BBA Report

BBA 71103

Effects of calcium and lead on potassium permeability of human erythrocyte ghosts

J.R. RIORDAN and H. PASSOW

Max-Planck-Institut für Biophysik, 6 Frankfurt (Main) 70, Kennedyallee 70 (Germany)

(Received September 27th, 1971)

SUMMARY

K^+ efflux from reconstituted erythrocyte ghosts was induced by Pb^{2+} or by Ca^{2+} in the presence or absence of F^- or iodoacetic acid *plus* adenosine. Oligomycin completely inhibited Ca^{2+}-induced K^+ loss and partially inhibited loss caused by maximally effective doses of Pb^{2+}. However, at submaximal Pb^{2+} concentrations oligomycin increased K^+ exit. Incorporation into ghosts of ATP or EDTA prevented Ca^{2+}-induced K^+ efflux but increased Pb^{2+}-induced K^+ efflux. ATP also prevented oligomycin from inhibiting Pb^{2+}-induced loss of K^+. The kinetics of Ca^{2+}-induced K^+ loss are similar to those previously observed with Pb^{2+} and suggest an 'all or none' effect.

Certain abnormal metabolic states of the red blood cell are associated with a considerable increase of the passive permeability of the membrane to K^+. The metabolic changes which are the prerequisites for the permeability change can be evoked by a variety of seemingly unrelated conditions, *e.g.* inhibition of glycolysis by F^- (ref. 1), the metabolic conversion of adenosine in the presence of iodoacetate[2], or prolonged substrate depletion[3,4]. However, in all instances, the metabolic disturbances are only followed by loss of K^+ if Ca^{2+} is present in the medium[5-7,4].

The described permeability change is confined to K^+. There is little effect on Na^+ transfer[8-10,4]. Some other agents, such as Pb^{2+} (ref. 9, 11) or propranolol[12] which do not primarily act as metabolic poisons, also evoke K^+ loss with little concomitant Na^+ uptake. Hence the effects produced by these substances may belong to the same class as those observed after the addition of Ca^{2+} to metabolically preconditioned erythrocytes.

Recently, it has been shown by Blum and Hoffman[4] that the effects of Ca^{2+} and iodoacetic acid on substrate-depleted cells can be inhibited partially by ouabain, or more completely by oligomycin. This suggests that, under the conditions used by these authors, the increased K^+ efflux is associated with an alteration of the system which serves as the K^+/Na^+ pump in normal red cells.

