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Increase of Intestinal Brush Border Hydrolases in Mucosa of Streptozotocin-Diabetic Rats*

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Summary. Experimental diabetes mellitus in rats was induced by streptozotocin. Five days after administration of streptozotocin intestinal brush border hydrolases (maltase, sucrase, trehalase, lactase) and alkaline phosphatase were markedly elevated at all levels of the small intestine as measured in the total homogenate and in the isolated brush border preparation. Insulin treatment beginning 15 h after administration of streptozotocin was able to decrease the increased disaccharidase activity due to streptozotocin diabetes. In experimental diabetes mellitus of rats trransport as well as digestive functions of the intestinal mucosa are stimulated.

Key words: streptozotocin-diabetes, insulin, intestinal enzymes, disaccharidase activity, alkaline phosphatase.

Increase of active intestinal transport for hexoses has been reported in alloxan-diabetic rats [2, 8, 14] and in diabetes mellitus in man [16]. Since there exists a close functional and structural relationship between intestinal brush border hydrolases (sucrase, maltase, lactase, trehalase) and the intestinal glucose transport system [3, 4], we studied the effect of streptozotocin-diabetes and insulin on the activity of intestinal brush border hydrolases in rat small intestine.

Methods

Female Wistar rats (200 ± 20 g) were injected intravenously via the saphenous vein 70 mg/kg of steptozotocin (Upjohn Company, Kalamazoo, Michigan, USA — LOT No. 9681-GGS-118FI U 9885) in citrate buffer (pH 4.5), controls received citrate buffer alone. Only rats with a heavy glucosuria, corresponding to blood glucose levels between 350—500 mg% were used for the experiments. Controls and streptozotocin-diabetic rats had free access to food and water. In one group insulin-treatment (5 units long acting insulin (Depot-Insulin, Hoechst) per day per animal) was commenced 12—15 h after injection of streptozotocin. Five days after administration of streptozotocin rats were fasted overnight and killed by decapitation. The intestine was removed, flushed with ice-cold saline, everted, washed again.

The mucosa was scraped off with a microscopic glass slide and was diluted 1:50 (weight/volume) in 5 mM EDTA (pH 7.4) and homogenized for 20 sec in a Warring Blender. Appropriate dilutions were assayed for disaccharidase activity according to the method of Dahlqvist [5] using 56 mM substrates (sucrose, maltose, lactose, trehalose) in maleate buffer (0.1 M, pH 6.0). Alkaline phosphatase was assayed using p-nitrophenylphosphate as the substrate (pH 10.5). Incubations for disaccharidase activity were for 60 min at 37°C, for alkaline phosphatase activity 10 min at 37°C. Isolated brush border fractions were obtained by the method of Forstner, Sabesin and Isselbacher [9]. Purity of the preparation was judged by light microscopy. The sucrase activity in this preparation was ten times greater than in the total homogenate (*Table 1*).

Results

Specific activities of brush-border located intestinal hydrolases (sucrase, maltase, trehalase, lactase) and alkaline phosphatase were markedly increased five days after inducing streptozotocin-diabetes compared to controls. The increase in disaccharidase activity could be observed in the total jejunal homogenate of streptozotocin-diabetic rats as well as in the isolated brush border preparation (Table 1). The increase in

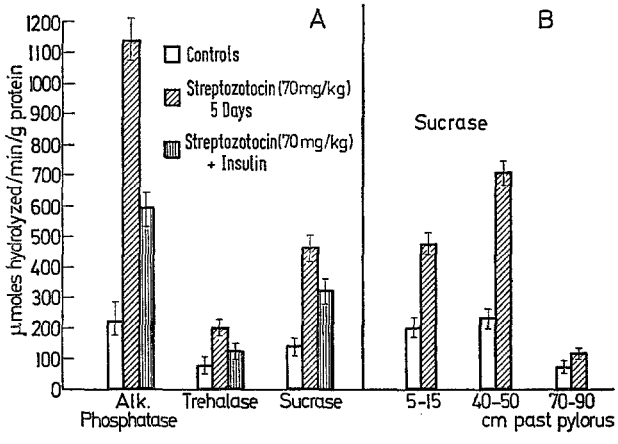

Fig. 1. Effect of streptozotocin-diabetes on intestinal brush border hydrolase activity and alkaline phosphatase. A. Effect of streptozotocin and insulin-treatment of diabetic rats on specific activity of alkaline phosphatase, trehalase and sucrase in isolated brush borders of rat small intestine. B. Sucrase activity along the small intestine of streptozotocin-diabetic rats. Specific activity is expressed in units (µmoles of disaccharide hydrolyzed)/g protein. Results are given as means ± S.E.M. (intestine from 6 animals for each group)

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disaccharidase activity could be seen at all levels of the small intestine (Fig. 1B). Insulin treatment commencing 12—15 h after steptozotocin injection significantly reduced increase of disaccharidase and alkaline phosphatase activity (Fig. 1A). Pair-feeding of streptozotocin-diabetic rats did not prevent the increase in enzyme activity.

