Biochimica et Biophysica Acta, 507 (1978) 178—181

BBA Report

BBA 71325

IS THE Ca^{2+}-SENSITIVE K^+ CHANNEL UNDER METABOLIC CONTROL IN HUMAN RED CELLS?

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(Received October 28th, 1977)

Summary

It is widely known that a rise in internal Ca^{2+} leads to an increased K^+ permeability of human red blood cells [1,2,3]. Binding of Ca^{2+} to some membrane receptors is required for the opening of the K^+ channel [4]. This requirement, however, seems to alter after "ageing" red cells in vitro in acid-citrate-dextrose solutions. Thus, the free Ca^{2+} concentration producing half-maximal effect on K^+ permeability ($[Ca^{2+}]_{K^+\text{-}50}$) of 4-weeks stored cells is approx. $2 \cdot 10^{-4}$ M (calculated from ref. 3 using 50% free Ca^{2+} according to Schatzmann [5]); nearly ten times lower than that reported for fresh cells [6]. This observation suggests the possibility that the K^+ channel may become more sensitive to Ca^{2+} on cold storage. The experiments described below support this idea.

Intact cells behave like a large-capacity Ca^{2+} buffer [7], where phosphorylated metabolic intermediates contribute appreciably. Alteration of metabolism of these cells would obviously influence Ca^{2+} binding to the membrane by both affecting the Ca^{2+} pump and regulating the amount of Ca^{2+} chelators. The use of ghosts, by contrast, circumvents direct interference of metabolites with Ca^{2+}, thus providing a way of exploring the influence of metabolism on channel sensitivity.

If the amount of free internal Ca^{2+} and other variables likely to affect Ca^{2+} binding such as temperature, pH and ionic composition are kept constant, any possible change of Ca^{2+}-sensitive K^+ efflux arising from various metabolic conditions would be due to alterations of membrane-bound Ca^{2+}. In the experiments reported here, high-K^+ ghosts containing very low ATP concentrations were prepared (see legend to Fig. 1) and the free Ca^{2+} content was kept constant at $1 \cdot 10^{-6}$ M using an EGTA-Ca^{2+} buffer. This concentration

was chosen since it is the minimal required for a maximal effect on K^+ permeability of ghosts from stored cells [4]. It is worth mentioning at this stage that the $[Ca^{2+}]_{K^+\text{-}50}$ of ghosts from fresh cells is approx. $2—5 \cdot 10^{-7}$ M [8,9], a concentration four orders of magnitude lower than that reported for intact cells [6]. This difference suggests that the affinity of Ca^{2+} receptors controlling K^+ permeability is altered by the osmotic shock.

Various metabolic conditions were obtained either by "ageing" in vitro (storing at 4° C in acid-citrate-dextrose solutions) or by incubating for 2 h at 37° C, with 5 mM adenine plus 10 mM inosine [10]. Ghost K^+ content was measured by flame photometry and nucleotides by gas-liquid chromatography [11]. The rate constant of Ca^{2+}-sensitive net K^+ efflux (k) was calculated from the slope obtained from a regression analysis of a family of curves relating K^+ loss to time divided by the ghost K^+ content after preincubation [12]. The ATP content of ghosts used in all experiments was below 3 μM (see Fig. 2). Since this concentration is much lower than the K_m of the Ca^{2+} pump for ATP (namely about 12 μM) [13], it can be safely assumed that the pump remained inactivated in these ghosts throughout the present work.

As "ageing" in vitro proceeds, k rises in a statistically significant way, thus increasing up to three-fold the initial value after 42 days of cold storage (Fig. 1).