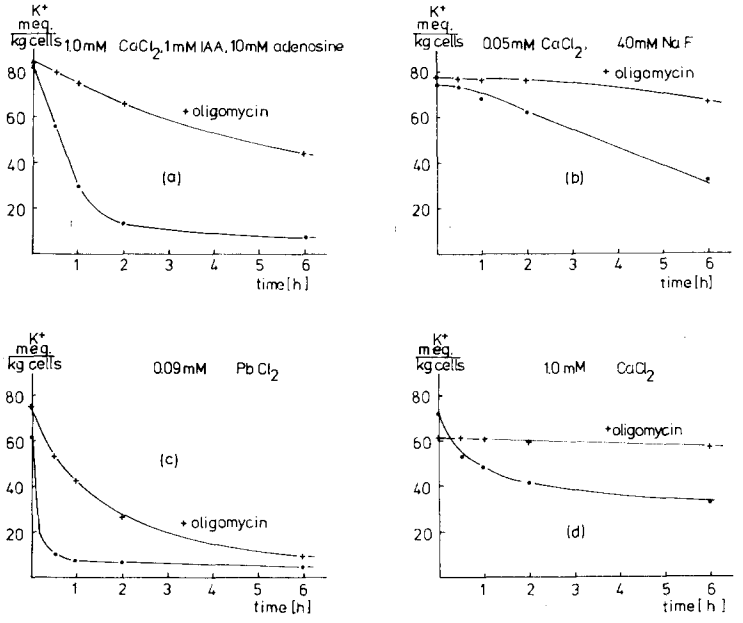

Fig. 1. Inhibition of K^+ loss from erythrocyte ghosts by oligomycin (20 mg/ml). Ghosts were prepared according to the method of Bodeman and Passow[13] and incubated in isotonic NaCl containing: (a) 0.05 mM $CaCl_2$ *plus* 40 mM NaF; (b) 1.0 mM $CaCl_2$; (c) 1 mM $CaCl_2$ *plus* 1 mM iodoacetate (IAA) *plus* 10 mM adenosine; and (d) 0.09 mM $PbCl_2$. K^+ levels in the ghosts were determined by flame photometry. All curves have been corrected for K^+ loss from the 'leaky' (Type III ghosts of Bodeman and Passow[13]) ghosts in each suspension.

The K^+ permeability of actively metabolizing erythrocytes is little if at all affected by extracellular Ca^{2+}. However, ghosts prepared from these cells lose K^+ when exposed to Ca^{2+} (Fig. 1d). In accordance with similar observations from other laboratories[15,16], they behave like substrate-depleted intact erythrocytes. This suggests that the cell contents protect the membrane against the action of Ca^{2+}. The ability of Ca^{2+} to induce the permeability change in the ghosts remained unaltered when the erythrocytes were preincubated in the absence of substrates for periods up to 24 h prior to the preparation of the ghosts.

Fig. 1b also shows that oligomycin inhibits Ca^{2+}-induced K^+ exit. Even if the sensitivity of the ghosts to the action of Ca^{2+} is augmented by the presence of either F^- (Fig. 1b), or a combination of iodoacetic acid and adenosine (Fig. 1a), the inhibitory effect of oligomycin is largely or fully preserved. Similar observations have been made with intact erythrocytes. The effects of oligomycin develop over a narrow concentration range. They are negligible at concentrations below 1 μg/ml and maximal at 10 μg/ml.

Interestingly enough, the K^+ exit produced by Pb^{2+} is also, at least partially, inhibited by oligomycin (Fig. 1c). This finding lends support to the assumption that Pb^{2+} and Ca^{2+} act on the same transfer system.

The metabolic disturbances preceding the onset of the permeability change are always associated with a dramatic decrease of intracellular ATP. The question has been asked, therefore, whether or not there exists a causal relationship between the intracellular ATP content and the effects of Ca^{2+} or lead on K^+ permeability (V.L. Lew, personal

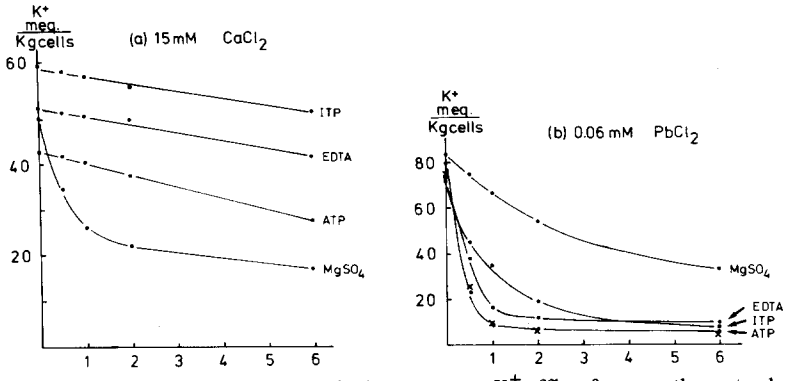

Fig. 2. Effects of intracellular complexing agents on K^+ efflux from erythrocyte ghosts. ATP, ITP, or EDTA at concentrations of 2 mmoles/l were incorporated into ghosts during osmotic hemolysis. Control cells contained 4 mmoles/l $MgSO_4$. Incubation was carried out as in Fig. 1 in isotonic NaCl containing (a) Ca^{2+} or (b) Pb^{2+} at the concentrations indicated. The symbol (X) refers to ATP-containing cells which were exposed to the simultaneous action of Pb^{2+} and oligomycin (20 mg/ml).

