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Low potassium-type but not high potassium-type sheep red blood cells show passive K^+ transport induced by low ionic strength

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Low potassium-type (LK) sheep red blood cells show a significant increase of the residual (i.e., ouabain-insensitive) K^+ influx when the ionic strength of the solution is decreased. This effect is absent from high potassium-type (HK) sheep red blood cells. The KCl cotransport system is not involved since three different manoeuvres to suppress the KCl cotransport (replacement of Cl^- by NO_3^-, volume-decrease, inhibition by anti-L_1 antibodies) have no effect on the low ionic strength-stimulated K^+ influx.

Introduction

It is now apparent that there are a number of separate transport pathways for monovalent cations in red blood cells [1,2]. K^+ transport through the red cell membrane can occur via the Na^+/K^+ pump, NaKCl cotransport, and the Ca^{2+}-activated K^+ (Gardos) channel. In addition, there is a residual (i.e., (ouabain + bumetanide + Ca^+)-insensitive) route, which may include mediated components [1,3]. In 'young' human and in low potassium type (LK) sheep red blood cells, as well as those of certain other species, this residual component includes a coupled KCl cotransport which is volume-dependent [3–5]. Under other experimental conditions, e.g., reduced ionic strength [6–9], or treatment with SH-reagents [10,11] an increased residual monovalent cation flux also occurs. Inhibitor studies have ruled out the involvement of the Na^+/K^+ pump, NaKCl cotransport and the Gardos channel in these induced fluxes [12], but it is difficult to decide precisely which mechanisms may be involved in the low ionic strength effect. The aim of the present paper is to take advantage of K^+ transport polymorphism in sheep red blood cells to try and correlate the low ionic strength effect with other transport processes. We have compared high potassium type (HK) and LK sheep red blood cells, and used anti-L_1 alloantibodies (the most specific inhibitor of KCl cotransport [13]) to demonstrate that the low ionic strength effect occurs only in LK but not HK sheep red blood cells, and does not involve the KCl cotransport system.

Abbreviations: LK, low potassium-type; HK, high potassium-type.

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Materials and Methods

Sheep blood was drawn by venipuncture with heparin as anticoagulant. The red blood cells were washed three-times by centrifugation and resuspension in solutions of physiological ionic strength containing (mM): NaCl or $NaNO_3$, 155; glucose, 5; 3-(*N*-morpholino)propanesulphonic acid (Mops), 10 (pH 7.4) and the buffy coat removed. Then the cells were washed once in the flux solution. The flux solution of high ionic strength contained (mM): NaCl or $NaNO_3$, 148; KCl or KNO_3, 7; glucose, 5; Mops, 10 (pH 7.4). The flux solution of low ionic strength contained (mM): KCl or KNO_3, 7; glucose, 5; sucrose, 270; Mops, 10 (pH 7.4).

Volume changes were achieved by adding either different amounts of distilled water to swell the cells, or different amounts of 1 M NaCl ($NaNO_3$) in solution of high ionic strength, or 1 M sucrose in solution of low ionic strength, to shrink the cells. Relative volumes were calculated from hematocrit and determination of cyanomethemoglobin (in Drabkin's solution), and are expressed relative to untreated cells in plasma [13]. The absorbance at 540 nm for LK cells was 261.71 ± 2.44 and for HK cells 240.09 ± 13.22 (mean ± S.E.) in plasma.

Unidirectional K^+ influx was measured as described by Dunham and Ellory [14]. ^{86}Rb (37 kBq/ml) was

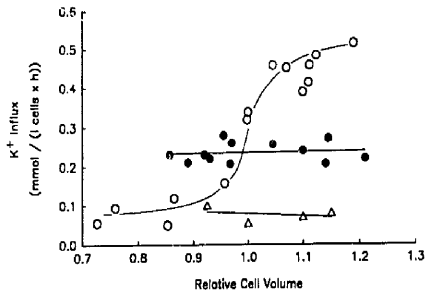

Fig. 1. Effect of alteration of cell volume on ouabain-insensitive K^+ influx of LK sheep red blood cells. The cells were shrunken by addition of different amounts of 1 M NaCl ($NaNO_3$) or 1 M sucrose. The cells were swollen by addition of different amounts of distilled water. Symbols represent single experiments (triplicate samples) as follows: ○, Cl^--containing high ionic strength solutions; ●, Cl^--containing low ionic strength solutions; △, NO_3^--containing high ionic strength solutions.

used in all experiments as a tracer for K^+ influx. The flux time was 30 min at 37°C and the hematocrit was about 5%. In all experiments ouabain (0.1 mM) and EGTA (1 mM) were present. All flux values were corrected to express them in mmol per litre of fresh cells (relative volume of 1) per h, irrespective of the measured cell volume during the experiment.

