Glucose Metabolism in the Mucosa of the Small Intestine

CHANGES OF HEXOKINASE ACTIVITY DURING PERFUSION OF THE PROXIMAL HALF OF RAT SMALL INTESTINE

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1. The effect of perfusion on the activities of hexokinase and lactate dehydrogenase was studied in the proximal half of the small intestine of fed and starved rats. 2. Perfusion of preparations from starved rats with a medium containing glucose caused a significant increase in hexokinase activity of the particle-free supernatant. The increase in activity was observed as early as 5 min after the start of perfusion and persisted for up to 66 min of perfusion. No increase in hexokinase activity of the particle-free supernatant was observed when a medium containing mannitol was used. As a further control, preparations from fed rats were perfused under the same conditions. With the medium containing glucose, the hexokinase activity of the particle-free supernatant remained unchanged during the first 15 min of perfusion and thereafter fell gradually until, after 66 min of perfusion, 73% of the original activity was retained. 3. The activity of lactate dehydrogenase in the particle-free supernatant prepared from the proximal half of the untreated small intestine of starved rats was significantly lower than in corresponding preparations from fed animals. However, it did not change significantly on perfusion with media containing either mannitol or glucose. 4. The distribution of hexokinase activity between total particulate fraction and particle-free supernatant was measured in preparations from starved rats after perfusion for 5–10 min. In preparations that had not been perfused the ratio of hexokinase activity in total particulate fraction/particle-free supernatant was significantly higher in starved than in fed animals. After perfusion with a medium containing glucose, the total homogenate activity had not changed significantly, whereas the ratio of hexokinase activity in total particulate fraction/particle-free supernatant decreased significantly and approached the value obtained with fed animals. 5. The results agree with the view that the glucose-dependent increase of hexokinase activity in the soluble cell compartment as observed *in vivo* and *in vitro* in the intestinal mucosa of starved rats is brought about by a release of hexokinase activity from a particulate subcellular structure(s).

As an extension of observations *in vivo* (Srivastava & Hübscher, 1966; Srivastava, Shakespeare & Hübscher, 1968) it has been shown that the hexokinase activity of the particle-free supernatant prepared from mucosal homogenates increased after sacs of the small intestine of starved rats had been incubated *in vitro* in a medium containing glucose (Shakespeare, Srivastava & Hübscher, 1969). The present work was undertaken to characterize further the changes in hexokinase activity brought about by incubation *in vitro*.

Perfusion of the small intestine was preferred to the previously used incubation of intestinal sacs to prevent any accumulation of metabolites in the

lumen of the small intestine and to ensure constancy of the concentration of glucose (or, in control experiments, mannitol) at the mucosal side throughout the experiment.

MATERIALS AND METHODS

Animals. Specific pathogen-free female rats of the Wistar strain weighing 150–200 g were used. The rats were obtained from the Scientific Products Farm, Ash, Canterbury, Kent, U.K.

Starvation. Rats were starved for 36 h in metabolic cages and had free access to water throughout this period. Starvation was started between 17.00 and 18.00 h.

Perfusion. The proximal half of the small intestine was isolated and irrigated with ice-cold 0.9% NaCl solution. The intestine was perfused at 37°C with a Delta Peristaltic

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Pump (Watson-Marlow, Marlow, Bucks., U.K.). The flow rate of the perfusate was maintained at 100–150 ml/h and as a perfusion medium Krebs–Ringer phosphate buffer, pH 7.4 (Umbreit, Burris & Stauffer, 1959), modified by the omission of CaCl_2 and MgSO_4 , was used. During the perfusion the intestine was suspended in a vessel containing approx. 100 ml of the perfusion medium. The medium outside the intestine and that passing through the lumen were gassed with pure oxygen throughout the experiment. At the end of the perfusion, the intestine was removed from the apparatus and the luminal contents were discarded by gently squeezing the intestine. This process resulted in the loss of less than 3% of lactate dehydrogenase related to the activity obtained subsequently in the particle-free supernatant.

