Diurnal Variation and Increase of Disaccharidase Activity in Diabetic Rats

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Summary The small intestinal disaccharidase activity and its daily variation in the diabetic rat have not been well described. Therefore, the small intestinal disaccharidase (maltase, lactase and sucrase) activity and its daily profile were studied in streptozotocin-induced diabetic rats under physiological conditions. In diabetic rats, a similar pattern of diurnal variation of disaccharidase activity to control rats was observed, while the relationships between daily change of disaccharidase activity and that of food consumption suggested that there was a different mechanism of diurnal variation in diabetic rats. On the other hand, a significant increase of mean 24-h lactase and sucrase activities was noted in diabetic rats, while that of maltase was not significant. Using the in vitro incubation method, a significant correlation between glucose concentration and lactase or sucrase activity but not maltase activity was observed. However, insulin showed no effect on disaccharidase activity. Thus we clarified the presence of a diurnal variation of disaccharidase activity and an increase in its activity in diabetic rats. This change was suggested to be derived from high plasma glucose level.

Key Words disaccharidase activity of the small intestine, maltase, lactase, sucrase, diabetes mellitus, diurnal variation, food consumption, plasma glucose, insulin, rats

It has been known that disaccharidase (DS) activity in the small intestine in normal rats shows daily rhythmic change related to the time of food ingestion (1, 2).

On the other hand, small intestinal DS activity in diabetes mellitus has not been well understood. Some authors have reported increased DS activity in diabetic rats (3–11), but conflicting observations also have been published (12, 13). In diabetic patients no consistent opinion has been presented concerning the small intestinal DS activity (14–17).

In the present study, we examined the DS activity and its daily profile in the
small intestine of streptozotocin (STZ)-induced diabetic rats. For the purpose of clarifying the mechanism of daily variation of DS activity, its relationship with the amount of food ingestion and plasma glucose level was examined. Furthermore, to study the mechanism of increased DS activity, direct effects of glucose or insulin on DS activity in the small intestinal mucosal cells of rats were also examined in vitro.

MATERIALS AND METHODS

Male Wistar rats were kept under conditions of a 12-h light-dark cycle (light from 7:00 h to 19:00 h, and dark from 19:00 h to 7:00 h), with 50±5% humidity and a room temperature of 24±1°C. Standard laboratory chow and water were given ad libitum. At the age of 8 weeks, rats were randomly divided into two groups. One group of rats received citrate buffer (control group) and the other group received streptozotocin (18) 80 mg/kg (Sigma Chemical Co., St. Louis, U.S. A.) dissolved in citrate buffer (diabetic group) by intraperitoneal injection. Plasma glucose levels were determined one week after injection, and those rats showing plasma glucose levels of 250 mg/dl or more, were defined as diabetic. Thus we had 42 diabetic and 48 control rats at the age of 12 weeks.

At first the daily profile of food ingestion was studied with 4 rats in each group. Then the small intestinal mucosa was collected from 42 diabetic and 42 control rats every 4 h to study the daily profile of DS activity. At the same time, blood was collected from inferior vena caval vein for plasma glucose and glycated hemoglobin (HbA1) determination. Furthermore, the direct effect of glucose or insulin on DS activity was investigated using 6 control rats in vitro.

1. Disaccharidase activity and its daily profile in diabetic and control rats. At 4 weeks after the treatment, the small intestine were excised every 4 h under pentobarbital anesthesia in 42 diabetic and 42 control rats. After washing the excised small intestine with chilled physiological saline, within 5 min the mucous membrane in the upper one-third of the small intestine was collected by scraping with a slide glass, and the collected specimens were stored at −40°C until analysis. Enzyme activity was determined by the modified method of Dahlqvist et al. (19) after thawing the frozen mucous membrane. Namely, 4 parts of chilled distilled water were added, and the tissue was homogenized by ultrasonication [Microson™ ultrasonic cell disruptor, Model MS-50 (Heat Systems-Ultrasonics, Inc., New York, U.S.A.)] for 90 s. Then the mixture was centrifuged at 3,000×g at 4°C for 10 min, and the supernatant was collected as the enzyme solution (diluted to less than 0.10 U/ml at measurement). Substrate solutions [maltose, lactose (Wako Pure Chemical industries Ltd., Osaka, Japan), sucrose (Nakarai Chemicals Ltd., Kyoto, Japan)] were prepared at 0.056 M in sodium maleate buffer (0.1 M, pH 6.0). Both the enzyme and the substrate solutions were incubated for 5 min at 37°C. Then 100 μl of both solutions were mixed and the mixture was incubated for 15 min at 37°C. The enzyme reaction was stopped by immersing the reaction mixture in a boiling water bath for 2 min. Liberated glucose from the substrates was determined by the

