GLUCOSE UTILIZATION BY THE POLYOL PATHWAY IN HUMAN ERYTHROCYTES

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SUMMARY

Sorbitol is present in human erythrocytes in concentrations exceeding that in plasma, and is linearly related to the plasma glucose concentration. When erythrocytes are incubated in media containing increasing glucose concentrations, the intracellular sorbitol and fructose concentrations increase and free fructose appears in the media. At a medium glucose concentration of 5 mM approximately 3% of the glucose uptake is utilized for sorbitol and fructose synthesis. Glucose appears to be a physiological substrate for alditol:NADP oxidoreductase in the erythrocyte.

INTRODUCTION

It has recently been demonstrated that alditol:NADP oxidoreductase (E.C.1.1.1.21) is widely distributed in mammalian tissues,[1] however, the physiological substrate(s) for this enzyme in tissues other than the seminal vesicles is unknown. The enzymes isolated from lens,[2] brain,[3] aorta,[4] sciatic nerve,[5] and pancreas[5] have higher Km's for glucose than for a number of other aldoses, and on this basis it has been suggested that glyceraldehyde and erythrose may be the physiological substrates in brain.[3] We have observed that sorbitol and fructose synthesis from glucose occurs in human erythrocytes at physiological plasma glucose concentrations, and that this process accounts for a small, but significant fraction of the glucose uptake.

METHODS

Heparinized samples of blood obtained from normal and diabetic males were centrifuged at 600 x g for 20 minutes at 4°C. The plasma and "buffy coat" were removed; the red cells were washed (3X) with 0.9% NaCl at 4°C. The final erythrocyte suspensions (hematocrit approx. 90%) contained less than 200 leukocytes per ml. The sorbitol content of the washed erythrocytes was determined

in neutralized perchloric acid filtrates by a fluorometric enzymatic assay.[4] Two ml aliquots of the red cell suspensions were added to 4.0 ml of Krebs-bicarbonate buffer pH 7.4, gas phase 5% CO_2 in air. Glucose was present in the medium in concentrations ranging from 5 to 50 mM. The erythrocytes were incubated for 20 minutes at 37°C, 80 oscillations per minute to permit equilibration. Aliquots were then removed for the determination of glucose, sorbitol, and free fructose in the erythrocytes and in the medium. The hematocrit was also determined. The incubation was then continued for an additional 2 hours and the sampling was repeated. Glucose uptake by the erythrocytes remained linear for at least 3 hours under these conditions. Glucose was determined spectrophotometrically by means of yeast hexokinase and glucose-6-phosphate dehydrogenase.[6] Fructose in the red blood cells and media was initially determined by means of gas-liquid chromatography of trimethylsilyl ethers prepared from lyophilized barium hydroxide-zinc sulfate filtrates.[7] The determinations were carried out by means of a Packard Instruments gas-liquid chromatographic apparatus using a 6-foot column of 3% JXR on Gas Chrom Q (Supelco Inc.) at 165°C with a hydrogen flame detector. α-Methyl mannoside was used as an internal standard. This technique permitted the demonstration of free fructose in the erythrocytes and incubation medium; however, the small quantities of free fructose that appeared in the medium could not be accurately quantified in the presence of 5 to 50 mM glucose. Subsequently fructokinase was prepared from rat liver[8] and the free fructose present in neutralized perchloric acid filtrates of erythrocytes and incubation media was determined by fluorometric assay of the ADP produced by the fructokinase reaction. The assay system contained potassium phosphate buffer pH 7.0 (50 mM), KCl (10 mM), $MgCl_2$ (5 mM), phosphenolpyruvate (0.8 mM), ATP (0.05 mM), NADH (0.02 mM), lactic dehydrogenase (Boehringer rabbit muscle) 7.5 I.U., and pyruvate kinase (Boehringer rabbit muscle) 1.5 I.U. in a final volume of 1.0 ml. The reaction resulting from the presence of pyruvate in the samples was completed within 2 minutes, at that point 1.0 I.U. of an ammonium sulfate suspension of rat liver fructokinase (20 I.U. per mg) was

