Regulation of Mucosal Phosphofructokinase in the Small Intestine of the Streptozotocin-Diabetic Rat

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Summary. The total activity of mucosal phosphofructokinase in the proximal jejunum of the streptozotocin-diabetic rat was found to be diminished compared with normal from 13.4 to 10.4 units/g wet weight mucosa and the activity ratio of the enzyme at pH 7 was diminished from 0.40 to 0.23, indicating that the enzyme is more susceptible to inhibition by ATP. The combination of these changes is, in principle, more than sufficient to account for the 29% decrease in glucose utilisation observed in the jejunum of diabetic rats. Insulin, when injected in vivo, has the ability to restore both the total activity and the regulatory properties of phosphofructokinase to normal in streptozotocin-diabetic rats. The action of insulin is blocked by the protein synthesis inhibitor cycloheximide, and is not mediated by changes in blood glucose levels.

Key words: Intestine, glucose utilisation, phosphofructokinase, diabetes, insulin.

It is well-established that glucose absorption by the epithelial cells of the small intestine is increased in rats with experimental diabetes compared with normal rats [1, 2]. Two factors that contribute to the enhancement of glucose transport are an increased mucosal to serosal flux resulting from the induction by hyperglycaemia of the synthesis of new carrier sites in the basolateral membrane [3], and a decreased rate of glucose utilization by the intestine [4]. The mechanism responsible for the latter is considered here.

In studies with a vascularly-perfused preparation of jejunum, Hanson and Parsons [4] found that the rate of glucose utilization in streptozotocin-diabetic rats was decreased by about 29% compared with normal rats. The rate of glucose utilization in the muscularis is relatively small compared with that of the epithelial cells [5]; hence the decrease in glucose utilization primarily reflects an inhibition of the glucose metabolism of the epithelial cells in streptozotocin-diabetic rats. The decrease in utilisation cannot be explained by changes in the levels of glycolytic enzymes, such as hexokinase [6] for the latter are increased in diabetic rats [7]; nor can it be explained by the large intramucosal accumulation of glucose that occurs in diabetic rats [3, 8] since the rates of utilisation by jejunum from normal and diabetic rats were determined in vitro at the same glucose concentration [4]. Some other factor is therefore implicated in the control of intestinal glucose metabolism in experimental diabetes.

We have suggested recently that the mechanism whereby glucose utilization is decreased in another condition in which the circulating levels of insulin are also depressed, that of starvation, is through the control of phosphofructokinase activity [9]. Thus, when rats are starved for 48h, the activity of mucosal phosphofructokinase is reduced in two ways. First, the regulatory properties of the enzyme are altered in that it is much more susceptible to inhibition by ATP and second, the total activity (units/g wet weight) is reduced by some 20%. The combination of these two changes is sufficient to account, in principle, for the 64% reduction in glucose utilization rate observed by Hanson and Parsons for a vascularly-perfused preparation of jejunum from rats starved for 48h compared with fed rats [4].

We report here that changes in the activity of mucosal phosphofructokinase, similar to those seen on starvation, also occur in the rat small intestine during the onset of experimental diabetes induced by streptozotocin.

Materials and Methods
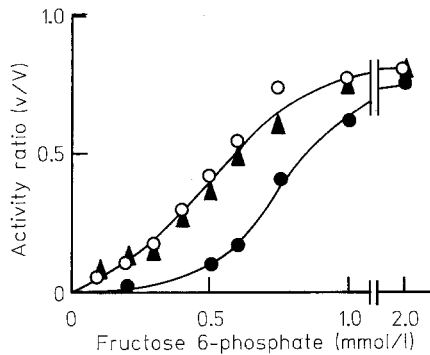
Fig. 1. The effect of streptozotocin diabetes on the regulatory properties of phosphofructokinase in crude extracts of rat jejunal mucosa from normal rats O—O), streptozotocin-diabetic rats (●—●) and streptozotocin-diabetic rats maintained on insulin for 25 h (▲—▲). The dependence of activity ratio on fructose 6-phosphate concentration was measured in the presence of 2.5 mmol/l ATP
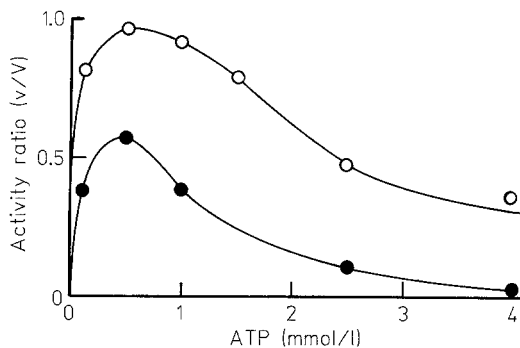
Fig. 2. The effect of streptozotocin-diabetes on the regulatory properties of phosphofructokinase. The dependence of activity ratio on ATP concentration was measured in the presence of 0.5 mmol/l fructose 6-phosphate. Other details and symbols as for Figure 1

