Biochimica et Biophysica Acta 938 (1988) 222–230
Elsevier

BBA 73874

Temperature dependence of glucose transport in erythrocytes from normal and alloxan-diabetic rats

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(Received 20 July 1987)

Key words: Glucose transport; Erythrocyte; Alloxan diabetes; Temperature dependence; (Rat)

Alloxan diabetes increased 3-*O*-methylglucose transport rates in rat red blood cells (RBC) at temperatures below 30 °C and decreased them above 30 °C. Preincubation of RBC from control rats with 20 mM glucose, 3-*O*-methylglucose, 2-deoxyglucose or xylose greatly elevated transport at 14 °C by increasing V_{max}. The effect was slight at 40 °C. Preincubation with glucose or deoxyglucose alone caused a 50% depression of transport rates at 40 °C as a result of a rise in the K_m, which is similar to findings in cells from alloxan-diabetic rats. Measurement of intracellular glucose metabolites suggested inhibition of glycolysis in cells from diabetic rats and a positive correlation between the level of intracellular hexose monophosphates and transport inhibition. Membrane fatty-acid and cholesterol composition and membrane lipid-ordering as monitored by electron paramagnetic resonance were not altered by alloxan diabetes. It is concluded that intracellular sugar and sugar metabolism alter the temperature dependence of glucose transport kinetics. Glucose metabolism can feed back to inhibit transport by increasing the transport K_m at physiological temperatures only.

Introduction

The temperature dependence of glucose transport has often been used as a tool to investigate the kinetics of the transport system [1,2], the mechanism of insulin action on glucose transport [3,4], or to infer on membrane function and fluidity [5]. In this report we have studied the temperature dependence of glucose transport in red blood cells (RBC) from normal or alloxan-diabetic rats. Rat RBC lack intracellular organelles, and temperature-mediated recruitment of new carriers, as thought to occur in adipose and muscle tissues [6–8], is not a complicating factor. We describe very different temperature curves for glucose transport in RBC from normal vs. alloxan-diabetic rats. These differences could be related to the level of intracellular glucose and to glucose metabolism. These results, obtained with a very simple cell system, will be discussed as to what they imply in general for studies of the temperature dependence of transport rates and kinetics.

Abbreviations: KRH, Krebs-Ringer solution buffered with Hepes; MeGlc, 3-*O*-methylglucose; dGlc, 2-deoxyglucose; G6*P*, glucose 6-phosphate; F6*P*, fructose 6-phosphate; F1,6P_2, fructose 1,6-diphosphate; GAP, glyceraldehyde 3-phosphate; DHAP, dihydroxyacetone phosphate; RBC, red blood cells; dGlc6*P*, deoxyglucose 6-phosphate.

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Methods

Animals and cell preparations. Normal and alloxan-diabetes rats (200–250 g) of the Sprague-Dawley strain (Harlan Industries) were used as

blood donors. Diabetes was induced by injection of alloxan (60 mg/kg) into the tail vein. The rats were maintained on insulin for 3 days and then used 35–40 h following the last insulin injection. This regimen was designed to allow recovery from the acute toxic effects of alloxan. Rats were considered diabetic if their blood glucose exceeded 400 mg/dl, as determined by an autoanalyser. Blood was drawn from the aorta of nembutal-anesthetized rats into heparinized syringes. Red cells were separated from white cells by centrifugation through a Percoll (Pharmacia)/ saline gradient ($d = 1.09$ g/ml) at 4° C, followed by a saline wash. For studies of the effect of glucose depletion, the cells underwent two successive 20 min incubations (5% suspension) and washed at 37° C in Krebs-Ringer solution buffered at pH 7.5 with Hepes (KRH) without substrate but containing 0.2% bovine serum albumin (Fraction V) (Sigma). This protocol was found to reduce intracellular glucose to less than 100 μM in cells from control animals. RBC from diabetic rats still contained 2–5 mM glucose following these incubations. To study the efefct of preincubation with various sugars, depleted cells were maintained for an additional 60 min at 37° C in KRH free of substrate (controls) or containing 20 mM of one of the following: glucose; 3-*O*-methylglucose (MeGlc); 2-deoxyglucose (dGlc); or xylose (all purchased from Sigma). Extracellular sugar was then removed by centrifugation at 4° C through the above Percoll gradient and the resuspended cells (40% (v/v) were kept on ice until assayed for glucose transport using the non-metabolizable analog, MeGlc.