Alloimmune sheep anti-L_1 antiserum was raised and prepared as described by Tucker and Ellory [15]. Before antiserum treatment, red cells were washed three-times in NaCl solution of physiological ionic strength (see above). To about 50 μl of packed cells 200 μl antiserum was added. The cells were resuspended and incubated at 32°C for 1 h. For the corresponding control experiments, 200 μl of the washing solution was added instead of the antiserum but otherwise the cells were treated identically. After sensitization the cells were washed twice in NaCl solution of physiological ionic strength (see above) and once in the flux solution of high or low ionic strength.

The results are presented either as the mean ± S.E. of at least three experiments (in which case an error is indicated) or represent data from a single experiment (triplicate samples).

Intracellular pH was measured at 37°C using a water-jacketed Radiometer pH glass capillary electrode linked to a Radiometer pH meter (PHM 52) to sample packed cell haemolysates. LK and HK red cells were washed three times and resuspended in buffers of high or low ionic strength containing chloride or nitrate. Packed cell haemolysates were obtained by centrifuging the cell suspensions (at 15000 × *g* for 30 s), aspirating the supernatant and the top layer of cells and then rapidly freezing and thawing the cell pellet three-times using liquid nitrogen.

Results

When LK sheep red blood cells are placed in high ionic strength solutions containing Cl^-, with different tonicities (240–400 mosM) there is a large volume-sensitive, ouabain-insensitive K^+ influx (Fig. 1), representing KCl cotransport [13,16]. If a low ionic strength medium is used, the resultant K^+ influx is very different. At low volumes (shrunken cells), there is a marked (2-fold) enhancement of the flux; in contrast, at high volumes (swollen cells) the K^+ influx is less than in cells in high ionic strength media (Fig. 1). Since the KCl cotransport pathway can be suppressed by replacing Cl^- with NO_3^- [13,17], we repeated this experiment in NO_3^--containing solutions of high and low ionic strength. In this case there is no volume-sensitive K^+ influx in high ionic strength NO_3^- media (Fig. 1) and the K^+ influx in low ionic strength media containing NO_3 (0.191 ± 0.013 mmol/l cells per h) is not significantly different from the K influx in Cl-containing low ionic strength media (0.236 ± 0.008 mmol/l cells per h).

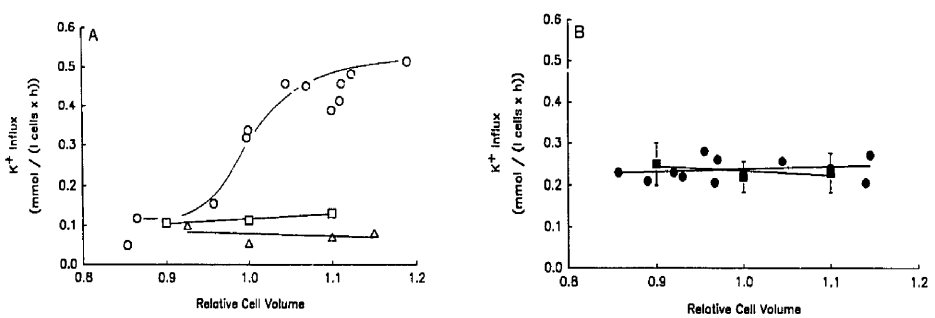

Fig. 2. Effect of anti-L_1 antibodies on the ouabain-insensitive K^+ influx of LK sheep red blood cells at different cell volumes. See Fig. 1 for volume manipulations. Symbols represent single experiments (triplicate samples) except where stated as follows: (A) high ionic strength conditions: ○, Cl^--containing solutions; △, NO_3^--containing solutions, □ Cl^--containing solutions with addition of anti-L_1 antibodies (data represent mean ± S.E. of three independent experiments, error bars are smaller than symbols); (B) low ionic strength conditions: ●, Cl^--containing solutions, ■, Cl^--containing solutions with addition of anti-L_1 antibodies (data represent mean ± S.E. of three independent experiments).

