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Hexokinase Activity of Various Mucosal Segments of the Gastrointestinal Tract in the Rat

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The hexokinase activity of different mucosae of the gastrointestinal tract of the male rats has been studied. The activity was definitely higher in the mucosa of the glandular part of the stomach than in the whole wall of the membranous part, but still somewhat lower than the activity in the upper part of the small intestine. The activity increased slightly along the first third of the small intestine, but then decreased continuously toward the large intestine. The mucosal activity was about the same in the appendix as in the middle part of the small intestine, but lower in the large intestine than in the last third of the small intestine. The activities of different parts of the mucosa of the gastrointestinal tract changed differently during storage at a low (4°C) temperature. The activity was markedly lower in the liver than in the mucosa of the small intestine.

Wilbrandt and Lazt [1] presented the phosphorylation mechanism for the absorption of sugars in the intestine after they had found that phosphate stimulates and monoiodoacetic acid inhibits the absorption. Several investigators have later claimed that this concept is supported by the observed inhibition of the absorption by phloridzine, [2] by the accumulation of organic phosphoric acid esters in the intestinal wall during the absorption [3,4] and by the specific capacity of hexokinase to promote the phosphorylation.[5–7]

The phosphorylation-dephosphorylation hypothesis has been proved to be untenable by means of absorption experiments with sugar derivatives.[8,9] Also studies on hexokinase have revealed several facts that speak against the hypothesis. The activity of this enzyme in the intestinal wall does not differ from its activity in other tissues,[10] actively absorbed sugars are not phosphorylated at the same rate as they are absorbed,[11] and different diets do not produce changes in the enzyme activity of the intestinal wall.[12]

Nevertheless, the reaction catalyzed by hexokinase represents an initial phase in the chain of oxidation of sugars and therefore also in the adenosine triphosphate (ATP) synthesis. The active absorption mechanisms of various nutrients as well as the synthetic and secretory functions of the intestinal wall all consume large amounts of energy. Various parts of the intestine are not

equal in respect of these functions. In the present study attempts were made to determine the hexokinase activity in the mucous membrane of the gastrointestinal tract as a function of its length.

METHODS

Ten male Wistar rats (260 ± 320 g) were employed in the study. The animals were fed once a day with a mixed diet consisting of nonhomogenized surplus food obtained from a large hospital. This diet has met the necessary nutritive requirements as judged by the absence of any manifestations of mineral, protein or vitamin deficiency and by normal fertility and reproduction rate over a period of 8 years. Specimens were taken 22 h after the last feeding. The rats were anaesthetized with ether, the abdominal cavity exposed in a cold room (4°C) and the stomach and intestine quickly removed and immersed in water (4°C). The organs were opened and the contents were rinsed with 4°C distilled water using a light brush. The stomach and the upper part of the intestine were always found to be empty. Sample (1) was a section from the membranous part of the stomach, the other samples were taken by scraping the mucosa with an ampoule file (discarding the muscular wall) from the following: (2) the glandular stomach, (3) the first 5-cm section of the small intestine, (4 – 7) successive 10-cm sections of the small intestine, (8) the fifth 10-cm section from the distal end of the small intestine, (9) the third 10-cm section from the distal end of the small intestine, (10) the last 10-cm section from the distal end of the small intestine, (11) the appendix, (12) the first 10-cm section of the large intestine. Control samples were taken from the livers of some of the animals. Each specimen was homogenized in 2.5 ml of 0.15 M KF solution in a Potter-Elvehjem all-glass homogenizer. These operations were carried out in 45 min. The sampling was started alternatively from the uppèr and the lower end of the gastrointestinal tract. A 0.5-ml sample of each homogenate was transferred to a tared centrifuge tube, kept at 190°C for 60 min, and weighed after it had stood at room temperature for 40 min. The homogenates were diluted to contain 20 mg/ml of dry matter. During the dryweight determinations the specimens were stored in an ice bath.

Enzyme analyses (*ca.* 165 min after the animal was sacrified). A 100-μl volume of the sample homogenate was added to a centrifuge tube containing 100 μl of the buffered substrate solution (15 mM ATP, (Sigma grade, Sigma Chemical Co.) 12 mM glucose, 10 mM $MgCl_2$, 50 mM potassium phosphate buffer, pH 7.9) and then incubated for 5 min in a water bath at 30°C. The reaction was terminated by transferring the tube to a boiling water bath for 20 sec. After the tube had cooled, 0.5 ml of 0.3 N $Ba(OH)_2$ and 3.5 ml of a 0.84 % $ZnSO_4 \cdot 6H_2O$ solution were added to remove the sugar phosphates. The precipitate was centrifuged. Copper reagent [13] (0.5 ml) was added to 0.5 ml of the supernatant. After the mixture had been kept for 10 min in a boiling water bath and cooled, 0.5 ml of chromogen reagent [14] and 2 ml of water were added. The color intensity was measured at 500 mμ after 30 min. Parallel determinations were made on samples to which ATP was added after the termination of the reaction and also on other samples to which glucose and ATP had been added after the incubation had taken place. Triplicate determinations were made on each sample.

