Increases in Gastric Histidine Decarboxylase Activity and Plasma Gastrin Level in Streptozotocin-induced Type 1 Diabetic Rats

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Summary When type 1 diabetes mellitus was induced in rats by injecting streptozotocin, histidine decarboxylase expression was abnormally up-regulated in a transcriptional level, and 7 d after the injection, the enzyme activity was increased about 3-fold over the control (p<0.05). When the diabetic rats were administered with insulin for 3 d, the increased histidine decarboxylase activity returned to a normal level in addition to normalization of the plasma glucose level. The plasma gastrin level in the fasting state was also significantly elevated in the diabetic rats, and the insulin treatment normalized the level. In the diabetic rats, fasting gastric acid output increased significantly and gastric pH was lowered. These results suggest that the gastric histidine decarboxylase activity and plasma gastrin level are increased in connection with the depletion of insulin in streptozotocin-induced diabetic rats, and gastric acid secretion is stimulated at a basal level, presumably due to increases in the concentrations of histamine and gastrin in oxyntic mucosa.

Key Words type 1 diabetes mellitus, histidine decarboxylase, gastrin, gastric acid secretion, rats

Materials and Methods

Animals. Male Wistar rats (6-wk-old), purchased from Kiwa Laboratory Animals (Wakayama, Japan) were induced with diabetes by a single injection of streptozotocin, as described in a previous paper (2). These rats were fed a standard diet (CE-2, Clea, Osaka, Japan) from 17:00 to 20:00 ad libitum, and kept at controlled temperature (24±2°C), humidity (65±5%) and lighting (from 8:00 to 20:00). Seven days later, to confirm the induction of diabetes, the plasma glucose level in the fasting state (16-h starvation) was determined by using a commercial kit (Blood Glucose Test, Boehringer-Mannheim). All experimental procedures involving laboratory animals were approved by the Animal Care and Use Committee of Osaka Prefecture University.

Insulin treatment. From 7 d after the injection of streptozotocin, the diabetic rats were treated with insulin for 3 d as follows: Rats were injected 2 times per...
day with regular and normal types of human recombinant insulin (each 5 units), donated from Shionogi Pharmacy (Osaka, Japan), into the tail vein (regular type) and subcutaneously (normal type). The first injection was done 30 min before feeding and the second injection 7.5 h after the first one.

Enzyme assay. Rats were starved for 16 h and killed after being anesthetized with sodium pentobarbital. The stomach, small intestine, pancreas, liver and kidney were obtained and homogenized in a 100 mM potassium phosphate buffer, pH 6.8, containing 1% polyethyleneglycol (average molecular weight: 300), 0.2 mM dithiothreitol, 10 μM leupeptin and 0.5 mg/mL peptatin A with a polytron homogenizer at 4°C. After centrifugation at 20,000×g for 15 min, the supernatant obtained was used as a crude enzyme solution. To induce the HDC reaction, 10 μL of 25 mM (0.24 μCi/μL) [carboxy-14C]histidine (Muromachi Yakuhin, Tokyo, Japan) was added into 40 μL of the enzyme solution and incubated at 37°C for 60 min. The reaction was stopped by the addition of 10% trichloroacetic acid (50 μL) and the radioactivity in CO₂ released during the reaction was measured to determine the HDC activity. The protein content was determined according to Bradford (8) using bovine serum albumin as the standard.

Northern blotting. The total RNA was extracted from the stomach by a published method (9), separated by electrophoresis through a 1% agarose gel containing 17% formaldehyde, and transferred to a nylon membrane. Hybridization was done at 42°C for 40 h and high stringency washing at 65°C. Part of the rat HDC cDNA (base position: 720-1,090) (10) was prepared by reverse transcription-polymerase chain reaction and used as a probe after labeling with [32P]dCTP. For control hybridization, part of the rat glyceraldehyde-3-phosphate dehydrogenase (base position: 320-1,760) (11), donated from Dr. J. M. Blanchard (Institut de Génétique Moléculaire de Montepellier, CNRS, France) was used. Radioactive probes on the membrane were detected by autoradiography.

Measurement of gastric acid output. Rats were deprived of food for 18 h but had free access to water up to 1 h before operation. A pylorus ligation was performed under light ether anesthesia as described previously (12). Rats that had regained the righting reflex 5–10 min after the operation were held in consciousness for 3 h. These rats were anesthetized and decapitated, and the stomach was removed. Rats were not used when either residual food or fecal material remained in their stomachs. The gastric content was collected and centrifuged at 15,000×g for 15 min at 4°C. The amount of acid output was determined by back-titration to pH 7.0 with 0.1 N NaOH.

Measurement of gastrin. After starving for 16 h, blood was collected by cardiac puncture under ether anesthesia. Plasma gastrin level was measured by radioimmunoassay using a commercial kit (Dinabot, Tokyo, Japan).