added and the subsequent decrease in fluorescence due to the oxidation of NADH determined. The reaction was monitored by means of an Eppendorf fluorometer modified according to the recommendations of Estabrook, et. al.[9] and was usually completed within 10 to 15 minutes. The assay is linear for fructose over the range of 0.125 to 1.5 nmoles in the presence of pyruvate concentrations as high as 5.0 nmoles per ml. In practice the pyruvate concentration of the samples was determined initially and the assay for fructose carried out with aliquots having a pyruvate concentration of less than 2.0 nmoles per ml. Glucose, galactose, and mannose in concentrations of 10 µmoles/ml did not interfere with the assay for fructose. Adelman, Ballard and Weinhouse[8] reported that D-xylulose and L-sorbose are also substrates for rat liver fructokinase; D-xylulose may therefore have contributed to the estimates of free fructose obtained by this enzymatic assay.

RESULTS AND DISCUSSION

Erythrocytes from 10 normal adult males contained 11.5 ± 0.5 nmoles of sorbitol per ml of erythrocyte while the plasma from the same subjects con-

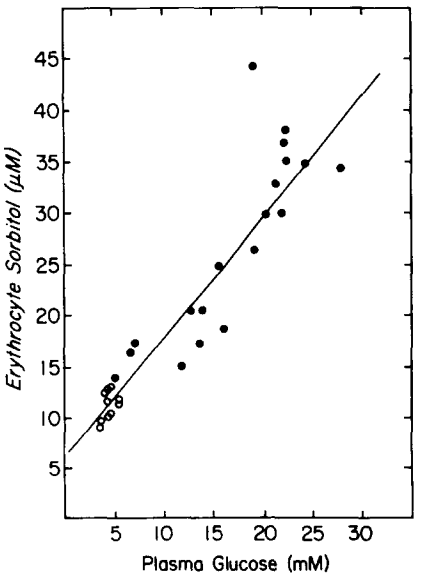

Figure 1 Comparison of erythrocyte sorbitol and plasma glucose concentrations in normal (O) and diabetic (●) humans. Line fitted by method of least squares (r = + 0.90).

tained 2.3 ± 0.2 nmoles per ml. The concentration of sorbitol in erythrocyte water would be appreciably greater than the estimate given above since the water content of the erythrocyte is approximately 66% by weight.[10]

As shown in Figure 1 there was a linear correlation (r = + 0.90) between plasma glucose and erythrocyte sorbitol concentrations in samples obtained from 10 normal and 19 diabetic males.

When washed erythrocytes from normal adults were incubated with increasing medium glucose concentration for 2 hours there was a progressive rise in the erythrocyte sorbitol concentration observed at the end of the incubation. (Figure 2) Since in the erythrocyte intracellular transport is not rate limiting for glucose utilization by pathways involving phosphorylated intermediates, increasing medium glucose concentration results in increased levels of free intracellular glucose.[11] It appears that the rate of sorbitol formation in the erythrocyte is regulated by the ambient glucose concentration over a wide range.

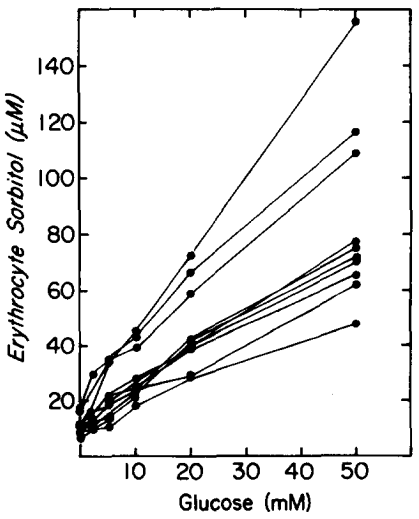

Figure 2 Erythrocytes from 10 normal male adults incubated for 2 hours in Krebs-bicarbonate buffer pH 7.4, gas-phase 5% CO_2 in air containing glucose in increasing concentrations.

