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Relationship between portal signal-induced liver glycogen synthesis and the vagus afferent nerve in unrestrained, conscious rats

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Abstract

Stimulation of hepatic glycogen synthesis by blood glucose is much efficient when glucose is supplied through the hepatic portal vein rather than through the liver artery. Earlier studies including us indicated that this phenomenon, namely "portal signal", was at least partly due to the modulation of afferent vagus nerve activities by glucose sensing mechanism(s) located in portal vein wall. To confirm the contribution of the afferent vagus nerve on the portal signal, using rats, we investigated the effects of blocking afferent vagus nerve activities by capsaicin, a selective blocker of unmyelinated nerves, on the hepatic glycogen content, and the plasma level of glucose, insulin and glucagon, since the subphrenic vagus nerve mainly consists of unmyelinated afferent fibers. Liver glycogen content was time dependently increased by time after continuous glucose infusion. In the control experiments, the liver glycogen content of the rats in which glucose was continuously infused for 24 hrs via portal vein was much higher than those with glucose infusion for 24 hrs via jugular vein, although the plasma level of glucose, insulin and glucagons were not different between the two groups. On the other hand, when the vagus nerve was treated with capsaicin, such significant difference of the liver glycogen level between the two groups was not observed, indicating loss of the portal signal by capsaicin.

The above findings suggested that the portal signal-induced liver glycogen synthesis is mediated through the afferent branch of vagus nerve.

Introduction

Liver glucose uptake and glycogen synthesis is regulated by brood flow route dependent mechanisms. When glucose was infused into the liver through the portal vein in dogs and rats, liver glucose uptake was promoted and the liver glycogen content increased compared to infusion of the same amount of glucose through other veins in dogs (1–9) and rats (10). This phenomenon is called the portal signal. Many studies of liver uptake were performed by the hyperglycemic hyperinsulinemic glucose clamp method, in which somatostatin was administered to inhibit endogenous pancreatic hormone secretion, instead insulin and glucagon were exogenously administered. This method is advantageous to easily control the plasma level of insulin, glucagons and glucose constant, but these levels exceed the physiological ranges. We have investigated portal signals in beagles and Sprague-Dawley rats under more physiological conditions (11), and found that liver glucose uptake and liver glycogen content may be affected by the amount of glucose reaching the liver, the route of administration, differences between arterial and portal glucose levels, and the insulin level.

The vagus nerve has been reported to be involved in portal signal transmission pathway. Shimazu et al.

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suggested that the vagus nerve stimulation promotes glycogen synthesis because it increased liver glycogen synthetase in vitro (12). Stumpel et al. also reported that in a liver perfusion experiment, addition of acetylcholine under hyperglycemic hyperinsulinemic conditions increased net hepatic glucose uptake (NHGU), while addition of atropine decreased it (13). In an in vivo study, Chap et al. reproduced an increase in blood glucose seen after oral glucose loading by infusion of glucose in dogs, and found that atropine decreased NHGU by 44% relative to the amount of glucose that reached the liver (14). Furthermore, Shiota et al. reported that acetylcholine increased NHGU in hyperglycemic hyperinsulinemic glucose clamp (15). However, acetylcholine and atropine were intravenously administered in these in vivo studies, and whether the actions on the vagus nerve hepatic branch were direct or indirect was unclear. Xue directly cut the hepatic branch of the vagus nerve, and found that the liver glycogen content decreased under conditions of free access to food compared to that without cutting the hepatic branch (16). Matsuhisa et al. also compared rat groups with and without vagectomy in normoglycemic hyperinsulinemic glucose clamping by the dual tracer method, and found a decrease in liver glucose uptake and an increase in endogenous glucose production in the group with vagectomy (17). These findings suggested that the vagus nerve is directly involved in portal signals.

In an electrophysiological study, Niijima et al. infused glucose via the portal vein in guinea pigs, and found that the electric pulse of the vagus nerve afferent branch in the portal wall decreased in a concentration-dependent manner, while the electric pulse of the pancreatic efferent branch increased (18).