Assay of sugar transport. Cells and medium containing 4000 cpm/μl of [^{14}C[MeGlc (ICN Radiochemicals) were warmed separately to the desired temperature for 2 min at $T \geq 23°$ C and for 4 min at $T < 23°$ C and mixed to initiate uptake. Contribution of internal sugar to extracellular hexose concentration in this time was calculated to be negligible ($\ll K_m$). Samples were taken at intervals and mixed into the upper layer of an ice-cold sucrose-KRH gradient containing phloretin (100 μM) to stop further transport, and then centrifuged. Contamination of the RBC pellet by radioactivity from the medium was eliminated through use of a special rinse apparatus as described by Whitesell et al. [9]. The cell pellet (usually 12 μl) containing about $1 \cdot 10^8$ cells was lysed at 4° C immediately with 0.5 ml H_2O followed by 0.5 ml of 1 M perchloric acid, and centrifuged at $2000 \times g$ for 5 min. An aliquot (0.7 ml) of the clear supernatant was transferred to a vial containing 6 ml aqueous scintillation fluid, (ACS, Amersham) and counted.

Time-courses were routinely run (data not shown) to determine that sugar influx did not depart significantly from a single exponential process in the range of concentrations used. In addition, uptake was established to be protein mediated by its sensitivity to the glucose transport inhibitors phloretin and cytochalasin B. In particular, cytochalasin (50 μM) inhibited influx rates by 88% at 40° C and by 98% at 14° C. The rates are expressed as μmol per 10^{10} cells per min. The quantity 10^{10} cells was found to contain approx. 1 ml of intracellular water. This was measured as the difference between the ^3H_2O space and the [^{14}C]sorbitol space, or by the [^{14}C]MeGlc space in RBC equilibrated for 90 min at 37° C and then washed in phloretin stop solution as described above.

Blanks (uptake at zero time) were obtained by adding the cells to cold KRH-sucrose gradients which were pre-mixed with phloretin and an appropriate quantity of isotope, and processed as above. Blanks were consistently low, and, together with background cpm, never exceeded 20% of intracellular radioactivity. Uptake values were routinely corrected for the blanks.

Study of sugar metabolism. For determination of intracellular metabolites, an aliquot (1 ml) of cell suspension (80% (v/v)) was lysed at 4° C with 3 ml water and 3 ml of 1 M perchloric acid, added together with vortexing. The clear extract was neutralized on ice with 0.5 M KOH containing 5 mM Hepes, and centrifuged to precipitate the perchlorate. The supernatant was then lyophilized and the residue redissolved in 1 ml water. The various metabolites were assayed according to established procedures [10,11]. All standards were purchased from Calbiochem.

In some experiments, RBC were preincubated with [U-^{14}C]2-deoxyglucose (ICN Radiochemicals). The cells were then extracted as described above, except that the residue following lyophili-

zation was taken up in 5 ml triethanolamine buffer (20 mM, pH 8) and applied to a 2.5 × 0.5 cm anion-exchange column (DEAE 25, Bio-Rad). This was eluted with 30 ml of a triethanolamine gradient (20–600 mM). Fractions (1 ml) were collected and counted. The column was standardized using radioactively labelled glucose 1- and 6-phosphate and fructose 1,6-diphosphate (New England Nuclear).

Determination of membane lipid composition. Washed RBC were added dropwise to the methanol portion of a Folch extraction mixture [12] with constant stirring. Chloroform was then added and the mixture was filtered before addition of water and layer separation. The chloroform layer was evaporated and the lipid extract was kept at −70 °C under a nitrogen atmosphere until use. Cholesterol content was estimated according to Brown et al. [13]. Fatty acid composition was determined, following saponification of the lipid, by gas chromatography of fatty acid methyl esters.

