Biochimica et Biophysica Acta 858 (1986) 317–320
Elsevier

BBA 70331

BBA Report

Evidence for the presence of volume-sensitive KCl transport in 'young' human red cells

A.C. Hall [a,*] and J.C. Ellory [b]

[a] *Physiological Laboratory, Downing Street, Cambridge CB2 3EG*
and [b] *University Laboratory of Physiology, Parks Road, Oxford OX1 3PT (U.K.)*

(Received March 17th, 1986)

Key words: K^+ transport; Volume sensitivity; Hydrostatic pressure; (Human erythrocyte)

'Young' human red cells are shown to possess a specific K^+ pathway which is dependent on Cl^- and sensitive to cell volume. This system was latent in 'mature' cells but was revealed by high hydrostatic pressure. This suggests the pathway is functionally active in 'young' cells but becomes masked with cell maturation.

Red cells from many species possess a specific KCl transport system which is insensitive to ouabain and 'loop' diuretics but is responsive to changes in cell volume produced by alterations to medium tonicity [1,2]. The presence of this pathway in human erythrocytes from normal donors under standard experimental conditions remains controversial with reports suggesting that altering red cell volume has no effect [2], decreases [3], or has 'complex' effects [4] on 'passive' K^+ transport. Nevertheless, three lines of evidence support the notion that the transporter is present in the membrane but latent under normal experimental conditions. Thus, volume-sensitive KCl transport in human red cells is observed (a) with the addition of the thiol-reactive agent, *N*-ethylmaleimide [1,5], (b) by the application of high hydrostatic pressure [2,6], or (c) in red cells from donors with certain haemolytic anaemias [2]. Interpretation of flux data obtained from the latter group is, however, complicated by large numbers of morphologically abnormal cells, and characteristically, a significant fraction of reticulocytes (frequently > 10%) and 'young' cells. Thus it is not clear which of these cell populations possess the functional volume-sensitive KCl transporters. If 'young' but not mature cells exhibited this flux then in normal human blood samples with average levels of reticulocytosis (0.2–2.0%, [7]) the fraction of cells possessing this pathway would be small and variable making detection of the KCl flux difficult under normal conditions. The purpose of the present report, therefore, is to investigate the possibility that the volume-sensitive KCl transporter operates in young red cells, but becomes masked with cell maturation.

Fresh blood obtained from normal, healthy human donors was washed in a buffered isotonic saline and the cells filtered through glass wool (twice) to remove residual platelets and leucocytes. Red cell fractions enriched with young or mature cells were obtained by the methods of Tucker and Young [8]. Briefly, red cells at about 80% haematocrit were centrifuged ($2500 \times g$, 60 min, 21°C, swing-out head) in polythene tubes and the top 1/4 clipped off with arterial forceps and pooled. The 'bottom' fraction represented the cell population enriched with mature erythrocytes. The 'top'

* To whom correspondence should be addressed.

