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VANADATE INCREASES OXYGEN AFFINITY AND AFFECTS ENZYME ACTIVITIES AND MEMBRANE PROPERTIES OF ERYTHROCYTES

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SUMMARY: Incubation of blood with vanadate markedly increases the affinity of hemoglobin for oxygen, decreases the deformability of erythrocytes, reduces their osmotic fragility and alters their morphology, determining the appearance of equinocytic forms. Since vanadate is easily taken up by the erythrocytes and binds hemoglobin, these effects might result from interactions of vanadate with hemoglobin and with membrane proteins at the glycerate-2,3-P_2 and/or ATP binding site. In addition, vanadate inhibits phosphoglycerate mutase, phosphoglucomutase and adenylate-kinase activities from hemolysates, suggesting a possible inhibitory effect on erythrocyte metabolism

INTRODUCTION

Interest in the biological actions of vanadium compounds has increased greatly in recent years as a result of the discovery that vanadate can act as a potent inhibitor of the (Na^+-K^+)-ATPase (1,2) and several other enzymes which catalyze phosphoryl transfer or release reactions (3,4). It has been suggested that vanadium ions compete with phosphate because they can easily adopt a trigonal bipyramidal structure which resembles the transition state of phosphate during the reactions (3,4). Moreover, it has been demonstrated that vanadate is rapidly transported across the red cell membrane by the anion-exchange system and inhibits the (Na^+-K^+)-ATPase from the cytoplasmic side(5). Much

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of the vanadate taken up by the red cell is reduced to the vanadyl form and binds to hemoglobin(6). Being the affinity of hemoglobin for oxygen regulated through its interaction with glycerate-2,3-P_2(7), it seems likely that binding with vanadyl could alter the functional properties of hemoglobin. Furthermore, the recently described modulation effect of glycerate-2,3-$P_2$and ATP on the lateral mobility of integral membrane proteins(8,9) constitutes the basis for the possible effects of vanadate on some red cell properties related with membrane structure and function. The study of the effects of vanadate on hemoglobin function and on properties of red cell membrane is the purpose of the present paper

MATERIAL AND METHODS

Anhydrous sodium metavanadate ($NaVO_3$) was from Merck Darmstad. Purified enzymes, substrates and cofactors were from either Boehringer Mannheim or Sigma Corporation Co. All other chemicals used were reagent grade.
Studies were performed on fresh heparinized venous human blood. Vanadate was dissolved in plasma two hours before its addition to whole blood to obtain the desired final concentrations.
Hemoglobin-Oxygen saturation curve was automatically recorded at 37°C from whole blood with an Hem-O-Scan system, after incubation with vanadate.
Red cell morphology was analyzed by light microscope observation after fixation of blood samples in 2% glutaraldehyde solution (pH 8.35;210 mOs).
Membrane flexibility (erythrocyte deformability), was measured by the filtration time of 1 ml of 44% red cell suspension in autologous plasma through a 5μm pore Nucleopore^{R} membrane with a 20 cm water column negative pressure(10).
Erythrocyte osmotic fragility test was performed according to Dacie and Lewis(11). Plasma viscosity and osmolarity, and pH of plasma and whole blood were measured before and after incubation with vanadate.
Red cell enzymes were determined after hemolysis by standard methods at 37°C (12).

RESULTS

Effect of Vanadate on Hemoglobin-Oxygen saturation curve

Fig. 1 demonstrates the effect of vanadate on hemoglobin-oxygen saturation curve. As shown, the P_{50} value was slightly decreased when whole blood was incubated for 120 min at room temperature with 2 mM vanadate, and was markedly reduced by incubation with 4 mM vanadate. No effect was observed after 30 min and 60 min incubation. Blood pH remained constant at a value of 7.4.

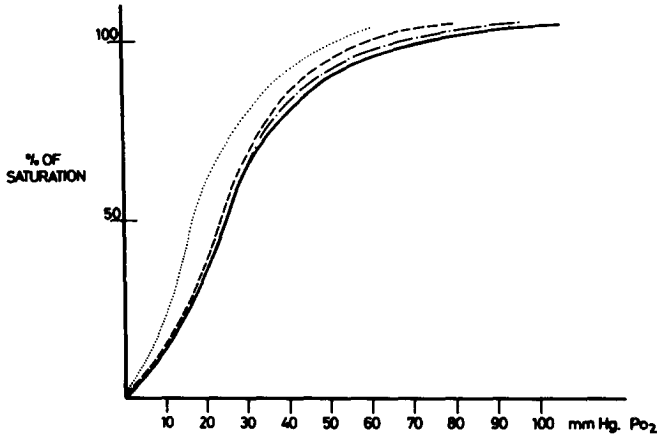

FIGURE 1

Effect of Vanadate on oxygen-dissociation curve of Hemoglobin. Whole blood was incubated with and without vanadate for 120 min at room temperature and the saturation curve was automatically recorded at 37° C (——), without vanadate (P_{50} = 26); (-.-.-), 1 mM vanadate (P_{50}= 26); (---) 2 mM Vanadate (P_{50} = 24); (...) 4 mM vanadate (P_{50} = 18).

