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THE INACTIVATION BY FLUORODINITROBENZENE OF GLUCOSE TRANSPORT ACROSS THE HUMAN ERYTHROCYTE MEMBRANE

THE EFFECT OF GLUCOSE INSIDE OR OUTSIDE THE CELL

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SUMMARY

The inactivation of glucose transport in human red cells by fluorodinitrobenzene is accelerated by 120 mM glucose outside the cell but retarded at least 50% by 120 mM glucose inside the cell. This suggests that the transport system is predominantly in one conformation when there is glucose inside the cell, and in another conformation when there is glucose outside the cell.

INTRODUCTION

1-Fluoro-2,4-dinitrobenzene (FDNB) irreversibly inactivates glucose transport in human red cells, and the rate of inactivation is increased in cells equilibrated with glucose[1,2], suggesting that glucose causes a conformational change in the transport system[2]. To investigate this conformational change I have determined the change in the rate of inactivation when there is glucose on only one side of the membrane. Glucose outside the cell accelerates the rate of inactivation but glucose inside the cell retards the reaction.

A preliminary account of this work has been given[3].

METHODS

To measure the rate of inactivation in cells with glucose inside, the cells were incubated with FDNB for only 1 min so that the glucose had not all left the cell before the end of the incubation. To inactivate a substantial proportion of the transport in 1 min, 8 mM FDNB was used and ethanol, which accelerates inactivation[4], added to 12.33% (by vol.). In the absence of glucose the half-time of inactivation was 0.5 min.

Blood 2–6 days old from a blood bank was washed in "buffer": saline containing 5 mM Tris–HCl (pH 7.7 at 25 °C). Cells were loaded with glucose or made glucose-free by incubating in buffer with or without 120 mM glucose at 37 °C for 30 min.

Abbreviation: FDNB, 1-fluoro-2,4-dinitrobenzene.

Ethanol was added at 25 °C to 12.33% (by vol.), and the cells were centrifuged. 0.1 ml packed cells were exposed to FDNB by suspending them at 25 °C in 5.7 ml buffer containing 12.33% (by vol.) ethanol and 8.1 mM (with respect to total volume) FDNB. After 1 min, 30 ml ice-cold buffer containing 10 mM mercaptoethanol was added. This stopped reaction in less than 2 s and did not reverse the inactivation of transport by FDNB. The cells were washed and incubated in buffer at 37 °C for 1 h to remove glucose. The inactivation of the glucose transport system was determined by measuring transport of the weak substrate sorbose. Cells were loaded with [^{14}C]-sorbose by incubation in 104 mM [^{14}C]sorbose for 1 h at 37 °C. They were washed in ice-cold buffer and suspended at 20 °C in 8 ml buffer containing 104 mM unlabelled sorbose, and the efflux of [^{14}C]sorbose measured by taking samples of the suspension immediately and 25 min later, centrifuging and taking 1 ml of the supernatant for scintillation counting. The total radioactivity in 1 ml suspension, T, was measured by mixing a sample with an equal volume of 5% trichloroacetic acid and counting the supernatant. The rate constant of efflux, a, was calculated from

$$\ln(1-s/T) = -at + \text{constant}$$

where s is the radioactivity in a sample taken at time t. A correction was made for cell lysis, which did not change the apparent rate of inactivation by FDNB, k, by more than 6%. k was calculated from

$$-\ln(a/a_0) = kt'$$

where a is the efflux rate from cells incubated for a time t' with FDNB, a_0 the rate from cells incubated under the same conditions but without FDNB.

To show the effect of glucose outside the cell, glucose-free cells were incubated in buffer containing FDNB, ethanol and glucose (120 mM with respect to the buffer before addition of ethanol) for 0.5 min. Methods were as above except that incubations contained 0.25 ml cells and 50 mM sorbose was used for the transport measurements. The same procedure was used to show the effect of glucose on both sides of the membrane at once, using glucose-loaded cells.

Materials. [^{14}C]Sorbose was obtained from the Radiochemical Centre Amersham; FDNB was from British Drug Houses Ltd; malonamide was from Ralph N. Emmanuel Ltd.

