Glucose Metabolism in the Rat Small Intestine: the Effect of Glucose Analogues on Hexokinase Activity

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The effect of intubation of starved rats with solutions of glucose, 3-*O*-methylglucose, mannose, 2-deoxyglucose and sorbitol on the subcellular location of hexokinase in the mucosa of the small intestine was studied. Glucose, 3-*O*-methylglucose and mannose caused the soluble hexokinase activity to rise to a value similar to that found in fed animals, whereas sorbitol and 2-deoxyglucose caused smaller increases.

It has been shown that hexokinase (EC 2.7.1.1) is the rate-limiting enzyme of the glycolytic pathway in the mucosa of the small intestine of the cat and rat (Srivastava & Hübscher, 1966) and of the guinea pig, pigeon and rabbit (Clark & Sherratt, 1967). It has also been shown that 80–107% of the glycolytic activity but only 35% of the hexokinase activity of homogenates of rat mucosa is recovered in the particle-free supernatant (Srivastava & Hübscher, 1966), which may suggest that only soluble hexokinase is involved in lactate production. It was further shown that the binding of hexokinase to subcellular particulate structures was affected by diet. Administration of a solution of glucose by stomach tube caused a rise in hexokinase activity in the particle-free supernatant subsequently prepared from homogenates of small-intestinal mucosa. Isoenergetic (isocaloric) amounts of amino acids or lipid administered by stomach tube, or of glucose administered intravenously, caused no change in hexokinase activity in the particle-free supernatant (Shakespeare *et al.*, 1966). Experiments *in vitro* indicated that the rise in soluble hexokinase activity was the result of the release of the enzyme from particulate structures (Shakespeare *et al.*, 1969; Mayer *et al.*, 1970). Thus an increase in the glucose concentration in the lumen of the intestine caused a solubilization of hexokinase and, presumably, an increase in glycolytic activity. The experiments now described were designed to delineate further the stimulus for hexokinase solubilization.

Materials and methods

Animals. Male Wistar rats weighing 190–210 g were obtained from the Breeding Unit, Nottingham School of Agriculture, Sutton Bonington, Leics., U.K. They

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were fed *ad libitum* on rat cake from Pilsbury's Ltd., Birmingham, U.K., or starved for 36 h before they were killed. Starvation was carried out in metabolic cages to minimize coprophagy. Where applicable, animals were intubated with 5 ml of 0.9% NaCl or 5 ml of a solution of a glucose analogue (10%, w/v) 2 h, 1 h and 10 min before death. The solutions were warmed to 37°C before use. All animals were allowed free access to water.

Chemicals. Bovine serum albumin (fraction V; fatty acid-poor) was obtained from Miles Laboratories, Kankakee, Ill., U.S.A.; dithiothreitol was obtained from Koch-Light Laboratories, Colnbrook, Bucks., U.K.; glucose 6-phosphate dehydrogenase (140 EC units/mg of protein), 6-phosphogluconate dehydrogenase (12 EC units/mg of protein), NADP⁺, NADH and the sodium salts of ATP and pyruvate were obtained from Boehringer Corp. Ltd., London W.5, U.K.; *N*-acetylcysteine, DNA, 3-*O*-methylglucose, 2-deoxyglucose, mannose and sorbitol were obtained from Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey, U.K. All other chemicals were of A.R. grade.

Assay systems. Protein and DNA were assayed by the methods described by Hübscher *et al.* (1965). Hexokinase and lactate dehydrogenase (EC 1.1.1.27) activities were assayed by the methods described by Srivastava *et al.* (1968).

Preparation of tissue. The animals were killed by cervical dislocation; the intestine of each was removed, irrigated twice with 200 ml of cold 0.9% NaCl and scraped gently with a wooden spatula, and the mucosal scrapings were suspended (20%, w/v) in 0.14 M-KCl containing 5 mM-Tris-HCl buffer, pH 7.4, and 1 mM-dithiothreitol. After homogenization in a Potter-Elvehjem homogenizer [580 rev./min; radial clearance 0.17 mm (0.0065 in)] samples were centrifuged at 100 000g (calculated at $r_{av.} = 5.84$ cm) for 1 h, yielding a particulate fraction and particle-free supernatant (Shakespeare *et al.*, 1969).