Effect of vanadate on erythrocyte morphology, deformability and osmotic fragility.

Incubation of whole blood for 120 min at 25°C with 20 mM vanadate produced a remarkable alteration on the morphology of the red cells characterized by the appearance of equinocytic forms in a proportion higher than 80%(Table 1). Much less effect was observed after incubation with lower concentrations of vanadate. As shown in Table 2, blood incubation for 30 min with 20 mM vanadate strikingly reduces the filterability of the red cells. Lower concentrations of vanadate had a minor effect.

The effect of vanadate on erythrocyte osmotic fragility is shown in Fig. 2. As can be appreciated, 20 mM vanadate strongly reduced the hemolysis produced by hypotonic salt solutions.

Control experiments showed that plasma viscosity and osmolarity remained within normal values after incubation with vanadate, even at the higher concentrations used.

Effect of vanadate on the erythrocyte enzyme activities

As shown in Table 3, 10μM vanadate inhibited phosphoglucomutase (EC,2.7.5.1) and phosphoglycerate mutase(EC,2.7.5.3) activities. Adenylate kinase(EC,2.7.4.3) activity was only partially reduced by 1 mM vanadate. Hexokinase(EC,2.7.1.1), phosphoglucoseisomerase (EC,5.3.1.9), phosphofructokinase(EC,2.7.1.11), aldolase(EC,4.1.2.13)

TABLE 1

EFFECT OF VANADATE ON ERYTHROCYTE MORPHOLOGY

ERYTHROCYTE MORPHOLOGY FORMS	PERCENTAGE OF EACH FORM				
	NORMAL VALUES (N:20)	INCUBATION WITH VANADATE			
		-	0.2 mM	2 mM	20 mM
DISCOCYTE	86.3 ± 4.4	89.4	88.6	85.5	4.5
KNIZOCYTE	3.1 ± 2.2	2.5	0.3	0.6	0
STOMATOCYTE	3.5 ± 1.58	3.7	2.4	2.5	0
SPHEROSTOMATOCYTE	2.4	0.7	0	0	0
SPHEROCYTE	0.8	1	0	0.2	0
EQUINOCYTE I	1.3	1.2	7.0	7.3	11.1
II	0.6	0.5	0.9	2.4	46.7
III	0.2	0	0	0.8	37.8
ELLIPTOCYTE	0.8	0.8	0.2	0.2	0
DACRIOCYTE	0.6	0.3	0.7	0.5	0
SQUIZOZYTE	0.5	0	0	0	0

Whole blood was incubated for 120 min at 25°C with $NaVO_3$. Red cell morphology was analyzed as described in "Material and Methods"

TABLE 2

EFFECT OF VANADATE ON ERYTHROCYTE FILTERABILITY

TIME IN SECONDS				
NORMAL VALUES	INCUBATION WITH VANADATE			
	-	0.2 mM	2 mM	20 mM
Males: 33.5 ± 9.5 Females: 29.9 ± 5.4	29	35	33	∞

Whole blood was incubated for 30 min at 25°C with $NaVO_3$ and filterability time was measured as described in "Material and Methods". Results are the mean of three determinations.

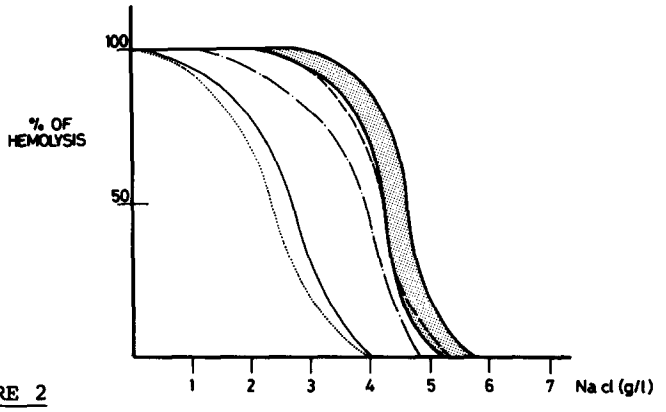

FIGURE 2

Effect of vanadate on osmotic fragility of erythrocytes. Whole blood was incubated with and wihtout vanadate for 120 min at room temperature and the osmotic fragility was measured acording to (11). Shadowed area, normal limits; (---), without vanadate (-.-.-) 0.2 mM vanadate (——), 2 mM vanadate (...) 20 mM vanadate.

phosphoglyceratekinase(EC,2.7.2.3),glyceraldehyde-3-phosphate dehydrogenase(EC,1.2.1.12),triosephosphate isomerase(EC,5.3.1.1) bisphosphoglycerate mutase(EC,2.7.5.3),enolase(EC,4.2.1.11), pyruvate kinase(EC,2.7.1.40),glucose-6-phosphate dehydrogenase (EC,1.1.1.49),6-phosphogluconate dehydrogenase(EC,1.1.1.44), glutathione reductase(EC,1.6.4.2) and glutathione peroxidase (EC,1.11.1.9) activities were not modified even with 1 mM vanadate.

