Glucose Suppresses the Activity of Rat Oxyntic Histidine Decarboxylase Without Affecting Gastrin Levels

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ABSTRACT Glucose suppressed the activity of oxyntic mucosal histidine decarboxylase within 2 h when given either intragastrically or intraperitoneally to rats fasted for 24 h. Serum levels of gastrin, secretin, glucagon, and somatostatin and oxyntic mucosal levels of gastrin, histamine, and somatostatin showed no significant changes after glucose. Glucose suppressed the aspirin-induced histidine decarboxylase activity without changing serum gastrin. It also suppressed the pentagastrin-induced histidine decarboxylase activity. Neither fructose nor mannitol had such an effect. These results suggest that glucose acts directly on the enterochromaffin-like cells in rat oxyntic mucosa to suppress histidine decarboxylase activation.

KEYWORDS enterochromaffin-like cell, gastrointestinal hormones, glucose, histidine decarboxylase

Glucose is known to affect various functions of the gastrointestinal tract. For example, glucose is reported to modify gastric acid secretion, gastric motility and emptying, and gastrin release [1–5]. It has not yet been clarified whether glucose acts directly on the digestive tract to influence its activity or works via other factors including gastrointestinal hormones. The increase in plasma glucose is normally followed by augmented insulin secretion, which in turn may increase gastric histidine decarboxylase (HDC) activity [6,7]. Fasting is known to increase the susceptibility to experimentally induced gastric erosions and ulcers [8], whereas hyperglycemia protects animals from stress erosions [9]. Furthermore, glucose is known to attenuate gastric lesions induced by aspirin [10] and phenylbutazone [11]. Glucose is thought to reduce gastric ulceration and hemorrhage by inhibiting abnormal gastric contractile activity [12]. In contrast, insulin [13] and 2-deoxy-D-glucose (2-DG) [14] have been shown to induce ulcers in rats. Both insulin and 2-DG are known to increase gastric HDC activity [6,7,13]. These data seem to suggest that glucose has a cytoprotective

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effect on gastric mucosa [15,16] and plays an important role in ulcer formation, but little is known about the mechanism by which glucose protects gastric lesions.

Because ulcer formation seems to be closely associated with gastric histamine levels [17], we thought it of interest to investigate the effect of glucose on gastric histamine metabolism. The present study deals with the suppressive effect of glucose on histidine decarboxylase activity.

MATERIALS AND METHODS

Animals

Male rats of Wistar strain weighing around 150 g were housed in a light-controlled room (light on 7 a.m.–7 p.m.) at 25 ± 1°C. They were fasted for 24 h prior to the experiments, and water was withdrawn 8 h before sacrifice. The rats were sacrificed by decapitation between 10 a.m. and 1 p.m. The stomach was opened along the greater curvature, and mucosal samples of the oxyntic gland region were carefully excised with scissors. Tissues were homogenized in 4 volumes of 50 m*M* sodium phosphate buffer (pH 7.2) containing 5 m*M* dithiothreitol. The whole homogenates were centrifuged at 12,000 × g for 20 min in a Kubota 6800 centrifuge, and the supernatants were used for enzyme assay.

In order to extract gastrin and somatostatin, gastric tissues were immediately homogenized in 10 volumes of 0.01 *M* phosphate-buffered saline (PBS) solution in a Teflon homogenizer. The whole homogenates were centrifuged at 1,000 × g for 20 min, and the supernatants were analyzed for their contents by radioimmunoassay.

Drugs

Aspirin was suspended in 0.5% methylcellulose and administrated intragastrically at a dose of 200 mg/kg at 4 h before the experiments, unless otherwise noted.

Pentagastrin was first dissolved in a minimum volume of 10% ammonium hydroxide and then diluted with saline solution. This hormone was usually administered intraperitoneally at a dose of 500 μg/kg 30 min before the experiments. Our preliminary experiment has revealed that pentagastrin stimulates gastric HDC activity maximally at between 30 and 60 min.