Preparation of subcellular fractions. The intestinal mucosa was collected, homogenized and fractionated as described by Hübscher, West & Brindley (1965), except that the medium used throughout was 0.15 M-KCl in 5 mM-tris-HCl buffer, pH 7.4, containing 2 mM-dithiothreitol.

Assays of hexokinase and lactate dehydrogenase. These were carried out as described previously (Srivastava *et al.* 1968).

Determinations of protein and DNA. These were carried out by the method of Hübscher *et al.* (1965).

EXPERIMENTAL AND RESULTS

Two series of experiments were performed. In the first series preparations of the proximal half of the small intestine were perfused for 15–66 min, and in the second a perfusion period of only 5–10 min was employed.

For the following reasons it was decided to include assays of lactate dehydrogenase in the first series of experiments. In rat intestinal mucosa lactate dehydrogenase is found mainly in the subcellular compartment containing the soluble proteins, whereas only 36% of the hexokinase activity of mucosal homogenates is found in this compartment

(Srivastava *et al.* 1968). Lactate dehydrogenase could therefore serve as an indicator for leakage of soluble proteins from epithelial cells during perfusion. Further, lactate dehydrogenase activity decreases in the intestinal mucosa during starvation and the effect of perfusion with a medium containing glucose on its activity may be compared with the changes on hexokinase activity obtained under the same experimental conditions.

The perfusion medium contained either 50 mM-glucose or 50 mM-mannitol. The latter is not metabolized in the intestinal mucosa, but ensured that the osmotic pressure of the perfusion medium was the same as that of the medium containing glucose.

During perfusion with the medium containing mannitol of the proximal half of the small intestine of fed and starved rats there were no significant changes in the DNA content of mucosal homogenates or in total protein content of the particle-free supernatant (see Tables 1 and 2). This result suggests that there was only a negligible loss of mucosal cells during perfusion. Further, the leakage of soluble proteins from mucosal cells must have been similarly unimportant. This latter contention is supported by the fact that lactate dehydrogenase activity, when related to the DNA content of the homogenate, remained constant throughout the period of perfusion. The progressive decrease in hexokinase activity in the particle-free supernatant seen during perfusion of preparations from fed rats cannot therefore be explained by loss of mucosal cells or leakage of soluble proteins. Preparations from starved rats did not show this decrease in hexokinase activity during perfusion. Finally, the hexokinase activity of the particle-free supernatant decreased on perfusion of preparations from fed rats to the activity seen in corresponding preparations from starved rats.

When preparations from fed and starved rats were

Table 1. *Effect on hexokinase and lactate dehydrogenase activities and on particle-free supernatant protein and DNA phosphorus during perfusion of the proximal half of the small intestine of fed animals with a medium containing mannitol*

The proximal half of the small intestine was perfused with a medium containing 50 mM-mannitol. The results are given as means $\pm$ S.D. Numbers in parentheses are the numbers of observations.

Time of perfusion (min)	Specific activity of hexokinase in particle-free supernatant (nmol/min per μmol of DNA P)	Total lactate dehydrogenase activity ($\mu\text{mol/min}$)	Specific activity of lactate dehydrogenase ($\mu\text{mol/min per } \mu\text{mol}$ of DNA P)	Particle-free supernatant protein (mg of protein in whole supernatant)	Total DNA content ($\mu\text{moles of DNA P}$ in homogenate)
Not perfused	62.8 $\pm$ 12.8 (10)	308 $\pm$ 91 (10)	13.1 $\pm$ 3.1 (10)	63.7 $\pm$ 8.2 (10)	23.5 $\pm$ 4.9 (10)
15	48.2 $\pm$ 9.5 (4)	303 $\pm$ 68 (4)	12.2 $\pm$ 1.9 (4)	59.6 $\pm$ 2.5 (4)	25.0 $\pm$ 4.5 (4)
30	32.4 $\pm$ 3.5 (4)	249 $\pm$ 77 (4)	8.5 $\pm$ 2.3 (4)	56.4 $\pm$ 5.3 (4)	29.2 $\pm$ 3.1 (4)
45	32.8 $\pm$ 5.0 (4)	258 $\pm$ 80 (4)	8.8 $\pm$ 1.3 (4)	58.4 $\pm$ 6.9 (4)	28.3 $\pm$ 4.6 (4)
66	25.0 $\pm$ 2.7 (4)	274 $\pm$ 64 (4)	11.0 $\pm$ 1.8 (4)	49.4 $\pm$ 10.5 (4)	24.9 $\pm$ 3.2 (4)