It has been shown previously that the Ca^{2+}-stimulated K^+ efflux requires external K^+, the effect being maximal at about 4 mM K^+ [12]. The dependence on external K^+ was studied in order to test whether cold storage affects the sensitivity for external K^+. As demonstrated in Fig. 1, the sensitivity is lost after storing for more than 20 days (see also below). The results show

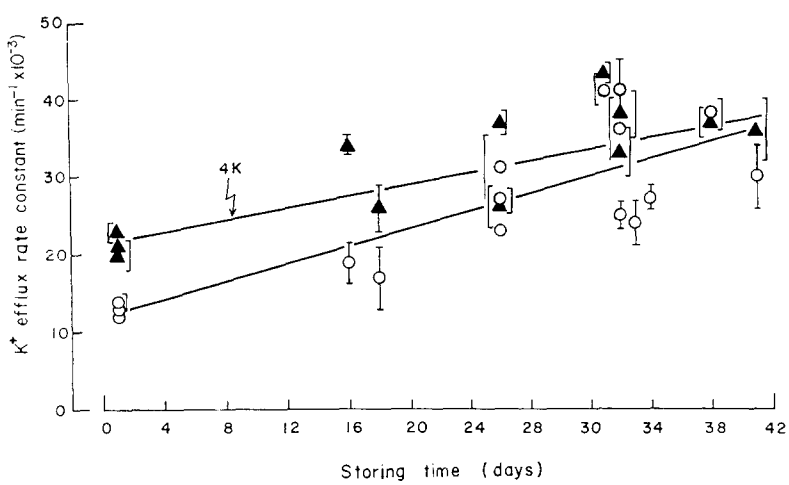

Fig. 1. Increased Ca^{2+}-sensitivity of K^+ channel on cold storage. One volume of human red cells, which had been stored at 4° C in acid-citrate-dextrose solutions for the length of time indicated above, was lysed with 30 vols. of a medium containing (mM): $MgCl_2$, 2; EGTA, 5; $CaCl_2$, 4.8; Tris-acetate, 10 (pH 6.5). After resealing, ghosts were preincubated for 10 min at 37° C in 160 mM NaCl + 20 mM Tris·HCl (pH 7.6), in order to remove K^+ from poorly-resealed ghosts. They were subsequently incubated at a 3% haematocrit in fresh Na-solution with (▲) and without (○) 4 mM K^+, for up to 50 min at 37° C and samples were taken at 10 min intervals. Thereafter, ghosts were washed twice in fresh Na-medium and lysed in water. The figure shows collected results from different experiments. Each point on the graph corresponds to a single experiment run in 3 replicates. Results are given as mean values ± 1 S.D.

first, that the K^+ channel becomes more sensitive to Ca^{2+} when the red cell "ages" in vitro. Secondly, the K^+ channel loses its sensitivity to external K^+. The effect described above is associated with a decreased metabolic activity, as shown by pilot experiments. Thus, when fresh cells are preincubated for 2 h at 37° C in the presence of iodoacetate (1 mM) and ghosts are made containing a fixed free Ca^{2+} concentration (conditions as in Fig. 1), k (in $min^{-1} \times 10^{-3}$) rises from about 11 (in controls) to 38.

To investigate whether the effect of cold storage on k is reversible, 4-weeks stored cells were preincubated with substrates and subsequently treated as described in legend to Fig. 1. This treatment produced a ten-fold decrease of k, which fell below the value found in ghosts prepared from 1-day stored cells. Thus, k (in $min^{-1} \times 10^{-3}$) was decreased from 30.6 ± 6.7 in controls to 3.2 ± 1.5 (mean of 6 experiments ± 1 S.D.) in ghosts from cells preincubated with substrates. The addition of 4 mM K^+ was without any effect. These findings indicate that the activation of metabolism of stored cells by substrates that give rise to ATP, markedly decreases the Ca^{2+}-sensitivity of the K^+ channel. This reduction of k is poorly related to the initial ATP content of ghosts (Fig. 2). It shows, however, a characteristic dependence on the initial ADP/ATP ratio (Fig. 3), suggesting that this ratio modulates the Ca^{2+}-sensitivity of the K^+ channel. This idea is in agreement with a recent paper, where the control of channel sensitivity is attributed to the action of some metabolites [6]. Regulation of channel sensitivity by the ADP/ATP ratio cannot be mediated by changes in Ca^{2+} chelating potential since the affinity of these nucleotides for Ca^{2+} is about the same.

As ageing in vivo is most likely to involve a change in ADP/ATP ratio [14], the increased Ca^{2+}-sensitivity of stored cells may be of physiological relevance.