Table 1. The total activity of mucosal phosphofructokinase and its susceptibility to inhibition by ATP in the proximal jejunum and distal ileum of normal and streptozotocin-diabetic CHFB Wistar rats

Experimental group	Intestinal segment	Phosphofructokinase activity		
		Total activity		
		Units/g wet weight	Units/mg protein	$v_{0.5}/V$
Normal	Proximal Jejunum	13.4 ± 1.4 (10)	179 ± 16 (4)	0.40 ± 0.5 (8)
	Distal Ileum	4.6 ± 0.5 (6)	70 ± 15 (4)	0.42 ± 0.05 (6)
Diabetic	Proximal Jejunum	10.4 ± 1.6 (10)[a]	96 ± 15 (4)[a]	0.23 ± 0.07 (8)[a]
	Distal Ileum	5.9 ± 0.8 (6)	62 ± 7 (4)	0.26 ± 0.05 (6)[a]

Values are given as mean ± SD with numbers of rats in parentheses. The total activity of mucosal phosphofructokinase was measured under optimal conditions at pH 8 as described in Materials & Methods; the susceptibility of phosphofructokinase to inhibition by ATP was measured by changes in the activity ratio ($v_{0.5}/V$) of the activity determined at pH 7.0 in the presence of 0.5 mmol/l fructose-6-phosphate and ATP (2.5 mmol/l) at pH 7.0. Significant differences from normal rats: [a] $p < 0.001$

positive glycosuria (> 400 mg/100 ml; Boehringer, London, UK test strip) 4 and 5 days after injection were used. The results obtained showed no obvious relationship to the period of diabetes and have been pooled.

In order to reverse the effects of diabetes, normal and 4-day streptozotocin-diabetic rats received either three or four IP injections of insulin (20 U/kg body weight in 50 mmol/l sodium phosphate buffer, pH 7.1) at 8 h intervals, commencing 17 h or 25 h, respectively before removal of tissue; thus rats were used in each case 1 h after the final injection of insulin.

The effect of cycloheximide was observed in streptozotocin-diabetic rats maintained on insulin for 25 h as described above. Cycloheximide solution (1 g/l in 0.9% saline) was injected IP (3 ml/kg body weight) at 13 h and 2.5 h before tissue removal as described by Crouzoulon and Dandrifosse [10].

Experimental hyperglycaemia was produced in normal rats under anaesthesia as follows. The femoral vein was cannulated and rats received a priming dose of 1.15 g of glucose in heparinised Krebs-Henseleit bicarbonate medium (0.31 g heparin/l), followed by an infusion of glucose (30% w/v) in saline (0.9% NaCl) at a rate of 2 ml/h for 4 h. The blood glucose level was thus maintained above 44 mmol/l for the whole of the infusion. Control rats received an infusion only of saline (0.9% NaCl).

The daily food intakes of normal and streptozotocin-diabetic rats were measured at 10.00 h using female Leeds Wistar rats (220–250 g).

Extracts of intestinal mucosa were prepared and phosphofructokinase activity assayed as described previously [9].

Data on the regulatory properties of mucosal phosphofructokinase are presented as the activity ratio (v/V) where v is the suboptimal activity at pH 7.0 in the presence of ATP (2.5 mmol/l) and a given concentration of fructose 6-phosphate under the conditions defined by Hussey et al. [11] and V is the maximal activity at pH 8.0, where the enzyme is not subject to allosteric regulation, determined according to Ling et al. [12]. In this way, the effects of streptozotocin diabetes on the regulatory properties and on the total activity of mucosal phosphofructokinase could be distinguished. One unit of activity is defined as the formation of 1 μmol of fructose 1,6-biphosphate per min at 27 °C.

