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INFLUENCE OF MONOVALENT IONS ON THE ACTIVITY OF THE (Ca^{2+} + Mg^{2+})-ATPase AND Ca^{2+}-TRANSPORT OF HUMAN RED BLOOD CELLS

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Summary

In reconstituted human red blood cells a difference was found in (Ca^{2+} + Mg^{2+})-ATPase activity and in Ca^{2+} efflux at 37°C, depending on the side of the membrane at which the monovalent cations K^+ and Na^+ were placed. Under the conditions used, (Ca^{2+} + Mg^{2+})-ATPase activity and Ca^{2+} efflux was highest when K^+ (35 ± 0.5 mM (± S.E.), mean of four experiments) was at the inside and Na^+ (130 mM) at the outside of the ghost membrane.

The (Ca^{2+} + Mg^{2+})-ATPase of human red blood cells and of the sarcoplasmic reticulum from skeletal and heart muscle is activated by monovalent cations, whereby K^+ activates more than does Na^+ [1—4]. It seemed interesting to test whether (Ca^{2+} + Mg^{2+})-ATPase and Ca^{2+} efflux changes when Na^+ or K^+ are placed either on the inside or outside of the cell membrane. Therefore, reconstituted ghosts from human red blood cells were either filled with K^+ (35 ± 0.5 mM, mean of four experiments) or with Na^+ (38 ± 0.75 mM, mean of four experiments) and then incubated either in a solution containing K^+ or Na^+ (130 mM).

Reconstituted human red cell ghosts were prepared according to Bodemann and Passow [5] with some variations. Human red blood cell concentrate was washed with Tris-HCl solution, pH 7.0, osmolarity 270 mosM. The washed cells were centrifuged at 4000 rev./min (1800 × *g*) for 15 min at 20° C. This procedure was repeated until the supernatant was clear. The washed red blood cells were slowly mixed with five parts of a hemolysing solution which comprised: 4 mM Tris-ATP, 1.5 mM $CaCl_2$, 1.0 mM EGTA, 2.5 mM *n*-tris-hydroxymethyl-methyl-2-aminoethanesulfonic acid (Tes) and 2.5 mM 2-*N*-morpholinoethanesulfonic acid (Mes). After 10 min at room

Abbreviation: EGTA, ethylene glycol bis (β-aminoethyl ether)-*N,N'*-tetraacetic acid.

temperature the mixture was centrifuged for 15 min at 10 000 rev./min (11 000 × *g*) at 20° C. The supernatant was discarded and the hemolysed cells were again suspended in twice the amount of the hemolysing solution. When Ca^{2+}-efflux was tested, 0.5 μCi/ml ^{45}Ca was added to the solution. In some experiments, the 4 mM Tris-ATP in the hemolysing solution was omitted. The suspension was quickly adjusted to 300 mosM with 3 M KCl or NaCl, whereby the ghosts resealed. After 5 min at room temperature, the resealed ghosts were centrifuged for 20 min at 10 000 rev./min (11 000 × *g*) at 0° C. The pellet was suspended at 0° C in an incubation medium comprising 1.5 mM $CaCl_2$, 1.0 mM EGTA, 130 mM KCl or 130 mM NaCl, 0.25 mM ouabain, 5 mM Tes and 5 mM Mes, and centrifuged as before and then again mixed with the incubation medium to give a hematocrit of 30%. One part of this suspension and one part of the same medium were mixed in a reaction vial and incubated at 37° C in a water bath or at 0° C on ice. All solutions used were adjusted with Tris to pH 7.0.

The total $[Ca^{2+}]$ in the cells was $(6.6 \pm 0.39) \cdot 10^{-4}$ mol (S.E., mean of four experiments) per l packed ghosts and the total $[Ca^{2+}]$ in the incubation medium was $1.5 \cdot 10^{-3}$ M. ATPase activity was determined by measuring the P_i liberated from ATP according to Post and Sen [6]. The size of the resealed ghosts was determined in a Coulter counter.

$[Na^+]$, $[K^+]$ and total $[Ca^{2+}]$ inside the resealed ghosts were measured in a flame photometer. The measuring was done after the resealing of the ghosts and a subsequent washing with Tris-HCl to remove adherent ions from the outside of the membrane. The samples were boiled with concentrated nitric acid or ashed at 400° C.

Ca^{2+} efflux was measured by determining the ^{45}Ca appearing in the incubation medium after loading the ghosts with ^{45}Ca as described above.

The results show differences in $(Ca^{2+} + Mg^{2+})$-ATPase activity and in Ca^{2+} efflux at 37° C with regard to the side of the membrane on which the

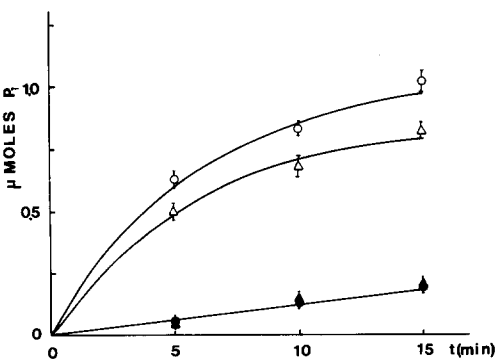

Fig. 1. Monovalent cation stimulation of the $(Ca^{2+} + Mg^{2+})$-ATPase activity of reconstituted human red cell ghosts. $(Ca^{2+} + Mg^{2+})$-ATPase activity was measured over a period of 15 min at 37° C and 0° C in the presence of K^+ and Na^+. K^+ was either at the inside of the ghost membrane and Na^+ at the outside (○——○, 37° C; ●——●, 0° C) or vice versa (△——△, 37° C; ▲——▲, 0° C). The monovalent ion concentration at the inside of the ghosts was 35 ± 0.5 mM (mean ± S.E. of four experiments) K^+ or 38 ± 0.75 mM(mean ± S.E. of four experiments) Na^+ and at the outside 130 mM for both ions. The ordinate shows the P_i liberated from 1 ml packed ghosts. The results are S.E. of the mean of five experiments.

