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ENZYMATIC EVENTS FOLLOWING THE INTERACTION OF FRUCTOSE-1, 6-DIPHOSPHATE WITH RED CELL MEMBRANES

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ABSTRACT

When fructose-1,6-diphosphate (FDP) is incubated with rat erythrocytes it is hydrolyzed by a membrane-bound phosphatase. The hydrolysis of FDP is associated with an uptake of K^+ ions and an extrusion of H^+ ions. The discharge of K^+ ions and the uptake of H^+ ions observed in the presence of valinomycin are decreased when FDP is present in the medium together with valinomycin.

KEYWORDS

Red cells, fructose-1,6-diphosphate, potassium uptake, proton ejection, valinomycin.

INTRODUCTION

Fructose-1,6-diphosphate (FDP) is used in parenteral nutrition and the administration of large amounts of FDP proved to be effective in the post-operative management of patients with adynamic ileus (Manani and colleagues, 1977). A double-blind clinical trial showed that FDP anticipates in fact the recovery of the intestinal peristalsis and decreases the incidence of nausea and vomiting in the post-operative period.

In a preceeding paper (Rigobello and colleagues, 1981) we have shown that FDP, incubated with rat erythrocytes, is enzymatically hydrolyzed and the released inorganic phosphate increases both in the incubation medium and inside the red cells. The enzymatic activity is dependent on a membrane-bound enzyme because it is present also in isolated red cell membranes. Other investigators (Magalini, Bondoli and Scrascia, 1977) have shown that, after incubation with FDP, both rat and human red cells increase their internal ATP level.

FDP was also shown to be able to decrease the toxicity of K^+ in mice (Cattani and colleagues, 1980) and, according to Lockwood and Lum (1973), such a protective effect on K^+ toxicity indicates an enhanced tissue uptake of K^+ and a reduction of the hyperkalemic state.

The enzymatic hydrolysis of FDP by red cells did not appear to be related to an activity of the FDP-bis phosphatase (EC 3.1.3.11) or FDP-aldolase (EC 4.1.2.13) and the lack of effects of ouabain on the FDP-stimulated K^+ uptake suggested that the latter is not strictly dependent on an ATPase-

mediated transport.
The "in vitro" studies hitherto carried out provide a rationale for the clinical use of FDP which, by stimulating K^+ uptake, should repolarize the intestinal smooth muscle and hence restore its function. The present study extends the preceeding ones and aims at providing further informations on the ability of FDP to regulate some transport phenomena of the red cell membrane.

RESULTS AND DISCUSSION

We have analyzed the influence of FDP (Biomedica Foscama, Rome) on the rates of disappearance of K^+ ions and appearance of H^+ ions in the suspending medium of rat erythrocytes.
Potassium was measured by atomic absorption (Perkin-Elmer 305B spectrophotometer) and protons by a glass electrode (Beckman Expandomatic recording pH-meter) while the red cells were obtained from 30 Wistar male rats (220-230 g) according to the already described procedure (Rigobello and colleagues, 1981).
The standard suspending medium contained 3.5 mg of hemoglobin (Hb), 2 mM FDP, 2 mM Tris-HCl and 140 mM NaCl at pH 7.5 in a final volume of 2.5 ml. Table 1 summarizes the results obtained after measuring the residual K^+ in the supernatant after spinning down the red cells (500 x g). The incubation was carried out at 37°C in the presence or absence of added valinomycin (2 ug).

TABLE 1: K^+ in the suspending medium of rat erythrocytes. Mean ± SD. The number of experiments is given within brackets.

	K^+ uEq/min/mg Hb
Control (7)	79.5 ± 13.4
+ FDP (7)	36.2 ± 16.2
+ Valinomycin (7)	116.2 ± 49.5
+ Valinomycin + FDP (7)	77.9 ± 18.8

From Table 1 it appears that the presence of FDP decreased the rate of discharge of K^+ from the red cells into the suspending medium. The presence of valinomycin greatly increases the efflux of K^+ from the red cells and such an efflux is reduced in the presence of FDP. In both cases the apparent reduction of K^+ efflux induced by FDP may indicate a reuptake of K^+ by the red cells.
Table 2 summarizes the results of the measurements of the pH of the external medium when the red cells were incubated with FDP in the presence or absence of valinomycin.
The conditions were similar to those used to measure the K^+ ions.

TABLE 2 H^+ ejection or uptake by red cells. Means ± SD. The number of experiments is given within brackets.

	H^+ nEq/min/mg Hb
Control (17)	- 9.55 ± 3.3
+ Na-phosphate (6)	-10.25 ± 2.8
+ FDP (17)	+20.60 ± 4.3
+ Valinomycin (12)	-15.45 ± 2.9
+ Valinomycin + FDP (10)	-10.60 ± 1.2

The negative sign in Table 2 indicates an alkalinization of the suspending medium and therefore an uptake of H^+ while the positive sign indicates an

acidification of the medium and therefore an ejection of H^+.
The results suggest that a strong ejection of H^+ is induced by FDP and does not depend on the release of inorganic phosphate as shown by control with Na-phosphate equimolecular to FDP. The net rate of H^+ extruded in the presence of FDP is therefore 30.15 nEq/min/mg Hb.
Valinomycin alkalinizes the medium but such an effect is brought to values close to the control in the presence of FDP.
In general ATP depletion and Ca entry into the red cells are phenomena known to induce a selective efflux of K^+ ions (Lew and Ferreira, 1977). FDP, on the contrary, when added to the medium of incubation, increases the ATP level within the red cell and stimulates an entry of K^+ against a gradient.
The red cell membrane is a Donnan-like system with relatively impermeant internal cations and any uptake of K^+ must be associated with an expenditure of energy while the transport of H^+ seems related to exchange processes of anions such as bicarbonate (Hladky and Rink, 1977).
The present data show that although the fluxes of K^+ and H^+ ions occur in opposite directions, either in the absence or in the presence of valinomycin, FDP is able to counteract both fluxes. In fact, in the presence of valinomycin, FDP decreases the efflux of K^+ (ca. 33%) and the H^+ uptake (ca. 32%), while in its absence the uptake of K^+ is increased (ca. 55%) and the ejection of H^+ appears as a typical response.
It is not possible to define, at present, whether the mechanisms of stimulation of the K^+ uptake or H^+ ejection which occur in the absence of valinomycin are a simple reversal of those of K^+ loss and H^+ uptake nor it is possible to suggest their mutual dependence or independence.
If the cellular accumulation of K^+ is controlled by specific absorption and desorption at negative sites throughout the whole cell, as advocated by the so-called association-induction hypothesis (Karreman, 1980), the present results could have an explanation.

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