For comparison, experiments were also performed with HK sheep red blood cells. For these cells, which have been shown previously to lack the KCl cotransport system [3,18,19], there is no difference between K^+ influx in Cl^--containing solutions of high ionic strength when compared to the K^+ influx in low ionic strength Cl^--containing media (0.148 ± 0.006 and 0.129 ± 0.005 mmol/l cells per h) over the relative volume range 0.8–1.2.

Intracellular pH was measured under the four experimental conditions used (low ionic strength with nitrate or chloride and high ionic strength with nitrate or chloride) in red cells from two LK and two HK sheep. For both LK and HK cells the intracellular pH in high ionic strength media was the same. Under low ionic strength conditions intracellular pH alkalinized by 0.4 of a pH unit, but again there was no difference between LK and HK samples.

Finally, it is possible to inhibit the KCl cotransport system of LK sheep red blood cells by sensitizing the cells with anti-L_1 antibodies [13,16,20]. Fig. 2a shows the efffect of anti-L_1 on the ouabain-insensitive K^+ influx in LK sheep red blood cells in Cl^--containing media of high ionic strength. The antibodies inhibit the KCl cotransport system almost completely, to the level found in shrunken cells in Cl^--containing high ionic strength media, or NO_3^--containing media of high ionic strength. However, anti-L_1 antibodies have no effect on the ouabain-insensitive K^+ influx measured over the whole volume range in low ionic strength media (Fig. 2b).

Discussion

Since the early work of Davson [6], it has been known that low ionic strength media can induce increased K^+ fluxes in red blood cells of different species [21,22]. The present paper indicates that LK, but not HK sheep red blood cells, also show enhanced fluxes in low ionic strength media. The magnitude is smaller in the sheep cells than in human red blood cells. Interestingly, HK sheep red blood cells fail to show volume-sensitive KCl cotransport, in contrast to LK sheep red blood cells. However, the present paper offers several distinct lines of evidence to suggest that the KCl cotransport pathway is not involved in the low ionic strength effect. The KCl cotransport system shows an absolute requirement for halide ions, nitrate replacement abolishing KCl cotransport [13,17]. Furthermore, it is highly volume-sensitive (swelling increases the flux [13,16]), and it is inhibited by anti-L_1 antibodies [13,20]. All these criteria confirm the separate identities of KCl cotransport and the low ionic strength-induced K^+ flux. The loss of volume-sensitivity of the K influx in Cl^--containing solutions of low ionic strength (Fig. 1) is probably due to the low chloride concentration under these conditions limiting KCl cotransport [13]. However, it cannot be completely ruled out that the increased intracellular pH of cells in low ionic strength media inhibits KCl cotransport [23]. If this were the case, then it would be further evidence that KCl cotransport is not involved in the low ionic strength effect.

The finding that LK but not HK sheep red blood cells show this effect rules out an exclusive role for differences in membrane lipids in this phenomenon, since the lipid composition of HK and LK sheep red blood cells has been shown to be identical [24]. A participation of the voltage-activated cation transport [25] in stimulating the K influx in low ionic strength solutions is unlikely, since these voltage-activated fluxes have been found in HK, but not LK, sheep red cells [26]. This is consistent with our explanation that the ionic strength of the solution rather than the membrane potential is responsible for the observed increase in cation fluxes [12].

Potassium polymorphism in sheep is well established as the product of a single gene. This gene has been associated with the L_1 antigen [15,27] and consequent alterations in Na^+/K^+ pump activity [28,29] as well as KCl cotransport [13,20]. The present paper identifies a third transport property which is different in the two phenotypes, an effect of low ionic strength media on the residual K^+ transport in LK sheep red blood cells. This effect is not sensitive to anti-L_1, and does not involve any other known transport system. In human red blood cells, where the low ionic strength effect has been best studied [6–9,12], there is still no clear indication of its mediator, with results not allowing a distinction between carrier or channel-like mechanisms. Jones and Knauf [9] implicated Band 3 in the low ionic strength effect, since their induced fluxes were partially inhibited by DIDS. Such an involvement seems intrinsically unlikely in the present work, since this would indicate a functional difference in Band 3 between HK and LK cells. In fact, there seems to be considerable variability in the response of human red cells to low ionic strength media (e.g., see Ref. 26 for discussion), and in the present paper we emphasize the difference in response between HK and LK cells.

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