Biochemicals were obtained from the Sigma, London or Boehringer and other reagents were of analytical grade.

Statistical Analysis

The data are expressed as mean ± SD, and Comparisons between sets of data were made using the Student's t-test.

Results

The regulatory properties of phosphofructokinase from rat intestinal mucosa are altered during the onset of streptozotocin diabetes. Figure 1 shows that in 4- and 5-day streptozotocin-diabetic rats, the fructose 6-phosphate saturation curve of jejunal phosphofructokinase, measured under suboptimal conditions in the presence of ATP (2.5 mmol/l) at pH 7.0, is displaced to higher fructose 6-phosphate concentrations. The apparent K_M of the enzyme for fructose 6-phosphate in normal rats was 0.47 mmol/l compared with 0.75 mmol/l in streptozotocin-diabetic rats. The increase in K_M is a reflection of an increased susceptibility to inhibition by ATP (Fig. 2). Figure 1 shows that the difference in the phosphofructokinase activity ratio at 0.5 mmol/l fructose 6-phosphate ($v_{0.5}/V$) between normal and streptozotocin-diabetic rats was about maximal. In further investiga-

Table 2. Daily food intake of normal and streptozotocin-diabetic rats

Experi-mental group	Food intake (g)				
	Day 1	Day 2	Day 3	Day 4	Day 5
Normal (6)	40.1 ± 0.4	34.7 ± 5.0	36.7 ± 5.0	39.6 ± 3.9	35.9 ± 4.5
Diabetic (6)	19.7 ± 1.7^a	18.4 ± 4.2^b	31.4 ± 3.2	34.5 ± 4.8	41.4 ± 5.7

Values are given as mean $\pm$ SD with numbers of rats in parentheses. Female Leeds Wistar rats (220–250 g) were made diabetic by the injection of streptozotocin and their daily food intake monitored against that of normal rats. Significant differences from normal rats are denoted: $^a p < 0.005$; $^b p < 0.01$

Table 3. The effect of insulin in vivo on mucosal phosphofructokinase activity in the proximal jejunum after intraperitoneal injection into normal and streptozotocin-diabetic Leeds Wistar rats

Experimental group	Phosphofructokinase activity	
	Total activity (units/g wet weight)	$v_{0.5}/V$
Normal	$14.4 \pm 2.2 (6)$	$0.39 \pm 0.04 (9)$
Normal + insulin (25 h)	$14.7 \pm 0.5 (3)$	$0.36 \pm 0.01 (3)$
Diabetic	$10.5 \pm 0.9 (5)^c$	$0.12 \pm 0.03 (5)^a$
Diabetic + insulin (17 h)	$11.1 \pm 1.7 (3)^d$	$0.21 \pm 0.02 (3)^b$
Diabetic + insulin (25 h)	$14.0 \pm 1.4 (3)$	$0.39 \pm 0.07 (3)$
Diabetic + cycloheximide + insulin (25 h)	$9.2 \pm 1.3 (4)^a$	$0.13 \pm 0.02 (6)^a$
Diabetic + cycloheximide (25 h)	$9.0 \pm 0.8 (3)^a$	$0.13 \pm 0.03 (3)^a$

Values are given as mean $\pm$ SD with numbers of rats in parentheses. Significant differences from normal rats are denoted: $^a p < 0.001$, $^b p < 0.005$, $^c p < 0.01$, $^d p < 0.05$

tions of the regulatory properties of phosphofructokinase using multiple samples of the proximal jejunal and distal ileal segments of intestine, $v_{0.5}/V$ was therefore determined.

Normal rats displayed a negative aboral gradient of total phosphofructokinase activity, falling from 13.4 ± 1.4 units/g wet weight mucosa, in the proximal jejunum to about 34% of that value in the distal ileum (Table 1). In streptozotocin-diabetic rats, the total activity in the proximal jejunum was diminished by about 23% (p < 0.001) in comparison with normal rats, whereas no significant change was observed in the distal ileum. In contrast to the changes in total activity, the activity ratio $v_{0.5}/V$ in normal rats was the same in the distal ileum as in the proximal jejunum and both were almost halved, from about 0.41 to about 0.23 (p < 0.001) in streptozotocin-diabetic rats.

