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THE EFFECT OF INORGANIC PHOSPHATE ON SODIUM FLUXES IN DOG RED BLOOD CELLS

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Summary

The effect of extracellular inorganic phosphate on Na^+ movements in dog red blood cells has been studied. As the phosphate concentration is increased from 0 to 30 mM, Na^+ efflux increases by 2- to 3-fold and Na^+ influx increases approximately 2-fold. This enhancement of Na^+ fluxes by phosphate can be prevented by the addition of iodoacetate (1 mM), an inhibitor of glycolysis, or 4-acetamido-4′-iso-thiocyantostilbene-2,2′-disulfonic acid (0.01 mM), which blocks anion transport, to the medium. The increases in Na^+ movements are not caused by changes in cell volumes. These results suggest that phosphate must enter the cell to enhance Na^+ fluxes and that the mechanism of action may be via a stimulatory effect on glycolysis.

Introduction

Dog red blood cells differ from human erythrocytes in that the intracellular sodium concentration is high and the potassium content is low. Furthermore, no ouabain-sensitive cation flux has been measured in these cells [1—3]. It has been recognized for some time that the values for Na^+ and K^+ fluxes in dog red cells are variable because of the heterogeneity even within a given sample of cells [4,5]. Recently, during the course of other experimental work in our laboratory we discovered another factor which may contribute to the variability in flux measurements. We found that the rate of Na^+ efflux was greater when dog red cells were incubated in medium containing phosphate than in non-phosphate medium. Since many investigators use media of varying phos-

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TABLE I

COMPOSITION OF THE INCUBATION MEDIA

The composition of the seven different experimental media used in this study. The approximate total phosphate concentration is shown in the left hand column. All numbers represent concentrations in mmol per l. In addition, glucose (5.5 mM) was present in all solutions.

Total phosphate concentration	NaCl	KCl	Na_2HPO_4	NaH_2PO_4	Na PIPES	Sucrose
0	145	5	0	0	5	12
5 mM	139	5	4.2	0.8	4.2	10
10 mM	133	5	8.4	1.6	3.3	8
15 mM	127	5	12.6	2.5	2.5	6
20 mM	121	5	16.8	3.3	1.7	4
25 mM	115	5	21	4.1	0.9	2
30 mM	109	5	25.4	5	0	0

phate concentrations, we decided to study this phenomenon more closely. The objective of this study is to report the dependence of Na^+ fluxes on the inorganic phosphate concentration of the incubation medium.

Materials and Methods

The procedures for preparation of the blood and for influx and efflux measurements were the same as those reported in earlier publications [2,6]. Sodium efflux was measured as the rate constant for efflux in h^{-1} and influx was expressed as mmol Na^+/l of cells per h. All flux measurements were made over a 2 h period in incubation media in which the inorganic phosphate concentrations were varied between 0 and 30 mM. When no phosphate was present, the solution was buffered with 5 mM NaPIPES (piperazine-*N*,*N*′-bis (2-ethanesulfonic acid)). The pH of all solutions was 7.4. In addition, sucrose was added to the medium whenever appropriate to maintain the volume of the cells relatively constant. The composition of the solutions is shown in Table I.

Results

The results from one typical experiment, in which the effects of 0, 10 mM and 30 mM phosphate on Na^+ efflux were determined, are shown in Fig. 1. Sodium efflux in dog red cells behaves as a three-compartment system [2,4, 6,7]. In this paper we will deal only with efflux from the second intracellular compartment (i.e., after 30 min of incubation) which represents more than 95% of the total Na^+ efflux. The figure shows that as the phosphate concentration is increased from 0 to 30 mM, the rate constant for this portion of the efflux also increases. In contrast, K^+ efflux seems to be unaffected by external phosphate (data not shown here).

The results of the Na^+ efflux experiments are summarized in Fig. 2 in which the relationship between Na^+ efflux and the phosphate concentration of the incubation medium is shown. The rate of Na^+ efflux increases in a somewhat linear manner with increasing phosphate concentration from 0.065 h^{-1} at 0 mM phosphate to 0.180 h^{-1} at 30 mM inorganic phosphate. There does not appear