We assume that the portal wall senses changes in blood glucose, and transmits the information to the central nervous system through the vagus nerve afferent branch, and the information is then transmitted to the liver through the efferent branch, changes liver enzymes, and promotes liver glycogen synthesis. In this study, we investigated the involvement of the vagus nerve afferent branch in the liver glycogen content using capsaicin, which using capsaicin, a selective inhibitor of unmyelinated nerve fibers (19), since the afferent branch of the vagus nerve mainly consists of unmyelinated nerve fibers.

Methods

<Animal management> All experimental procedures conformed to the "Guideline Principles of the Care and Use of Animals in the Field of Physiological Science" approved by the Council of the Physiological Society of Japan, and were carried out under the "Rules and Regulation of the Animal Studies Committee of Tokyo Medical University". Female Sprague-Dawley (SD) rats aged 8–12 weeks and weighing 180–240 g each were randomly divided into 4 groups based on the route of glucose infusion and the presence or absence of capsaicin treatment to the vagus nerve : a control group receiving glucose via the portal vein (ContPo), a control group receiving glucose via the jugular vein (ContPe), a capsaicin treated group receiving glucose via the portal vein (CapPo), and a capsaicin treated group receiving glucose via the jugular vein (CapPe). The rats were incised approximately 3-cm from the site below the xiphoid process to the left abdominal region along the 12th rib under anesthesia with an intraperitoneal injection of pentobarbital (50 mg/kg ip, Abbott Laboratories, Tokyo, Japan). The esophagus was exposed, and the vagus nerve located on the right side of the esophagus was detached, then a 8×15 mm strip of parafilm® (Pechiney Plastic Pckaging, Chicago, IL, USA) was inserted between them. A twisted cotton string soaked in 5% wt/vol capsaicin dissolved in olive oil was placed on the parafilm® strip (Figure 1) to avoid capsaicin application to the organs other than the vagus nerve. In the control groups, olive oil without capsaicin was applied by the same procedure. After 30 min application of capsaicin or olive oil to the vagus nerve, the cotton string and the parafilm® strip were removed. In the cases of the ContPe and CapPe, approximately 10-mm incision was made in the upper region of the right calvicle, and after fat cleansing using forceps, the right jugular vein was exposed, then a polyethylene catheter was inserted for about 15 mm. In the cases of ContPo and CapPo, the region from the cecum to the large bowel was pulled out through the

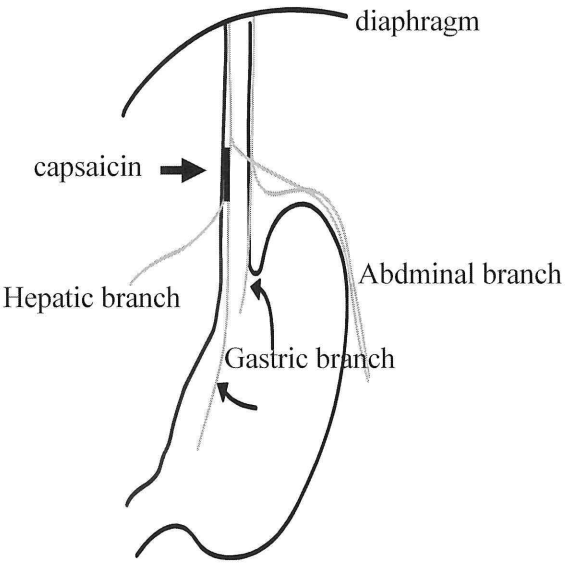

Fig. 1 Applied site of capsaicin

incision described above, and a polyethylene catheter was inserted for about 20 mm through the mesenteric vein over the portal vein using a 20 G Surflow® (Terumo, Tokyo, Japan). The catheter was connected to an infusion pump (Atom medical corporation, Tokyo, Japan). The rats were individually housed in cages immediately after surgery, and maintained under conditions of constant ventilation, constant room temperature at 20°C, and 12-hour lighting cycle. Drinking water and food were given ad libitum. During the housing and the glucose infusion, the rats were cared without restraint or anesthesia.