Determination of Gastric Histamine and HDC Activity

The HDC activity was measured as reported previously [18]. Briefly, the reaction mixture in a total volume of 200 μl contained 50 m*M* sodium phosphate buffer (pH 7.2), 1 m*M* aminoguanidine, 1 m*M* histidine, 0.05 m*M* pyridoxal 5-phosphate, 0.1 m*M* ethylenediaminetetraacetic acid, and 100 μl of the oxyntic mucosal supernatant. The incubation was carried out at 37°C for 3 h. The reaction was linear for at least 3 h. The reaction was stopped by adding 10 μl of 6 *N* perchloric acid. To this mixture 590 μl of 0.34 *N* potassium citrate buffer (pH 5.49) was added. After centrifugation, the supernatants were passed through microfilters with 0.45-μm pores. In order to test the increase in histamine during incubation, histamine was measured before and after the incubation. Histamine was determined by high-performance liquid chromatography as described previously [19]. The enzyme activity was expressed as nanomoles of histamine produced per gram wet tissue weight per hour.

Glucose Assay

Glucose levels of the rat sera were determined by the glucose oxidase method with a glucose assay kit (Wako, Saitama, Japan). Glucose levels were expressed as milligrams per 100 ml of serum.

Serum Gastrin, Secretin, Glucagon, and Somatostatin Assay

Serum levels of gastrin were determined by radioimmunoassay with a Gastrin RIAKIT II (Dainabot, Tokyo, Japan). Gastrin levels were expressed as picogram equivalents of human gastrin 17 per milliliter of serum. Secretin, glucagon, and somatostatin were determined by radioimmunoassay with a Daiichi secretin kit, Daiichi glucagon kit (Daiichi, Tokyo,

Japan), and somatostatin ^{125}I radioimmunoassay (RIA) kit (INCSTAR, MN), respectively. The hormone levels were expressed as picogram equivalents of human secretin, glucagon, and somatostatin per milliliter of serum.

Mucosal Gastrin and Somatostatin Assay

Gastrin and somatostatin levels in extracts of oxyntic mucosa were determined by RIA with respective assay kits (Daiichi, Tokyo, Japan, and INCSTAR, MN). The hormone levels were expressed as picograms per milligram tissue protein content.

Effect of Glucose on Serum Glucose, Serum Hormones, Mucosal Hormones, and Oxyntic HDC Activity

Fifty percent glucose was given intragastrically through a gastric tube at a dose of 1.5 g/kg body weight. Serum levels of glucose and oxyntic HDC activity were measured at 0, 2, 4, 8, and 16 h after glucose ingestion treatment. At the same time, serum levels of gastrin, secretin, glucagon, and somatostatin and oxyntic levels of gastrin, histamine, and somatostatin were measured. The number of determinations for each point was six. One rat was used for each determination.

Effects of Various Doses of Glucose

Various doses of glucose (0, 0.15, 0.45, and 1.5 g/kg body weight) were given intragastrically at 4 h before experiment. Levels of serum glucose and gastrin, oxyntic HDC activity, and histamine levels were measured. The number of determinations for each point was seven.

Effects of Monosaccharides on HDC Activity

Fifty percent glucose, 50% fructose, and 20% D-mannitol were given intragastrically at a dose of 1.5 g/kg body weight through a gastric tube 4 h before experiment. Levels of oxyntic HDC activity were measured. The number of determinations for each point was seven.