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glucose oxidase method, and one unit of DS activity was defined as the activity of the enzyme hydrolyzing 1 μmol substrate in a minute, and the enzyme activity per g of protein was expressed as the specific activity. The amount of protein was measured by the Lowry method (20) using BSA (bovine serum albumin, Fraction V, Nakarai Chemicals Ltd., Kyoto, Japan) as the standard. Thus the daily pattern of DS activity was examined and the mean 24-h DS activity was also estimated.

2. Daily profile of food ingestion in diabetic and control rats. Four rats in each group were isolated in individual cages, and they were kept for 3 days of acclimatization. Then, the mean amount of food consumed every 4 h starting from time 0:00 h was considered to be the ingested amount of food at times 04:00, 08:00, 12:00, 16:00, 20:00, 24:00 h. The daily pattern of food ingestion expressed per gram of body weight per hour was compared in both groups.

3. Daily profile of plasma glucose level in diabetic and control rats. Blood of rats in each group was collected every 4 h, at the same time when the intestinal mucosa was collected, from the vena cava inferior vein using a heparinized syringe. Plasma glucose was determined by the glucose oxidase-mutarotase method (GLU Neo-Shino Test, Shino Test Co., Tokyo, Japan), and glycated hemoglobin (HbA1) by the affinity column method (21, 22) (Glyc-Affin·GHB, Seikagaku Kogyo, Tokyo, Japan).

4. Effect of glucose or insulin on DS activity of the intestinal mucosal cells from control rats. The mucous membrane of the upper 1/3 of the small intestine of 6 control rats was obtained by the method described above. It was immediately minced in chilled Krebs-Henseleit bicarbonate buffer, pH 7.4 (KHBB) containing 3.3 mM glucose.

The minced tissue was treated in 10 ml of KHBB containing 0.5% BSA, 3.3 mM glucose and 1 mg/ml collagenase (Boehringer Manheim, GmbH, Germany) for 15 min at 37°C. After centrifugation, the precipitate was washed 4 times with KHBB containing 0.5% BSA and 3.3 mM glucose. An aliquot of this cell suspension was pre-incubated in KHBB containing 0.5% BSA and 3.3 mM glucose for 20 min, and its was then incubated at 37°C for 3 h under the gas phase of 95% O2 and 5% CO2 in 0.5% BSA·KHBB containing various concentrations of glucose (3.3, 8.3, 16.7 and 33.3 mM). The cell suspension was also incubated with various concentrations of insulin (Actrapid Human Insulin, Novo Nordisk A/S, Bagsvaerd, Denmark; 0, 10, 100, and 1,000 μU/ml) in the same manner as above, after pre-incubation in KHBB containing 0.5% BSA and 16.7 mM glucose for 20 min. After incubation, DS activity was measured by the method described above. These incubations were carried out between 17:00 h and 20:00 h, when the diurnal variation of DS activity was found to be relatively small.

The cell viability of the small intestinal mucosal cells throughout the incubation periods was examined using dye-exclusion test by trypan blue.

5. Statistical analysis. The Student’s t-test was used to compare mean values. The daily profile of values was compared by analysis of variance. All calculated p values are two-tailed. All p values less than 0.05 were considered to
indicate statistical significance. All group data are reported as mean±SE.