Ordering of membrane lipids. Washed RBC were spin-labelled with the fatty acid derivatives 5-, 12- and 16-nitroxylstearates according to the procedure of Wilkerson et al. [14]. Labeled intact cells were placed in a standard WG-812 flat cell (Wilmad) for spectral recording. EPR spectra were obtained with a Varian E-109 EPR system operating at x-band with an E-238 microwave cavity. Sample temperature was regulated during measurements by an E-257 variable temperature accessory by passing pre-cooled air into the cavity through the radiation slots in the front. This resulted in a 1 C° temperature gradient over the sample as monitored by a digital thermometer (Baily BAT 12R) fitted with a microprobe. Spectra were recorded at a microwave power of 10 mW using a 100 kHz field modulation of 1G (peak-to-peak).

Results

Transport kinetics of RBC from alloxan diabetic rats

As indicated under Methods, cells from control and alloxan-diabetic rats were centrifuged through a Percoll gradient and then washed with Krebs-Ringer Hepes buffer at 4 °C, prior to the transport assay. This protocol eliminated contamination of the cell pellet with plasma glucose. As shown in Fig. 1, the temperature dependence of transport activity was altered by diabetes. In cells from diabetic rats, activity was increased above control levels at temperatures between 4 and 30 °C; it was the same between 30 and 32 °C, but was depressed from 34 to 40 °C. Determinations of the transport kinetics at 14 and 40 °C are shown in Fig. 2 and the coefficients are presented in Table I. At 14 °C, the K_m for glucose transport in diabetic RBC was the same as that for control cells (3 to 4 mM), whereas the transport V_{max} was several-fold higher (left panel). On the other hand, at 40 °C the V_{max} was the same for both cell types (about 2 μmol/ml cell water per min), but transport activity was depressed in diabetic cells because of a doubling of the K_m (right panel).

Effect of glucose and glucose analogs on the kinetics of MeGlc transport measured at 14 °C

To determine whether these alterations in tem-

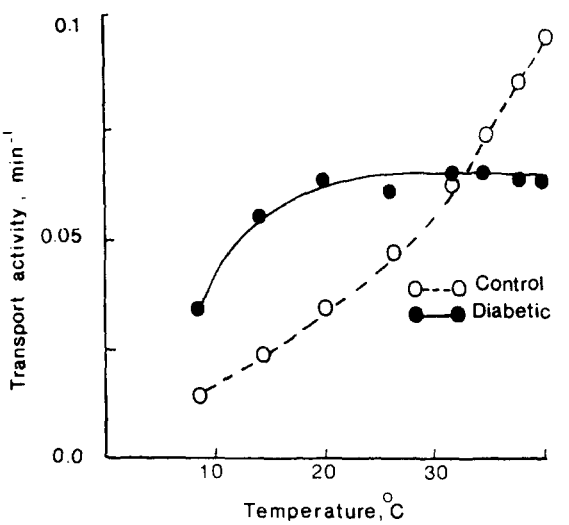

Fig. 1. Temperature dependence of [^{14}C]MeGlc transport in RBC from control and alloxan diabetic rats. RBC were depleted of glucose as described under Methods. Intracellular glucose was then measured to be 50 μM in control cells and 3 mM in cells from alloxan-diabetic animals. Uptake of [^{14}C]MeGlc was measured, adjusting sampling time to remain within the linear portion of the time-course at each temperature. The concentration of [^{14}C]MeGlc (0.2 mM) was well below the K_m. Transport activity plotted on the y-axis represents v/S where $S \ll K_m$.

TABLE I

KINETICS OF MeGlc TRANSPORT IN ERYTHROCYTES FROM CONTROL AND ALLOXAN-DIABETIC RATS

The table summarizes the data in Fig. 2. Means of four observations are presented ± S.E.

RBC donor	Transport assay			
	14 °C		40 °C	
	K_m (mM)	V_{max} (μmol/ml per min)	K_m (mM)	V_{max} (μmol/ml per min)
Control	3.0 ± 0.6	0.03 ± 0.004	10 ± 1.4	1.8 ± 0.4
Diabetic	4.0 ± 0.6	0.25 ± 0.02	21 ± 2.2	1.5 ± 0.4

