Serum Glucose and Insulin Response in Rats Administered with Sucrose or Starch Containing Adenosine, Inosine or Cytosine

Yasunori Fukumori,† Norio Maeda, Hiroyuki Takeda, Shuichi Onodera,* and Norio Shiomi*

Department of General Foods, The Hokuren Federation of Agriculture Corporation, Sapporo 060-8651, Japan
*Department of Food Science, Faculty of Dairy Science, Rakuno Gakuen University, Ebetsu 069-8501, Japan

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Blood glucose and insulin responses and gastric emptying were examined in rats intubated with sucrose or soluble starch that contained adenosine, inosine and cytosine.

The increase in serum glucose and insulin levels in the rats following loading with sucrose (2.5 g/kg of body weight) or soluble starch (1.875 g/kg of body weight) was significantly reduced by the administration of adenosine, inosine and cytosine (0.0625–0.125 g/kg of body weight).

The gastric emptying rates were only marginally affected by the nucleoside administration. The activities of sucrase, maltase, isomaltase and glucoamylase in a crude preparation from the small intestinal mucosa of rats were mildly inhibited by the nucleosides.

The decrease in blood glucose and insulin levels may have been in response to a decrease in glucose absorption caused by the inhibiting effect of the nucleosides on the mucosal enzymes that digest sucrose, maltose, and malto- and isomalto-oligosaccharides.

Key words: rats; nucleosides; blood glucose and insulin; α-glucosidase inhibition

Water-soluble polysaccharide such as guar gum,† pectin† and konjac mannan‡ administered with glucose are known to decrease the postprandial blood glucose and insulin concentrations. The guar gum-induced lowering of blood glucose is known to be related to the viscosity of the polysaccharide.† Isomaltotriose,¶ pullulan,* and indigestible dextrin,¶ which are low-viscosity saccharides, have also been shown to lower the blood glucose and insulin levels in rats administered with sucrose and starch in tolerance tests. In addition, peptidoglycans from fruiting bodies of Ganoderma lucidum Karsten possess hypoglycemic activity.¶ The mechanism by which these saccharides reduce blood glucose and insulin remains to be clarified in detail.

On the other hand, α-glucosidase inhibitors that reduce postprandial hyperglycemia such as Acarbose,† Voglistat§ and Miglitol¶ have been investigated in clinical trials as effective methods for the glycemic control of diabetes. However, these substances are essentially xenobiotic and thus have associated safety concerns; for this reason, the administration protocol has been strictly regulated.

We have been investigating several kinds of α-glucosidase inhibitor having the action of gradually inhibiting α-glucosidase from the rat small intestine, thereby delaying the digestion of sucrose or starch, so that the inhibitors suppressed a rapid increase in blood glucose level and also suppressed insulin secretion at a low level. We have recently reported fructosylxyloside,† xylitol,¶ erythritol¶ and various nucleotides¶ that mildly inhibited α-glucosidase activity in the microvilli of the rat small intestine leading to a repression of the blood glucose and insulin levels. In these studies, we found that several nucleosides and their base such as adenosine, inosine and cytosine inhibited α-glucosidase from the rat small intestine more strongly than the nucleotides, adenosine 5'-monophosphate, inosine 5'-monophosphate and guanosine 5'-monophosphate.

The effect of adenosine, inosine and cytosine administered with sucrose or soluble starch on blood glucose and insulin responses and gastric emptying in rats is reported here.

Materials and Methods

Chemicals. Adenosine (98%), inosine (99%), and cytosine (97%) were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Animal care. The study was approved by the Animal Use Committee of Rakuno Gakuen University, and the animals were maintained in accordance with the guidelines for the care and use of laboratory animals that have been established by Rakuno Gakuen University.

† To whom correspondence should be addressed. Tel: +81-11-232-6145; Fax: +81-11-232-0068; E-mail: fukumori@rakuno.ac.jp
Animals. Six-week-old male Sprague-Dawley rats (Japan SLC, Hamamatsu, Japan) were housed in a room in individual cages with stainless steel wire screen bottoms. The room was maintained at 23 ± 1°C and illuminated from 0700 to 1900. The rats were standardized by feeding with a purified casein-sucrose diet containing 250 g of casein, 652.3 g of sucrose, 50 g of corn oil, 35 g of a mineral mixture (AIN 1977), 10 g of a vitamin mixture (AIN 1977, 1980), and 2.7 g of choline bitartrate for 7 days.

