Effects of celiac superior mesenteric ganglionectomy on glucose homeostasis and hormonal changes during oral glucose tolerance testing in rats

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Abstract. The liver plays an important role in maintaining glucose homeostasis in the body. In the prandial state, some of the glucose which is absorbed by the gastrointestinal tract is converted into glycogen and stored in the liver. In contrast, the liver produces glucose by glycogenolysis and gluconeogenesis while fasting. Thus, the liver contributes to maintaining blood glucose level within normoglycemic range. Glycogenesis and glycogenolysis are regulated by various mechanisms including hormones, the sympathetic and parasympathetic nervous systems and the hepatic glucose content. In this study, we examined a rat model in which the celiac superior mesenteric ganglion (CSMG) was resected. We attempted to elucidate how the celiac sympathetic nervous system is involved in regulating glucose homeostasis by assessing the effects of CSMG resection on glucose excursion during an oral glucose tolerance test, and by examining hepatic glycogen content and hepatic glycogen phosphorylase (GP) activity. On the oral glucose tolerance test, CSMG-resected rats demonstrated improved glucose tolerance and significantly increased GP activity compared with sham-operated rats, whereas there were no significant differences in insulin, glucagon or catecholamine levels between the 2 groups. These results suggest that the celiac sympathetic nervous system is involved in regulating the rate of glycogen consumption through GP activity. In conclusion, the examined rat model showed that the celiac sympathetic nervous system regulates hepatic glucose metabolism in conjunction with vagal nerve innervations and is a critical component in the maintenance of blood glucose homeostasis.

Key words: Glycogen phosphorylase (GP), Sympathetic nervous system, Celiac superior mesenteric ganglion (CSMG), Liver glycogen

THE LIVER plays an important role in the maintenance of whole-body glucose homeostasis. In the prandial state, some of the glucose which is absorbed by the gastrointestinal tract is converted into glycogen and stored in the liver. In contrast, the liver produces glucose by glycogenolysis and gluconeogenesis while fasting. Thus, the liver contributes to maintaining blood glucose level within normoglycemic range. Magnusson et al. using the ^13^C-nuclear magnetic resonance system [1, 2] observed that both hepatic glycogenolysis and glycogenesis occur simultaneously in both prandial and fasting states. Hepatic glycogenesis and glycogenolysis are regulated by various mechanisms including hormones, the sympathetic / parasympathetic nervous systems and the hepatic glucose content [3-5]. The involvement of the celiac sympathetic nervous system on the regulation of blood glucose has also been reported. A hypoglycemic clamp study of celiac superior mesenteric ganglion (CSMG)-resected rats reported impaired compensatory elevations in the levels of catecholamines by two-thirds compared with sham-operated non-CSMG-resected rats [6]. These results suggest that the celiac sympathetic nervous system plays an important role in preventing hypoglycemic conditions. On the other hand, the role of the celiac sympathetic nervous system in hyperglycemic conditions remains to
be investigated.

In order to elucidate the role of the celiac sympathetic nervous system in hepatic glucose metabolism, we established a rat model in which the CSMG was resected. We investigated the effects of the celiac sympathetic nervous system on glucose homeostasis and hepatic glucose metabolism during hyperglycemia by measuring glucose excursion during the oral glucose tolerance test (OGTT), and examined the hormonal changes, hepatic glycogen content and hepatic glycogen phosphorylase (GP) activity.

Materials and Methods

Animal surgery

We used 7-week-old male Sprague-Dawley rats (Clea Japan, Tokyo, Japan). The animals had free access to water and normal rodent chow (Clea Japan). The rats were randomly divided into 2 groups: one was CSMG-resected group and the other was non-CSMG-resected sham-operated one. CSMG resection was performed as described previously [6]. The rats were fasted overnight and were then intraperitoneally anesthetized with pentobarbital sodium (50 mg / kg). The abdominal cavity was exposed through a ventral midline incision and the intestines were moved to the right side. The CSMG, which is located on the descending aorta at the branch points between the celiac and superior mesenteric arteries, was identified and resected under a microscope. Wounds were closed after all surgical procedures. The resected tissue was histologically examined and confirmed as CSMG. The sham-operated animals were subjected to an identical surgical procedure, but without resection of the CSMG. All animals were given free access to food and water after surgery.