＜Experimental design＞ Only rats that recovered to the preoperative body weight by 5 days after surgery were used for the experiments. To remove liver glycogen before the experiment, the animals were fasted for 24 hours, and only drinking water was given. After fasting for 24 hours, glucose solution (20% glucose, Hikari Pharmaceutical Co., Ltd. Tokyo, Japan) was continuously infused through the catheter at a dose of 78 μmol/kg/min, established by the method reported by Ogihara et al. (11), for 0, 4, and 24 hours. The animals were then anesthetized with pentobarbital, and blood was collected from the portal vein and inferior vena cava. After blood collection, the liver was excised. Glucose, insulin, and glucagon were measured in the blood samples, the liver glycogen content was measured in the liver samples, and the results were compared among the 4 groups.

＜Measurement＞

Glucose measurement

To a 1.5-ml Eppendorf tube containing 30 μl of heparin, 1 ml of the blood sample was added, and plasma was separated by centrifugation. The plasma glucose level was measured by the Glucose mutarotase-GOD method. (Wako Pure Chemical, Osaka, Japan)

Insulin measurement

To a 1.5-ml Eppendorf tube containing 30 μl of heparin, 1 ml of the blood sample was added, and plasma was separated by centrifugation. The plasma insulin level was measured using the EIA sandwich method. (Morinaga Biochemical Laboratories, Yokohama, Japan)

Glucagon measurement

To an Eppedorf tube containing 20 μl of Trasylol and 20 μl of EDTA2Na, 1 ml of the blood sample was added, and plasma was separated by centrifugation. The plasma glucagon level was measured by the RIA 2-antibody method.

Measurement of liver glycogen content

The liver was homogenized, glycogen was decomposed to glucose by amyloglucosidase under acidic conditions, and glucose was measured by mutarotase-GOD method. (Wako Pure Chemical, Osaka, Japan)

Statistical analysis

The values were presented as means±SE. Non-repeated measures ANOVA was used for comparisons among the 4 groups, and when a significant difference was noted, the SNK test was used for intergroup comparison.

Results

Rats with and without application of capsaicin received glucose administration at 78 μmol/kg/min through the portal vein and jugular vein, and the plasma glucose, insulin, and glucagon levels and the liver glycogen content were measured after infusion for 0, 4, and 24 hours. The plasma glucose level after infusion for 4 hours was higher than that at 0 hours, but the level after infusion for 24 hours was similar to that at 0 hours in all groups (ContPo, ContPe, CapPo, and CapPe). No significant difference was noted among the 4 groups after infusion of glucose for 0, 4, or 24 hours (Fig. 2). The plasma insulin level was significantly increased after the 4-hour glucose infusion, but returned to the 0-hour level after the 24-hour infusion. No significant difference was noted in the insulin level among the 4 groups after

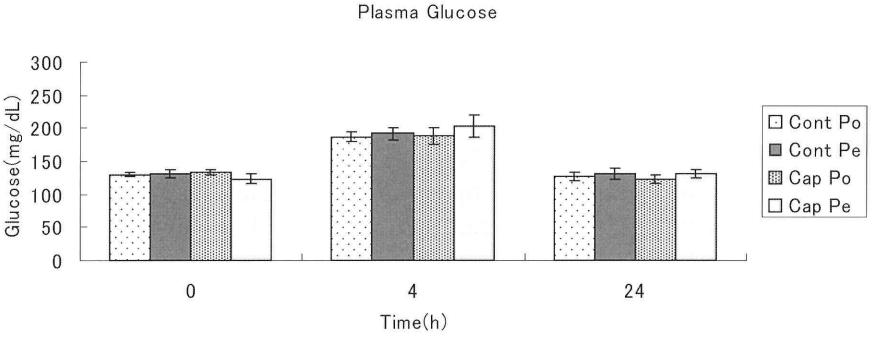

Fig. 2 Plasma glucose level at 0, 4, and 24 h during Cont Po, Cont Pe, Cap Po and CapPe
Data represent means±SE. The plasma glucose level was increased after glucose infusion for 4 hours, but decreased after infusion for 24 hours to a level similar to that at 0 hours. No significant difference was noted in the plasma glucose level among the 4 groups.

