriate, choline was replaced by an osmotically equivalent amount of NaCl (0–160 mM) or KCl (0–40 mM). Ghosts were further treated as described under method 'b' by Romero [11].

Due to unbalanced Na^+ or K^+ movements, ghosts shrank after incubation. In order to correct for these volume changes, the cation content of ghosts was referred to the original volume, using ghost haemoglobin as a volume index.

The Na^+ concentration of high-K^+ ghosts increased more or less linearly with time after incubation with Na^+ (Fig. 1a; lower graph). The rate of entry being about 0.2, 0.4 and 0.6 μequiv. Na^+/ml ghosts per min at 40, 80 and 160 mM NaCl, respectively. These ghosts also decreased their K^+ content by about 20 μequiv./ml ghosts when incubated for 6 min with or without 40 mM Na^+ (Fig. 1a; upper graph). Increasing Na^+ above

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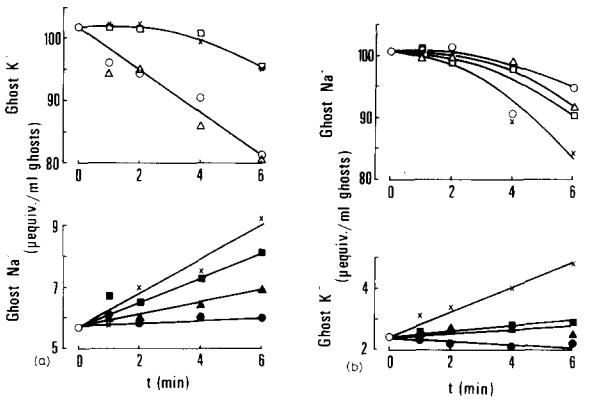

Fig. 1. Maintenance of effective Na^+ and K^+ gradients by dextran-resealed ghosts. Human erythrocyte ghosts (0.5 vol.), resealed to alkaline cations in dextran solutions, were incubated with 9.5 vol. of a medium containing (mM): choline chloride, 170; $CaCl_2$, 5; adenine, 5; inosine, 10; imidazole-HCl, 20 (pH 6.8 at 37°C) and increasing NaCl (0–160 mM) or KCl concentrations (0–40 mM). Isotonicity was maintained by replacing choline for an osmotically equivalent amount of Na^+ or K^+. The Na^+ or K^+ concentration of high-K^+ ghosts after incubation in the presence of 0(○,●); 40 (△,▲); 80 (□,■) and 160 mM NaCl (X) for the time indicated above, is shown in part a. The corresponding cation content of high-Na^+ ghosts after incubation with 0(○,●); 10 (△,▲); 20 (□,■) and 40 mM KCl (X) is given in part b. Results from a typical experiment are presented.

40 mM markedly reduced K^+ loss.

The K^+ content of high-Na^+ ghosts was slightly raised after 6 min incubation with 10 or 20 mM K^+, whilst in the presence of 40 mM K^+ it was almost doubled (Fig. 1b; lower graph). Under the latter condition, by contrast, internal Na^+ decreased by about 20 μequiv./ml ghosts (Fig. 1b; upper graph).

The increased Na^+ loss found at high external K^+ cannot be accounted for by Na^+ pump activity, for the following reasons. First, the pump rate is relatively slow (about 3 μequiv./ml cells per h). Secondly, it should be inhibited by the high Ca^{2+} concentration attained in ghosts.

The above results demonstrate that Na^+ or K^+ ions leak into the medium during incubation. However, as the incubation haematocrit was 5%, an external contamination of 1 mM at most is expected due to leakage from ghosts. On the other hand, the concomitant entry of Na^+ or K^+ was relatively small. Therefore, it can be safely assumed that a gradient of alkaline cations was effectively maintained across the ghost membrane.

The Ca^{2+} concentration of high-K^+ ghosts was decreased linearly with time during incubation in the absence of Na^+, being reduced by about 0.6 μmol/ml ghosts after 6 min (results not shown). Addition of Na^+ did not alter linearity but further diminished ghost Ca^{2+}, which was decreased by about 1.1 μmol Ca^{2+}/ml ghosts over the same period. Essentially identical results were obtained with high-Na^+ ghosts by increasing K^+ up to 40 mM.