RESULTS

The effect of glucose on the rate of inactivation of glucose transport by 8 mM FDNB is shown in Table I.

Glucose inside the cell can at least halve the rate of inactivation. During the 1-min incubation with FDNB the internal glucose concentration would have fallen rapidly, so these experiments underestimate the protective effect. (At 25 °C the maximum rate V of glucose efflux from red cells is about 170 mM/min[5], but 5% (by vol.) ethanol inhibits glucose efflux by 33%[4], so 12.33% ethanol will perhaps halve the V of efflux.)

Glucose outside the cell increased the rate of inactivation by about 30% compared to both the rate in the absence of glucose and the rate with glucose both sides of the membrane, which was measured in the same experiments. This is an

TABLE I

THE EFFECT OF GLUCOSE ON THE RATE OF INACTIVATION OF GLUCOSE TRANSPORT BY FDNB

The relative rate of inactivation is the rate of inactivation in the presence of glucose divided by the rate in the absence of glucose. Each effect was measured on at least two separate occasions. Results are mean ±S.E. with total number of determinations.

120 mM glucose		*Relative rate of inactivation*
Inside	*Outside*	
+	−	0.50 ±0.01 (5)
−	+	1.26 ±0.03 (10)
+	+	0.96 ±0.02 (7)

underestimate of the effect of glucose outside as the internal glucose concentration would have risen rapidly during the 0.5-min incubation with FDNB. (The V of net influx of glucose at 20 °C is about 40 mM/min[6] and the internal concentration of glucose at which net influx is half of V is about 3 mM[7]; the rate of influx at 25 °C and its inhibition by ethanol are not known.)

To show that these effects of glucose were not due to osmotic swelling or shrinking of the cells during exposure to FDNB, the experiments were repeated both with 100 mM glycerol and with 100 mM malonamide replacing the glucose. Glycerol[8] crosses the red cell membrane faster than glucose, and malonamide (Widdas, W. F., personal communication) crosses at much the same rate as glucose and therefore they cause similar osmotic swelling and shrinking during the exposure to FDNB, but they are not substrates of the glucose transport system. In neither case was there any change in the rate of inactivation. Nor was the effect of internal glucose an artifact caused by a large "leak" flux of sorbose in the transport measurement, since all the sorbose fluxes could be at least 90% inhibited by phloridzin. The effect of glucose outside the cell was specific, as sorbitol, a hexose which is not transported by the glucose transport system, had no effect on the rate of inactivation when outside the cell.

In the presence of 120 mM glucose on both sides of the membrane at once the rate of inactivation by FDNB was the same as in the absence of glucose (Table I). This is unexpected since, when cells are treated with 2 mM FDNB in the presence of 5% (by vol.) ethanol, glucose on both sides of the membrane causes a 2.5-fold increase in the rate of inactivation[2]. This discrepancy was confirmed by measuring the increase in inactivation rate produced by 120 mM glucose both sides of the membrane when cells were treated with 2, 4 and 8 mM FDNB in 12.33% ethanol; the increase fell from 2.2-fold to 1.2- and 1.0-fold, respectively (averages of duplicates).

DISCUSSION

These results show that glucose can "protect" against inactivation by FDNB. FDNB crosses the red cell membrane rapidly, so the different effects of glucose outside and inside the cell suggest that the glucose transport system is asymmetrical.

The simplest interpretation of the results is that, under the conditions of these experiments, the transport macromolecule can exist in two conformational states of low and high reactivity to FDNB, most of the transport macromolecules being in the low reactivity conformation when there is glucose inside the cell, most in the high reactivity conformation when there is glucose outside. These two conformations might well be "outward-facing carrier" and "inward-facing carrier", respectively. This hypothesis is suggested because: (i) it also explains the protective effect of competitive inhibitors outside the cell[2,9] and (ii) in an analogous system, the inactivation of choline transport in red cells by *N*-ethylmaleimide, this hypothesis accounts quantitatively for the effects of substrate and competitive inhibitors[3,10,11].

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