perature dependence were a result of high levels of intracellular glucose in diabetic RBC, we attempted to deplete these cells of glucose by two successive 20 min incubations as a dilute suspension (5% (v/v)) followed by washes at 37 °C. It was determined, however, that although this protocol successfully depleted normal cells, diabetic RBC still retained between 2 and 5 mM intracellular glucose as a result of their very high starting level of intracellular sugar. The changes observed in Fig. 1 were still present. We then adopted the alternate approach of studying the effect of sugar preloading on the temperature dependence of 3-*O*-methylglucose transport in control RBC. Cells were depleted of glucose (to less than 100 μM) and then incubated for 1 h at 37 °C with or without 20 mM glucose, methylglucose (MeGlc), 2-deoxyglucose (dGlc) or xylose. The cells were then centrifuged through a Percoll gradient at 4 °C to remove extracellular sugar, washed once in cold KRH buffer, and kept on ice until assayed for transport. Transport rates were measured at two temperatures, 40 and 14 °C. The effects of preincubating RBC at 37 °C with glucose, MeGlc, dGlc or xylose on transport kinetics measured at 14 °C are shown in the left panel of Fig. 3. It can be seen that preincubation with each of the sugars increased the transport rate several-fold over that in cells preincubated without substrate. The increase was mainly due to a rise in V_{max} (Fig. 3, left panel and Table II) since the K_m was not altered. A similar 'trans effect' has been described before in thymocytes [15] and human erythrocytes [16,17].

Effect of glucose and glucose analogs on transport kinetics measured at 40 °C

Transport kinetics determined at 40 °C for glucose-depleted cells and for cells preloaded with

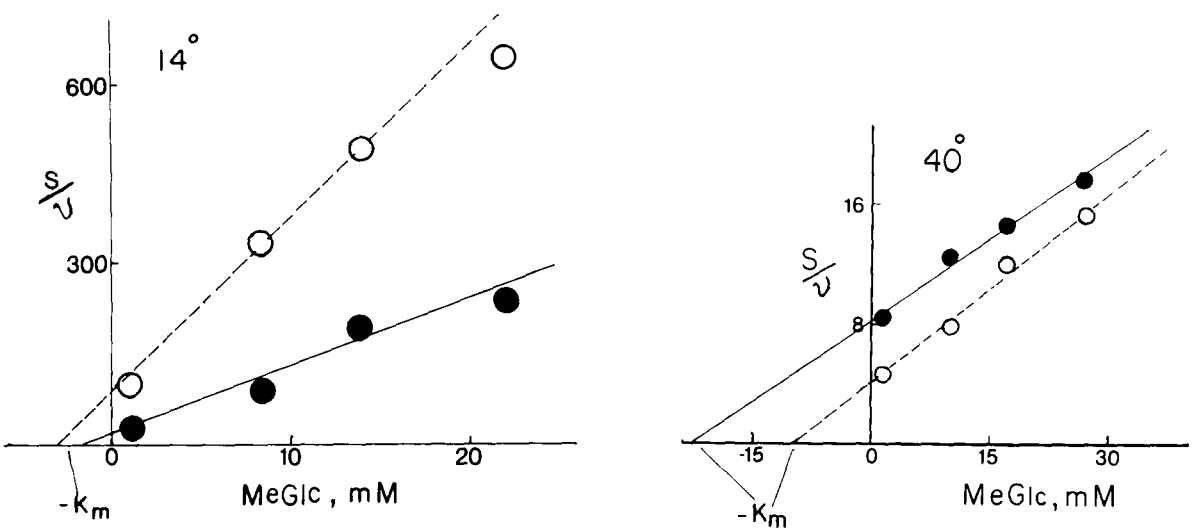

Fig. 2. Hanes (S/v vs. S) plots of MeGlc transport in RBC from control and diabetic rats at 14 °C (left panel) and 40 °C (right panel). RBC were treated and assayed as described for Fig. 1. Initial rate of uptake of [^{14}C]MeGlc was estimated from 6 and 12 min samples at 14 °C (left panel) and at 0.5 and 1 min at 40 °C (right panel). At these time periods, uptake was virtually linear and the fraction of cell water equilibrated was less than 0.2. K_m (mM) is the absolute value of the x-intercept. V_{max} (μmol/min per ml cell water or 10^{10} cells) is the slope^{-1}. The units for the ordinate, S/v, are min. The data shown are a composite of three experiments. See Table I for statistical analysis. The symbols used are the same as in Fig. 1.