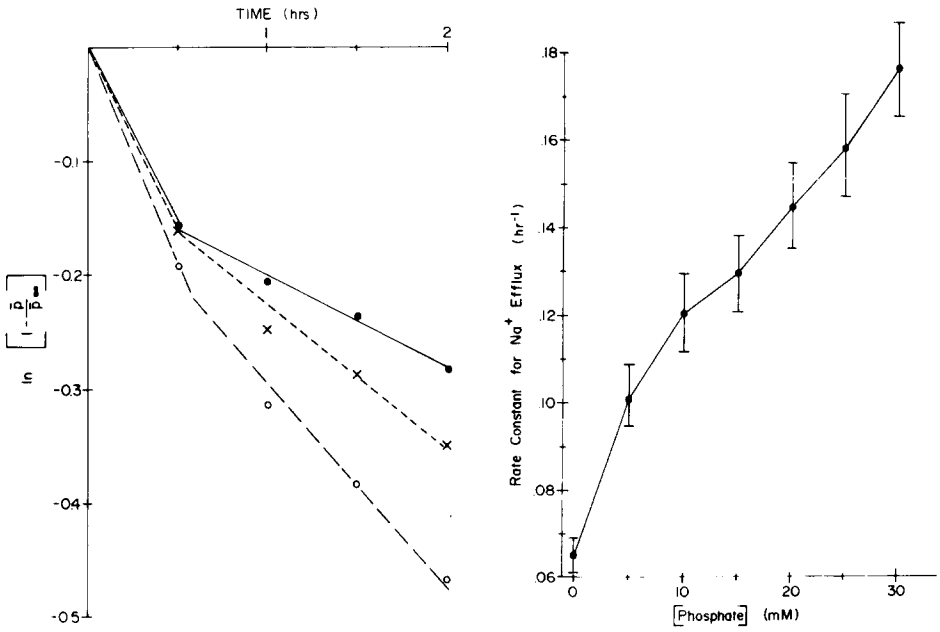

Fig. 1. Sodium efflux measurements in media containing 0 (closed circles), 10 (crosses), and 30 mM (open circles) inorganic phosphate. The data in this figure represent results obtained from one typical experiment, i.e., in red blood cells from one animal.

Fig. 2. The relationship between Na^+ efflux and the inorganic phosphate concentration of the medium. Sodium efflux is measured as the rate constant which is plotted on the ordinate. The points represent the mean values for 9 experiments and the bars are the standard errors of the mean.

to be any tendency toward saturation. Thus, over the range of 0—30 mM external phosphate the rate of Na^+ efflux increases 2.5- to 3-fold. It should also be pointed out that there was a rather large variability in the efflux measurements. Nevertheless, there was always a greater rate of efflux in the presence of phosphate. A possible explanation for the variability in these measurements will be presented later in this paper.

The relationship between Na^+ influx and the extracellular phosphate concentration is shown in Fig. 3. The rate of Na^+ influx definitely increases with an increase in the external phosphate concentration. The increase in Na^+ influx over the entire range of phosphate concentrations studied is about 2-fold, which is slightly less than the increase that occurs in the efflux rate. Nevertheless, these results do show that Na^+ permeability, i.e., both influx and efflux, is dependent upon the inorganic phosphate concentration of the incubation medium.

It is possible that the results reported above could have been caused by changes in the volumes of the cells. The Na^+ permeability of dog red blood cells is dependent upon cell volume, i.e. Na^+ permeability is increased in shrunken cells and decreased in swollen cells [8]. Therefore, in each of the experiments reported above the mean cell volumes were determined in the usual manner by dividing the hematocrit by the cell count (determined with a Coulter Counter, Model B; Coulter Instrument Co., Hialeah, Florida). In the nine experiments

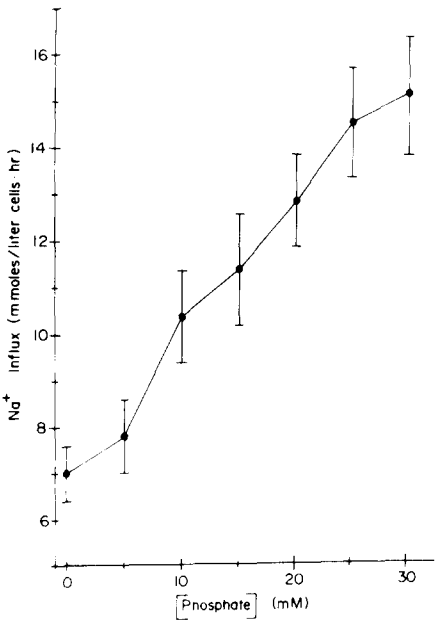

Fig. 3. The relationship between Na^+ influx and the inorganic phosphate concentration of the medium. The points represent the mean values for 9 experiments and the bars are the standard errors of the mean.

reported, the mean change in cell volume as the phosphate concentration was increased from 0 to 30 mM was a 0.6% swelling (±0.8%). Therefore, the change in Na^+ permeability does seem to be caused by inorganic phosphate and not by a change in the volume of the cells.

The phosphate-induced enhancement of Na^+ permeability can be prevented by the addition of either iodoacetate or SITS (4-acetamido-4′-iso-thiocyanto-stilbene-2,2′-disulfonic acid) to the medium. These data are shown in Table II. Over the range of 0—30 mM external phosphate, the rate constant for Na^+ efflux increases about 2.5-fold (Fig. 2). In the presence of 1 mM iodoacetate the rate constants for Na^+ efflux in 0 and 30 mM phosphate media are identi-

TABLE II

EFFECT OF IODOACETATE AND SITS

The concentration of iodoacetate used in these experiments was 1 mM and the concentration of SITS was 0.01 mM. The numbers to the right of the rate constants are the standard errors of the mean and *n* represents the number of experiments performed.