DISCUSSION

It has been shown that vanadate enters into the erythrocytes by the same anion-exchange transport system as phosphate. Most

TABLE 3

EFFECT OF VANADATE ON ERYTHROCYTE ENZYME ACTIVITIES

ENZYME	RESIDUAL ACTIVITY (%) VANADATE FINAL CONCENTRATION			
	10 μM	20 μM	100 μM	1 mM
PHOSPHOGLUCOMUTASE	62	48	13	7
PHOSPHOGLYCERATE MUTASE	94	98	26	18
ADENYLATE KINASE	100	100	86	63

$NaVO_3$ was added to the assay mixture and the residual activity measured at 37°C (12)

of the vanadate taken up is then converted to the +4 oxidation state and binds to hemoglobin(5,6). The results presented in this paper demonstrate that incubation of whole blood with 4 mM vanadate for two hours markedly increases the affinity of hemoglobin for oxygen. Binding of vanadyl ions at the glycerate 2,3-P_2 binding site of hemoglobin could result in a competition which might explain the observed decrease in oxygen affinity. Furthermore, our results show that incubation of whole blood with vanadate severely decreases the deformability of erythrocytes, reduces their osmotic fragility and affects the morphology of the cells increasing the equinocytic forms. These effects could not be attributed to possible modifications of plasma pH,viscosity and osmolarity by vanadate, since these properties remained unchanged under experimental conditions.Therefore it seems reasonable to assume that membrane flexibility was modified by vanadate.Recently has been shown that the lateral mobility of the erythrocyte membrane proteins is modulated through interactions with polyphosphates such as glycerate-2,3-P_2, ATP and GTP. It has been postulated that through the regulation of glycerate-2,3-P_2 and ATP levels the red cell could control membrane skeleton organization and those cell properties affected by the skeleton (8,9). On this basis it seems reasonable to suggest that the effect of vanadate upon the properties of the erythrocyte membrane reported in this paper could result from vanadate interfering the interaction of polyphosphates with the protein of the membrane skeleton. The ability of vanadate taken up by erythrocytes to interact with the membrane has been demonstrated in the case of (Na^+-K^+) ATPase ,which is inhibited by vanadate from the cytoplasmic side(5).

Finally, the possibility that vanadate could affect the metabolism of the erythrocytes has to be considered since our results show that vanadate at the μM range inhibits phosphoglucomutase and phosphoglycerate mutase activities from the hemolysates. Inhibition of such enzymes from yeast and rabbit muscle has been already reported(13,14). In this context, it is interesting to notice that recently has been reported a significative increase in plasma vanadate levels in chronic renal disease(15). Some of the abnormalities on erythrocyte structure and funcion present in chronic renal failure probably could be explained through vanadate effects.

REFERENCES

1. Josephson,L. and Cantley,L.C.Jr.(1977).Biochemistry 16,4572-4578
2. Cantley,L.C. Jr. Josephson,L., Warner,R., Yanaguishawa,M., Lechere,C. and Guidotti,G. (1977).J.Biol.Chem. 252,7421-7424.
3. Simons,T.J.B.(1979) Nature 281,337-338.
4. Macara,I.A.(1980) Tibs 5,92-94.
5. Cantley,L.C.Jr., Resh,M.D., and Guidotti,G.(1978) Nature 272, 553-554
6. Cantley,L.C., Jr., and Aisen Ph.(1979) J.Biol. Chem.254,1781-1784
7. Benesh,R.E., and Benesh R.(1974). Advan. Protein. Chem. 28, 211-237
8. Schindeler,M., Koppel, D.E., Sheetz,M.P.(1979). Proc. Natl. Acad. Sci. USA 77, 1457-1461.
9. Sheetz,M.P., Casaly J.(1980) J. Biol. Chem. 255,9955-9960
10. Reid,H.L., Barnes,A.K., Lock,P.J., Dormandy,J.A., Dormandy,T.L. (1976). J. Clin. Pathol. 29,855-858
11. Dacie,J.V., Lewis,S.M. (1975). Practical Haematology. Churchill and Livingstone.London
12. Beutler,E.(1975) Red cell metabolism.A manual of biochemical methods. 2nd Ed. Grune & Stratton. New York.
13. Carreras, J. Bartrons R., Grisolia S.(1980). Biochem. Biophys. Res. Commun. 96, 1267-1273
14. Climent,F., Bartrons,R., Pons,G., Carreras,J.(1981). Biochem. Soc. Trans. 9(2),179P
15. Bello-Reus,E., Grady,T., Mazumbar,D.(1979) Ann.Int. Med. 91, 743-745