RESULTS

In the diabetic group, the body-weight decreased from 208.8±1.0 g to 170.9±3.4 g 4 weeks after treatment. On the other hand, in the control group, body-weight increased from 210.8±1.6 g to 294.1±3.2 g in the same period. Final body-weight in the diabetic group was significantly (p<0.01) lower than the control group. HbA1 was 12.5±0.4% and 4.4±0.0% in diabetic and control group, respectively (p<0.001) (Table 1).

1. Disaccharidase activity and its daily profile in diabetic and control rats

Maltase activity in both diabetic and control groups showed diurnal variation (p<0.001). In both groups the maximum maltase activity was observed at 4:00 h (485.8±32.4 U/g protein in the diabetic group and 562.2±24.2 U/g protein in the control group) and the minimum activity was noted at 08:00 h (273.4±22.0 U/g protein in the diabetic group and 201.1±16.8 U/g protein in the control group) (Fig. 1).

Lactase activity in both diabetic and control groups also showed diurnal variation (p<0.01 and p<0.001, respectively). In both groups the maximum lactase activity was observed at 04:00 h (34.1±3.5 U/g protein in the diabetic group, and 23.5±1.0 U/g protein in the control group). The minimum activity was found at 12:00 h (23.9±1.5 U/g protein in the diabetic group and 13.0±0.8 U/g protein in the control group) (Fig. 1).

Similarly, a diurnal variation of sucrase activity in both the diabetic and control groups was also observed (p<0.001 in both groups). The maximum sucrase activity was at 16:00 h in the diabetic group (127.3±11.1 U/g protein) and at 20:00 h in the control group (101.2±7.7 U/g protein). The minimum sucrase activity was observed at 08:00 h in both groups (92.9±5.3 U/g protein in the diabetic group and 52.4±3.2 U/g protein in the control group) (Fig. 1).

In the diabetic group, maltase activity was significantly higher during from 08:00 to 16:00 h (p<0.05 at 08:00 h and 16:00 h, p<0.01 at 12:00 h), and a

| Group       | HbA1 (%) (Age: 12 w) | Body weight (g) |      |          |
|-------------|----------------------|-----------------|------|----------|
|             |                      | Initial         | Terminal    |          |
|             |                      | body-weight    | body-weight |          |
| Control (48) | 4.4±0.0              | 210.8±1.6      | 294.1±3.2   |          |
| Diabetic (42) | 12.5±0.4            | 208.8±1.0      | 170.9±3.4   |          |
| Significance of difference | p<0.001          | n.s.           | p<0.01      |          |

Results are expressed as mean±S.E. Numbers of animals are given in parentheses. p values are statistical differences from control rats.

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Fig. 1. Daily profile of disaccharidase activity of the small intestine, food intake and plasma glucose values in diabetic and control rats fed ad libitum. Each points represents M±SE. Enzyme activity is expressed as units per gram protein (units of activity = μmol disaccharide hydrolyzed/min). Numbers of animals are given in parentheses. * (p < 0.05), ** (p < 0.02), *** (p < 0.01), ++ (p < 0.001): significantly different from control rats. ●, diabetic rats; ○, control rats. p values indicate the results of analysis of variance in each daily profile of values. Vertical bars indicate standard errors.
significant increase of lactase activity was observed during from 00:00 h to 20:00 h (p<0.05 at 00:00 h, 04:00 h and 20:00 h, p<0.001 at 08:00 h, 12:00 h and 16:00 h). Similarly, significantly higher sucrase activity was found at from 00:00 h to 16:00 h in the diabetic group (p<0.02 at 04:00 h, and p<0.01 at 16:00 h, p<0.001 at 00:00 h, 08:00 h and 12:00 h) (Fig. 1).

Mean 24-h maltase activity in both groups was not statistically significant (400.9±31.8 U/g protein, 358.7±58.1 U/g protein in the diabetic and the control group, respectively). On the other hand, the mean 24-h lactase activity was significantly higher in the diabetic group (28.1±1.8 U/g protein in the diabetic group, 18.6±1.5 U/g protein in the control group) (p<0.003). The mean 24-h sucrase activity was 112.4±5.8 U/g protein in the diabetic group and 79.0±7.7 U/g protein in the control group, which was statistically significant (p<0.01) (Fig. 2).