Table 1. HDC activities in various tissues in streptozotocin-induced diabetic rats.

| Tissue             | Diabetic rats | Non-diabetic rats |
|--------------------|---------------|-------------------|
| Stomach            | 19.78±2.141*  | 5.91±0.680        |
| Small intestine    | 1.61±0.287    | 2.02±0.708        |
| Pancreas           | 8.72±2.260    | 8.14±2.688        |
| Liver              | 8.91±2.022*   | 3.09±1.667        |
| Kidney             | 1.64±0.332*   | 0.76±0.264        |

Diabetes mellitus was induced by injecting streptozotocin, and 7 d later the HDC activities in various tissues were determined. Values are means±SE (n=4). *: p<0.05 compared with the non-diabetic rats.

Results and Discussion

Type 1 diabetes mellitus was induced in rats by a single injection of streptozotocin and 7 d later histidine decarboxylase (HDC) activities in various tissues were compared between the diabetic rats and the non-diabetic control group (plasma glucose levels in the fasting state were 554±65.1 and 117±10.6 mg/dL, respectively; p<0.05). In the streptozotocin-induced diabetic rats, a remarkable increase (3.3-fold; p<0.05) in HDC activity was observed in the stomach (Table 1). The amount of HDC protein in the stomach, when examined by Western blotting with anti-rat HDC antibodies (7), was substantially high in the diabetic rats compared to the control group (data not shown). In addition, the HDC activities in the liver and pancreas, as well as the stomach, were significantly increased in the diabetic rats, whereas no significant difference in the activity was observed in the small intestine and pancreas (Table 1). The increase in HDC activity in the kidney supports a previous report showing that the renal histamine level was elevated in the streptozotocin-induced diabetic rats (13). Plasma histamine level has also been reported to be elevated in diabetic patients (14) and in experimental type 1 diabetic rats (15), presumably due to increased HDC activity in the aortic endothelial and smooth muscle cells (15, 16). The elevation of the plasma histamine level is thought to be important for mediating many of the initial events associated with microangiopathies and accelerated atherosclerosis in diabetic mellitus (14, 15). However, detailed mechanisms by which the HDC activities in the aortic endothelial and smooth muscle cells are abnormally increased in diabetic mellitus have not yet been well elucidated, although it has been reported when type 1 diabetic rats are treated with insulin. The increased HDC activities are normalized with a concomitant decrease in the plasma histamine level (15, 16).
Rats were randomly divided into control (open bars), diabetes (solid bars) and diabetes-insulin (shaded bars) groups, and streptozotocin was injected in the diabetes and diabetes-insulin groups. From 7 d after the injection of streptozotocin, rats in the diabetes-insulin group were treated with insulin for 3 d. Then, 10 d after the injection of streptozotocin, the HDC activity in the stomach, and the plasma gastrin and glucose levels in the fasting state were determined in the three groups. Values are means±SE (n=5). Bars with different letters are significantly different (p<0.05).

As shown in Fig. 1B, 10 d after injecting streptozotocin, the plasma gastrin level in the fasting state was increased about 2-fold over the control level (p<0.05). Furthermore, the plasma gastrin level, as well as gastric HDC activity, was normalized when the diabetic rats were administered insulin for 3 d. Similar results have been reported by Schedl et al. (18), who examined post-prandial levels of gastrin in serum and pyloric antrum in type 1 diabetic rats treated with or without insulin.

It has been reported that gastrin regulates HDC expression at the transcriptional level in the stomach in addition to the stimulation of histamine release, and that the amount of HDC mRNA is increased in oxyntic mucosa in response to the increase in plasma gastrin concentration when gastrin is administered intravenously (17, 19). In addition, Miyazaki et al. (20) reported that gastric HDC activity was markedly increased with a concomitant increase in gastric histamine content in patients with hypergastrinemia. In contrast, Kaneko et al. (21) reported that glucose suppressed the HDC activity in oxyntic mucosa in non-diabetic rats when given intragastrically. In addition, they showed that glucose also suppressed the induction of HDC activity by gastrin. On the basis of our findings, there may exist two possibilities. One possibility is that the increased plasma gastrin level results in the up-regulation of gastric HDC expression without being affected by glucose in streptozotocin-induced diabetic rats in which the plasma glucose level is continuously increased by the depletion of insulin, in contrast to non-diabetic rats given glucose intragastrically. Alternatively, the depletion of insulin itself, regardless of the increase in the plasma levels of gastrin and glucose, may be a potent factor causing the up-regulation of gastric HDC expression in type 1 diabetic rats. However, it

Fig. 1. HDC activity in stomach (A), and plasma gastrin (B) and glucose (C) levels in streptozotocin-induced diabetic rats. Rats were randomly divided into control (open bars), diabetes (solid bars) and diabetes-insulin (shaded bars) groups, and streptozotocin was injected in the diabetes and diabetes-insulin groups. From 7 d after the injection of streptozotocin, rats in the diabetes-insulin group were treated with insulin for 3 d. Then, 10 d after the injection of streptozotocin, the HDC activity in the stomach, and the plasma gastrin and glucose levels in the fasting state were determined in the three groups. Values are means±SE (n=5). Bars with different letters are significantly different (p<0.05).