Table 2. *Perfusion of the proximal half of the small intestine of starved animals with a medium containing mannitol*

The experimental conditions and parameters measured were the same as those indicated in Table 1. The results are given as means $\pm$ S.D. Numbers in parentheses are the numbers of observations.

Time of perfusion (min)	Specific activity of hexokinase in particle-free supernatant (nmol/min per μ mol of DNA P)	Total lactate dehydrogenase activity (μ mol/min)	Specific activity of lactate dehydrogenase (μ mol/min per μ mol of DNA P)	Particle-free supernatant protein (mg of protein in whole supernatant)	Total DNA content (μ mol of DNA P in homogenate)
Not perfused	35.8 $\pm$ 4.5 (11)	154 $\pm$ 39 (11)	7.7 $\pm$ 1.0 (11)	44.2 $\pm$ 5.5 (11)	20.4 $\pm$ 4.9 (11)
15	33.3 $\pm$ 3.8 (4)	138 $\pm$ 9 (4)	6.3 $\pm$ 0.2 (4)	41.0 $\pm$ 7.6 (4)	21.7 $\pm$ 3.3 (4)
30	25.2 $\pm$ 3.1 (4)	145 $\pm$ 17 (4)	6.7 $\pm$ 0.2 (4)	33.5 $\pm$ 2.1 (4)	21.5 $\pm$ 1.5 (4)
45	27.5 $\pm$ 3.8 (4)	158 $\pm$ 11 (4)	8.2 $\pm$ 1.6 (4)	43.5 $\pm$ 1.3 (4)	19.1 $\pm$ 3.1 (4)
66	31.9 $\pm$ 4.5 (4)	133 $\pm$ 23 (4)	7.8 $\pm$ 0.7 (4)	32.0 $\pm$ 6.1 (4)	16.9 $\pm$ 2.4 (4)

Table 3. *Effect on hexokinase and lactate dehydrogenase activities and on particle-free supernatant protein and DNA phosphorus during perfusion of the proximal half of the small intestine of fed animals with a medium containing glucose*

The proximal half of the small intestine was perfused with a medium containing 50 mM-glucose. The results are expressed as means $\pm$ S.D. Numbers in parentheses are the numbers of observations.

Time of perfusion (min)	Specific activity of hexokinase in particle-free supernatant (nmol/min per μ mol of DNA P)	Total lactate dehydrogenase activity (μ mol/min)	Specific activity of lactate dehydrogenase (μ mol/min per μ mol of DNA P)	Particle-free supernatant protein (mg of protein in whole supernatant)	Total DNA content (μ mol of DNA P in homogenate)
Not perfused	62.8 $\pm$ 12.8 (10)	308 $\pm$ 91 (10)	13.1 $\pm$ 3.1 (10)	63.7 $\pm$ 8.2 (10)	23.5 $\pm$ 4.9 (10)
15	62.2 $\pm$ 7.7 (4)	442 $\pm$ 84 (4)	16.8 $\pm$ 3.1 (4)	55.8 $\pm$ 9.4 (4)	26.4 $\pm$ 3.5 (4)
30	55.3 $\pm$ 5.6 (4)	442 $\pm$ 45 (4)	16.0 $\pm$ 1.7 (4)	56.2 $\pm$ 6.0 (4)	27.8 $\pm$ 2.6 (4)
45	46.3 $\pm$ 4.5 (4)	401 $\pm$ 89 (4)	12.8 $\pm$ 4.0 (4)	50.5 $\pm$ 7.2 (4)	32.1 $\pm$ 3.0 (4)
66	45.7 $\pm$ 4.3 (4)	387 $\pm$ 40 (4)	13.0 $\pm$ 1.5 (4)	45.7 $\pm$ 1.5 (4)	28.7 $\pm$ 3.0 (4)