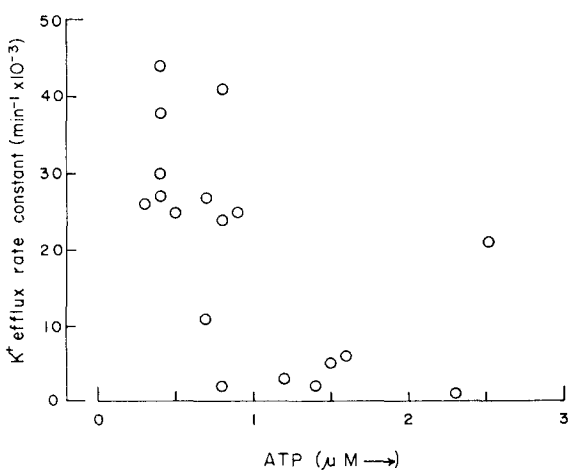

Fig. 2. Poor correspondence between ghost ATP and Ca^{2+}-sensitive net K^+ efflux. Cells which had been either stored in the cold for 1—42 days or stored for 3—4 weeks and then preincubated with substrates, were lysed in the presence of $1 \cdot 10^{-6}$ M free Ca^{2+}. Ghosts were preincubated as described in Fig. 1 and divided into two lots. One portion was incubated for up to 50 min at 37° C in order to assess the net K^+ efflux rate constant. The other portion was deproteinized with 5% perchloric acid. Thereafter, the acid was precipitated as $KClO_4$, the supernatant solution was lyophilized and the residue was dissolved in 20 μl water for analyses of nucleotides. These were determined by high pressure liquid chromatography, using a pellicular anion-exchange resin and a linear gradient of eluents. Collected results from different experiments are shown in the graph. Each point corresponds to a single experiment run in 3 replicates.

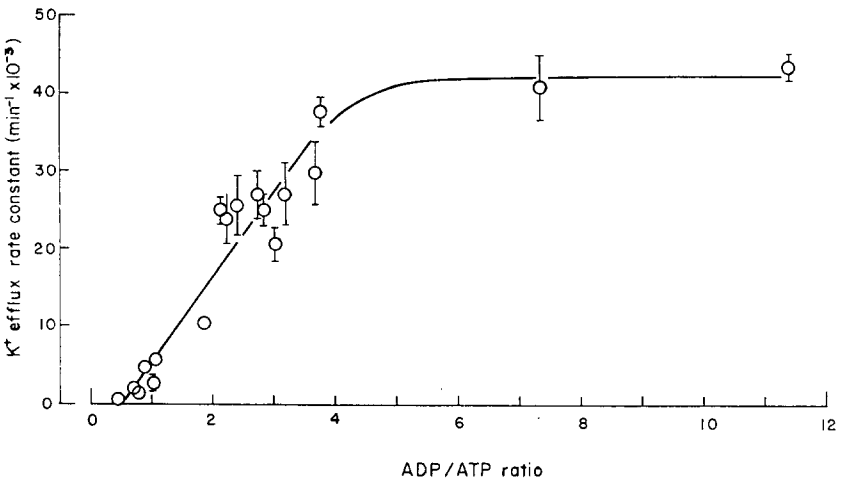

Fig. 3. The dependence of channel sensitivity on the initial ADP/ATP ratio. The ADP/ATP ratio was determined for each experiment reported in Fig. 2. Vertical bars show ± 1 S.D. and where not drawn, they are enclosed by the circle.

Only minute amounts of free Ca^{2+} within senescent cells would be required to alter membrane permeability and presumably, red cell deformability. Such rigid cells could be selectively trapped at the narrow splenic orifices, thus facilitating phagocytosis by reticulo-endothelial cells [15]. On the other hand, the above findings could also explain why metabolic inhibitors such as fluoride or iodoacetate potentiate the action of Ca^{2+} on K^+ permeability [3].

The author is indebted to Miss Eneida Romero for skilled technical assistance, to Dr. Itala de Becemberg for valuable help in analyses of nucleotides and to the Blood Bank Unit of Vargas Hospital. The author is also grateful to Dr. H.J. Schatzmann for valuable discussion. Financial support from CONICIT (Grant DF-S1-0214) and CDCH from UCV (Grant 365) is also acknowledged.

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