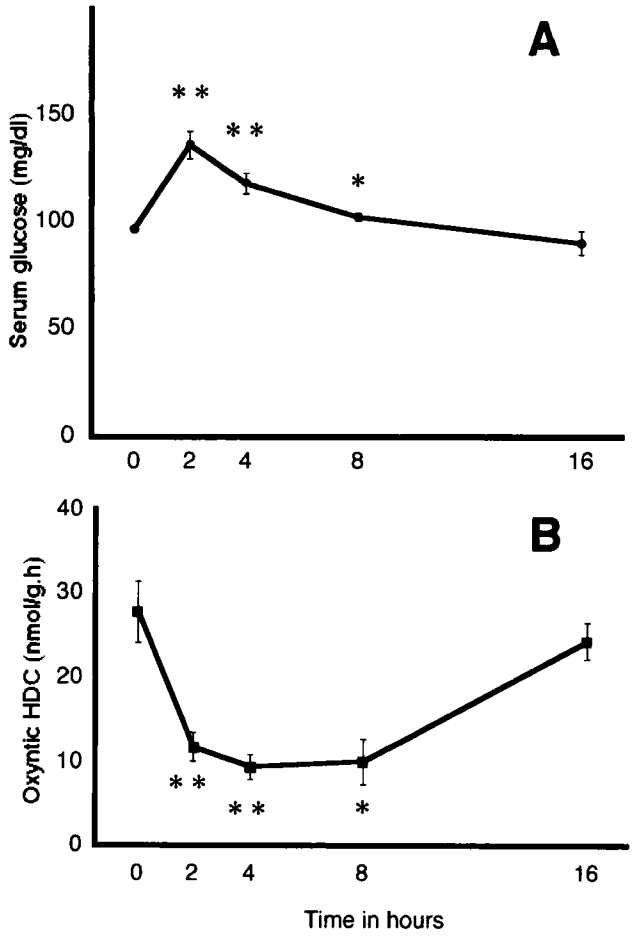

FIGURE 1 Changes in (A) serum glucose levels and (B) oxyntic histidine decarboxylase activity after glucose ingestion treatment. Glucose was given intragastrically at a dose of 1.5 g/kg body weight. The number of determinations for each point was six. All data are shown as the mean ± SEM. Statistical significance: $*p < .05$, $**p < .01$ compared with control.

Effect of Glucose Pretreatment on Pentagastrin-Induced HDC Activity

Pentagastrin was administered intraperitoneally at a dose of 500 µg/kg 30 min before experiments. Fifty percent glucose was given intragastrically at a dose of 1.5 g/kg body weight 3.5 h before pentagastrin. Levels of oxyntic HDC activity were measured. The number of determinations for each point was seven.

Effect of Glucose Pretreatment on Aspirin-Induced HDC Activity

Aspirin was administrated intragastrically at a dose of 200 mg/kg 4 h before experiments. Fifty percent glucose was given intragastrically at a dose of 1.5 g/kg body weight 2 h before aspirin. Levels of serum gastrin, oxyntic HDC activity, and histamine were measured. The number of determinations for each point was seven.

Statistical Analysis

All data were expressed as the mean ± SEM. The data sets from the experimental groups were compared using analysis of variance (ANOVA) with a multiple comparison test. In all cases a probability of error of less than .05 was selected as the criterion for statistical significance.

RESULTS

When a 50% glucose solution was given intragastrically to fasted rats at a dose of 1.5 g/kg body weight, blood glucose levels were significantly higher for 8 h than in the initial control (Figure 1A). The activity of oxyntic HDC was significantly decreased at 2 h, and remained suppressed for 8 h after glucose (Figure 1B). Serum levels of gastrin, secretin, glucagon, and somatostatin showed no significant change throughout 16 h after glucose ingestion (Table 1). Mucosal levels of gastrin, histamine, and somatostatin showed no significant change after glucose ingestion (Table 2).

When various doses of glucose were given intragastrically, the activity of oxyntic HDC was decreased in a dose-related manner (Figure 2). Essentially the same changes were observed when glucose (1.5 g/kg) was administered intraperitoneally (data not shown). Neither fructose nor mannitol was effective in suppressing the HDC activity at the dose of 1.5 g/kg body weight (Figure 3).

Aspirin increased the serum gastrin levels, oxyntic HDC activity, and histamine concentrations at a dose of 200 mg/kg body weight 4 h after oral administration. Glucose (1.5 g/kg) reduced the aspirin-induced HDC activity without a significant change in gastrin or histamine concentrations (Table 3). It also significantly reduced the pentagastrin-induced HDC activity at the same dose (Figure 4).

TABLE 2 Changes in mucosal hormones after glucose ingestion

Time after glucose (h)	n	Gastrin (pg/mg protein)	Histamine (nmol/g)	Somatostatin (pg/mg protein)
Control 0	6	50.4 ± 4.5	296.4 ± 24.9	19.7 ± 3.0
Glucose 2	6	60.0 ± 8.9	290.7 ± 22.0	22.5 ± 3.2
Glucose 4	6	43.6 ± 4.0	304.8 ± 42.9	23.0 ± 4.0
Glucose 8	6	44.3 ± 2.4	297.5 ± 18.0	15.3 ± 2.4
Glucose 16	6	51.5 ± 9.4	251.6 ± 28.6	26.6 ± 9.4

Note. Glucose was given intragastrically at a dose of 1.5 g/kg body weight. All data are shown as the mean ± SEM; *n*, number of determinations. There is no significant difference between any groups.

