Reprint from

EXPERIENTIA

Proton and potassium fluxes in rat red blood cells incubated with sugar phosphates

L. Galzigna and M. P. Rigobello, Padova (Italy)

Vol. 42
No. 2
1986
pages 138–139

Birkhäuser Verlag
Basel · Boston · Stuttgart

Experientia 42 (1986), Birkhäuser Verlag, CH-4010 Basel/Switzerland

Proton and potassium fluxes in rat red blood cells incubated with sugar phosphates

L. Galzigna and M. P. Rigobello

Institute of Biological Chemistry, University of Padova, I-35131 Padova (Italy), 2 August 1984

Summary. Fructose-1,6-diphosphate counteracts potassium ejection and proton uptake induced in rat red blood cells by valinomycin and an uncoupler. The effect on potassium ejection is reduced in the presence of ouabain and divalent cations.
Key words. Rat erythrocytes; K^+-flux; H^+-flux; fructose-1,6-diphosphate; potassium ejection.

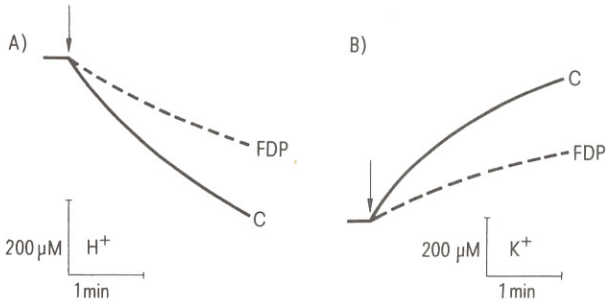
A previous study[1] showed that fructose-1,6-diphosphate (FDP) induces an uptake of K^+ and an extrusion of H^+ ions detectable, in rat erythrocytes, both in the presence and absence of valinomycin. Further work[2] showed that FDP increases the internal pH and K^+ concentration, and acts as an activator of Ca/Mg-ATPase of human red blood cells. This seems to be a rather specific effect of FDP, which is also bound by the red cell membrane.

An increase of the internal FDP concentration has been observed when FDP is incubated with human red blood cells[3], and FDP is more active than other sugars in protecting the mouse from potassium toxicity[4], since it enhances the uptake of K^+ by the tissues and therefore reduces the hyperkaliemic state[5].

This paper reports the results obtained by measuring K^+ and H^+ fluxes in rat erythrocytes incubated with FDP, fructose + phosphate (F + P), fructose-6-phosphate (F6P) fructose-2,6-diphosphate (F2,6P), an endogenous stimulator of phosphofructokinase[6], and cyclic FDP (FDPc), an intermediate in the chemical synthesis of F2,6P.

Materials and methods. FDP trisodium salt (Biomedica Foscama, Roma), F + P (Boehringer, Mannheim), F6P, FDPc and F2,6P (Sigma, St Louis) were used without further purification. Proton flux was measured with a Beckman glass electrode and potassium flux with a Beckman K^+-sensitive electrode, connected to a Beckman Expandomatic pH-meter and an Omni-Scribe recorder (Houston Instrument). All measurements were carried out at 25 °C, under constant magnetic stirring.

Whole blood was collected from Wistar male rats (280 g), after decapitation, and 5 ml of it was diluted with 30 ml of 125 mM NaCl, 30 mM Tris-HCl pH 8.0 and 10 mM EDTA. After 10 min centrifugation at 2000×g, supernatant and white cells were discarded and the sedimented red cells washed three times with 30 ml of the diluting solution. Hemoglobin (Hb) content was measured according to Beutler[7]. 3–4 mg/ml of Hb were used in each assay and concentration of sugar phosphates ranged between 1 and 4 mM. The experiments were carried out in the presence of 0.3 µg/ml valinomycin and 2 µg/ml carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP) (Sigma, St Louis), used together to induce outward K^+ and inward H^+ movements[8] and also in the presence of 66 µM ouabain (Siguama, St Louis), 4 mM $MgCl_2$ and 0.5 mM $CaCl_2$, to inhibit Na/K-ATPase[9] and activate Ca/Mg-ATPase[10]. H^+ translocation was measured in an



Tracing of H^+ uptake, with disappearance of H^+ from the medium (A) and K^+ ejection, with increase of K^+ in the medium (B), after addition of valinomycin/FCCP (arrow). Control (C) and 4 mM FDP (FDP).

incubation medium of 100 mM NaCl, 30 mM KCl, 5.5 mM glucose and 2.5 mM Tris-HCl pH 8.0; K^+ translocation in a medium of 250 mM sucrose, 5.5 mM glucose and 5 mM Tris-HCl pH 8.0, by adding 1 mM KCl before the addition of valinomycin/FCCP.

Results and discussion. The figure shows typical tracings of H^+ and K^+ movements obtained in the presence and absence of FDP: the inhibitory effect of FDP on both fluxes, as documented in table 1 and table 2, seems rather specific.