communication; and refs. 14, 16, 17). Fig. 2a shows that the protective action of the cell content against Ca^{2+} can be accounted for by the presence of intracellular ATP or related phosphoric acid esters. In accordance with observations by Hoffman[15] and Romero and Whittam[16] we find that in ghosts loaded with ATP, extracellular Ca^{2+} produces no K^+ loss. If in place of ATP another chelating agent such as EDTA or ITP is incorporated into the ghosts, the effect of Ca^{2+} is also abolished. This finding suggests that ATP could exert its protective action simply by its capacity to form complexes with Ca^{2+}. In starved cells, the effects of Ca^{2+} can be augmented by the addition of iodoacetic acid[4]. This is also true for ghosts prepared from fresh erythrocytes. Again, the action of Ca^{2+} is prevented by the incorporation of either ATP or EDTA into the ghosts. These protective effects of complexing agents are similar to those obtained with red cell ghosts which are exposed to very low concentrations of $CaCl_2$ (0.05 mmole/l) in the presence of F^- in the medium[10]. V.L. Lew (personal communication) has suggested that even the inhibition of the Ca^{2+} effect by ouabain as described by Blum and Hoffman[4] is the direct consequence of the preservation of some ATP in cells whose K^+/Na^+ sensitive pump enzyme had been inhibited by the glycoside.

In contrast to the inhibitory action of incorporated complexing agents on Ca^{2+}-induced K^+ loss, intracellular ATP, ITP, or EDTA considerably increase the effect of lead on K^+ efflux (Fig. 2b). However, the presence of intracellular ATP not only enhances the response to a given concentration of lead but also prevents the inhibition of Pb^{2+}-induced K^+ exit by oligomycin (Fig. 2b; compare the locations of dots with crosses on the curve marked 'ATP'). This results shows clearly that, although there are marked similarities between the action of Pb^{2+} and Ca^{2+}, there are also distinct differences in detail which will be significant for a future understanding of the mechanism by which the two metals modify K^+ permeability. In this context, it is noteworthy that the inhibitory action of oligomycin is only observed when the Pb^{2+} concentration is high enough to produce a maximal rate of K^+ loss, as in the experiment represented in Fig. 1c. At submaximal concentrations, oligomycin enhances the action of Pb^{2+}, thereby acting somewhat similarly to intracellular ATP (Fig. 3).

Fig. 4a shows the relationship between Ca^{2+} concentration in the medium and its effect on the K^+ content of red cell ghosts. After an initial rapid phase, before reaching

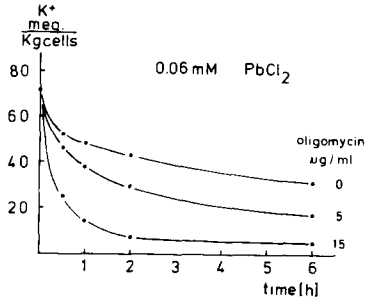

Fig. 3. Stimulation by oligomycin of K^+ loss caused by a submaximal dose of Pb^{2+} (0.06 mmole/l).

diffusion equilibrium, K^+ loss slows down and continues at a much lower rate. The time interval elapsing between the beginning of the experiment and the transition from the rapid to the slow phase of K^+ loss seems to be nearly independent of the Ca^{2+} concentration. This behaviour is similar to that observed in Pb^{2+} poisoning of intact human erythrocytes[11,18] where the individual cells respond in an 'all or none' fashion. In a given red cell suspension, increasing the lead concentration has little effect on K^+ efflux until a threshold value is exceeded. A slight further increase of the concentration of the poison leads to the maximal rate of K^+ exit. The measured K^+ content of the cell population represents the average of nearly unchanged and maximally altered cells. The curves relating K^+ loss to time level off when the cells whose threshold values are exceeded reach diffusion equilibrium. The K^+ content measured after that time primarily reflects the K^+ content of those cells whose threshold was not surpassed.