DISCUSSION

The present study has shown that glucose specifically suppresses the activity of oxyntic HDC

TABLE 1 Changes in serum hormoes after glucose ingestion

Time after glucose (h)	n	Gastrin (pg/ml)	Secretin (pg/ml)	Glucagon (pg/ml)	Somatostatin (pg/ml)
Control 0	6	102.7 ± 11.2	63.4 ± 5.0	92.5 ± 6.2	110.6 ± 15.1
Glucose 2	6	112.7 ± 11.5	74.3 ± 13.3	86.8 ± 11.0	105.6 ± 12.2
Glucose 4	6	107.0 ± 9.0	47.8 ± 12.7	85.8 ± 14.5	111.6 ± 9.6
Glucose 8	6	90.5 ± 13.1	66.5 ± 5.3	86.3 ± 8.0	108.0 ± 14.3
Glucose 16	6	92.1 ± 5.9	51.4 ± 5.1	86.8 ± 14.5	102.6 ± 9.5

Note. Glucose was given intragastrically at a dose of 1.5 g/kg body weight. All data are shown as the mean ± SEM; *n*, number of determinations. There is no significant difference between any groups.

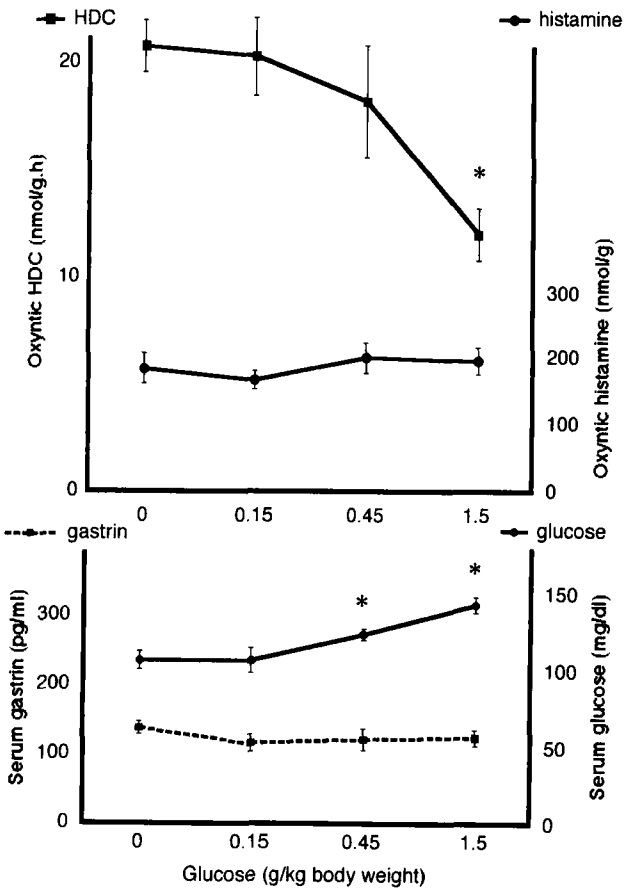

FIGURE 2 Effect of various doses of glucose on serum glucose and gastrin, oxyntic histidine decarboxylase activity, and histamine levels after glucose. Glucose was given intragastrically 4 h before the experiment. The number of determinations for each point was seven. All data are shown as the mean ± SEM. Statistical significance: $*p < .05$ compared with control.

without affecting serum or mucosal gastrin levels. Oxyntic HDC is known to be localized in enterochromaffin-like (ECL) cells in the rat [20]. ECL cells respond sensitively to gastrin to increase the oxyntic HDC activity [21]. The gastric HDC activity is mainly regulated by circulating gastrin [21,22]. Recently we have observed that aspirin induces not only gastric lesions in the rat but also oxyntic HDC activity and that the increased histamine formation is associated with ulcer formation [23]. In the present study, the activity of both aspirin-induced and pentagastrin-induced HDC was suppressed by glucose with little change in serum gastrin. Other monosaccharides such as fructose and mannitol were without effect, which may mean that glucose acts specifically on ECL cells through its glucose receptor. These results raise the possibility that high doses of glucose suppress acid secretion by inhibiting histamine formation in the oxyntic mucosa. Furthermore, it is suggested that the beneficial effect of glucose during ulcerogenesis induced by aspirin [10] and phenylbutazone [11] may partly be due to its suppression of HDC activity. These effects of glucose cannot be explained by the increase in serum levels of insulin following hyperglycemia, because this hormone activates the oxyntic HDC [6,7,24].