Sugar tolerance tests. Three hundred and forty two rats were separated into fifty seven experimental groups of six rats each on the basis of body weight and were starved for 18 h before being administered with a 20% sucrose solution (200 g/liter of water, 2.5 g/kg of body weight) or 15% soluble starch solution (150 g/liter of water, 1.875 g/kg of body weight) containing 0.5-1% (5-10 g/liter of water, 0.0625-0.125 g/kg of body weight) of each nucleoside (12.5 ml per kg of body weight) or with no nucleoside by gastric intubation. After the sucrose, starch and nucleoside administration, the rats were decapitated at 0, 15, 30, 60, 120 and 180 min and their blood samples and stomachs were obtained. Serum was obtained, and an aliquot (20 μl) of each serum sample was analyzed for glucose concentration with the glucoseoxidase-peroxidase method, and a GL-5 kit (Kainos Laboratories, Tokyo, Japan). The remaining serum (100 μl) was used for an insulin assay with the EIA kit ("Mitsui" II, Mitsui Seiyaku, Tokyo, Japan).

Determination of gastric emptying. The amount of sucrose remaining in the stomach was determined by HPLC (Bio LC-300, DIONEX) with a carbohydrate column (PA1, CarboPack, Sunnyvale, CA, U.S.A.).

Preparation of the carbohydrate-digesting enzyme from rat intestine. To prepare the enzyme, 44 Sprague-Dawley rats fed on rat-feed (Nihon Nosankogyo K. K., Yokohama, Japan) were used. The rats were killed by decapitation, and the jejunum was excised. Unless otherwise stated, all subsequent steps were carried out at 4°C. The excised jejunum was washed with phosphate-buffered saline (pH 7.0). A mucosal fraction containing the brush border membrane was scraped with a glass slide from the everted jejunum. The mucosal fraction (44 ml) was homogenized with a Polytron mixer (Kinematica AG, Littau, Switzerland) in 200 ml of a potassium phosphate buffer (0.1 M, pH 7.0) containing 5 mM ethylenediaminetetraacetic acid (buffer A). The homogenate was centrifuged (21,000 × g, 60 min), and the precipitate obtained was suspended in 10 ml of buffer A. After adding of 50 ml of buffer A containing Triton X-100 (1 g/liter) to the suspension, the enzymes bound to the brush border membrane were rendered soluble at 0°C for 12 h. A crude disaccharidase solution was obtained by ultracentrifugation (110,000 × g, 90 min), and dialyzed against a potassium phosphate buffer (10 mM, pH 7.0).

The resulting dialyze was used for measuring the sucrase, isomaltase, maltase and glucoamylase activities.

Determination of the enzyme activities of sucrase, isomaltase, maltase and glucoamylase. A reaction mixture containing 0.1 ml of the dialyze and sucrose (20 mm, 0.5 ml), isomaltase (20 mm, 0.5 ml), maltose (20 mm, 0.5 ml) or soluble starch (2 g/100 ml, 0.5 ml) was incubated at 37°C for 30 min in the presence of 0, 1, 2, 3, 4, 5, 6, 7 or 8 mM adenosine, inosine or cytosine dissolved in a potassium phosphate buffer (0.1 M, pH 6.1, 0.4 ml). Subsequently, a Tris-HCl buffer (2 ml, pH 7.0, 2 ml) was added to terminate the reaction. The liberated glucose was measured by the glucoseoxidase-peroxidase method with a Glucose AR-II kit (Wako Pure Chemical Industries, Osaka, Japan).

Statistical analysis. Each graphical data point is presented as the mean ± SEM (n = 6). The data were analyzed by Stat View 5 software for the Macintosh (SAS Inc., Cary, NC, U.S.A.). Two-way ANOVA was employed with the concentrations of serum glucose or insulin and the administered nucleoside as the main effects. This analysis was followed by Fisher’s least significant difference test to compare treatment means. Differences are considered to be statistically significant if the associated P value is <0.05.