EXPERIMENTS AND RESULTS

Under the experimental conditions the glucose contents decreased linearly during the first 10 min in reaction mixtures containing intestine and stomach homogenates. Upon incubation without ATP present an increase in the amount of reducing substances occurred in the reaction system as compared to the incubation without ATP and glucose. The increase amounted to about 0.03 – 0.04 μmole of glucose/5 min. The appendix was an exception, the increase in this case being almost negligible.

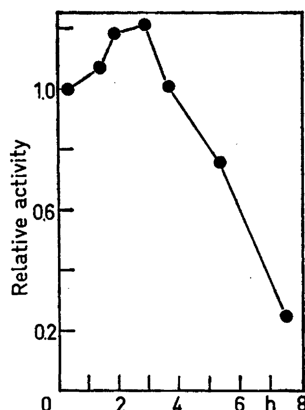

Fig. 1. Hexokinase activity in a homogenate of the duodenal mucosa of rat as a function of storage time at 4°C. Time is in hours from the animal's death, relative enzyme activities, when 15 min activity is denoted by 1.0.

Little hydrolysis of glucose-6-phosphate took place under the conditions of the hexokinase determinations. Release of glucose from glucose-6-phosphate even after a prolonged incubation was still minimal.

Since the glucose determinations were based on a relatively unspecific method, the changes in ATP contents during the incubation were controlled by ion exchange chromatography (modified method of Hurlbert *et al.*[15]). The nucleotides in incubated and unincubated reaction systems were determined employing twice the original volumes and the usual hexokinase activity analyses were made simultaneously. The found decrease in ATP content was 0.284 μmoles and the same of glucose 0.310 μmoles. Furthermore, chromatographic analyses showed that no demonstrable amounts of adenosine-5-monophosphate were produced during the incubation.

Preliminary trials revealed that the enzyme activity of the homogenates did not remain constant with time. As Fig. 1 shows, the enzyme activity in

Table 1. Hexokinase activities in the gastrointestinal mucosa of the rat expressed as a decrease in glucose content (± standard error) and the activities relative to that of sample No. 3.

Sample	Glucose decrease μmoles/5 min · g	Relative activities
1	55.3 ± 17.8	0.30
2	160 ± 33.1	0.87
3	184 ± 31.0	1.00
4	193 ± 26.4	1.05
5	204 ± 31.4	1.09
6	209 ± 33.1	1.13
7	199 ± 32.9	1.08
8	176 ± 32.5	0.96
9	157 ± 32.7	0.85
10	137 ± 24.3	0.74
11	167 ± 40.9	0.90
12	125 ± 36.7	0.68

homogenates prepared from the mucosa of the upper part of the small intestine increased slightly during the first 150 min on storage in the 4°C water bath; after this the activity decreased. After 24 h no activity was found. Changes in activity occurred also in the mucosa homogenates of the latter part of the small intestine although the increase was smaller. The activity in samples of the glandular stomach mucosa was about 20 % higher 130 min than 20 min after death. At 230 min the activity was still 85 % of the value at 20 min. Decrease in the activity was lower in the appendix than in the homogenates of the small intestine mucosa and the activity after 30 h was still 43 % of the activity 60 min after the animal was killed. Storage of the homogenates in an ice mixture clearly retarded the inactivation of the enzyme.

The lowest enzyme activity in the studied gastrointestinal samples was in the membranous part of the stomach (Table 1). The activity was also slightly lower in the mucosa of the glandular stomach than in the small intestine in general. A slight increase in the hexokinase activity of the intestinal mucosa was first observed with increasing distance from the stomach (Fig. 2). Further on the activity decreased continuously so that at the end of the small intestine the activity was only 3/4 of the activity of the upper part. The activity in the appendix was the same as in the small intestine. The enzyme activity in the large intestine was lower than in the end section of the small intestine. — The activities differed greatly in different animals.

The hexokinase activity of the liver was only about 10 % of that of the intestinal mucosa homogenates.

DISCUSSION

Melchior and Melchior [16] have shown that the hexokinase activity of yeast is inhibited by fluoride since the concentration of the ATP-Mg complex is diminished if fluoride is added before ATP into the reaction mixture. On the other hand, neither Berger *et al.*[17] with yeast nor Long [10] working with an intestinal enzyme observed such an inhibition by fluoride. In our own work ATP

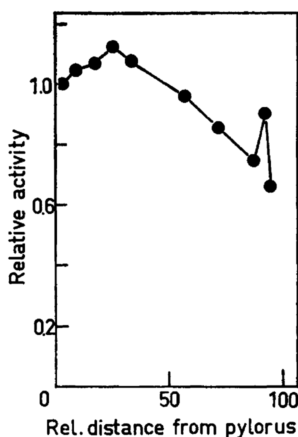

Fig. 2. Relative activities of hexokinase in the intestinal mucosa of rat. The activity of sample No. 3 is denoted by 1.0, and the length of the intestine from the pylorus to the anus by 100.

and Mg^{2+} were added together into the reaction mixture so that the ATP—Mg complex had time to form and furthermore the number of equivalents of Mg^{2+} was greater than that of the fluoride ion. According to Melchior and Melchior the precipitation of magnesium fluoride is slow.