2. **Daily profile of food ingestion in diabetic and control rats**

Both the diabetic and control groups showed diurnal variation of food consumption (p<0.01 and p<0.001, respectively). In the 2 groups the maximum food ingestion was noted at 00:00 h (8.3±1.1 mg/g body weight/hour in the diabetic group and 2.9±0.2 mg/g body weight/h in the control group). On the other hand, minimum food consumption was observed at 08:00 h (2.3±1.1 mg/g body weight/h) in the diabetic group and at 16:00 h (0.7±0.3 mg/g body weight/h) in the control group (Fig. 1).

The amount of food consumption was significantly more in the diabetic group than in the control group at each time measured except 08:00 h (p<0.02 at 04:00 h, p<0.01 at 00:00 h, p<0.001 at 12:00 h, 16:00 h and 20:00 h, respectively) (Fig. 1).

3. **Daily profile of plasma glucose level in diabetic and control rats**

The plasma glucose level was significantly higher in the diabetic group than in the control group throughout 24 h (p<0.01 at 20:00 h and p<0.001 at from 00:00
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h to 16:00h) (Fig. 1).

The daily profile of plasma glucose level showed no significant statistical variation in either the diabetic or control groups, while the differences between the peak and the trough values in both groups were significant ($p<0.05$ and $p<0.02$, respectively). The maximum plasma glucose level was noted at 12:00h in the diabetic group (549.0±15.2 mg/dl) and at 04:00h in the control group (221.0±23.5 mg/dl). The minimum level was observed at 20:00h in the diabetic group (435.0±47.0 mg/dl) and at 12:00h in the control group (135.0±7.2 mg/dl) (Fig. 1).

4. Effect of glucose or insulin on DS activity of the intestinal mucosal cells from normal rats

The cell viability of the small intestinal cells throughout the incubation periods was confirmed using dye-exclusion test by trypan blue.

Although maltase activity was not altered, a significant correlation between glucose concentration and lactase or sucrase activity was observed. By simple regression analysis, the correlation coefficients between glucose concentrations and DS activity were determined, i.e. in lactase, at 1 h's incubation $r=0.61$ ($p<0.003$), at 3 h of incubation $r=0.46$ ($p<0.03$); in sucrase, at 1 h's incubation $r=0.52$ ($p<0.03$), at 3 h of incubation $r=0.44$ ($p<0.05$). Furthermore, sucrase activity was enhanced after 3 h of incubation, from 34.3±6.6 to 76.2±13.0 U/g protein ($p<0.02$) by increasing the glucose concentration from 3.3 mM to 33.3 mM (Fig. 3).

On the other hand, maltase, lactase and sucrase activities were not affected by incubation with insulin (Fig. 3).

DISCUSSION

In diabetic rats there have been no consistent results concerning DS activities in the small intestine. Some authors have reported increased activities (3, 4, 8), and others have observed no alteration of the activities in terms of the sites of sampling or duration of diabetes (12, 13). This may be due to differences of times of sampling, which would affect DS activity due to the presence of diurnal variation in normal rats (1, 2). Furthermore, DS activity itself (2, 5) or the amplitude of diurnal variation (2) are known to be different depending on the sites of the small intestine. Therefore, in this study, we measured the enzyme activity at a consistent site.

In the present study, we were able to prove the presence of diurnal variations of maltase, lactase and sucrase activities in diabetic rats. These observations have not been previously reported.

Concerning the relation between DS activity and time of food ingestion in normal rats, increasing tendency of DS activity before food ingestion (1, 2, 23), and the maximum activities related to the time of food ingestion (24, 25) have been reported. However, these previous observations were made under unphysiological
Fig. 3. Effects of glucose and/or insulin on disaccharidase activity of the small intestine. The direct influence of glucose and/or insulin on disaccharidase activities were studied using the small intestinal mucosal cells of normal rats incubated with various concentrations of glucose (left panels) or insulin (right panels). The specific activity was expressed in units per gram protein (units of activity = $\mu$mol disaccharide hydrolyzed/min). The results are given as $M \pm SE$. Numbers of animals are given in parentheses. Vertical bars indicate standard errors.

conditions such as constant light or dark conditions, or modified feeding schedules.