Discussion

Recent observations have shown an increase of intestinal sucrase activity in alloxan-diabetic rats [13] as well as an increase of maltase activity [10]. We found that merely all brush-border-located intestinal hydrolases (sucrase, maltase, trehalase, lactase) and alkaline phosphatase were markedly increased in small intestinal mucosa of rats five days after inducing streptozotocin-diabetes.

The increase of brush border alkaline phosphatase in streptozotocin-diabetic rats paralleled with the disaccharidase increase leading to the assumption of a common underlying mechanism of enzyme induction by experimental diabetes mellitus. The function of alkaline phosphatase in brush border membranes is, however, still inknown.

The observed increase of disaccharidase activity per unit protein (specific activity) suggests that in the diabetic jejunum more enzymatic activity per cell was present. A close functional and spatial relationship between disaccharidases and the transport system for glucose exists in the intestine resulting in a "kinetic advantage" [3] for absorption of glucose derived from disaccharidase hydrolysis [3, 4]. As experimental diabetes mellitus stimulates transport capacity for actively transported hexoses [1, 1a, 2, 8, 13, 16] as well as digestive functions of disaccharidases [1a, 10, 13] a common mechanism may be assumed. Increases in transport capacity [1a] and digestive enzyme activity (this investigation) due to streptozotocin diabetes can be reduced by insulin treatment. Thus the underlying mechanism is assumed to be sensitive to insulin and cannot be attributed to a direct effect of streptozotocin on functions of the mucosal epithelial cell. The bulk of ingested carbohydrates has to be absorbed by the digestive-absorptive surface of the small intestine after pancreatic digestion as maltoses, sucrose and lactose. Only minimal amounts of their constituing monosaccharides occur in the small intestinal lumen.

Table 1. *Effect of streptozotocin-diabetes on rat intestinal brush border hydrolase activity*

	Controls		Streptozotocin-diabetes	
	Specific activity (Units */g of protein)		Specific activity (Units */g of protein)	
	Homogenate	Isolated brush borders	Homogenate	Isolated brush borders
Maltase	281±29	2430±195	381±20[a]	3950±212[b]
Sucrase	52± 7	556± 34	90± 8[a]	998± 72[b]
Trehalase	20± 3	112± 8	42± 3[a]	343± 28[b]
Lactase	5± 1	64± 8	10± 2[a]	86± 4[b]
alkaline Phosphatase	74± 5	496± 21	111± 6[a]	786± 38[b]

Rat small intestine was assayed for disaccharidase activity 5 days after administration of streptozotocin (70 mg/kg). 8 rats were used for each group.

[a] = $p < 0.001$ comparing total homogenates from controls and streptozotocin diabetic rats.

[b] = $p < 0.001$ comparing isolated brush border disaccharidase activity from controls and diabetic rats. Specific activity is given as means ± S.E.M.

* Units of disaccharidase activity = μmoles of disaccharide hydrolyzed/minute.

As altered diets are known to influence sucrase activity [15] in the intestine pair-feeding experiments were performed. There was no change either in increased active transport of hexoses in streptozotocin diabetic small intestine nor an effect on the increases of intestinal disaccharidase activities in streptozotocin diabetic rat small intestine (own unpublished results) which is in agreement with the results of Olson *et al.* [13] obtained in alloxan diabetic rats.

The reason for the rise in disaccharidase activity is unknown. Although rat intestinal sucrase may be precociously induced by steroids [7] and its appearance delayed in adrenalectomized rats [11], sucrase and maltase levels in the adult rat were not affected by adrenalectomy or steroid administration [6]. The periods of observation after steroid administration, however, were rather short. As steroids are able to promote intestinal glucose transport [1] and elevated steroid hormone levels have been observed in experimental diabetes mellitus, there still might be the possibility that the increase in disaccharidase activity is due to elevated steroid levels in experimental diabetes mellitus.

Digestive and transport functions are very closely coupled at the mucosal surface. Thus, an important question is whether the observed increase in disaccharidase activity and the reported increase in hexose transport capacity [2, 8, 14] in experimental diabetes mellitus produces an additive effect upon glucose transport from disaccharides.

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(J. lab. clin. Med. 79 (1972) 579–586) reporting on increases of intestinal disaccharidase activities in alloxan diabetic rat small intestine. Our results confirm their findings.

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