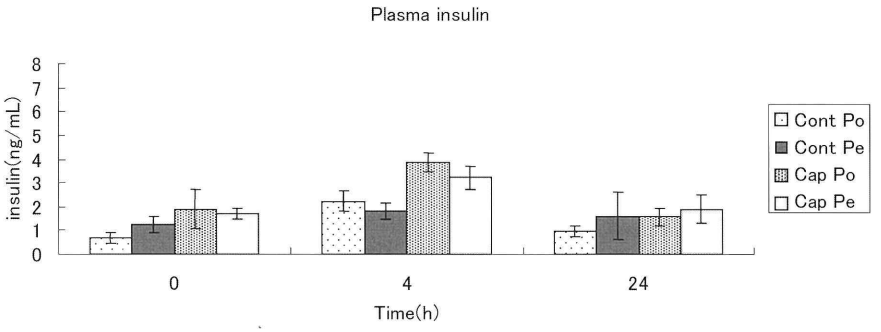

Fig. 3 Plasma insulin concentration at 0, 4, and 24h during Cont Po, Cont Pe, Cap Po and Cap Pe
Data represent means±SE. The plasma insulin level was increased after glucose infusion for 4 hours, but the level after infusion for 24 hours was similar to that at 0 hours. The plasma insulin level after infusion for 4 hours was slightly higher in the capsaicin-applied groups (Cap), but no significant difference was noted among the 4 groups.

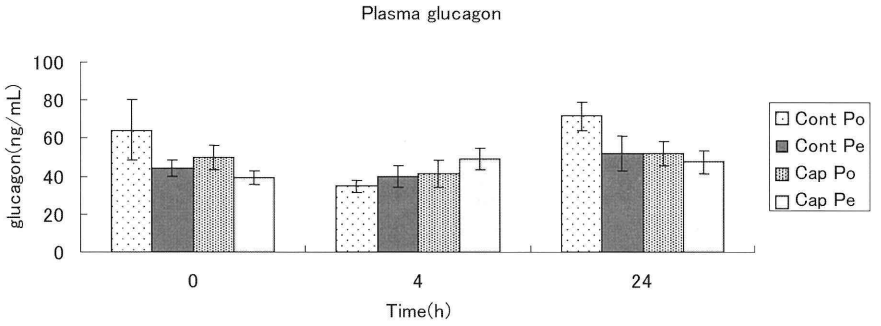

Fig. 4 Plasma glucagon concentration at 0, 4, and 24 h during Cont Po, Cont Pe, Cap Po and Cap Pe
Data represent means±SE. Changes in the plasma glucagon level were opposite to changes in the plasma glucose and insulin levels. The level was slightly decreased after the 4-hour glucose infusion. No significant difference was noted in the plasma glucagon level among the 4 groups.

any duration of glucose infusion. The insulin level after the 4-hour infusion was slightly higher in the 2 capsaicin groups (CapPo and CapPe) than in the groups without capsaicin treatment (Fig. 3). Changes in the plasma glucagon level were opposite to the changes in the plasma glucose and insulin levels, and levels were slightly decreased after the 4-hour infusion. No significant difference was noted in the plasma glucagon level among the 4 groups (Fig. 4). The liver glycogen content was nearly 0 at 0 hours, and significantly increased after glucose infusion for 4 hours. The content was further increased after the 24-hour infusion. The increase at 4 hours was not significantly different among the 4 groups. However, the liver glycogen content was significantly higher in the ContPo group than in the ContPe group after infusion for 24 hours, suggesting the action of the portal signal. In contrast, the liver glycogen contents of the CapPo and CapPe were not significantly different, suggesting that the portal signal did not act.

Discussion

When glucose was continuously infused for 24 hours in the groups without application of capsaicin, the liver glycogen content was significantly higher in the ContPo group than in the ContPe group. In the capsaicin-applied groups, no significant difference was noted in the liver glycogen content between the CapPo and CapPe groups, and the content was similar to that in the ContPe group. Therefore, the vagus nerve afferent branch seems to involve the pathway through which the information of portal glucose contents transmits to the regulating mechanisms of the liver glycogen synthesis.