Gastric mucosal histamine is thought to influence the HDC induction [17], but no significant change in histamine levels was observed following glucose treatment. Some gastrointestinal hormones are known to affect various aspects of gastric function. For example, serum secretin has been known to affect G cells and to suppress acid secretion [25]. Somatostatin is thought to have a suppressive effect on G cells, parietal cells, and ECL cells [26–28]. Glucagon is known to suppress gastric motility and acid secretion [1,3]. These hormones, which may possibly affect histamine metabolism in gastric mucosa, showed no significant change after glucose

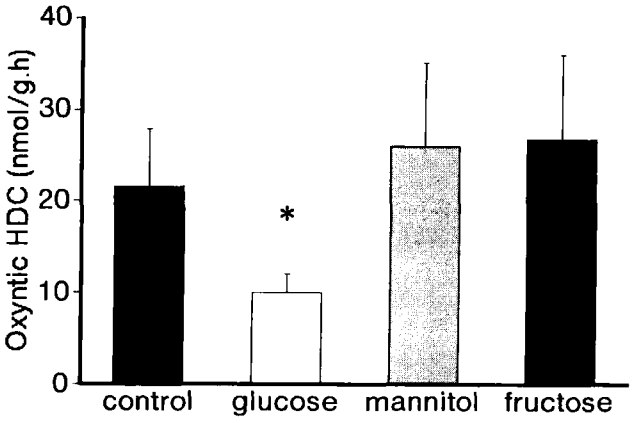

FIGURE 3 Histidine carboxylase activity 4 h after administration of glucose, fructose, and mannitol. Each monosaccharide was given intragastrically at a dose of 1.5 g/kg body weight. The number of determinations for each point was seven. All data are shown as the mean ± SEM. Statistical significance: $*p < .05$ compared with control.

TABLE 3 Effect of glucose pretreatment on aspirin-induced HDC activity

Treatment	n	Gastrin (pg/ml)	Histamine (nmol/g)	HDC (nmol/g · h)
Control	7	78.0 ± 7.4	288.7 ± 29.6	20.2 ± 1.9
Glucose	7	78.7 ± 7.4	313.2 ± 23.3	10.0 ± 1.5*
Aspirin	7	142.3 ± 21.4	386.8 ± 34.8*	45.5 ± 2.8**] ††
Glucose + aspirin	7	110.8 ± 14.5	368.8 ± 13.7*	29.2 ± 4.8]

Note. Glucose was given intragastrically at a dose of 1.5 g/kg body weight 2 h before aspirin. Aspirin was given intragastrically at a dose of 200 mg/kg body weight 4 h before sacrifice. All data are shown as the mean ± SEM; *n*, number of determinations. Statistical significance: $*p < .05$, $**p < .01$ compared with control; $\dagger p < .05$, $\dagger\dagger p < .01$ aspirin with and without glucose.

treatment. These findings suggest that the suppression of oxyntic HDC activity by glucose is not induced by the agency of these gastrointestinal hormones. We have already suggested that the change in endogenous prostaglandins in rat oxyntic mucosa may play a role in cytoprotection [29]. The suppressive effect of glucose on histamine metabolism in rat oxyntic mucosa may be associated with the change in endogenous prostaglandins induced by hyperglycemia. Endogenous prostaglandins, however, are not necessarily essential for the cytoprotective effects of hyperosmolar glucose [30]. These findings suggest that prostaglandins are not involved in the suppressive effects of glucose on the stomach.

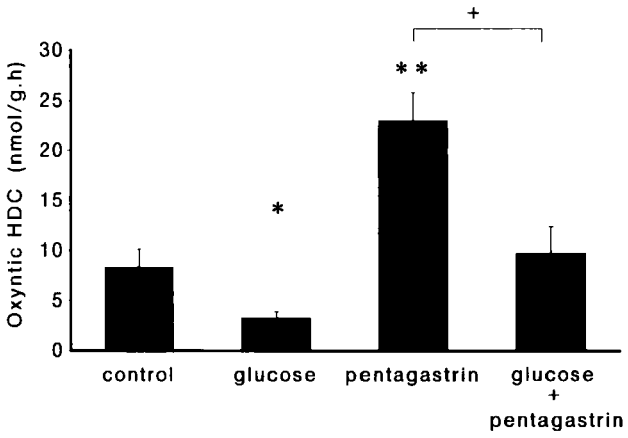

FIGURE 4 Effect of glucose pretreatment on pentagastrin-induced histidine decarboxylase activity. Glucose was given intragastrically 3.5 h before pentagastrin. Pentagastrin (500 μg/kg) was given intraperitoneally 30 min before sacrifice. The number of determinations for each point was seven. All data are shown as the mean ± SEM. Statistical significance: $*p < .05$, $**p < .01$ compared with control; $\dagger p < .05$, glucose with and without pentagastrin.

Taken all together, it is likely that glucose acts directly on the ECL cells to reduce HDC activity. ■

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