Table 1. *Effect of intubation with glucose analogues on the activities of hexokinase and lactate dehydrogenase in rat small-intestinal mucosa*

Values are the means $\pm$ s.d. for five animals. *P* values were calculated by using Student's *t* test; N.S., not significant ($P > 0.02$). Rats were starved for 36 h, then saline or glucose analogue was administered by stomach tube over 2 h. Further experimental details are given in the text.

	Hexokinase			Lactate dehydrogenase		
	Homogenate activity (EC unit/min per μ mol of DNA P)	Soluble activity (% of total)	Particulate activity (% of total)	Homogenate activity (EC units/min per μ mol of DNA P)	Soluble activity (% of total)	Particulate activity (% of total)
Diet						
Fed	0.384 $\pm$ 0.023 <i>P</i> < 0.001	35.3 $\pm$ 2.7 <i>P</i> < 0.001	61.8 $\pm$ 2.2 <i>P</i> < 0.001	33.2 $\pm$ 5.3 <i>P</i> < 0.001	80.0 $\pm$ 3.0 <i>P</i> < 0.001	17.7 $\pm$ 2.7 <i>P</i> < 0.001
Saline	0.270 $\pm$ 0.031 Null	19.8 $\pm$ 2.9 Null	78.1 $\pm$ 5.4 Null	18.3 $\pm$ 2.0 Null	91.1 $\pm$ 1.7 Null	8.5 $\pm$ 1.1 Null
Glucose	0.255 $\pm$ 0.033 N.S.	34.1 $\pm$ 3.0 <i>P</i> < 0.001	60.6 $\pm$ 3.0 <i>P</i> < 0.001	17.2 $\pm$ 1.5 N.S.	86.2 $\pm$ 3.6 N.S.	10.3 $\pm$ 2.8 N.S.
3- <i>O</i> -Methylglucose	0.258 $\pm$ 0.025 N.S.	36.2 $\pm$ 3.7 <i>P</i> < 0.001	59.7 $\pm$ 3.4 <i>P</i> < 0.001	20.7 $\pm$ 4.2 N.S.	85.5 $\pm$ 1.9 <i>P</i> < 0.01	12.2 $\pm$ 5.1 N.S.
2-Deoxyglucose	0.223 $\pm$ 0.022 N.S.	28.9 $\pm$ 5.7 <i>P</i> < 0.02	67.9 $\pm$ 6.5 <i>P</i> < 0.05	17.2 $\pm$ 2.3 N.S.	90.6 $\pm$ 8.6 N.S.	8.7 $\pm$ 2.9 N.S.
Mannose	0.233 $\pm$ 0.039 N.S.	33.3 $\pm$ 3.1 <i>P</i> < 0.001	61.9 $\pm$ 5.7 <i>P</i> < 0.001	16.4 $\pm$ 2.8 N.S.	84.7 $\pm$ 7.2 N.S.	9.9 $\pm$ 1.5 N.S.
Sorbitol	0.287 $\pm$ 0.058 N.S.	26.2 $\pm$ 2.0 <i>P</i> < 0.01	74.7 $\pm$ 3.5 N.S.	18.5 $\pm$ 2.8 N.S.	90.3 $\pm$ 4.6 N.S.	8.5 $\pm$ 2.6 N.S.

Results and discussion

The increase in soluble hexokinase at the expense of particle-bound hexokinase may be triggered by some factor dependent on the active transport of glucose into the epithelial cells or may result from an increase in the intracellular concentration of glucose, glucose 6-phosphate or a metabolite of glucose 6-phosphate. The glucose analogues selected to investigate this problem were: (1) 3-*O*-methylglucose, which is actively transported across the intestine (Campbell & Davson, 1948) but is not phosphorylated (Elsas & MacDonnell, 1972; Sols, 1956); (2) mannose, which is passively transported (Wilson *et al.*, 1960) and can be phosphorylated by hexokinase and the product further metabolized (Sols & Crane, 1954; Grossbard & Schimke, 1966); (3) 2-deoxyglucose, which is passively transported (Elsas & MacDonnell, 1972; Wilson *et al.*, 1960) and phosphorylated by hexokinase type II isoenzyme (Grossbard & Schimke, 1966) and intestinal hexokinase (Elsas & MacDonnell, 1972), although the phosphorylated product is not a substrate for glucose 6-phosphate dehydrogenase (EC 1.1.1.49) or for phosphohexose isomerase (EC 5.3.1.9) (Crane & Sols, 1953); (4) sorbitol, which is not transported into cells (Wilson *et al.*, 1960; Wilson & Landau, 1960) and is not a substrate for hexokinase type II isoenzyme (Grossbard & Schimke, 1966). In rat small-intestinal mucosa 95% of hexokinase is the type II isoenzyme (Srivastava *et al.*, 1968).