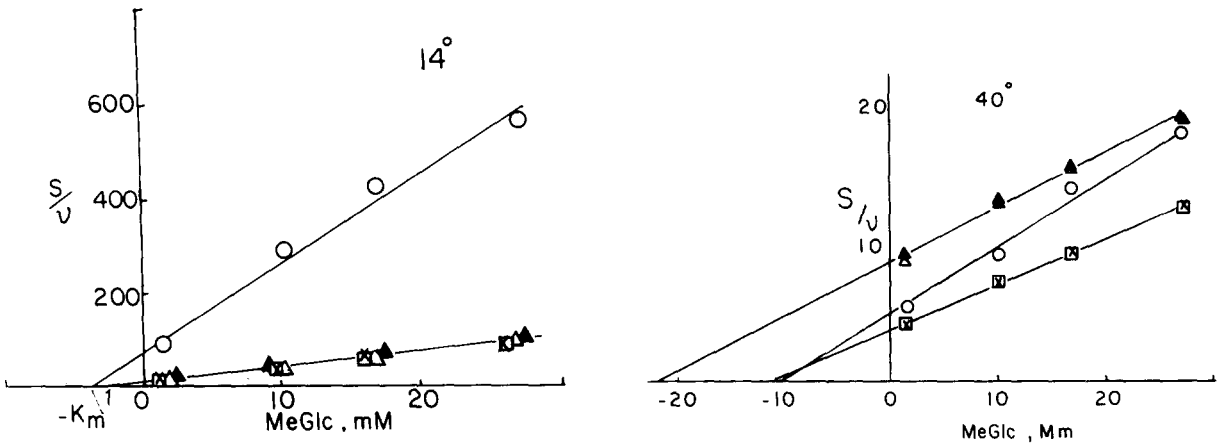

Fig. 3. Effect of preincubation with glucose or glucose analogues on transport kinetics of [^{14}C]MeGlc in rat RBC. Hanes plots (S/v vs. S) of MeGlc transport are shown for RBC preloaded with 20 mM glucose (△), MeGlc (□), dGlc (▲) or xylose (×). (Control 0) RBC were depleted of glucose then preincubated for 1 h at 37 °C with or without the test sugar, as described under Methods. Transport of [^{14}C]MeGlc was assayed at 14 °C (left panel) and 40 °C (right panel) as described in the legend to Fig. 2.

glucose, dGlc, MeGlc and xylose are shown in the right panel of Fig. 3 and in Table II. Preincubating cells with dGlc or glucose caused a 50% decrease in transport activity. This was due to a doubling of the K_m, while the V_{max} was not altered. On the other hand, loading with MeGlc and xylose only slightly increased the V_{max}. The large trans effect previously observed with these sugars when the preloaded cells were assayed at 14 °C, was slight at 40 °C.

The effect of temperature per se on transport kinetics can be seen by comparing the control curves in both panels of Fig. 3. A decrease in temperature from 40 to 14 °C significantly decreased transport activity. It induced a very large decrease in V_{max}, which outweighed the effect of a concomitant decrease in K_m *.

TABLE II

EFFECTS OF PREINCUBATION WITH GLUCOSE AND GLUCOSE ANALOGS ON THE KINETICS OF MeGlc INFLUX IN ERYTHROCYTES ASSAYED AT TWO TEMPERATURES

This table summarizes data from Fig. 3.

Sugar in preincubation (1 h at 37 °C)	Transport assay			
	14 °C		40 °C	
	K_m (mM)	V_{max} (μmol/ml per min)	K_m (mM)	V_{max} (μmol/ml per min)
None	4.4±0.3	0.05±0.005	10±1.5	2.0±0.5
Glucose	4.2±0.2	0.35±0.02	23±2.5	2.5±0.7
dGlc	4.1±0.2	0.35±0.02	23±2.4	2.9±0.8
MeGlc	4.1±0.3	0.35±0.02	11±1.3	2.5±0.6
Xylose	4.0±0.3	0.35±0.02	11±1.3	2.9±0.8

Glucose metabolites in RBC following preincubation with glucose or induction of alloxan diabetes

