CORRELATION BETWEEN THE LEVEL OF MOTOR ACTIVITY ON THE SMALL INTESTINE AND HEXOKINASE ACTIVITY IN ITS MUSCULAR LAYER

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Correlation between the motor activity of the small intestine and the intensity of energy formation in its muscular layer was studied. Significantly higher hexokinase activity was observed in the muscles of the duodenum than of the jejunum and ileum. Statistically significant differences in hexokinase activity were not found between the jejunum and ileum. Hexokinase activity was found to vary considerably and to correlate directly with the level of motor activity of the muscular layer, which forms the contractile system of the intestine.

KEY WORDS: hexokinase; motor activity of the small intestine; correlation.

An organ can perform its specific function only if the level of its activity is matched by the level of energy formation within the organ. This problem has been studied most completely with respect to the activity of striated muscle [2, 4-6, 8, 9], although it has received only little study in the smooth muscles of the intestine. The physiological properties of these muscles are known to differ in certain respects from those of striated muscles, and one difference is the ability of smooth muscle to reproduce its proper and relatively constant frequency of rhythmic contractions [10, 15]. The gradient of distribution of this frequency in the gastrointestinal tract shows a distinct fall in the caudal direction [1, 11]. By contrast with the frequency of the intestinal contractions, their amplitude is less constant and more variable, and this is naturally accompanied by changes in the quantity of work done by the motor apparatus of the intestine.

The object of this investigation was to study the intensity of glycolytic energy formation in the muscular layer of different parts of the small intestine and to compare it with the motor activity of the organ.

Hexokinase activity was chosen as the indicator of the intensity of energy formation, for this enzyme is a key factor in carbohydrate metabolism in all organs and tissues [3, 7, 12 13]. The velocity of the hexokinase reaction determines the intensity of glucose utilization in the energy metabolism of the cell.

EXPERIMENTAL METHOD

Experiments were carried out on 18 sexually mature male Wistar rats weighing 160-180 g and on 12 cats. In the experiments on rats pieces of intestine were excised immediately after decapitation of the animals in the following regions: in the middle of the duodenum, at the end of the anterior third of the jejunum, and in the middle of the ileum. Cats were anesthestized by intravenous injection of chloralose (80 mg/kg body weight). Biopsy was performed in the same region of the jejunum as that in which contractile reactions had previously been recorded (for 30-40 min) by means of a balloon and graphic method, using an electromanometer incorporating mechanotrons. Motor activity was expressed in conventional units of work: the mean sum of the amplitudes (in mm) of contractions per minute with a constant amplification factor of the instrument in all the experiments. In experiments on both rats and cats, special attention was paid to speed of taking the biopsy material and placing it in an ice-cooled chamber, in which the speciments of muscle tissue were prepared for biochemical analysis. Pieces of muscle tissue were cleaned and weighed (usually they weighed about 200 mg) and an extract in 0.15 M KCl (ratio 1: 15) was obtained from them by grinding the tissue in a mortar with quartz sand. The homogenate was centrifuged at 12,000g for 20 min at 4°C. Hexokinase activity was measured in the

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TABLE 1. Hexokinase Activity in Muscular Layer of Rat Intestine (in μmoles NADPH/mg protein/h)

No. Expt.	Duodenum	Jejunum	Ileum
1	6,8	5,7	5,3
2	7,7	6,3	5,4
3	12,0	8,4	7,3
4	11,0	9,7	8,1
5	5,0	4,5	3,5
6	6,8	4,5	3,7
7	4,2	3,4	3,3
8	5,4	4,7	4,6
9	5,2	4,2	4,1
10	7,2	6,5	6,5
11	9,1	4,4	5,7
12	6,2	4,8	5,3
13	4,9	3,5	4,7
14	4,8	2,1	3,4
15	4,0	2,7	3,7
16	5,3	3,1	3,7
17	4,0	4,2	4 1
18	3,4	5,2	2,2
$M \pm m$	6,2±0,56	4,9±0,42	4,7±0,37