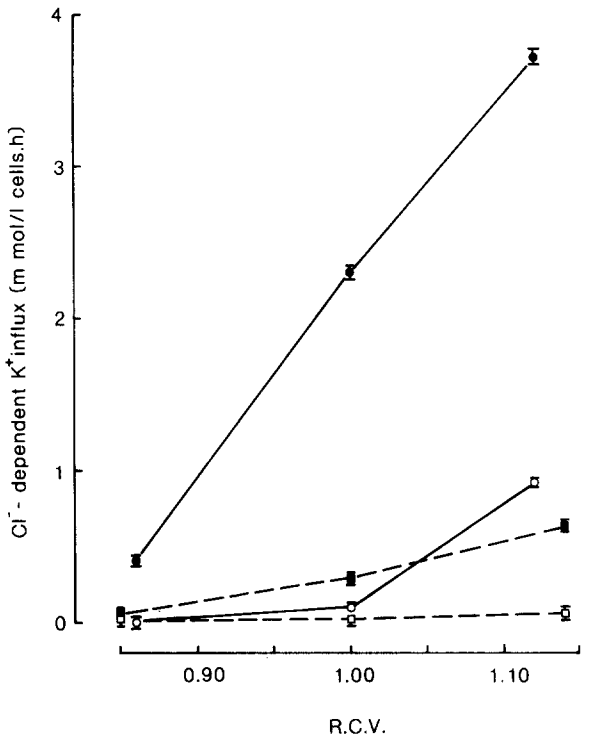

Fig. 1. The effects of cell volume on 'passive' K^+ uptake in red cells from different fractions of centrifuged human blood. Cl^--dependent K^+ (^{86}Rb) uptake, i.e. (K^+ influx in Cl^- medium) − (influx in $CH_3SO_4^-$ medium), was measured using standard techniques (5% haematocrit; 37°C; [9]) in incubation media of the following composition (mM); Na (Cl or CH_3SO_4) 130; K (Cl or CH_3SO_4) 7.5; glucose 5; Mops (morpholinepropanesulphonate) 15; ouabain (Sigma Chemical Co.) 0.5; bumetanide (Leo Pharmaceuticals, Princes Risborough, U.K.) 0.1; EGTA 0.1 (adjusted to 290 mOsm/kg with sucrose; pH 7.4 at 37°C). The inhibitors and Ca^{2+} chelator were used at concentrations sufficient to inhibit the Na^+/K^+ pump [13], ($Na^+ + K^+$)-cotransport [11], and Ca^{2+}-activated K^+ channel [14], respectively. At the end of the incubation period (usually 20 min) the cells were washed free of extracellular isotope by centrifugation (15000 × *g*, 10 s), aspiration and resuspension in ice-cold medium comprising (mM); $MgCl_2$ 106; Mops 15; (pH 7.4 at 5°C). The cell pellet was then treated with 0.5 ml of 0.1% (v/v) Triton X-100 in water followed by 0.5 ml 5% (w/v) trichloroacetic acid. The precipitate was removed by centrifugation (15000 × *g*, 5 min) and the supernatant counted in a scintillation spectrophotometer. Relative cell volume (R.C.V.) was determined relative to cells of 'normal' volume (i.e. suspended in the above saline with R.C.V. = 1.00), and was increased or decreased by varying medium tonicity using distilled water or sucrose, respectively, and monitored as described [9]. The substitution of Cl^- by $CH_3SO_4^-$ was performed as previously reported [15]. The measurement of K^+ influx at 400 ATA (ATA = atmospheres absolute; 1 ATA = 0.101 MPa) was performed on red cell suspensions contained within syringes (see Ref. 16) including mixing bars for cell agitation. The fractions were recentrifuged, and the upper cell layers saved as before, representing the young red cell-enriched population.

Fig. 1 shows that at 'normal' cell volume (i.e. R.C.V. = 1.00; 290 mosM/kg) and at atmospheric pressure, cells from the 'top' fraction exhibited a Cl^--dependent 'passive' (i.e., (ouabain + bumetanide + EGTA)-insensitive) K^+ flux which was increased or decreased by cell swelling or shrinking, respectively. In contrast, cells from the 'bottom' fraction showed no significant Cl^--dependent K^+ flux at any cell volume. At high pressure, the K^+ flux in cells from the 'top' fraction was markedly elevated at all cell volumes. In cells from the 'bottom' fraction, a significant increase in K^+ flux was detected at 'normal' volume which was enhanced by a reduction in medium osmolarity. In the absence of Cl^- ($CH_3SO_4^-$ replacement) there was no significant effect of cell swelling (R.C.V. 0.85–1.14) on K^+ uptake in red cells from either 'top' or 'bottom' fractions (results not shown). This shows that in both cell populations the volume-sensitive K^+ flux is dependent on the presence of Cl^- ions.

In other species, the volume-sensitive KCl transport system is selective for K^+ over Na^+ [1,9], so it is an important control to compare the effects of cell volume on Na^+ or K^+ uptake in the two cell populations. In such experiments in both 'top' and 'bottom' cell fractions, increasing cell volume (R.C.V. 0.84–1.13) had no significant effect on 'passive' Na^+ uptake in a Cl^- medium. Thus, with $[Na^+]_o = 65$ mM (K^+ replacement), Na^+ influx in shrunken cells was 1.27 ± 0.04 (mean ± S.E., $n = 3$) and 1.18 ± 0.01 mmol/l cells per h, for cells obtained from 'top' and 'bottom' fractions, respectively. In swollen cells the comparable values were 1.24 ± 0.20 and 1.08 ± 0.01. The replacement of Cl^- by $CH_3SO_4^-$ was without significant effect on Na^+ influx in both cell fractions regardless of cell volume.

syringes were placed in a water-filled pressure bomb, and there was no significant change in temperature on compression. Following decompression, the intracellular accumulation of radioisotope was determined in the same manner as for the unpressurised controls. Symbols: cells from 'top' fraction (○, ●) 1 and 400 ATA, respectively; cells from the 'bottom' fraction (□, ■) 1 and 400 ATA, respectively. Data throughout are means of triplicate determinations ± S.E.