perfused with a medium containing glucose, the same results as those reported in Tables 1 and 2 were obtained with respect to DNA content of mucosal homogenates, total protein content of the particle-free supernatant and lactate dehydrogenase activity (see Tables 3 and 4). However, hexokinase activity of the particle-free supernatant from preparations from starved rats increased significantly ($P < 0.001$) within the first 15 min of perfusion and remained thereafter at this level. The maximum specific activity of hexokinase, with respect to DNA, reached after perfusion of preparations from starved rats with a medium containing glucose was not significantly different ($P > 0.10$) from the activity of preparations from fed rats that were either not perfused or perfused for 15 min with a medium containing glucose.

Preparations from fed rats, on perfusion with a medium containing glucose, behaved differently from starved rats. Preparations from fed rats, which had a significantly higher hexokinase activity before perfusion than had corresponding preparations from

starved rats ($P < 0.001$), did not show a change of hexokinase activity during the first 15 min of perfusion. On further perfusion there was a slight decrease in hexokinase activity to 73% (at 66 min) of that observed in preparations that had not been perfused.

The changes in hexokinase activity can also be seen when expressed in terms of protein. This has been done in Fig. 1, where the values quoted are those of the experiments summarized in Tables 1–4. In preparations from starved rats the specific activity of hexokinase increased significantly when the perfusion fluid contained glucose but remained unchanged when glucose was replaced by mannitol. In contrast, the hexokinase activity of preparations from fed rats did not change when the perfusion fluid contained glucose but fell progressively when glucose was replaced by mannitol.

The rapidity of the change in hexokinase activity of the particle-free supernatant prepared from the mucosa of the small intestine after perfusion with a

Table 4. *Perfusion of the proximal half of the small intestine of starved animals with a medium containing glucose*

The experimental conditions and parameters measured were the same as those indicated in Table 3. The results are given as means $\pm$ s.d. Numbers in parentheses are the numbers of observations.

Time of perfusion (min)	Specific activity of hexokinase in particle-free supernatant (nmol/min per μ mol of DNA P)	Total lactate dehydrogenase activity (μ mol/min)	Specific activity of lactate dehydrogenase (μ mol/min per μ mol of DNA P)	Particle-free supernatant protein (mg of protein in whole supernatant)	Total DNA content (μ mol of DNA P in homogenate)
Not perfused	35.8 $\pm$ 4.5 (11)	154 $\pm$ 39 (11)	7.7 $\pm$ 0.96 (11)	44.2 $\pm$ 5.5 (11)	20.4 $\pm$ 4.9 (11)
15	52.6 $\pm$ 13.2 (12)	138 $\pm$ 22 (6)	8.5 $\pm$ 2.6 (6)	38.8 $\pm$ 3.9 (6)	17.9 $\pm$ 3.0 (6)
30	54.5 $\pm$ 5.6 (6)	122 $\pm$ 21 (6)	7.6 $\pm$ 1.8 (6)	29.7 $\pm$ 4.7 (6)	16.3 $\pm$ 3.7 (6)
45	48.4 $\pm$ 3.6 (6)	131 $\pm$ 19 (6)	6.9 $\pm$ 1.4 (6)	35.3 $\pm$ 3.2 (6)	19.8 $\pm$ 5.4 (6)
66	55.2 $\pm$ 1.0 (6)	117 $\pm$ 13 (6)	7.1 $\pm$ 1.4 (4)	36.9 $\pm$ 5.1 (6)	17.0 $\pm$ 1.5 (4)