Wiebelhaus and Lardy [18] have shown that sodium inhibits the phosphorylation of glucose catalysed by brain hexokinase. Potassium, on the other hand, activates it as Long [10] has been able to demonstrate with an intestinal preparation. Sodium was also present in our reaction mixture (added as the salt of ATP). Potassium chloride added to the reaction mixture had no effect.

The mucous membrane of the intestine contains also specific glucose-6-phosphatase;[19] the pH optimum of this enzyme is, however, considerably lower than the pH used in our study.[20] Fluoride inhibits phosphatases; possibly it is bound by the enzyme [22] or by the activator, the magnesium ion. Glucose has been found to inhibit liver glucose-6-phosphatase.[22] Non-specific alkaline and acid phosphomonoesterases are also found in the intestinal mucosa. The pH optima, however, are rather far from the pH used in this study.[23] All these facts may explain the observed slow hydrolysis of glucose-6-phosphate. The rate of hydrolysis may also be affected by the phosphate buffer. On the other hand, it is known that phosphate does not inhibit, *e.g.*, liver glucose-6-phosphatase.[22]

Long [10] mentions that the hexokinase activity of stomach homogenates is completely transferred to the supernatant whereas in the case of similar preparations made from the intestinal mucosa only a little more than half is transferred to the supernatant. The present experiments may have led to a possible liberation of the enzyme from the subcellular structures; microscopic examination of the homogenate did not reveal any intact cells. The enzyme of the glandular stomach seemed to have been released almost completely during the homogenisation. Inactivation of hexokinase appears to be slower in the glandular stomach and in the appendix than in the small intestine. This might be an indication that the small intestine contains some inactivating factor in larger amounts than other parts of the mucosa or that the activity in preparations made from different parts of the gastrointestinal tract is due to different enzymes.

The hexokinase activity of the membranous part of the stomach was lower than in any other part of the gastrointestinal tract. Long [10] studied the enzyme activity of rat stomach, but he did not state from which parts the samples were taken. If his results are expressed as ratios of the activity of the stomach to that of the mucous membrane of the small intestine, this ratio is 1.2. In our own study the activity ratio of the glandular stomach is 0.87. The difference in hexokinase activity in the membranous and glandular parts of the stomach is easily understandable if the amount of energy consumed by different biosynthetic and secretory mechanisms in considered.

No previous systematic studies have been carried out on the hexokinase activity in the small intestine. Phosphorylation rates found in this study are greater than those found by Hele,[5] Long,[10] and Sols.[11] The latter carried out enzyme activity determinations 30 min after the death of the animal; the activity was still increasing slowly in our experiments at this time. The enzyme activity in the small intestine was markedly lower than in the stomach in the experiments of Long. It was even lower than in the large intestine; the activity

in the mucosa of the small intestine was about 68 % of this. The enzyme activity of the appendix was about the same as that of the mucosa of the small intestine. In our study we found that the enzyme activity in the mucosa of the small intestine varied along the latter.

The higher enzyme activity found in the upper part of the small intestine may be a sign of a higher energy consumption. Various absorption mechanisms are most active here.[24–29] Of the biosyntheses carried out in the mucosa, at least the biosynthesis of β-D-glucopyranosiduronic acids appears to be most effective in the upper part of the small intestine.[30] The rate of cell regeneration, on the other hand, is approximately the same in various parts of the mucosa of the small intestine.[31] Indications of a high energy consumption in the upper part of the small intestine are also the lower oxygen consumption in lower parts of the small intestine,[32] the decrease in the adenosine triphosphate content of the mucosa along the small intestine as well as the changes in the activity of the enzyme system hydrolyzing adenosine triphosphate.[33]

The obtained results may, on the other hand, also be taken as evidence supporting the phosphorylation mechanism of absorption of sugars, since the absorption of sugars is greatest in the area where the enzyme activity is greatest.[24,28,29]

The mucosal hexokinase activity was found to be lower in the large intestine than in the lower end of the small intestine. The high activity, even higher than that in the mucosa of the small intestine, found by Long [10] could not be confirmed. Perhaps Long used the whole wall of the large intestine in his study. The low hexokinase activity found in our study supports the view that the energy consumption in the mucosa is smaller in the large intestine than in the small intestine, since active absorption of nutrients has not been found to occur in the large intestine and cell material synthesis also is slower there.[31]

Like Long [10] we found only a low hexokinase activity in the liver homogenates as compared to the gastrointestinal samples. This may point to different mechanisms of energy transfer in these organs.

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