Results

Reduction in the serum glucose and insulin levels by adenosine, inosine or cytosine

Figure 1 shows the serum glucose levels in rats administered with sucrose (2.5 g/kg of body weight) or sucrose (2.5 g/kg of body weight) with adenosine (0.0625 and 0.125 g/kg of body weight), inosine (0.0625 and 0.125 g/kg of body weight) or cytosine (0.0625 and 0.125 g/kg of body weight). The increases in glucose level (209.4 ± 3.6, 221.1 ± 6.8 and 219.7 ± 6.1 mg/dl) in the serum of the rats 15 min after sucrose intubation with adenosine, inosine and cytosine (0.0625 g/kg of body weight) were significantly less than that of the control group (245.4 ± 3.1 mg/dl) at 15 min. However, the glucose levels in the rats 30 and 60 min, or 60 and 180 min after sucrose intubation with inosine or cytosine, respectively, tended to be higher than that of the control group. The suppressive effects of adenosine, inosine and cytosine on the serum glucose concent-
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Fig. 1. Changes in the Serum Glucose Level after Administering Sucrose alone or Sucrose with Adenosine, Inosine or Cytosine.
Symbols represent the serum glucose level in rats administered with sucrose alone (●), sucrose with adenosine (●), inosine (■) or cytosine (▲). Sucrose (200 g/l of water) or sucrose (200 g/l of water) with a nucleoside (5 g/l (-----) or 10 g/l (——) of water) was intubated to rats at 12.5 ml per 1 kg of body weight. Each value is the mean ± SEM (n = 6). Values at time points that have different letters are significantly different (p < 0.05).

Fig. 2. Changes in the Serum Insulin Level after Administering Sucrose alone or Sucrose with Adenosine, Inosine or Cytosine.
Symbols represent the serum insulin level in rats administered with sucrose alone (●), sucrose with adenosine (●), inosine (■) or cytosine (▲). Sucrose (200 g/l of water) or sucrose (200 g/l of water) with a nucleoside (5 g/l (-----) or 10 g/l (——) of water) was intubated to rats at 12.5 ml per 1 kg of body weight. Each value is the mean ± SEM (n = 6). Values at time points that have different letters are significantly different (p < 0.05).

tration were stronger at the higher concentration (0.125 g/kg) of adenosine, inosine and cytosine. Similar findings were observed longer after intubation in the rats intubated with sucrose containing cytosine, but not with adenosine or inosine, except for the respective glucose levels at 120 min or 120 and 180 min.

Figure 2 shows the serum insulin response of the rats administered with sucrose alone, or sucrose with adenosine, inosine or cytosine. The respective rise in serum insulin (31.31 ± 4.02, 28.17 ± 1.65 and 33.85 ± 6.55 μIU/ml) 15 min after the administration of sucrose with adenosine, inosine, or cytosine (0.0625 g/kg of body weight) was also significantly less than that of the control group (46.87 ± 4.16 μIU/ml) after 15 min, although the serum insulin levels after 30, 60 and 180 min (adenosine) and after 120 and 180 min (inosine) tended to be a little higher than those of the control group. Adenosine, inosine and cytosine (0.125 g/kg of body weight) respectively reduced the elevation of serum insulin at 15, 30, 120 and 180 min, 15-180 min and 15, 120 and 180 min, respectively when compared with the control group levels.
Figure 3 shows the effect of adenosine, inosine or cytosine on gastric emptying as estimated from the amount of sucrose remaining in the stomach of the rats that had been administered with sucrose alone or with sucrose containing adenosine, inosine or cytosine. Gastric emptying of the rats administered with sucrose plus adenosine, inosine or cytosine (0.0625 and 0.125 g/kg of body weight) did not significantly differ from that of the control group, although it tended to be prolonged 15, 30, 60 and 120 min after loading the sucrose containing nucleosides (10 g/l) when compared with the control group.

Figures 4 and 5 show the serum glucose and insulin responses of rats given starch alone (1.875 g/kg of body weight) and starch (1.875 g/kg of body weight) with adenosine (0.125 g/kg of body weight), inosine (0.0625 g/kg of body weight) or cytosine (0.125 g/kg of body weight). Thirty minutes or 15 and 30 minutes after respectively loading starch containing inosine or cytosine, the serum glucose levels were significantly lower than those of the control group. At 15, 30 and 60 min after loading starch containing...
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Fig. 5. Changes in the Serum Insulin Level after Administering Starch alone or Starch with Adenosine, inosine or Cytosine. Symbols represent the serum insulin level in rats administered with starch alone (O), or starch with adenosine ( ), inosine ( ) or cytosine ( ). Starch (150 g/l of water) or starch (150 g/l of water) with adenosine (10 g/l of water), inosine (5 g/l of water) or cytosine (10 g/l of water) was intubated at 12.5 ml per 1 kg of body weight. Each value is the mean ± SEM (n = 6). Values at time points that have different letters are significantly different (p < 0.05).

Fig. 6. In Vitro Inhibitory Effects of Adenosine, Inosine and Cytosine on the Activities of Sucrase, Isomaltase, Maltase and Glucoamylase in a Crude Preparation from Rat Intestine.