Fig. 1. Changes of hexokinase activity in the particle-free supernatant isolated from mucosal cells after perfusion of the proximal half of the intact small intestine *in vitro*. (a) Perfusion of preparations from starved rats with media containing 50 mM-glucose (●) or 50 mM-mannitol (○). (b) Perfusion of preparations from fed rats.

medium containing glucose suggested that this change may be brought about by an alteration in the subcellular distribution of hexokinase activity. For this

reason a series of experiments was carried out to study the effect of perfusion with a medium containing glucose on the distribution of hexokinase activity between the particle-free supernatant and the total particulate fraction of the intestinal mucosa.

The results presented in Table 5 show the changes in the subcellular distribution of hexokinase activity. Total hexokinase activity of mucosal homogenates was the same in starved and fed rats when the preparations were not perfused. On perfusion of preparations from starved rats the total hexokinase activity of homogenates and of total particulate fractions decreased but these changes were statistically insignificant. However, hexokinase activity in the particle-free supernatant increased significantly after 5 and 10 min of perfusion. The ratio of hexokinase activity in total particulate fraction/particle-free supernatant was taken as a measure of the subcellular distribution of hexokinase activity. In preparations from starved rats that were not perfused this ratio was more than twice that of corresponding preparations from fed animals. After perfusion of preparations from starved rats the ratio decreased significantly and approached that obtained with preparations from fed rats that had not been perfused. These results are compatible with the view that the glucose-dependent increase of hexokinase activity in the soluble cell compartment is brought about by a release of hexokinase activity from particulate subcellular structure(s).

DISCUSSION

The results presented in this paper confirm and extend previously published reports on the effects of starvation and of dietary glucose on the hexokinase activity of the mucosa of rat small intestine (Srivastava *et al.* 1968; Shakespeare *et al.* 1969). Similar observations *in vivo* were made on rats by Stifel, Rosensweig, Zakim & Herman (1968). However, these workers reported an increase in hexokinase

Table 5. *Changes in the subcellular distribution of hexokinase activity during perfusion of the proximal half of the small intestine with a medium containing glucose*

Activities are expressed as μmol of glucose phosphorylated/min and the results are given as means $\pm$ S.D. for the numbers of rats indicated in parentheses. N.S., Not significant.

	(a) Activity of total homogenate	(b) Significance (P)	(c) Activity of total particulate fraction	(d) Significance (P)	(e) Activity of particle-free supernatant	(f) Significance (P)	(g) Recoveries (e+f) $\times$ 100 (a)	(h) Activity of total particulate fraction/activity of particle-free supernatant	(i) Significance (P)	(j) Total DNA content (μmol of DNA P in homogenate)
Starved (7)	5.68 $\pm$ 2.20	Null	3.86 $\pm$ 1.70	Null	0.89 $\pm$ 0.20	Null	88.2 $\pm$ 7.1	4.39 $\pm$ 1.69	Null	29.3 $\pm$ 6.8
Fed (5)	5.79 $\pm$ 1.00	N.S.	3.21 $\pm$ 0.90	N.S.	1.91 $\pm$ 0.50	<0.001	88.4 $\pm$ 6.2	1.76 $\pm$ 0.60	<0.01	23.9 $\pm$ 6.2
Starved; perfused for 5 min (6)	3.96 $\pm$ 0.90	N.S.	2.51 $\pm$ 0.35	N.S.	1.21 $\pm$ 0.08	<0.01	98.6 $\pm$ 16.4	2.29 $\pm$ 0.55	<0.01	25.6 $\pm$ 3.9
Starved; perfused for 10 min (5)	4.29 $\pm$ 0.76	N.S.	2.37 $\pm$ 0.60	N.S.	1.25 $\pm$ 0.10	<0.01	86.8 $\pm$ 8.7	1.94 $\pm$ 0.50	<0.01	24.2 $\pm$ 3.4