Table 1

Effect of increasing medium glucose concentration on erythrocyte sorbitol and fructose

Exp. No.	SORBITOL μM medium glucose mM				FRUCTOSE μM medium glucose mM			
	5	10	20	50	5	10	20	50
1	33	45	73	156	75	97	94	222
2	18	25	43	71	51	40	70	116
3	35	43	67	117	20	28	38	72
4	35	39	59	109	26	23	48	72

As shown in Table 1 the rise in erythrocyte sorbitol concentration that occurs on incubation with media containing increasing glucose concentration is accompanied by a progressive increase in the free fructose content of the erythrocyte. The data presented in Table 2 indicate that significant quantities of free fructose appeared in the medium under these conditions, and the quantities recovered increased with increasing medium glucose concentration. However, no sorbitol was detected in the media.

From the data presented in Table 2, it is possible to derive a minimal estimate of the quantity of glucose utilized for sorbitol and fructose synthesis during the incubation of erythrocytes with glucose. The sum of the nmoles of sorbitol and fructose recovered in the erythrocytes and medium at the end of the incubation minus the sum of that present at the end of the equilibration period provides a rough estimate of the total quantity of glucose utilized for sorbitol and fructose synthesis. This is a minimal estimate since it does not consider the free fructose that may have been phosphorylated by hexokinase under these conditions. As shown in Table 2, glucose utilization via the polyol pathway accounts for approximately 3% of the glucose uptake when the medium glucose concentration is 5 mM. When the medium glucose concentration is increased there is both an absolute increase in the quantity traversing the polyol pathway, and in the fraction of the glucose uptake that

Table 2

Effect of increasing medium glucose concentration on percentage of glucose uptake metabolized by the polyol sequence

Medium Glucose mM	GLUCOSE UPTAKE µmoles/ml RBC /2 hr	ERYTHROCYTE SORBITOL µmoles/ml	ERYTHROCYTE FRUCTOSE µmoles/ml	FRUCTOSE in medium µmoles/ml RBC/2 hr	% Glucose Uptake
I*	-----	.010	.017	.022**	-----
5	2.68	.014	.031	.102	3.7%
50	2.66	.116	.065	.160	11 %
I*	-----	.004	.008	.011**	-----
5	3.32	.013	.035	.080	3.2%
50	4.20	.069	.055	.167	6.4%
I*	-----	.007	.004	.006**	-----
5	2.66	.012	.025	.070	3.4%
50	3.04	.075	.075	.127	7.9%

I* Concentration of sorbitol and fructose found in erythrocytes (RBC) at the end of a 20-minute equilibration period. **The value for fructose appearing in medium during the 20-minute equilibration period are expressed as µmoles/ml of erythrocytes.

it represents. (Table 2) This effect of increasing ambient glucose concentration presumably reflects a high Km glucose for the erythrocyte aldose reductase.

The demonstration that alditol:NADP oxidoreductase is widely distributed in the tissues of the rabbit and rat, and reports of the presence of significant concentrations of sorbitol in the lens, brain, nerve, kidney, and aorta of normal animals, led us to speculate that glucose may be a physiological substrate for the enzyme in many mammalian tissues.[1] The observation that approximately 3% of the glucose uptake is utilized for sorbitol and fructose synthesis in human erythrocytes at a physiological ambient glucose concentration supports this speculation. Interest in the activity of alditol:NADP oxidoreductase in mammalian tissues other than the seminal vesicles derived

in large part from evidence suggesting that increased reduction of glucose and galactose to their respective polyols is related to the development of pathological lesions in the lens and neural tissues in diabetes and galactosemia.[12,13,14,15] It is possible that this emphasis on the potential pathological significance of increased activity of the polyol pathway has obscured the recognition that sorbitol and fructose synthesis occur in many mammalian cells under physiologic conditions. However, the function subserved by this pathway of glucose metabolism in tissues other than the seminal vesicles remains to be determined.

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