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On the ATP dependence of the Ca^{2+} -induced increase in K^+ permeability observed in human red cells

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SUMMARY

When red cells are starved or incubated in the presence of metabolic poisons, with or without substrates, a large increase in K^+ permeability is observed which depends on the presence of Ca^{2+} in the medium. The production or removal of a metabolite which controls the K^+ permeability has been proposed to explain these effects. In the present experiments, a parallelism is found to exist between the rate of ATP depletion and the increase in Ca^{2+} uptake and K^+ loss when red cells are depleted by different methods. The results support the view that the intracellular concentration of ATP may be the main factor on which the rate of Ca^{2+} uptake and the subsequent increase in K^+ permeability depend.

When red cells are starved for 24 h (ref. 1) or incubated in the presence of iodoacetic acid and adenosine², a large increase in K^+ permeability is observed which depends on the presence of Ca^{2+} in the medium and is prevented or reversed by excess EDTA³. Passow⁴ suggested that under these conditions, a metabolite accumulates which controls the K^+ permeability. Hoffman¹ supported the alternative view that a protective substance that normally controls the K^+ permeability in red cells is removed by prolonged starvation or by the action of metabolic poisons. The existence of a lag period before the onset of the K^+ permeability change¹ suggests that it is necessary for ATP to be depleted before the actual mechanism responsible for the K^+ permeability change can be switched on. However, the possibility that the fall in the intracellular concentration of ATP might be the only relevant event in inducing the permeability change has been overlooked. If ATP were only required to keep Ca^{2+} out of the cell⁵ or out of some kind of receptor site on the membrane, either by means of a Ca^{2+} pump⁶ or by some other mechanism, one would expect the variations in Ca^{2+} uptake⁷ and the associated changes in K^+ permeability to correlate with the changes in ATP concentration, independently of the way in which they

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are produced. The experiments reported here were designed to provide only a qualitative test of this hypothesis.

Fig. 1 summarizes results obtained with this kind of experiment. It can be seen that the higher the efficiency of the method used to deplete the red cell ATP, the larger the magnitude of the Ca^{2+} uptake and K^+ loss and the shorter the duration of the lag period. According to the ATP hypothesis proposed here, the only action of the substrates in the presence of iodoacetamide is to speed up the rate at which the red cells are being depleted, since ATP is used in the conversion of the substrate to triose phosphate while the synthesis of ATP is blocked⁸. Inosine is more effective than glucose possibly because ATP is rate limiting for the hexokinase, a step bypassed by inosine in its conversion to fructose phosphate, and triose phosphate⁹. In inosine-fed cells, both the initial ATP concentration and the depletion rate after the addition of iodoacetamide were higher in the P_i -loaded cells