All animal experiments were approved by the Institutional Animal Care and Ethics Committee of our institution, and all surgical procedures followed the “Guiding Principles for Biomedical Research Involving Animals” of The Physiological Society of Japan, and the “Rules for Animal Experiments” of Tokyo Medical University Animal Experiment Committee.

Oral glucose tolerance test (OGTT)

Seven days after surgery, the animals were fasted overnight and glucose at 2 g / kg was administered orally via gavage. To determine blood glucose levels, blood specimens were collected from the tail vein of the animals before the OGTT and 30 min, 60 min, and 120 min after the OGTT. To measure the insulin, glucagon and catecholamine levels, blood specimens were obtained from the heart under ether anesthesia before the OGTT and 60 min and 120 min after the OGTT. The liver was then removed, weighed, frozen with liquid nitrogen and stored for further measurement. To measure insulin and glucagon levels, blood plasma samples were obtained by transferring 1 mL of blood to a test tube chilled with 30 µL of heparin for insulin testing, and to a test tube chilled with 20 µL each of aprotinin and EDTA sodium salt (EDTA·2Na) for glucagon testing. Subsequently, the test tubes were centrifuged, and the collected plasma was subjected to further assays.

Acetaminophen absorption test

Seven days after surgery, another set of animals was used to assess whether CSMG resection alters splanchnic blood circulation that reflect systemic blood acetaminophen concentration. A solution that contained 1% (w/v) acetaminophen (Sigma-Aldrich) was administered orally via gavages at a dose of 100 mg / kg [11]. Tail vein blood (50 μL) was collected into EDTA-coated tubes at 0, 30, 60, 90 and 120 minutes after acetaminophen administration. Plasma was separated by centrifugation at 4°C and stored at −20°C until measurement of acetaminophen levels using an enzymatic-spectrophotometric assay (Cambridge Life Sciences, UK).

Analytical procedures

Blood glucose levels were determined using a glucometer (Sanwa Kagaku Kenkyusho Co. Ltd., Nagoya, Japan). Plasma insulin concentration was determined by sandwich enzyme immunoassay (Morinaga Seikagaku, Yokohama, Japan). Plasma glucagon concentration was determined by radioimmunoassay (Daiichi Glucagon Kit, TFB, Inc., Tokyo, Japan). Blood adrenaline and noradrenaline concentrations were determined by high-performance liquid chromatography using deproteinized blood plasma samples. Hepatic glycogen was analyzed using a standard enzymatic assay as described previously [7].

To measure hepatic GP activity, the liver was homogenized in buffer containing 20 mM Tris HCl, pH 7.2, 250 mM sucrose, 50 mM NaF, 4 mM EDTA and 0.5 mM dithiothreitol at 2°C. A supernatant obtained by centrifugation of the homogenate was mixed with assay buffer (50 mM BES, pH 6.8, 1 mM EDTA, 2 mM MgCl₂, 10 μM glucose 1,6-diphosphate, 4 mg/mL glycogen and 0.7 mM β-NAD⁺ at final concentrations),
and the assay mixture was left to stand for 3 min at 37°C. Subsequently, an enzyme reaction mixture containing 800 U of phosphoglucomutase, 6000 U of glucose-6-phosphate dehydrogenase, 1.6 mM KH₂PO₄ and 2.4 mM Na₂HPO₄ was added to the assay mixture. GP activity was determined by measuring changes in absorbance at 340 nm.

Data analysis

All data were expressed as means ± standard errors (SE) of the mean. The area under the curve (AUC) was calculated using the trapezoidal rule. Student’s unpaired t-test was used for analysis between the 2 groups, and non-repeated measures analysis of variance with post-hoc Student Newman-Keuls was applied to analyze the 3 groups. A p-value of less than 0.05 was considered to indicate a statistically significant difference.

Results

Changes in body weight after surgery

The mean body weights before surgery were 225.3 ± 2.63 g vs. 229.3 ± 0.98 g for the CSMG-resected and sham-operated groups, respectively. The body weight changes after surgery were 237.8 ± 1.82 g vs. 243.7 ± 1.66 g on day 4 and 268.5 ± 2.49 g vs. 279.0 ± 0.47 g on day 8 for the CSMG-resected and sham-operated groups, respectively. The CSMG-resected group tended to show less body weight gain after surgery, but there was no statistically significant difference between the 2 groups (Fig. 1).