The amount of glucose that reaches the liver is a factor that affects liver glycogen synthesis, and it is calculated from the blood glucose level and blood flow. Although the blood flow was not measured in this study, no significant difference in the blood flow was noted between glucose administrations via the jugular vein and portal vein in our previous study performed using the same procedure (11). The plasma glucose levels in the ContPo and ContPe groups were similar (Fig. 2), suggesting that the amounts of glucose that reached the liver in these 2 groups without capsaicin treatment were similar. Since no significant differences were noted in the insulin and glucagon levels between these 2 groups after any duration of glucose infusion, the difference in the glycogen content between these 2 groups may have been

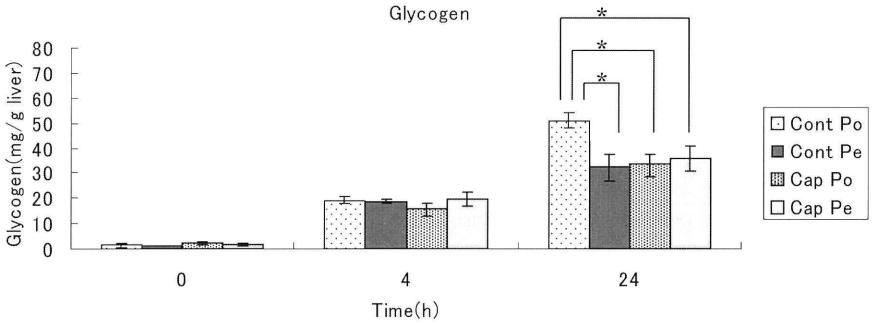

Fig. 5 Net hepatic glycogen synthesis at 0, 4, and 24 h during Cont Po, Cont Pe, Cap Po and Cap Pe
Data represent means±SE. $*P>0.05$
A significant difference was noted in the glycogen content after the 24-hour glucose infusion between the 2 routes of administration in the group without capsaicin treatment, and the content was significantly higher in the portal administration group (ContPo). However, the difference between the 2 routes of administration was lost in the capsaicin-applied group, and the content in the capsaicin-applied portal administration group (CapPo) was similar to that in the jugular administration group without capsaicin treatment (ContPe).

Table 1

		Glucose (mg/dL)	Insulin (ng/mL)	Glucagon (ng/dL)	Glycogen (mg/g liver)
0 h	ContPo	129.5±3.3	0.7±0.2	64±15.8	1.4±0.6
	ContPe	130.8±5.6	1.2±0.3	44±4.1	0.9±0.4
	CapPo	133.2±4.0	1.9±0.8	49.8±6.2	2.5±0.5
	CapPe	123.1±7.7	1.7±0.2	39±3.6	1.6±0.8
4 h	ContPo	186.7±7.8	2.2±0.4	34.7±3.2	19.2±1.5
	ContPe	191.3±10.4	1.8±0.3	39.7±5.5	18.5±0.9
	CapPo	188.6±12.7	3.9±0.4	41.3±7.1	15.5±2.5
	CapPe	203.4±16.9	3.2±0.5	48.8±5.8	19.6±2.6
24 h	ContPo	127.1±6.8	1±0.2	71.5±7.5	51.1±2.9
	ContPe	131.5±8.8	1.6±1.0	52±9.2	32.3±5.3
	CapPo	122.0±6.7	1.6±0.4	51.8±6.6	33.8±3.9
	CapPe	131.0±6.7	1.9±0.6	47.2±5.8	35.6±5.0

due to the portal signal. Although the blood flow with capsaicin treatment was not measured in this study, it has been reported that the liver blood flow was not significantly different between the groups with and without vagectomy (20), suggesting that the amount of glucose that reached the liver was not significantly different between the 2 capsaicin groups. Furthermore, the liver glycogen contents in the ContPe and CapPe groups were similar, suggesting that capsaicin did not change the blood flow.