Although the increased susceptibility of mucosal phosphofructokinase to inhibition by ATP in streptozotocin-diabetic rats was similar to that seen in starved rats, it was not the consequence of semi-starvation during the induction of diabetes by streptozotocin. Thus, whereas food intake was significantly reduced on days 1 and 2 after injection of rats with streptozotocin compared with normal rats, as was the case with mice [13],

the intake had returned to normal by day 3 (Table 2). Moreover, as refeeding of starved rats for only 16 h was sufficient to restore both the total activity and the activity ratio of mucosal phosphofructokinase to those characteristic of normal, fed rats [9], the diminished values observed in 4- and 5-day streptozotocin-diabetic rats could not have been caused by the initial reduction in food intake on days 1 and 2.

CHFB Wistar rats were used for the early experiments of this study (Table 1). In subsequent experiments a stress-resistant strain, Leeds Wistar rat, was used. When the latter rats were made diabetic, the susceptibility of mucosal phosphofructokinase from the proximal jejunum to inhibition by ATP was even greater than for CHFB Wistar rats, the activity ratio, $v_{0.5}/V$, being diminished from a normal value for Leeds Wistar rats of 0.39 ± 0.04 to as little as 0.12 ± 0.03 (p < 0.001) rather than the value of 0.23 for CHFB Wistar rats (Table 3). As was the case for CHFB Wistar rats, the total activity of phosphofructokinase was significantly reduced in Leeds Wistar rats that were streptozotocin-diabetic.

The effects of streptozotocin-diabetes on the total activity and on the regulatory properties of mucosal phosphofructokinase from the jejunum could be completely reversed by the administration of insulin in vivo (Table 3). In particular, the IP injection of 4-day streptozotocin-diabetic rats with insulin (20 U/kg body weight) at 8 h intervals resulted in an increase in the value of $v_{0.5}/V$ from 0.12 ± 0.03 to 0.21 ± 0.02 after 17 h and then to 0.39 ± 0.07 after 25 h when the fructose 6-phosphate saturation curve was indistinguishable from that for normal rats (Fig. 1). The ability of insulin to restore both the total activity of phosphofructokinase and its susceptibility to inhibition by ATP to normal in streptozotocin-diabetic rats was completely blocked by cycloheximide; no changes were seen in diabetic rats injected with insulin and cycloheximide compared with diabetic rats injected only with cycloheximide or untreated diabetic rats (Table 3). The treatment of normal rats with insulin for 25 h did not produce any chance in $v_{0.5}/V$ or in total activity (Table 3).

A 4 h period of experimental hyperglycaemia during which blood glucose was maintained above 44 mmol/l by IV glucose infusion into an anaesthetised, normal rat was without effect on either the total activity or the activity ratio of mucosal phosphofructokinase in the proximal jejunum and distal ileum (Table 4).

Discussion

Previous studies with starved rats led us to suggest that mucosal phosphofructokinase plays an important role in the regulation glucose utilization [9] and further support for this suggestion is provided in the present study. Thus, in streptozotocin-diabetic CHFB Wistar rats, the total activity of mucosal phosphofructokinase in the je-

Table 4. The effect of hyperglycaemia on the total activity of mucosal phosphofructokinase and its susceptibility to inhibition by ATP in the proximal jejunum and distal ileum in normal rats

Experi- mental group	Intestinal segment	Phosphofructokinase activity	
		Total activity (units/g wet weight)	$v_{0.5}/V$
Normal control	Proximal Jejunum	$14.9 \pm 1.1\,(4)$	$0.39 \pm 0.03\,(4)$
	Distal Ileum	$7.0 \pm 0.9\,(4)$	$0.45 \pm 0.05\,(4)$
Normal hypergly- caemic	Proximal Jejunum	$15.4 \pm 1.6\,(4)$	$0.45 \pm 0.08\,(4)$
	Distal Ileum	$6.7 \pm 0.8\,(4)$	$0.42 \pm 0.06\,(4)$

Values are given as mean $\pm$ SD with numbers of rats in parentheses. Anaesthetised normal Leeds Wistar rats were maintained in a hyperglycaemic state for 4 h by glucose infusion; controls were infused with 0.9% saline

junum is diminished by about 23% and the susceptibility to inhibition by ATP is increased so that the activity ratio is diminished by about 43%. Hence, when both changes are taken into account, the overall decrease in activity under the specified conditions of the assay is some 56% for CHFB Wistar rats (Table 1); in the case of Leeds Wistar rats, the overall decrease is as much as 78% (Table 3). Although it is not possible to choose assay conditions in vitro that reflect physiological conditions in precise detail, it is apparent that changes in phosphofructokinase activity can, in principle, more than account for the 29% reduction in intestinal glucose utilization in streptozotocin-diabetic rats [4].