A blank with NaCl was carried out to see whether the effect was due to the added Na^+ counterions or to osmotic factors. The samples with added NaCl showed a difference of less than 5% from the controls.

The only sugar with an action comparable to, albeit lower than that of FDP is F2,6P. If one considers the K^+/H^+ ratio (table 3), with an active Na/K-ATPase only FDP lowers the ratio by increasing the external H^+ and decreasing the external K^+. In the presence of Ca/Mg/ouabain, only FDP increases the ratio.

The presence of Ca/Mg/ouabain modifies the homeostasis of K^+ and H^+ fluxes induced by valinomycin/FCCP, and only FDP seems to be able to counteract such a modification.

The demonstrated interaction of FDP with the red cell membrane[2] might be related to this phenomenon and FDP could act as a trigger of a stimulus-response-recovery cycle of the red cell according to Roth et al.[11]. The clinical effects of FDP cannot, in fact, be explained on the basis of a penetration of the compound through the cell membranes, which is not possible, and FDP must therefore exert its intracellular effects by acting from outside. The present data, together with those obtained with human red cells[2] indicate that, by affecting ion translocation, FDP influences phosphofructokinase and hence glycolysis on one side and membrane polarization on the other.

Table 1. Inhibitory effect of 1.5 mM sugars, in the presence and absence of Ca/Mg/ouabain (CaMgOu), on the valinomycin/FCCP-induced K^+ ejection from rat red blood cells

		CaMgOu
Control	79.6 ± 1.1 (100)	44.7 ± 0.8 (100)
FDP	44.3 ± 0.9** (55)	31.5 ± 0.7* (70)
F2, 6P	64.0 ± 0.8* (80)	35.1 ± 0.4* (78)
F6P	67.0 ± 0.6 (84)	40.1 ± 0.6 (90)
F + P	68.5 ± 0.7 (86)	38.0 ± 0.5 (85)
FDPc	69.6 ± 0.8 (87)	37.6 ± 0.4 (84)

Mean ± SE of six experiments. Values expressed as nion/min/mg Hb. Percent in brackets. ** Statistically significant by Student's t-test ($p < 0.01$). * Statistically significant by Student's t-test ($p < 0.05$).

Table 2. Inhibitory effect of 1.5 mM sugars, in the presence and absence of Ca/Mg/ouabain (CaMgOu), on the valinomycin/FCCP-induced H^+ uptake by rat red blood cells

		CaMgOu
Control	46.7 ± 2.1 (100)	58.0 ± 1.8 (100)
FDP	29.0 ± 1.8** (62)	30.0 ± 1.6** (51)
F2, 6P	32.8 ± 1.6* (70)	43.0 ± 1.5* (74)
F6P	40.6 ± 1.4 (87)	56.0 ± 1.8 (96)
F + P	38.3 ± 1.2 (82)	49.8 ± 2.1 (86)
FDPc	38.5 ± 1.6 (83)	49.6 ± 1.3 (85)

Mean ± SE of six experiments. Values expressed as nion/min/mg Hb. Percent in brackets. ** Statistically significant by Student's t-test ($p < 0.01$). * Statistically significant by Student's t-test ($p < 0.05$).

Table 3. K^+/H^+ ratio calculated from tables 1 and 2 data, in the presence and absence of Ca/Mg/ouabain (CaMgOu)

		CaMgOu
Control	1.70	0.77
FDP	1.52	1.05
F2, 6P	1.95	0.81
F6P	1.65	0.71
F + P	1.78	0.76
FDPc	1.80	0.75

1 Rigobello, M.P., Deana, R., and Galzigna, L., in: Advances in Pathology, p. 215. Ed. E. Levy. Pergamon Press, Oxford 1982.
2 Galzigna, L., Rigobello, M.P., Scutari, G., and Burlina, A., in press 1985.
3 Lazzarino, G., Cattani, L., Costrini, R., Mulieri, L., Candiani, A., and Galzigna, L., Clin. Biochem. *17* (1984) 42.
4 Cattani, L., Costrini, R., Cerilli, C., Rigobello, M.P., Bianchi, M., and Galzigna, L., Agressologie *21* (1980) 263.
5 Lockwood, R.V., and Lum, B.K.B., J. Pharm. exp. Ther. *189* (1974) 119.
6 Hers, H.G., and Hue, L., A. Rev. Biochem. *52* (1983) 617.
7 Beutler, E., in: Red Cell Metabolism, p. 11. Grune & Stratton, New York 1971.
8 Harris, E.J., and Pressman, B.C., Nature *216* (1967) 918.
9 Isern, M., and Romero, P.J., J. Physiol. *265* (1977) 411.
10 Roufogalis, B.D., Can. J. Physiol. Pharmac. *57* (1979) 1331.
11 Roth, Z., Chayen, N., and Dikstein, S., Int. Rev. Cytol. *85* (1983) 39.

0014-4754/86/020138-02$1.50 + 0.20/0