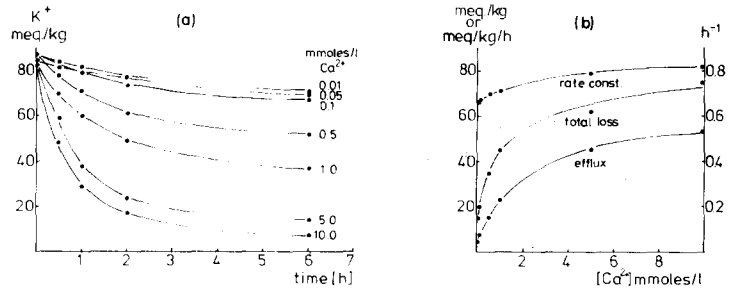

Fig. 4. Effects of varying Ca^{2+} concentrations in the medium on the decrease in K^+ content of erythrocyte ghosts. (a) Time course of K^+ loss. (b) Dependence of initial rates of K^+ loss ('efflux'), total K^+ loss during rapid early phase ('total loss'), and rate constants from curves in (a) on Ca^{2+} concentration.

The interpretation of the present experiments with Ca^{2+} is not yet clear. If one assumes that all of the ghosts of the population are similary affected, the results of Fig. 4a can be summarized by plotting the initial rates of K^+ efflux *versus* the Ca^{2+} concentration in the medium (Fig. 4b). However, if one assumes that the curves primarily represent an all or none effect, it would seem preferable to plot the rate constant which describes the time course of the transition from the fast to the slow phase of K^+ loss against Ca^{2+} in the medium. This rate constant primarily reflects the response of those ghosts whose

threshold was exceeded. Fig. 4b shows that this quantity varies only little with Ca^{2+} concentration. The curve marked 'total loss' represents the amount of K^+ lost during the initial rapid phase of K^+ exit. If an all or none effect is involved, this amount of K^+ is proportional to the number of ghosts whose threshold was surpassed. An all or none response suggests that the alteration of the permeability sites in the erythrocyte membrane brought about by Pb^{2+} and, possibly, Ca^{2+} are cooperative phenomena.

This work was supported by the Deutsche Forschungsgemeinschaft and by a Fellowship from the Canadian Cystic Fibrosis Foundation to J.R.R.

REFERENCES

1 W. Wilbrandt, *Arch. Ges. Physiol.*. 243 (1940) 519.
2 G. Gardos, *Biochim. Biophys. Acta*, 30 (1958) 653.
3 R.M. Blum and J.F. Hoffman, *Fed. Proc.*, 29 (1970) 663a.
4 R.M. Blum and J.F. Hoffman, *J. Membrane Biol.*, (1971) in the press.
5 G. Gardos, *Acta Physiol. Acad. Sci. Hung.*, 14 (1958) 1.
6 G. Gardos, *Acta Physiol. Acad. Sci. Hung.*, 16 (1959) 121.
7 S. Lepke and H. Passow, *Arch. Ges. Physiol.*, 271 (1960) 389.
8 E. Dunker and H. Passow, *Arch. Ges. Physiol.*, 252 (1950) 609.
9 H. Passow, in H.D. Brown, *Cell Interface Reactions*, Scholars Library, New York, 1963, p. 58.
10 H. Passow, in Ch. Bishop and D.M. Surgenor, *The Red Blood Cell*, Academic Press, New York, 1964, p. 113.
11 H. Passow, in J. Maniloff, J.R. Coleman and M.R. Miller, *Effects of Metals on Cells, Subcellular Elements, and Macromolecules*, C.C. Thomas, Springfield, Ill., 1970, p. 291.
12 A. Ekman, V. Manninen and S. Salminen, *Acta Physiol. Scand.*, 75 (1969) 333.
13 H. Bodeman and H. Passow, *J. Membrane Biol.*, (1971) submitted for pubication.
14 V.L. Lew, *Biochim. Biophys. Acta*, 233 (1971) 827.
15 J.F. Hoffman, *Am. J. Med.*, 41 (1964) 666.
16 P.J. Romero and R. Whittam, *J. Physiol.*, 214 (1971) 481.
17 S. Lepke and H. Passow, *J. Gen. Physiol.*, 51 (1968) 365s.
18 H. Passow and K. Tillman, *Arch. Ges. Physiol.*, 262 (1955) 23.