Therefore, we studied the enzyme activity in relation to food consumption in both diabetic and control rats under physiological conditions.

In control rats, the amount of food ingestion decreased in the morning.

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together with a decrease in DS activity. Then, preceding an increase of food consumption, an increase in DS activity was observed.

On the other hand, in diabetic rats, the diurnal variation of maltase or sucrase activity and food consumption suggested a relationship between them. Lactase activity showed similar changes to the daily profile of food consumption, with a lag phase of 4 h.

Thus, some correlation between the amount of food ingestion and DS activity in terms of diurnal variation is present in both groups, although the mechanisms might be different between diabetic and control groups. Furthermore, it is suggested that the mechanism of daily change of lactase activity may be different from that of maltase or sucrase activities in the diabetic rat.

In both groups, daily profiles of plasma glucose levels showed no significant daily variation, suggesting no direct effect on the formation of diurnal variation of DS activity.

In our study, synchronized daily changes of DS activity between diabetic and control groups was observed. This may suggest the presence of a common mechanism for the induction of daily variations in both groups, but the precise mechanism was not demonstrated in the present study.

Saito et al. reported the continuation of daily changes of DS activity after at least 2 days of cessation of food ingestion, after which time the diurnal variation became gradually more obscure and was finally lost after 5 days of fasting in normal rats (26, 27). This data suggests the role of some intrinsic factor for the induction of daily change. In their reports they also observed no recovery of diurnal variation until after more than 8 to 9 days of refeeding (26).

Concerning the mechanism of higher DS activity in diabetic rats, the role of hyperglycemia as a casual agent has been postulated (11, 28), while that of polyphagia has been thought to be less important (3, 4, 8, 29). In diabetic rats Olsen, W. A. and Korsmo, H. reported increased DS activity in Thiry-Vella fistula whose each end of a jejunal segment is open to the abdominal wall with intact nerve and blood supply (6). Furthermore, DS activity in the small intestine of diabetic rats has been found not to be different under conditions during which nutrients containing glucose and amino acids were administered intravenously or conditions during which they were infused directly to the stomach for 4 days (30). These findings suggest that DS activity in diabetic rats was not directly influenced by the contact of food or digestive fluids with the small intestinal mucous membrane. Takeguchi et al. observed increased DS activity under acute hyperglycemia induced by i.v. administration of glucose in normal rats (11, 28), and which was normalized by the correction of hyperglycemia with insulin administration (28).

On the other hand, the direct influence of glucose and insulin on DS activity had not been previously examined. In the present study, it was documented that lactase and sucrase activities were increased in vitro in the presence of high glucose. Meanwhile, any concentration of insulin did not alter DS activity in vitro. These results suggest the role of glucose in the regulation of both lactase and sucrase
activities. Furthermore, it may explain the background of high lactase and sucrase activities in diabetic rats.

Lower DS activity in malnourished rats was reported by Firmansyah et al. (31). Although similar metabolic conditions like malnutrition may be present at the cellular level in diabetes, increased lactase and sucrase activities were detected. As a consequence, these changes of DS activity were interpreted to be characteristic of the diabetic condition.

These observations in diabetic rats might suggest not only the presence of diurnal variation of DS activity but also an increase of enzyme activity in human diabetics.

Concerning small intestinal DS activity in human diabetic patients, this is still controversial (14–17). The reason might be due to the difference of sampling time in which daily changes of DS activity were not taken into consideration.

A positive correlation between the DS activity and the digestion and absorption of disaccharide is known (10). Therefore, increased DS activity in the diabetic state might influence the metabolic states through the increase of digestion and absorption of disaccharide.

In conclusion, in the diabetic rat, a diurnal variation of maltase, lactase and sucrase activities was documented and a pattern of food consumption was shown to correlate with these changes. Increased lactase and sucrase activities were also demonstrated in the diabetic rat; furthermore, the role of hyperglycemia on the enhancement of these enzyme activities was suggested in part to be important. More precise studies are necessary to elucidate the DS activity itself, its diurnal variation and the regulatory mechanisms of them in the diabetic state.

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