Ca^{2+} extrusion from high-Na^+ or $-K^+$ ghosts occurred at a rate of about 0.10–0.14 μmol Ca^{2+}/ml ghosts per min in the absence of external

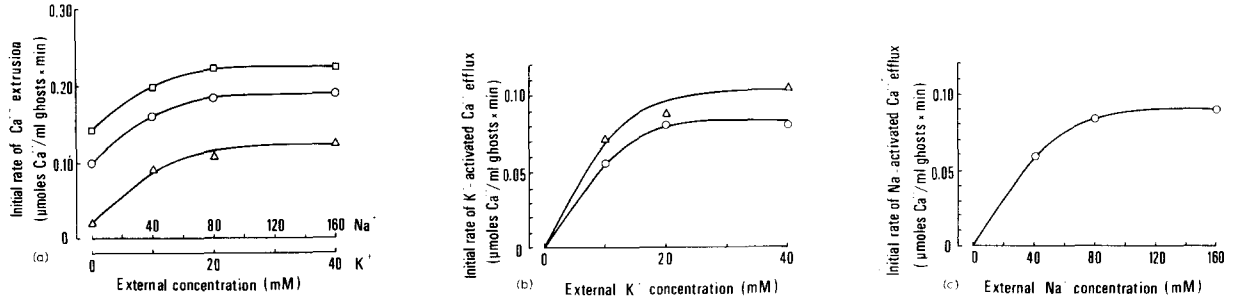

Fig. 2. Affinity of the Ca^{2+} pump for external Na^+ or K^+. The initial rate of Ca^{2+} extrusion from high-Na^+ (□) or -choline ghosts (△) was calculated from the linear decrease with time of internal Ca^{2+}, after incubating in the presence of increasing K^+ concentrations, as indicated in part a. In addition, this graph also shows the rate of extrusion from high-K^+ ghosts (○), which were incubated with the increasing Na^+ concentrations depicted on the second abscissa. The specific fraction of Ca^{2+} rate stimulated by K^+ is presented in part b, while that activated by Na^+ is shown in part c. Results are mean values of at least two different experiments.

Na^+ or K^+. Increasing Na^+ up to 160 mM or K^+ up to 40 mM stimulated by nearly 90% the rate of extrusion (upper two curves in Fig. 2a). These results confirm earlier observations that Na^+ or K^+ ions activate the Ca^{2+} pump from outside [10,11]. In addition, they show that K^+ is more effective than Na^+ at low concentrations.

Replacement of ghost Na^+ or K^+ by choline, markedly reduced the rate of extrusion (Fig. 2a; bottom curve), thus demonstrating an internal requirement for either cation. This finding is in conflict with the asymmetrical action of Na^+ suggested earlier [11].

By relating the rate of cation-stimulated Ca^{2+} pumping to the concentration of activating cation, some interesting points are revealed. First, the activation is maximal at about 20 mM K^+ (Fig. 2b) or 80 mM Na^+ (Fig. 2c). Second, it occurs along a rectangular hyperbola, as inferred from linear regression analyses of reciprocal plots ($r^2 = 0.96$ for Na^+ activation; $r^2 = 0.89$ or 0.99 for K^+ activation in high-Na^+ or -choline ghosts, respectively), thus implying a Michaelis-Menten type of kinetics.

The above analyses led to K_m and V_{max} (in μmol Ca/ml ghosts per min) values of 34.7 mM for Na^+ ($V_{max} = 0.115$); 6.9 or 8.2 mM for K^+ in high-choline ($V_{max} = 0.121$) or -Na^+ ghosts ($V_{max} = 0.104$), respectively. These results clearly demonstrate that the pump affinity for K^+ is not altered by a high internal choline concentration. In addition, they show that the affinity for K^+ is nearly 5-times that for Na^+.

On the other hand, there was a linear correlation between the rate of cation-activated Ca^{2+} extrusion and the influx rate of the stimulating cation (Fig. 3). Furthermore, addition of La^{3+} (0.5 mM) considerably decreased both fluxes (results not shown). As external La^{3+} is more or less a selective inhibitor of the Ca^{2+} pump, the above findings seem to indicate an exchange of Na^+ or K^+ for Ca^{2+} during active extrusion. The results do not allow us to discriminate whether such an exchange results from either a direct ionic coupling with the pump mechanism or an electric coupling as consequence of electrogenic Ca^{2+} extrusion [12].

The K_m values given above agree closely with those reported for Na^+ or K^+ activation of the

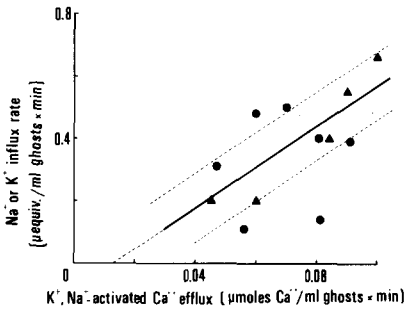

Fig. 3. Correspondence between cation-stimulated Ca^{2+} efflux and net influx of activating cation. The initial rates of cation-stimulated Ca^{2+} extrusion and concomitant Na^+ or K^+ net influx presented above are collected results from different experiments. The curve drawn corresponds to a regression line of equation $y = 6.55x - 0.085$ ($r^2 = 0.59$). Dotted lines represent ± 1 S.E. of estimate y on x.

($Ca^{2+} + Mg^{2+}$)-ATPase of human erythrocyte membranes, namely: $K_d^{Na^+} = 33$ mM; $K_d^{K^+} = 5.8$ mM [5]. Such findings lend further support for a direct involvement of the Na^+, K^+-dependent enzyme activity in active Ca^{2+} transport, as suggested earlier [11].

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