Blood glucose excursion during the oral glucose tolerance test

We first examined whether the CSMG is involved in the regulation of glucose homeostasis during hyperglycemia by performing the OGTT. The pre-dose blood glucose levels did not show any statistically significant difference between the CSMG-resected and sham-operated groups (70.1 ± 2.58 mg / dL vs. 70.9 ± 4.17 mg / dL, respectively). The blood glucose levels after oral glucose dosing were 138.9 ± 8.3 mg / dL vs. 167.0 ± 15.8 mg / dL at 30 min, 131.1 ± 5.9 mg / dL vs. 168.2 mg / dL at 60 min, 118.2 ± 4.6 mg / dL vs. 134.9 ± 10.5 mg / dL at 90 min and 122.2 ± 11.4 mg / dL at 120 min for the CSMG-resected and sham-operated groups, respectively (Fig. 2A). The CMSG-resected group demonstrated better glucose tolerance than the sham-operated group. In particular, a statistically significant reduction in the blood glucose level was observed in the CSMG-resected group at 60 min after the OGTT (p < 0.05, Fig. 2A).

Although no significant difference in the AUC was observed between the 2 groups at both 0-30 min and 90-120 min, the AUC at both 30-60 min and 60-90 min of the CSMG-resected group was significantly lower than that of the sham-operated group (p < 0.05, Fig. 2B).

Changes in hormonal parameters during the oral glucose tolerance test

Several hormones are known to be affected by elevated blood glucose levels. We assessed the changes in plasma insulin, glucagon and catecholamine levels dur-
The CSMG-resected rats showed relatively but not significantly lower plasma noradrenaline concentrations than the sham-operated rats both before and 120 min after oral glucose load. On the other hand, a trend of higher noradrenaline concentration was observed in the CMSG-resected group than in the sham-operated group at 60 min after oral glucose load (Fig. 3B, no statistical significance).

**Effect of CSMG resection on splanchnic circulation**

There is a potential that CSMG resection alters splanchnic blood circulation. If such change occurs, systemic blood circulation level of a particular substance that is absorbed from intestine would alter by CSMG resection. We therefore conducted acetaminophen absorption test using another set of animals under the same conditions as OGTT in order to assess whether CSMG resection alters temporal profile of systemic blood acetaminophen concentration, which is caused during the OGTT. There was no significant difference in the pre-dose plasma insulin levels between the CSMG-resected and sham-operated groups (0.62 ± 0.12 ng / mL vs. 0.44 ± 0.09 ng / mL, respectively; n = 5). On the other hand, the CSMG-resected group showed significantly lower plasma insulin levels than the sham-operated group 60 min after the OGTT (1.88 ± 0.52 ng / mL vs. 3.99 ± 0.6 ng / mL, respectively, p<0.05; Fig. 2C). However, no significant difference was observed at 120 min post-glucose loading (0.78 ± 0.17 ng / mL and 0.81 ± 0.15 ng / mL in the CSMG-resected and sham-operated groups, respectively). Plasma glucagon and adrenaline levels did not significantly differ between the 2 groups at 60 min post-glucose loading, however, pre-dose and 120 min post-dose plasma glucagon and adrenaline levels were tended to be lower in the CSMG-resected group than in the sham-operated one, although statistical significance was not observed (Figs. 2D and 3A).

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Effects of CSMG on glucose homeostasis

Hepatic glycogen content and glycogen phosphorylase (GP) activity

In order to elucidate the effects of CSMG resection on hepatic glucose metabolism, hepatic glycogen content and GP activity were examined in both fasting and OGTT conditions. Fasting hepatic glycogen content was significantly higher in the CSMG-resected group than in the sham-operated group (0.27 ± 0.08 mg/g liver vs. 0.08 ± 0.03 mg/g liver, p < 0.05; Fig. 4A). The CSMG-resected group also showed significantly higher glycogen content than the sham-operated group 60 min after oral glucose load (3.12 ± 1.03 vs. 1.08 ± 0.27 mg/g liver, p < 0.05). However, no significant difference was observed 120 min after oral glucose load. Hepatic GP activity in the fasting animals was significantly lower in the CSMG-resected group than in the sham-operated one (Fig. 4B), which is consistent with the elevated fasting glycogen level observed in the CSMG-resected group. GP activity at 60 min and 120 min after oral glucose load was not statistically significantly different between the 2 groups. When we observed the temporal changes in GP activity in each group during the OGTT, GP activity was significantly decreased at 60 min after oral glucose load (p < 0.05), and returned to a level comparable to that of the fasting state. A statistically significant reduction in GP activity induced by the OGTT was also observed in the sham-operated rats until 120 min following oral glucose load (p < 0.05).