Total phosphate concentration	Treatment	Rate constant for Na^+ efflux (h^{-1})	*n*
0	Control	0.065 (± 0.004)	9
30 mM	Control	0.178 (± 0.013)	9
0	Iodoacetate	0.056 (± 0.007)	4
30 mM	Iodoacetate	0.059 (± 0.005)	4
0	SITS	0.070 (± 0.019)	3
30 mM	SITS	0.063 (± 0.014)	3

cal. Iodoacetate is known to be a potent inhibitor of glycolysis in red blood cells [9]. Therefore, inhibition of the phosphate effect by iodoacetate suggests that the action of external phosphate on Na^+ permeability may be mediated through cellular metabolism.

The addition of 0.01 mM SITS to the medium completely blocks anion transport in dog red blood cells [10], but it does not affect cation permeability when used in this low concentration [11]. The fact that SITS blocks the phosphate-induced increase in Na^+ efflux is shown in Table II. These data suggest that phosphate must enter the cells in order to enhance Na^+ permeability. If the action of phosphate were mediated through metabolism, one would expect that it must enter the cell to cause an increase in Na^+ permeability. Therefore, blocking phosphate entry would prevent the phosphate-induced enhancement of Na^+ fluxes. Finally, it should be pointed out that neither iodoacetate nor SITS exerts its effect by changing the cell volumes.

Discussion

The results of these experiments indicate that the Na^+ permeability of dog red blood cell membranes is dependent upon the extracellular concentration of inorganic phosphate, but the exact mechanism by which phosphate acts is not clear. One possibility is that the phosphate effect is mediated via an effect on the metabolic state of the cells. When metabolism is blocked by iodoacetate, extracellular phosphate no longer causes an increase in Na^+ efflux. Therefore, the increase in Na^+ permeability caused by phosphate could be due to stimulation of glycolysis. Previous studies have shown that inorganic phosphate stimulates glucose utilization in human red cells and, that over the same concentration range as used in the present study, enhances glycolysis [12,13]. Furthermore, results of experiments from our laboratory (not reported here) show that the level of lactate production in dog red cells is approximately two times greater in the presence of 25 mM phosphate than in 0.2 mM phosphate. Thus, it seems likely that inorganic phosphate exerts its effect via glycolysis. This would also explain the large variability in the magnitude of the phosphate effect (see Figs. 2 and 3) since red cells drawn for different experiments would likely be in different metabolic states.

The suggestion that Na^+ permeability is somehow related to glycolysis is supported by several other studies. Miles and Lee [2] have shown that there is a decrease in the Na^+ permeability of glucose-starved dog erythrocytes, and that there is a concomitant decrease in the ATP content of these cells. Romualdez et al. [16] reported that phloretin causes a decrease in lactate production while also causing a decrease in Na^+ permeability. These investigators have suggested a model in which Na^+ movement and glycolysis are linked in dog red cells. In addition, there are other reports in which a connection between cellular metabolism and volume-dependent changes in sodium permeability has been established [1,14—16]. We have not yet investigated the effect of external phosphate on the volume dependence of Na^+ permeability in these cells.

In summary, the Na^+ permeability of dog erythrocytes is dependent upon the inorganic phosphate concentration of the extracellular fluid. The experiments indicate that phosphate must penetrate to the interior of the cell in

order to cause an increase in the Na^+ fluxes. It is suggested that the action of phosphate on Na^+ permeability is a result of an increase in the rate of glycolysis.

References

1 Hoffman, J.F. (1966) Am. J. Med. 41, 666–680
2 Miles, P.R. and Lee, P. (1972) J. Cell. Physiol. 79, 367–376
3 Parker, J.C. (1973) J. Gen. Physiol. 61, 146–157
4 Lange, Y., Lange, R.V. and Solomon, A.K. (1970) J. Gen. Physiol. 56, 438–461
5 Castranova, V. and Hoffman, J.F. (1977) Biophys. Soc. Abstr. 17, 151a
6 Castranova, V. and Miles, P.R. (1976) J. Gen. Physiol. 67, 563–578
7 Sha'afi, R.I. and Lieb, W.R. (1967) J. Gen. Physiol. 50, 1751–1764
8 Parker, J.C. and Hoffman, J.F. (1976) Biochim. Biophys. Acta. 433, 404–408
9 Necas, E. and Neuwirt, J. (1972) J. Lab. Clin. Med. 79, 388–396
10 Castranova, V., Weise, M.J. and Hoffman, J.F. (1976) Biophys. Soc. Abstr. 16, 170a
11 Castranova, V. and Miles, P.R. (1977) J. Membrane Biol. 33, 263–279
12 Rose, I.A., Warms, J.V.B. and O'Connell, E.L. (1964) Biochem. Biophys. Res. Comm. 15, 33–37
13 Minakami, S. and Yoshikawa, H. (1965) Biochim. Biophys. Acta. 99, 175–178
14 Elford, B.C. and Solomon, A.K. (1973) J. Physiol. 231, 37P
15 Elford, B.C. and Solomon, A.K. (1974) Biochim. Biophys. Acta. 373, 253–264
16 Romualdez, A., Sha'afi, R.I., Lange, Y. and Solomon, A.K. (1972) J. Gen. Physiol. 60, 46–57