TABLE 2. Hexokinase Activity and Motor Activity of Cat Small Intestine

No. Expt.	Hexokinase activity μmoles NADPH/mg protein/h	Motor activity, conventional units
1	2,9	267
2	2,8	244
3	2,8	240
4	2,6	180
5	2,5	186
6	2,4	250
7	2,4	168
8	2,2	163
9	2,2	130
10	2,0	168
11	1,9	126
12	1,8	126

resulting extract from the quantity of NADPH formed [14]. The reaction mixture consisted of the following components: 1.5 ml 0.2 M Tris-HCl, pH 8.0, 0.3 ml 0.1 M $MgCl_2$, 0.3 ml of 0.1 M ATP, 0.3 ml of 0.55 M glucose, 0.3 ml of 0.005 M NADP, 0.1 ml of glucose-6-phosphate dehydrogenase (1:500), and 0.2 ml of muscle extract. Enzyme activity was measured in the SF-4 spectrophotometer at a wavelength of 340 nm every 2 min for 18 min. The mixture of reagents without the muscle extract served as the control. Protein was determined by the biuret method.

EXPERIMENTAL RESULTS

In the experiments of series I on rats hexokinase activity was studied in the muscular layer of the three parts of the small intestine and compared with the frequency gradient of their contractile reactions.

The results are given in Table 1. Clearly the hexokinase activity was very variable in all parts of the small intestine: in the duodenum from 3.4 to 12.0 μmoles/mg/h, in the jejunum from 2.1 to 9.7 μmoles/mg/h, and in the ileum from 2.2 to 8.1 μmoles/mg/h. Despite this wide range of individual variations in hexokinase activity in the overwhelming majority (in 16 of 18) of the experiments, its highest values were observed in the muscular layer of the duodenum. Statistical analysis of the data showed that hexokinase activity in this part of the intestine was significantly higher than in the jejunum and ileum (Table 1). Comparison of the hexokinase activity in the jejunum and ileum showed that it was higher in the jejunum in 11 of 18 cases, and in the rest it was either the same in both regions or was higher in the ileum (Table 1). On average, the hexokinase activity in these two parts of the intestine was almost the same.

The results of these experiments thus showed that the activity of the key enzyme of glucose metabolism in the muscular layer of the small intestine of rats varies from one experiment to another within wide limits.

As a rule it is higher in the duodenum than in the jejunum and ileum. However, the character of distribution of the activity of this enzyme along the length of the intestine does not always correspond to the frequency gradient of the contractions of the organ. The results are in agreement with those of Alvarez [11], according to whom the presence of a frequency gradient of contractile reactions of the small intestine is due to inborn and unchanged features of metabolism of the smooth muscles. However, the question of the presence of a frequency gradient of rhythmic intestinal contractions in rats has not yet been settled. The results nevertheless emphasize the fact that the enzyme activity of energy-forming processes in the smooth-muscle cells of the same part of the intestine is in a dynamic state, possibly in connection with the changing amount of work done by its muscular apparatus.

To test this hypothesis a series of experiments (II) was carried out on cats. In these experiments correlation between the hexokinase activity of the intestine and the intensity of work performed by its contractile apparatus was investigated. Since tonic intestinal contractions make it difficult to assess the work of its contractile apparatus quantitatively, the results of experiments in which only rhythmic contractions were observed were used for analysis. It should be pointed out that biopsy of the intestine itself affected the motor activity of the region of intestine studied, as was shown by brief spastic contraction of adjacent segments after the reaction.

The results of this series of experiments (Table 2) show that individual differences in hexokinase activity of the smooth muscles of the cat intestine were observed between 1.8 and 2.9 μmoles/mg/h. There was an equally wide range of values of motor activity of the region of intestine from which the biopsy was taken: from 126 to 276 conventional units. On account of this variability of the results, they could be subjected to correlation analysis. This showed that the processes studied in this investigation correlate closely and directly, with a coefficient of 0.85.

The results of these experiments thus show that the activity of this key enzyme of glucose metabolism in the tissues is distributed irregularly in the muscular layer of the small intestine. In rats it is higher in the duodenum. They also show that hexokinase activity in the muscle tissue of the intestine is very dynamic and correlates clearly with its motor activity.

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