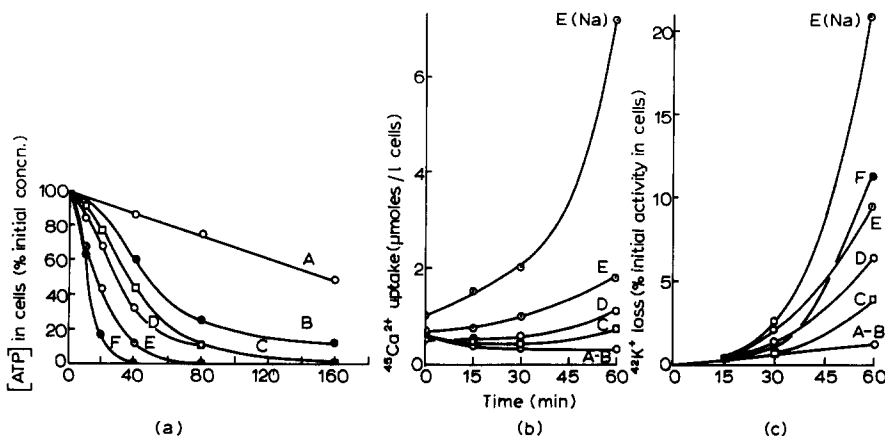


Fig. 1. The change with time of ATP concentration (a), $^{45}\text{Ca}^{2+}$ uptake (b) and $^{42}\text{K}^+$ loss (c) when human red cells are being depleted by different methods. Freshly drawn human red cells were washed and preincubated for 2–4 h at 37° in a medium containing some $^{42}\text{K}^+$ and either no substrate or the same substrate at the same concentration as during the final incubation (see below). After the preincubation the cells were thoroughly washed in a medium containing 140 mM KCl, 10 mM NaCl, and 10 mM Tris-HCl (pH 7.4 at 37°) and resuspended at a haematocrit of about 5% in the same medium to which 0.5 or 1 mM CaCl_2 with some $^{45}\text{Ca}^{2+}$ was added. The specific activity of $^{45}\text{Ca}^{2+}$ was about 1 mCi/mole. The procedure was somewhat different in the high- P_i cells which were loaded with P_i by incubating for about 20 min in a phosphate-citrate solution¹⁴, after the preincubation and before the washes. The additions to the final incubation medium were as follows: A, none; B, 5 mM 2-deoxyglucose; C, 5 mM iodoacetamide; D, 5 mM glucose and 5 mM iodoacetamide; E, 5 mM inosine and 5 mM iodoacetamide; F, same as E but containing the phosphate-loaded cells; E(Na), same as in E but the cells, after the preincubation, were washed and resuspended in a medium containing 150 mM NaCl and 2.5 mM KCl instead of 140 mM KCl and 10 mM NaCl. At the end of the final incubation, at the indicated times, the cell suspensions were cooled and centrifuged for 3 min at $2500 \times g$. The supernatant was assayed for $^{42}\text{K}^+$ activity and $^{45}\text{Ca}^{2+}$ was counted in the trichloroacetic acid supernatant of the washed cells. The wash solution contained 150 mM NaCl and either 5 mM CaCl_2 or 2 mM Tris-EGTA. ATP was determined in identically treated batches of cells by the firefly-extract method (C.L. Slayman via I.M. Glynn, personal communication). ATP concentration is expressed as a percentage of the initial concentration in cells at the beginning of the final incubation. The initial concentrations of ATP ranged between 650 and 900 $\mu\text{moles/l cells}$. Ca^{2+} uptake in phosphate-loaded cells is not reported since P_i leaking from the cells forms insoluble salts with external Ca^{2+} which precipitate with the cells during the washes (H. Passow, personal communication) thus masking the "true" uptake. In the conditions A and B the increase in Ca^{2+} uptake and K^+ loss occurred after the first hour and, within this period, the behaviour was identical with substrate-containing or Ca^{2+} -free controls.

than in the low- P_i cells. A possible explanation is that P_i is rate limiting for the nucleoside phosphorylase, which catalyses the conversion of inosine to ribose phosphate, as well as for the triose-phosphate dehydrogenase¹⁰. The K^+ -loss curves from inosine-fed cells in the presence of inosine and iodoacetamide show an increased lag period followed by a larger leak in the P_i -loaded cells compared with the low- P_i cells. This correlated well with the changes in ATP concentration. These results are illustrative of the high sensitivity of the mechanism involved in the K^+ permeability change to the intracellular ATP levels. This, rather than a chelating action of P_i on the intracellular Ca^{2+} , as suggested before by the author⁷, seems the most likely explanation for the effects of P_i on the K^+ -loss curve, since sulphate, with similar chelating properties as P_i (ref. 11) but without its effects on the cell ATP levels, fails to show any effect on the lag period. Once depleted, the Ca^{2+} -dependent K^+ leak is observed, irrespective of whether the depleting agents are present or have been washed out.

It was accidentally found that a substance with the spectroscopic and chromatographic characteristics of IMP accumulates in the red cells during acute ATP depletion in amounts roughly equivalent to the total adenine loss. This is probably produced by the increased activity of the AMP deaminase, no longer inhibited by 2,3-diphosphoglyceric acid¹² which runs down during depletion⁹. IMP, which could have made an ideal candidate to account for the Ca^{2+} -dependent changes in K^+ permeability according to Passow's⁴ views, had no effect on the K^+ fluxes in the presence of Ca^{2+} , when incorporated into ATP-free or ATP-containing resealed ghosts, in relation to the IMP-free controls.

Other agents, such as Pb, fluoride or triose reductone⁴, are known to produce a selective increase in the K^+ permeability in red cells, some of them even at high intracellular levels of ATP and in the absence of Ca^{2+} . Although the channel involved may be the same, the molecular mechanism by which the permeability change is actually produced may be entirely different in each case. A similar situation is illustrated in Figs. 1b and 1c, Curves E and E(Na). At the same intracellular level of ATP, a high concentration of K^+ in the medium decreases Ca^{2+} uptake and K^+ loss. This finding provides further support for the concept of a cause-effect relationship between Ca^{2+} uptake and the increase in K^+ permeability and at the same time underlines the existence of interactions other than those involving ATP at the Ca^{2+} site.

The results presented here raise the interesting possibility that the ATP-dependent mechanism that keeps the red cells virtually Ca^{2+} -free¹³ exerts a physiological control on the K^+ permeability.

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