The liver glycogen content after glucose infusion for 24 hours was significantly lower in the CapPo group than in the ContPo group. The insulin level after infusion for 4 hours was slightly higher in the CapPo group than in the ContPo group (Fig. 3). The glucagon level after infusion for 24 hours was slightly lower in the CapPo group than in the ContPo group (Fig. 4). Since insulin increases liver glycogen, and glucagon decreases liver glycogen, the lower liver glycogen content in the CapPo group than in the ContPo group may not have been due to the effects of insulin and glucagon, but due to loss of the portal signal.

These findings suggested that blockage of the vagus nerve afferent branch by the application of capsaicin blocked a portal signal-induced increase in glycogen synthesis.

There have been many reports of the involvement of the vagus nerve in the portal signal, but its transmission pathway has not been clarified, and there has been no previous report of the portal signal being investigated by blockage of the vagus nerve through the application of capsaicin. Chellington and Chardin et al. performed an experiment involving selective blockage of the vagus nerve efferent branch by cooling the vagus nerve in dogs, based on the electrophysiological study reported by Niijima et al. (18), and found that the blockage did not change NHGU (19). Thus, the involvement of the central nervous system and the vagus nerve efferent

branch was negative in their study, although the involvement of the vagus nerve afferent branch in the portal signal could not be ruled out.

We previously confirmed that the firing of the portal signal induced intrahepatic translocation of glucokinase (Tonyobyo 0021-437X, vol. 48, Suppl. 2, page S157, 2005.04), and this change was also caused by an increase in the cerebral glucose level, suggesting the involvement of the central nervous system in portal signal transmission. We assume that the portal signal is transmitted to the central nervous system through the vagus nerve afferent branch, acts on the liver through the efferent branch, and induces changes in intrahepatic enzymes (such as glucokinase).

To confirm the involvement of central nervous system in the portal signal transmission, further studies physiological studies measuring hypothalamus functions during the portal signal occurrence is awaited.

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門脈シグナルによる肝グリコーゲン合成促進作用と迷走神経求心枝の関係

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【要旨】 肝臓に到達する血管は、消化管から来る門脈と、再循環を経て来る肝動脈の二つの経路がある。門脈と肝動脈からそれぞれブドウ糖を投与し、同量のブドウ糖が肝臓に到達するとき、門脈投与の方がより多くのブドウ糖を取り込み、またより多くのグリコーゲンを合成する。この生理現象を門脈シグナルと呼んでいる。

我々の以前の検討においては、ビーグル犬、ラットで迷走神経を遮断 (ビーグル犬はアトロピン投与、ラットは切断) した場合に、犬では同量のブドウ糖注入を行っても、肝糖放出量から肝注入量を引いた、net hepatic glucose uptake (NHGU) が、ラットでは、肝グリコーゲン量が減少するという結果を得ている (未発表)。したがって迷走神経が門脈シグナルと関係していると考えられる。新島らの研究においては、門脈にブドウ糖を注入した場合にのみ門脈壁の迷走神経求心枝の電気的パルスが濃度依存性に減少し、膵遠心枝ではパルスが増加するという報告がなされている。この研究からも門脈シグナルと迷走神経の関係が示唆される。

横隔膜下迷走神経は 90% が髄鞘を持たない求心繊維 (感覚神経) で構成されており、カプサイシン感受性である。そこで我々は、迷走神経求心枝を選択的に遮断するカプサイシンを用い、門脈シグナルによる肝グリコーゲン合成と迷走神経との関係をラットを用いて検討した。

カプサイシン非塗布群においては、24 時間のブドウ糖持続注入は、門脈経由群が、頚静脈経由群よりも有意に多く肝グリコーゲンを合成した。カプサイシン塗布群において、門脈経由群と頚静脈経由群の肝グリコーゲン量には有意差はなく、カプサイシン非塗布群における頚静脈経由群の肝グリコーゲン量と同程度であった。

以上より、門脈シグナルによる肝グリコーゲン合成には、迷走神経求心枝を介する事が示唆された。

〈**キーワード**〉 肝グリコーゲン、門脈シグナル、迷走神経求心枝、カプサイシン