Although it is known that 3-*O*-methylglucose is not phosphorylated, a preliminary experiment was performed to determine whether the sugar binds to hexokinase. The effect of the presence of 3-*O*-methylglucose on the rate of phosphorylation of glucose by soluble hexokinase was measured. In the assay system hexokinase activity was linked with glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. The production of NADPH with 3-*O*-methylglucose as substrate was 1.4% of that observed with glucose as substrate when the glucose or 3-*O*-methylglucose concentrations were 1 mM, and 2.6% when the substrate concentrations were 10 mM. The K_m (glucose) was 0.105 mM, in agreement with Srivastava *et al.* (1968), and K_i (3-*O*-methylglucose) was 8 mM.

The activities and subcellular distributions of hexokinase and lactate dehydrogenase in the mucosa of the small intestine after intubation with saline or solutions of glucose analogues are shown in Table 1. The homogenate activities of both enzymes (expressed relative to DNA P) fell significantly on starvation, and did not rise on intubation with any of the glucose analogues. Starvation caused a fall in the proportion of hexokinase activity and a rise in the proportion of lactate dehydrogenase activity in the particle-free supernatant. Intubation with all the glucose ana-

logues caused the soluble hexokinase activity to rise to a value significantly different from that in animals intubated with saline. Glucose, mannose and 2-deoxyglucose caused no change in the soluble activity of lactate dehydrogenase, and 3-*O*-methylglucose caused a slight fall in the soluble activity. Since with all analogues the change in soluble hexokinase activity was inversely related to the change in soluble lactate dehydrogenase activity, it is unlikely that the rise in soluble hexokinase activity observed on intubation with the sugars was due to more extensive homogenization.

Glucose, 3-*O*-methylglucose and mannose caused the soluble hexokinase activity to rise to a value not significantly different from that found in fed animals, whereas the values obtained after intubation with sorbitol and 2-deoxyglucose were significantly different from those for fed animals ($P < 0.01$ and $P < 0.02$ respectively).

Intubation with 2-deoxyglucose caused a rise in soluble hexokinase activity, so that the release of hexokinase from particulate structures cannot be mediated by a rise in the concentration of a metabolite of a phosphorylated sugar. Since the greatest rise in soluble hexokinase activity occurred on intubation with 3-*O*-methylglucose it is likely that an increase in the intracellular concentration of a phosphorylated sugar is not of primary importance in the solubilization of the enzyme. This is in contrast with the mechanism of release of mitochondrial hexokinase in brain (Wilson, 1968) or ascites cells (Rose & Warms, 1967).

Since it was shown that 3-*O*-methylglucose has a very low affinity for hexokinase, it may be that the ability of this sugar to cause a solubilization of the enzyme can be attributed to the initiation of the active-transport process, to an effect on the enzyme at some site other than the substrate sites or to an effect of the sugar on subcellular membranes. The finding that an increase in intracellular glucose caused a solubilization of enzyme only when the glucose was presented to the lumen of the intestine (Shakespeare *et al.*, 1969) suggests that solubilization of hexokinase is connected with a vectorial process. However, the finding that mannose, sorbitol and 2-deoxyglucose all caused a rise in soluble hexokinase activity implies that the phenomenon is dependent on a rise in the intracellular sugar concentration. The requisite increase in intracellular sugar concentration must be very low, since cells are normally considered to be impermeable to sorbitol. It is possible that both mechanisms, i.e. the initiation of active transport and the rise in intracellular glucose concentration, are involved in hexokinase solubilization.

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