The finding that glucose transport kinetics at 40 °C are altered in RBC preincubated with glucose or dGlc, but not with MeGlc or xylose, suggested that accumulation of a glucose metabolite (possibly hexose 6-phosphate) was responsible for these effects. Fig. 4 shows that dGlc was recovered quantitively as the free and monophosphorylated sugar from an extract of RBC preequilibrated at 37 °C with 20 mM dGlc. No radioactivity was detected in the diphospho sugars which elute five or six fractions later than the monophospho sugars. This would suggest that transport

* The acute effect of the assay temperature on the transport K_m was independent of metabolism, since it was always observed regardless of preincubation conditions. This is further supported by the finding that treatment with arsenate (10 mM for 1 h at 37 °C) did not interfere with the temperature-induced K_m change.

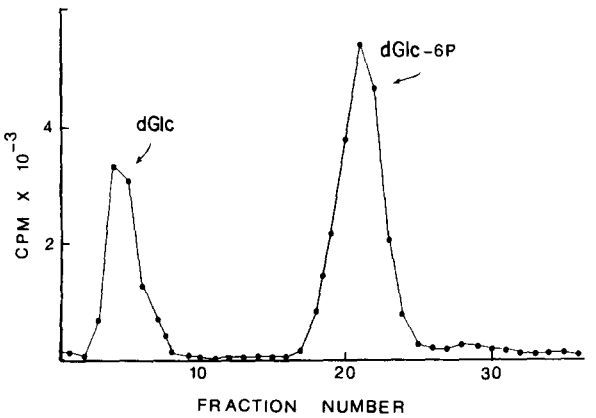

Fig. 4. Anion-exchange chromatography of intracellular radioactivity after 90 min incubation of RBC with 20 mM [^{14}C]dGlc (4000 cpm/μmol). The perchlorate extract of RBC was titrated to pH 8 with 0.5 M KOH containing 5 mM Hepes (precipitating the perchlorate), and then brought to 2 ml in 20 mM triethanolamine buffer (pH 8), and applied to the column (DEAE-25, 2.5×5 cm.) 3 ml triethanolamine followed the sample, and then a 30 ml gradient of triethanolamine (20–600 mM) was applied. 1 ml fractions were collected and counted. The column was standardized using [^{14}C]dGlc which ran with the first peak, [^{14}C]Glc6P and Glc1*P*, which ran with the second peak and [^{14}C]F1,6P_2, which eluted after the 30 ml fraction.

inhibition in this case might have been caused by accumulation of dGlc6*P*. Tables III and IV show the intracellular levels of various metabolites in control RBC preincubated with and without glucose and in RBC from alloxan-diabetic rats. It can be seen that preincubation of RBC with 20 mM glucose caused an increase in the levels of all the metabolites of glucose as the result of the availability of substrate (Table III). In RBC from alloxan-diabetic rats, however, the levels of F1,6P_2 and glyceraldehyde 3-phosphate (GAP) were markedly lowered (Table IV). All other metabolites measured were found in amounts similar to those in control cells preincubated with glucose.

Membrane lipid composition and ordering in RBC from normal and alloxan-diabetic rats

To check whether the transport disturbance in cells from diabetic animals could be explained by factors unrelated to glucose metabolism, we compared lipid composition and ordering of the membrane in cells from normal and diabetic rats. Cholesterol measurements gave the same value of 1.66 mg ± 0.02 per cell × 10^{10} ($n = 9$) for cells from control or alloxan-diabetic animals. Fatty-acid composition was also found to be similar in both cell types, as shown in Table V. Ordering of membrane lipids was investigated by EPR. RBC were spin-labelled with the three fatty acid probes 5-, 12- and 16-nitroxylstearates, and studied over the range of temperatures from 4 to 40 °C. The order parameters for membrane lipids were measured at selected temperatures, as detailed by Hubbell and McConnell [18]. Plots of order parameter vs. temperature for cells from normal

TABLE III

INTRACELLULAR METABOLITES IN NORMAL RAT ERYTHROCYTES PREINCUBATED FOR 1 H AT 37 °C WITHOUT OR WITH GLUCOSE

Means and S.E. are given for 3–5 experiments in each case. n.d., no detectable.