as well as glucokinase activity in the intestinal mucosa of starved rats when they were re-fed with a diet containing glucose as sole source of carbohydrate. The changes in glucokinase activity reported by Stifel *et al.* (1968) are not in agreement with our observations that rat intestinal mucosa lacks glucokinase and that it is the activity of hexokinase type II that changes during starvation and re-feeding (Srivastava *et al.* 1968).

In the present investigation it was shown that the changes of hexokinase activity in the soluble cell compartment of the intestinal mucosa depended on the presence or absence of glucose in the perfusion fluid. In view of the known permeability properties of the brush border (Alvarado & Crane, 1962) it is likely that the increase in hexokinase activity was caused by the presence of glucose or of a metabolite of glucose in the cells of the intestinal mucosa. Support for this contention is obtained by comparing the results of the experiments in which the intestines from fed rats were perfused with media containing either glucose or mannitol. In the intestines perfused with media containing glucose, hexokinase activity was unchanged but it decreased progressively to the level of activity observed in intestines of starved rats when the perfusion medium contained mannitol. As the experiments were conducted with the intact organ severed from its blood supply it would seem that the activity of hexokinase in the mucosa of the small intestine of the rat, although influenced by the availability of glucose from the lumen of the intestine, is not affected by the presence or absence of insulin.

The results obtained from the short-term perfusions indicate but do not prove unequivocally that glucose or a metabolite of glucose influences the activity of hexokinase in the particle-free supernatant. In view of the rapidity with which the hexokinase activity increased it would seem unlikely that the turnover of hexokinase is influenced by glucose or a metabolite of glucose in a way that has been described for the effect of cortisone on gluta-

mate-pyruvate transaminase of rat liver (Segal & Kim, 1963) and reviewed by Schimke, Ganschow, Doyle & Arias (1968). There is little conclusive evidence in the literature on the turnover of any of the components of the intestinal epithelial cell during the lifetime of this cell, and the half-life of the hexokinase in the soluble cell compartment would have to be of the order of 1 min in order that glucose, by merely altering the rate of turnover of the enzyme, could virtually double the hexokinase activity within 5 min of perfusion. The more likely mechanism is therefore based on a change in the intracellular distribution of hexokinase between soluble and particulate fractions caused by a change on the intracellular concentration of glucose or a metabolite of glucose. This view is in agreement with the experimental observations made during short-term perfusions where the ratio of hexokinase activity in total particulate fraction/particle-free supernatant showed significant changes.

If the increase of hexokinase activity in the particle-free supernatant were in fact due to a release of particle-bound hexokinase then it is unlikely that glucose itself would cause this release. In experiments investigating the binding of hexokinase to subcellular fractions of ox heart (Hernandez & Crane, 1966) and Sarcoma 37 ascites-tumour cells (Rose & Warms, 1967) it has been shown that the release of particle-bound hexokinase activity was brought about by glucose 6-phosphate.

The interpretation of the effect of glucose or a metabolite of glucose on hexokinase activity is complicated by the smallness of the increase in hexokinase activity in the particle-free supernatant compared with the large amounts of particle-bound hexokinase activity. Srivastava *et al.* (1968) have shown that the particle-bound hexokinase of the intestinal mucosa probably does not fulfil a glycolytic function. It is possible that the glucose-dependent change of hexokinase activity, which is the rate-limiting step in the sequence of glycolytic reactions

in the soluble cell compartment (Srivastava & Hübscher, 1966), is of importance in the regulation of glucose metabolism in the intestinal mucosa as a source of energy for active transport process.

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