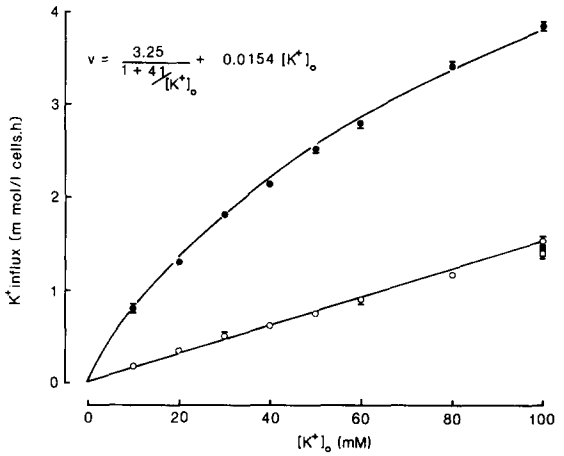

Fig. 2. The effect of cell volume on the concentration dependence of K^+ uptake in red cells obtained from different fractions of centrifuged human blood. 'Passive' K^+ uptake was determined as described in the legend to Fig. 1, with Na^+ as replacement cation. Symbols: (○, ●) cells from the 'top' fraction shrunken and swollen, respectively, and (□, ■) cells from the 'bottom' fraction shrunken and swollen, respectively. The insert shows the equation (see Ref. 15) of the K^+ influx data in swollen cells from the 'top' fraction, with the apparent kinetic constants computed from a Hanes-Wolf plot ($[S]/v$ vs. $[S]$). The slope of K^+ uptake as a function of $[K^+]_o$ in shrunken cells was 0.0153 ± 0.0005 (mmol/l cells per h per mM).

To characterise the volume-sensitive KCl system further, the kinetics of K^+ influx as a function of external $[K^+]_o$ were studied (Fig. 2). 'Passive' K^+ uptake in cells from the 'bottom' fraction, whether swollen or shrunken, exhibited linear concentration dependence over the range 10–100 mM $[K]_o$. In contrast, K^+ uptake in cells from the 'top' fraction was sensitive to cell volume with a saturable component having a relatively high apparent K_m and V_{max} being observed in swollen cells. The 'passive' K^+ uptake in cells from the 'top' fraction when shrunken was a linear function of $[K^+]_o$ and at $[K^+]_o = 100$ mM the absolute value of K^+ influx was the same as that of cells from the 'bottom' fraction which was volume-independent.

The volume-sensitive K^+ flux in cells from the 'top' fraction was strongly dependent on the presence of metabolic substrates. Thus, when cells were incubated (37°C) in a buffered isotonic saline containing iodoacetamide (6 mM) and inosine (10 mM) the volume-sensitive component of K^+ uptake (i.e., K^+ influx in cells swollen to 1.12 minus the K^+ influx in cells shrunken to 0.86) measured as described (see legend to Fig. 1), was 0.837 ± 0.008 mmol/l cells per h at $t = 0$, and fell to 0.020 ± 0.010 after 3h. Ouabain-sensitive K^+ influx ($[K^+]_o = 7.5$ mM) which was taken as a measure of the presence of metabolic substrates (see Ref. 10), was 2.857 ± 0.030 mmol/l cells per h, before depletion and 0.027 ± 0.006 following depletion indicating their almost complete removal.