Metabolite measured	Intracellular metabolites (nmol/ml packed cells)	
	No glucose	20 mM glucose
Glucose	260 ± 7	18000 ±130
Glc6*P*	4.8± 0.9	15 ± 1
F6*P*	n.d.	3.0± 0.8
F1,6P_2	4 ± 0.4	25 ± 5
GAP	n.d.	16 ± 2
DHAP	n.d.	60 ± 10
ATP	220 ±10	195 ± 10

TABLE IV

MEASUREMENT OF INTRACELLULAR METABOLITES IN CELLS FROM CONTROL OR ALLOXAN DIABETIC RATS IMMEDIATELY FOLLOWING COLLECTION

Means and S.E. are given for 3–5 experiments.

Metabolite measured	RBC donor Intracellular metabolites (nmol/ml packed cells)	
	Normal	Diabetic
Glucose	1800 ±85	19000 ±120
Glc6P	13 ± 1	20 ± 1
F6P	2.0± 0.5	2.4± 0.7
F1,6P	15 ± 0.5	2.0± 0.5
GAP	5.0± 0.4	0.1± 0.04
DHAP	50 ± 0.6	40 ± 4
ATP	230 ± 5	267 ± 8

TABLE V

FATTY ACID COMPOSITION OF RBC FROM NORMAL AND ALLOXAN-DIABETIC RATS

Rats were maintained on insulin for 3 days following alloxan injection and killed 36 h following insulin withdrawal. Values shown are % of total fatty acid and are means ± S.E. $n = 5$ for controls and 7 for diabetics.

Rat	Fatty acid (% total fatty acid)							
	14:0	16:0	16:1	18:0	18:1	18:2	20:4	22:6
Normal	1.5 ± 0.4 [b]	24.6 ± 1.3	2.2 ± 1	10.9 ± 0.6	11.6 ± 0.2	18.0 ± 0.8	29.8 ± 0.9	1.3 ± 0.6
Diabetic	1.5 ± 0.3	25.6 ± 1.0	2.5 ± 0.3	10.8 ± 0.9	11.4 ± 0.9	18.0 ± 0.5	28.7 ± 1.1	1.4 ± 0.1

and alloxan-diabetic rats were superimposable, with subtle bending at about 22 °C (data not shown).

Discussion

Red blood cells from alloxan-diabetic rats exhibited markedly increased 3-*O*-methylglucose transport rates at 14 °C as compared with cells from control rats. This effect was similar to that exerted by preloading control RBC with non-metabolizable glucose analogs suggesting that it was due to intracellular accumulation of free sugar. Such a trans-stimulation has been reported [15,16,18], and indicates that resistances for the cycling of empty and loaded carriers are dissimilar at 14 °C, with that for orientation of empty carrier outward probably being rate limiting. Thus, preloading the cells with free sugar facilitates this orientation and, in the present case, brings about a several-fold increase in activity. This trans effect, however, becomes insignificant at 40 °C (Fig. 3, right panel), indicating that warming has facilitated outward orientation disproportionately with respect to other steps in carrier function. This could have resulted from temperature-induced changes in the ordering of the membrane lipid bilayer. However, our studies using electron paramagnetic resonance probes make this possibility unlikely. On the other hand, temperature could have induced a conformational change in the carrier protein itself. Such change could explain why glucose transport at 40 °C is sensitive to inhibition by products of glucose metabolism, whereas it is not affected at 14 °C.

The inhibitory effect of alloxan diabetes or of sugar preloading observed at 40 °C must reflect an allosteric interaction, since it is exerted by an intracellular metabolite(s) on the K_m of sugar entry. In this context it is worth noting the report by Carruthers et al. [19] describing the inhibitory effects of a low molecular weight cytosolic factor on glucose transport in human RBC. The regulation of transport by temperature has been reported before. Lacko et al. [20] described the effect of temperature on transport K_m in human RBC. Metabolic regulation of glucose transport has also been suggested in many cell systems like thymocytes, adipocytes, chinese hamster fibroblasts and beef erythrocytes [21–24]. This study, however, points out an important new aspect, namely, the interaction between the effect of metabolic substrate and temperature on transport rate and K_m. This interaction is described in the rat RBC which lacks a nucleus, mitochondria and an apparatus for protein synthesis, so that effects mediated through carrier translocation or turnover can be ruled out.