Our results show that the total activity of mucosal phosphofructokinase decreases aborally from the proximal jejunum to the distal ileum in normal rats, whereas the susceptibility to inhibition by ATP is unaltered. The same is true for streptozotocin-diabetic rats, although the gradient of total activity is diminished, entirely as a result of a decrease in the proximal jejunum but not the distal ileum, and the susceptibility to inhibition by ATP is much greater in both jejunum and ileum. Exactly equivalent changes in total activity and susceptibility to inhibition by ATP were seen when rats starved for 48 h were compared with fed rats and imply that the mechanism that controls the total activity of mucosal phosphofructokinase is distinct from that controlling changes in regulatory properties, although both are mediated by humoral factors [9]. The changes in phosphofructokinase activity reported here for 4- and 5-day streptozotocin-diabetic rats were not caused by semi-starvation of rats during the onset of streptozotocin-diabetes (Table 2).

The ability of insulin to reverse the effects of streptozotocin diabetes on the total activity and on the susceptibility of mucosal phosphofructokinase to inhibition by ATP (Table 3) implicates insulin as one humoral factor, whether directly or indirectly. An obvious mechanism

for the indirect control of phosphofructokinase activity by insulin is through changes in blood glucose levels. Thus, the inhibition of mucosal phosphofructokinase in streptozotocin-diabetic rats could be caused by hyperglycaemia, particularly in view of the fact that the intracellular concentration of glucose in epithelial cells is determined by and is similar to that of blood glucose [8] and the report by Porteous [14] that glucose utilization by chick enterocytes is diminished by about 21% when the concentration of glucose in the incubation medium is doubled to 10 mmol/l.

However, no change was observed in either total mucosal phosphofructokinase activity or in the activity ratio when hyperglycaemia was produced in normal rats by glucose infusion for 4 h (Table 4). In such a situation, hyperglycaemia markedly stimulates insulin secretion and it is possible that an increase in total activity or activity ratio caused by insulin directly might have masked a corresponding decrease caused by hyperglycaemia. However, insulin injected into normal rats in the absence of hyperglycaemia did not affect either the total activity or the activity ratio (Table 3). In starved rats, which show a similar depression in the total activity and activity ratio of phosphofructokinase to that reported here for streptozotocin-diabetic rats, the blood glucose is depressed compared with normal [9].

The fact that cycloheximide completely blocks the ability of insulin to restore the total activity and activity ratio of mucosal phosphofructokinase to normal values in streptozotocin-diabetic rats indicates that protein synthesis is involved in the control of activity. In the case of changes in total phosphofructokinase activity, synthesis of new enzyme is most probably involved. Bazaes et al. [15] have reported recently that phosphofructokinase from the muscle of genetically diabetic mice (strain C57BL/KsJ) is more phosphorylated and slightly more susceptible to inhibition by ATP than from normal mice. It is possible that a similar explanation is involved in the changes in activity ratio of mucosal phosphofructokinase reported here. Equally, it is possible that the changes stem from alterations in the synthesis or activity of a different enzyme responsible for the production of an effector of phosphofructokinase; such a situation occurs in the regulation of phosphofructokinase in liver [16–20]. Hyperglycaemia is unlikely to be a factor in protein synthesis since the 4 h infusion, which results in a large increase in glucose carrier synthesis [3], was without effect on phosphofructokinase and diabetes results in a reduction in synthesis.

The intestine has traditionally been regarded as an insulin-insensitive tissue when studied in vitro while studies on the effect of insulin in vivo have been conflicting [1, 2]. Recently, however, evidence has been presented that enterocytes possess insulin-binding sites [21]. The present study clearly demonstrates that the injection of insulin into streptozotocin-diabetic rats restores the depressed activity of mucosal phosphofructokinase to normal. Although an indirect effect cannot be

excluded, the balance of the present evidence inclines towards a direct effect of insulin on the epithelial cells of the intestine.

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