From 7 d after the induction of diabetes mellitus by injecting streptozotocin, the diabetic rats were administered insulin for 3 d. Due to the insulin treatment, the increased gastric HDC activity in the diabetic rats returned to a normal level (Fig. 1A), in addition to the normalization of the plasma glucose level (Fig. 1C). As shown in Fig. 2, Northern blots of RNA obtained from stomachs by using a HDC probe gave a single mRNA band with 2.7 kb, which corresponded to the major HDC mRNA species in a previous paper (17) (the minor species observed in the previous paper (3.5 kb) was barely detected under our experimental conditions). The HDC mRNA level in the diabetic rats (lane 2) was obviously high compared to the non-diabetic control group (lane 1). Moreover, the abnormal increase in the HDC mRNA level was reversed when the diabetic rats were treated with insulin for 3 d (lane 3). These results suggest that gastric HDC expression is up-regulated at a transcriptional level in the streptozotocin-induced diabetic rats, and the depletion of insulin appears to be related to the up-regulation of HDC expression.

As shown in Fig. 1B, 10 d after injecting streptozotocin, the plasma gastrin level in the fasting state was increased about 2-fold over the control level (p<0.05). Furthermore, the plasma gastrin level, as well as gastric HDC activity, was normalized when the diabetic rats were administered insulin for 3 d. Similar results have been reported by Schedl et al. (18), who examined post-prandial levels of gastrin in serum and pyloric antrum in type 1 diabetic rats treated with or without insulin.

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has been reported that insulin, when administered into
donor diabetic rats at a high dose, induces an increase in
HDC activity in oxyntic mucosa together with an in-
crease in serum gastrin level (22). However, details con-
cerning the relationship between gastric HDC expres-
sion and plasma gastrin in type 1 diabetic rats remain
to be elucidated.

Gastric acid secretion in the fasting state in con-
sciousness was examined at 10 d after the induction of
diabetes mellitus by streptozotocin. As shown in Table
2, the amount of gastric acid output was about 1.5-fold
greater in the diabetic rats compared to the non-dia-
betic control group (p<0.05). As a result of the increase
in gastric acid output, gastric pH was significantly lower
in the diabetic rats. There was very little difference in
stomach weight between the diabetic and control rats,
although the body weight of the diabetic rats was signif-
ificantly lower compared to the control group. In the dia-
betic rats, obvious changes were not observed in their
stomaches.

In patients with long-standing diabetes mellitus, it is
often observed that fasting and postprandial gastric
concentrations in the plasma are substantially high
compared to non-diabetic subjects, but basal and food-
stimulated gastric acid output are not increased (rather
reduced) despite the increase in the gastrin levels (23–
25). It is thought that the hypergastrinemia in these pa-
tients is a reflection of a high prevalence of vagal dys-
function, which correlates with diabetic gastroparesis
and results inconstantly in reduced gastric acid secre-
tion (23–26). In a like manner, the frequent co-exis-
tence of hyposecretive autoimmune atrophic gastritis is
also thought to be closely related to hypergastrinemia
(26, 27). Data obtained in the present paper indicate that
the plasma gastrin level in the fasting state is ab-
normally increased in rats induced with type 1 diabetes
mellitus by streptozotocin. However, it is not reasonable
to conclude that the abnormal increase in the gastrin
level in the experimental diabetic rats results from vagal
dysfunction with diabetic gastroparesis or from at-
rophic gastritis with loss of parietal cells, since basal
gastric acid output increased with a concomitant de-
crease in gastric pH in these rats. Since the increased
 gastrin level in the diabetic rats was reversed by treat-
ment with insulin for 3 d, it is thought that when type 1
diabetes mellitus is caused by streptozotocin in rats, at
least in the early phase, gastrin release in the pyloric
antrum is stimulated in a basal level in connection with
the depletion of insulin. In addition, gastric HDC expres-
sion was also up-regulated in these rats, although it is
not clear whether gastrin participates in the up-regula-
tion. Consequently, gastric acid output would be stimu-
lated at a basal level in these diabetic rats due to in-
creases in the concentrations of gastrin and histamine
in oxyntic mucosa; however, details, including the rea-
son why feedback regulation is not induced in gastrin
release, remain to be clarified.

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Table 2. Gastric acid secretion in streptozotocin-induced diabetic rats.

| Variable                  | Diabetic rats | Non-diabetic rats |
|---------------------------|---------------|-------------------|
| Body weight (g)           | 142±6.6*      | 208±1.1           |
| Stomach weight (g)        | 1.71±0.081    | 1.83±0.057        |
| Gastric acid output (μeq/3 h) | 507±43.1*   | 332±55.9          |
| Gastric pH                | 1.12±0.038*   | 1.34±0.147        |

Diabetes mellitus was induced by injecting streptozotocin, and 10 d later the stomach weight, amount of gastric acid output and gastric pH were examined. Values are means±SE (n=4). *; p<0.05 compared with the non-diabetic rats.
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