These data demonstrate that a significant fraction of the red cell population in blood samples obtained from normal donors, corresponding to the less dense or 'young' cells, exhibits 'passive' K^+ transport dependent on the presence of Cl^- and strongly influenced by cell volume. The fact that this flux is not observed in fractions enriched with 'mature' erythrocytes, but that a volume-sensitive Cl^--dependent K^+ pathway is unveiled in these cells at pressure (Fig. 1) supports the hypothesis that the KCl transport system is functionally operative in 'young' red cells, but becomes masked with cell maturation. This specific KCl flux shows the properties of a low affinity, high capacity carrier-mediated system which can be distinguished from the Cl^--dependent ($Na^+ + K^+$)-'cotransport' pathway, by the latter's high sensitivity to 'loop' diuretics [11].

In donors with certain haemolytic anaemias where blood samples will, characteristically, contain a large proportion of reticulocytes and 'young' red cells, the involvement of this potent K^+ transport pathway must now be taken carefully into consideration, and consequently K^+ transport measurements interpreted with caution. The low affinity but relatively high capacity (Fig. 2) of this pathway indicate that its contribution at a single value of $[K^+]_o$, particularly when the osmotic pressure of the experimental medium is not measured, is extremely difficult to assess. Furthermore, when it is remembered that the density separation method only leads to enrichment and does not provide a homogeneous young cell sample, it is likely that the capacity for volume-sensitive KCl transport in a sub-population of that fraction must be considerable. These results also emphasize the dangers of measuring K^+ uptake over a limited range of $[K^+]_o$ and the frequently-held assumption that the slope of this relationship

solely represents the electrodiffusional 'leak'.

The present paper resolves several previous inconsistencies in the literature. The principal conclusion is that the volume-sensitive KCl transport pathway seen in many other species' red cells, and previously demonstrated as being cryptic in human cells, is operational in a certain fraction normally present in the human red cell population identified as the 'young' cells or reticulocyte-rich component. In anaemias, or states where the immature cell population is enhanced, there will be potentially large volume-sensitive K^+ movements. This may well play a role in volume-regulation and cell survival following perturbations in medium osmolarity or pH. However, in some situations the cell shrinkage following the activation of a rapid KCl efflux with osmotically obliged water would raise MCHC, so that for certain abnormal haemoglobin types there may be dramatic consequences in terms of cell morphology and survival [12].

This work was supported by the M.R.C. (U.K.).

References

1 Ellory, J.C., Dunham, P.B., Logue, J. and Stewart, G.W. (1982) Philos. Trans. Roy. Soc. B 299, 483–495
2 Ellory, J.C., Hall, A.C. and Stewart, G.W. (1985) Mol. Physiol. 8, 235–246
3 Poznansky, M. and Solomon, A.K. (1975) J. Membrane Biol. 10, 259–266
4 Adragna, N.C. and Tosteson, D.C. (1984) J. Membrane Biol. 78, 43–52
5 Lauf, P.K. (1984) Am. J. Physiol. 246, C385–C390
6 Ellory, J.C. and Hall, A.C. (1985) J. Physiol. (Lond.) 362, 15P
7 Dacie, J.V. and Lewis, S.M. (1975) Practical Haematology p. 13, Churchill Livingstone, Edinburgh
8 Tucker, E.M. and Young, J.D. (1982) in Red Cell Membranes; A Methodological Approach (Ellory, J.C. and Young, J.D., eds.), pp. 31–41, Academic Press, London
9 Dunham, P.B. and Ellory, J.C. (1981) J. Physiol. (Lond.) 318, 511–530
10 Hall, A.C. and Willis, J.S. (1984) J. Physiol. (Lond.) 348, 629–643
11 Ellory, J.C. and Stewart, G.W. (1982) Br. J. Pharmacol. 75, 183–188
12 Fabry, M.E., Kaul, D.K., Raventos-Suarez, C., Chang, H. and Nagel, R.L. (1982) J. Clin. Invest. 70, 1315–1319
13 Schatzmann, H.J. (1953) Helv. Physiol. Pharmacol. Acta 11, 346–354
14 Lew, V.L. and Ferreira, H.G. (1978) in Current Topics in Membranes and Transport, Vol. 10 (Kleinzeller, A. and Bronner, F., eds.), pp. 217–277, Academic Press, New York
15 Dunham, P.B., Stewart, G.W. and Ellory, J.C. (1980) Proc. Natl. Acad. Sci. USA 77, 1711–1715
16 Hall, A.C., Ellory, J.C. and Klein, R.A. (1982) J. Membrane Biol. 68, 47–56