The most immediate extrapolation of our studies is the practical consideration that when studying the temperature dependence of glucose transport kinetics, it is important to define intracellular glucose and the metabolic state of the cell studied. Intracellular sugar and sugar metabolism alter, as shown, the effect of temperature on transport rates and on transport kinetics. This would imply that they modulate the way the rate-limiting steps with respect to carrier function are affected by temperature. From a mechanistic point of view, this interaction could also provide a new direction for the study of the regulation of carrier function in the RBC and other cell types. Although it is possible that accumulation of intracellular sugar and sugar metabolites can be demonstrated more readily in the rat RBC due to their low rates of

sugar utilization, there are numerous examples in other cell types pointing to an effect of glucose metabolism on glucose transport. For example, inhibition of glucose transport through an apparent K_m increase by a glucose metabolite, postulated to be lactate, has been reported in beef erythrocytes [24]. More recently a temperature-dependent metabolic regulation of the glucose transport K_m has been documented in adipocytes. A decrease in temperature from 37 to 23°C reduces the K_m in that cell preparation, and, as a consequence, an effect of insulin to lower the K_m and of substrate to increase the K_m can be observed at 37°C to a much greater extent than at 23°C [22]. Similarly, in the RBC, preincubation with substrate increases the transport K_m, and this effect can be observed at 40°C but not at 14°C. A better understanding of the interaction between the effects of metabolism and temperature on transport in a simple system like the RBC might provide the basis for extending a similar approach to more complex cells like adipocytes and thymocytes, where metabolic regulation has more physiological importance.

In our study, transport inhibition above 30°C was observed with glucose and deoxyglucose, indicating a possible link to sugar metabolism. The inhibitory metabolites could be Glc6*P* and Foley and Hueckstead [25] briefly described an inhibitory effect of Glc6*P* on MeGlc transport in adipocyte membrane vesicles.

Evidence from earlier work indirectly links transport of glucose to its phosphorylation. Randle and Smith [26] and Morgan et al. [27] showed that anoxia increased both the transport and metabolism of glucose in muscle tissue; alloxan diabetes decreased both [28]. The mechanism of these effects on glucose phosphorylation (but not on transport) was later investigated by Regen et al. [29] who found that the stimulatory effect of anoxia was associated with a 50% drop in Glc6*P*. Small reductions (about 20%) were also seen in the levels of F6*P* and ATP while F1,6P_2 levels doubled. These changes were consistent with activation of glycolysis at the level of phosphofructokinase. Alloxan diabetes, on the other hand, doubled Glc6*P* and F6*P* and lowered F1,6P_2, consistent with inhibition of phosphofructokinase. Our results (Table III) would also suggest a correlation between inhibition of glycolysis at the phosphofructokinase level and transport inhibition. These findings, taken together with the inhibitory effect of deoxyglucose 6-phosphate, might be interpreted to indicate that hexose monophosphates exert a negative influence on glucose transport.

In summary, alloxan diabetes alters the temperature dependence of glucose transport in the rat RBC. This alteration was related to the increase in intracellular sugar and in some of its metabolites as a result of high circulating glucose, and possibly of inhibited glycolysis. This effect did not appear to result from changes in membrane composition or order which were comparable to those found in cells from control rats. In general terms, metabolic regulation of glucose transport seems to be a basic property of the glucose carrier which applies to a variety of cell types including the simple rat RBC. It can only be observed at physiological temperature, and is exerted through an increase in the transport K_m. Where present, hormone sensitivity, such as to insulin or growth factors, may constitute in part a superimposed mechanism to regulate transport by overriding metabolic feedback. The metabolic state of the cell as well as residual intracellular glucose, considering the difficulty of its complete removal, are thus important variables which should be carefully defined in studies of transport kinetics and regulation.

Acknowledgments

The authors are grateful to Drs. Charles R. Park and David M. Regen for many helpful discussions and to Patricia Perry and Saul Juliao for their efficient technical assistance. This work was supported by an American Diabetes Association grant, by a grant from the Juvenile Diabetes Foundation 82R604 and by NIH grant R01 HL34737.

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