Adenosine, significantly lower levels of serum glucose were observed. After 15 min, the rats administered with starch plus adenosine or cytosine had significantly lower insulin levels than those administered only with starch.

In vitro inhibition of sucrase, isomaltase, maltase and glucoamylase by adenosine, inosine or cytosine

The inhibitory effects of adenosine, inosine and cytosine on the activities of sucrase, isomaltase, maltase and glucoamylase in the crude preparation from rat intestine were investigated in vitro.

As shown in Fig. 6, the sucrase activity was inhibited by 61%, 39% and 41% by inosine (8 mM), adenosine (8 mM) and cytosine (8 mM), respectively. In addition, the maltase activity was inhibited by 18%, 12% and 21% by inosine (8 mM), adenosine (8 mM) and cytosine (8 mM), respectively.

Glucoamylase activity was also inhibited to the same extent as maltase by the nucleosides, while isomaltase activity was not greatly affected.

Discussion

The results of the present study suggest that the administration of adenosine, inosine or cytosine with
sucrose or starch to rats led to a reduction in the normal postprandial rise in the blood-glucose and -insulin levels that were observed with sucrose or starch alone.

In addition, these nucleosides had a small inhibitory effect \textit{in vitro} on the activities of sucrase, isomaltase, maltase and glucoamylase in the crude preparation from rat small intestine. The concentration of each nucleoside required to reduce the blood-glucose and -insulin levels in the rats was of an approximately similar order to that required \textit{in vitro} to inhibit the activities of sucrase, isomaltase, maltase and glucoamylase in the crude preparation from rat small intestine, although the nucleoside concentrations in the former case was higher than that in the latter by 4.7–11 times.

As the gastric emptying of rats given sucrose with a nucleoside did not significantly differ from that of the control group, except at 60 min, lowering the serum glucose and insulin levels in rats by administering a nucleoside and related substance hardly affected the gastric emptying in the rats.

We have previously tested the serum glucose and insulin responses in rats intubated with a 20% glucose solution and 20% glucose solution containing 1% inosine. Inosine was not effective for suppressing the rapid increase in serum glucose and insulin levels.

Thus, the reduction in serum glucose level in the rat may have been in response to glucose malabsorption caused by inhibition of the nucleosides of the mucosal enzymes which digest sucrose and starch, and not caused by any influence of the nucleosides on the glucose transport system in the small intestine.

Phenformin\textsuperscript{4,10} (a biguanide drug) and the sulfonylureas\textsuperscript{4,18} are known to be effective in lowering the blood glucose level when orally administered. Phenformin delays the rate of glucose absorption from the intestine, which inhibits the rise in blood glucose level that follows the administration of sugar with the drug. However, phenformin occasionally may be the cause of lactic acidosis\textsuperscript{18-20} accompanying the increases in lactic acid, alanine and pyruvic acid in the blood.

In a previous study,\textsuperscript{21} the level of lactic acid in the blood of rats intubated with sucrose containing adenosine, inosine or cytosine was not significantly different from that of the control rats, although the blood-alanine level in these rats tended to be less than that of the control rats. Sulfonylureas are known to stimulate the release of insulin from pancreatic cells. One of the sulfonylureas, chlorpropamide, is likely not to be excreted from the body of an elderly person with a poor diet or who has a low blood sugar level resulting from a kidney disorder.\textsuperscript{18} In the previous study,\textsuperscript{21} when rats were allowed free access to a diet containing inosine or adenosine (10 g, 25 g or 50 g/kg of diet) for 7 days, no difference in the blood-glucose or -insulin levels after starvation for 3 hours was apparent between the control group and the nucleoside diet groups. Thus, these nucleosides and a related base can be expected to be safe and healthy as artificial sweeteners or as food additives given to suppress the increase in blood-glucose and -insulin levels.

When used in combination with one, two or more digestible sugars selected from sucrose, starch and starch-derived oligosaccharides, nucleosides mildly suppress the action of digestive enzymes in the small intestine, which in turn suppresses the rapid increase in blood glucose level and then reduces insulin secretion to a low level. Sweeteners and food that contain these nucleosides may be useful for the prophylaxis of such adult diseases as obesity and diabetes mellitus in otherwise healthy people.

Overweight people and patients with diabetes mellitus can be provided with a wide range of sweeteners and food suitable for dietary treatment by using these nucleosides which have been shown to relieve regulating the intake of such sugars as sucrose, starch and starch-derived oligosaccharides.

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