![Fig. 3](image)

**Fig. 3** Plasma adrenaline (A) and noradrenaline (B) levels during the OGTT in the sham-operated group (circle) and CSMG-resected group (square). Data are expressed as means ± SE (n = 5). No statistical significance was observed between two groups.

![Fig. 4](image)

**Fig. 4** Hepatic glycogen content (A) and hepatic glycogen phosphorylase activity (B) during the OGTT in the sham-operated group (black bar) and CSMG-resected group (white bar). Data are expressed as means ± SE (n = 5). *p < 0.05 compared with the sham-operated group. #p < 0.05 and 8p < 0.05 compared with the 0-min value of the sham-operated and CSMG group, respectively.
**Discussion**

In the present study, we set out to investigate the role of the celiac sympathetic nervous system on glucose homeostasis, hormonal changes and hepatic glucose metabolism during hyperglycemia in a CSMG-resected rat model. Since CSMG resection significantly improved glucose tolerance in the present study, this suggests that the CSMG plays an important role in maintaining glucose homeostasis.

Several reports have indicated that the sympathetic nervous system directly affects hepatic glucose metabolism, and that catecholamines are slightly involved in the induction of hepatic GP activity, which is elicited by stimulation of the sympathetic nervous system [8, 9]. On the other hand, the present data demonstrated that CSMG resection reduced fasting GP activity, plasma glucagon and catecholamine levels, suggesting a potential relationship between reduced GP activity and these hormones. The CSMG-resected rats demonstrated reduced GP activity and a concomitant increase in hepatic glycogen content under a fasting state, suggesting that CSMG resection suppressed the glycogen breakdown pathway, which is associated with the relative enhancement of the glycogen synthesis pathway. This resulted in the rapid induction of glycogen synthesis after oral glucose loading, which in turn resulted in a significantly elevated hepatic glycogen level and a reduced blood glucose level at 60 min after oral glucose load. We could not determine whether the change was due to the direct involvement of the celiac sympathetic nerve or an indirect contribution via hormones that would be released by stimulation of the celiac sympathetic nerve. However, the present results strongly suggest that the CSMG plays a pivotal role in hepatic glucose metabolism. In previous studies, the electrical stimulation of either VMH or peripheral splanchnic nerves increased in the activity of hepatic glycogen phosphorylase [12-14]. Proost C et al. have reported in rabbit study that the activation of glycogen phosphorylase (phosphorylase α) was increased by electrical stimulation of splanchnic nerve. They also reported that phentolamine, a pharmacological agent that blocks α-adrenergic receptor inhibited noradrenaline-induced activation of phosphorylase α [15]. Resection of celiac superior mesenteric ganglion (CSMG) demonstrated reduced GP activity observed in the present study further support previously reported findings with providing more direct evidence that CSMG innervates the liver.

It is believed that the CSMG regulates the rate of hepatic glycogen consumption. Thus, a major reason for the improved glucose tolerance by the CSMG resection would be the reduced glycogen breakdown. We have previously reported the effects of vagal afferent nerve denervation by capsaicin application on portal glucose infusion-induced change in hepatic glycogen content in rats [10]. The denervation of the vagal afferent nerve demonstrated a significant reduction in hepatic glycogen content, suggesting that the vagal nerve is also involved in the regulation of hepatic glycogen content. In the present study, the livers of the CSMG-resected rats appeared to be more innervated by the vagal nerve than those of the sham-operated rats, which is complementary to our previous findings. Unfortunately we did not measure the activity of glycogen synthase in the present study, but it is also possible that a greater elevation of the glycogen synthase activity may occur in the CSMG-resected rats than in the sham-operated rats, which may also contribute to keeping higher hepatic glycogen content by CSMG resection. A comprehensive analysis of hepatic enzymes and intermediate substrates of glucose metabolism that account for the hepatic glycogen and/or glucose metabolisms will lead to more understanding of the importance of celiac sympathetic nervous system on the maintenance of glucose homeostasis through the regulation of hepatic glucose metabolism.

In conclusion, the CSMG plays an important role in regulating the hepatic glycogen consumption rate in the rat model examined in this study. This suggests that both the CSMG and hepatic vagal nerve regulate hepatic glucose metabolism, and that the CSMG plays an important role in maintaining glucose homeostasis through the regulation of glycogen metabolism.

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