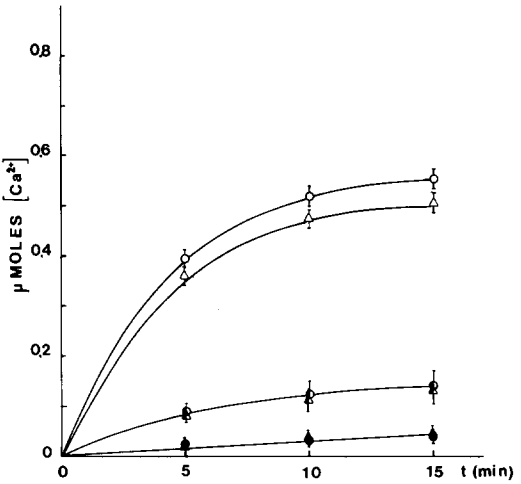

Fig. 2. Influence of Na^+ and K^+ on the outward transport of Ca^{2+} out of reconstituted human red cell ghosts. Ca^{2+} efflux was measured at 37°C and 0°C by the increase in ^{45}Ca in the incubation medium. The ordinate shows the µmol Ca^{2+} which were transported out of 1 ml packed ghosts. The ions used were K^+ and Na^+. The monovalent ion concentration at the inside of the ghost membrane was 35 ± 0.5 mM (mean ± S.E. of four experiments) K^+ or 38 ± 0.75 mM (mean ± S.E. of four experiments) Na^+ and at the outside 130 mM for both ions. The figure shows the S.E. of the mean of five experiments. Open symbols 37°C with ATP; half-closed symbols 37°C without ATP; closed symbols 0°C with ATP; circles K^+ inside and Na^+ outside the ghost membrane; triangles Na^+ inside and K^+ outside the ghost membrane.

monovalent cations were located. Under the conditions used $(Ca^{2+} + Mg^{2+})$-ATPase activity and Ca^{2+} efflux at 37°C was highest when K^+ (35 ± 0.5 mM, S.E., mean of four experiments) was at the inside and Na^+ (130 mM) at the outside of the cell membrane. When Na^+ (38 ± 0.75 mM, S.E., mean of four experiments) was at the inside and K^+ (130 mM) at the outside of the membrane the $(Ca^{2+} + Mg^{2+})$-ATPase activity was 27% lower (Fig. 1) and the Ca^{2+}-outward transport about 10% lower (Fig. 2) within 15 min incubation. The ratio between liberated inorganic phosphate and outward transported Ca^{2+} was 1:0.7 after 10 min incubation at 37°C. At 0°C the $(Ca^{2+} + Mg^{2+})$-ATPase activity and the Ca^{2+} efflux was much lower than at 37°C and there was no dependence on the side at which Na^+ or K^+ was placed. The same applied to the Ca^{2+} efflux at 37°C without addition of ATP (Fig. 2). The experiments at 0°C and those at 37°C without addition of ATP indicate that the difference in outward transport of $^{45}Ca^{2+}$ depending on the side on which the monovalent cations were placed is caused by an active transport and not by exchange between unlabelled Ca_o^{2+} and $^{45}Ca_i^{2+}$.

Measurements in a Coulter counter after the resealing of the ghosts showed no difference in distribution of size between Na^+ or K^+ filled ghosts; therefore the reason for the differences in $(Ca^{2+} + Mg^{2+})$-ATPase activity and Ca^{2+} efflux at 37°C could not be due to a different size between Na^+ filled and K^+ filled resealed ghosts. The hematocrit before and after incubation at 37°C was the same which means that no shrinking of the ghosts took place. The pH was constant over the whole incubation period.

To exclude any interference of the $(Na^+ + K^+)$-ATPase, 0.25 mM ouabain was added to all incubations [7—9].

Gardos [10] showed that Ca^{2+} leads to an increase in the rate of efflux of K^+ from red cells. The K^+ loss depends mostly on the free $[Ca^{2+}]$ inside the cells. The free $[Ca^{2+}]$ in our ghosts was approx. $2 \cdot 10^{-4}$ mol/l cells and the influence on the K^+ loss seemed to be within acceptable limits [11, 12].

These results show that for maximal $(Ca^{2+} + Mg^{2+})$-ATPase activity and for maximal Ca^{2+}-outward transport in human red blood cells at 37° C it seems necessary that K^+ is at the inside and Na^+ at the outside of the cell membrane. Whenever the distribution of these monovalent cations is changed the $(Ca^{2+} + Mg^{2+})$-ATPase activity and the Ca^{2+} efflux in human red blood cells decreases.

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