Mechanism of rapid-phase insulin response to elevation of portal glucose concentration

Makiko Fukaya,1* Akira Mizuno,2* Hidekazu Arai,1 Kazusa Muto,1 Takashi Uebanso,1 Kaoru Matsuo,1 Hironori Yamamoto,1 Yutaka Taketani,1 Toshio Doi,2 and Eiji Takeda1

1Departments of Clinical Nutrition, and 2Clinical Biology and Medicine, University of Tokushima School of Medicine, Tokushima, Japan

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To clarify these relationships, we examined whether the pattern of insulin response during continuous infusion of low amounts of glucose into the portal vein was different from the response after infusion into a peripheral vein, whether the sensing for specific monosugars in the hepatoportal region could induce a rapid-phase insulin response, and what stimulates insulin response via glucose sensing.

It has been suggested that glucose sensors representing the hepatoportal region detect a glucose gradient between the portal vein and arterial blood (16, 17, 23) and stimulate glucose utilization and glycogen storage in the liver (34, 35) during glucose perfusion into the portal vein, and that these responses are mediated by autonomic nerves. Guarino et al. (20) also suggested that parasympathetic nerves in the liver control peripheral insulin sensitivity via an NO-dependent pathway. Some reports have shown that normalizing glucose concentration in the portal vein in the presence of systemic hypoglycemia severely blunted the sympathoadrenal counterresponse to hypoglycemia (14, 22, 29). Furthermore, Carlsson et al. (9) demonstrated that intraportal glucose infusion induced elevation of islet blood flow in anesthetized rats. Thus, it would appear that glucose sensing in the hepatoportal region as well as insulin sensitivity of peripheral tissues are important for glucose regulation via insulin secretion from the endocrine pancreas.

To clarify these relationships, we examined whether the pattern of insulin response during continuous infusion of low amounts of glucose into the portal vein was different from the response after infusion into a peripheral vein, whether the sensing for specific monosugars in the hepatoportal region could induce a rapid-phase insulin response, and whether this glucose-sensing system could be affected by the autonomic nervous system.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats weighing ~350 g were obtained from a local breeding colony (Japan SLC, Shizuoka, Japan) and used in all studies. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

* M. Fukaya and A. Mizuno contributed equally to this work.

Address for reprint requests and other correspondence: A. Mizuno, Depts. of Clinical Biology and Medicine, Univ. of Tokushima School of Medicine, 3-18-15 Kuramoto, Tokushima, 770-8503, Japan (e-mail: mizuno@clin.med.tokushima-u.ac.jp or fukaya@nutr.med.tokushima-u.ac.jp).

THE MAINTENANCE OF GLUCOSE HOMEOSTASIS, which is essential for daily life, is regulated by the hormonal and autonomic nervous systems, especially by insulin secretion (43). In healthy subjects, intravenous glucose infusion induces an immediate increase in plasma insulin concentration that peaks from 3 to 5 min after infu-
sion and is called “first-phase insulin secretion” (18). Furthermore, continuous infusion of glucose leads to a rapid and simultaneous insulin response observed at 3 min after intraportal infusion of a small amount of glucose but not after intrajugular infusion. Furthermore, this insulin response was also induced by intraportal fructose infusion but not by nonmetabolizable sugars. The rapid-phase insulin response at 3 min during intraportal infusion did not differ between rats that had undergone hepatic vagotony or chemical sympathectomy with 6-hydroxydopamine compared with control rats, but this response disappeared in rats that had undergone chemical vagotomy with atropine. We conclude that the elevation of glucose concentration in the hepatoportal region induced different signals from undetectable sensors and that these signals stimulate pancreas to induce the rapid-phase insulin response via cholinergic nerve action. The maintenance of glucose sensing is mediated by autonomic nerves. Guarino et al. (20) also suggested that parasympathetic nerves in the liver control peripheral insulin sensitivity via an NO-dependent pathway. Some reports have shown that normalizing glucose concentration in the portal vein in the presence of systemic hypoglycemia severely blunted the sympathoadrenal counterresponse to hypoglycemia (14, 22, 29). Furthermore, Carlsson et al. (9) demonstrated that intraportal glucose infusion induced elevation of islet blood flow in anesthetized rats. Thus, it would appear that glucose sensing in the hepatoportal region as well as insulin sensitivity of peripheral tissues are important for glucose regulation via insulin secretion from the endocrine pancreas.
experiments. The rats had free access to water and food (MF; Oriental Yeast, Tokyo, Japan) at all times. All rats were cared for in accordance with the National Institutes of Health (NIH) Guidelines for Care and Use of Laboratory Animals. The rats that recovered their preoperational weight and exhibited normal activity were used.

**Surgical Preparation and Pretreatment**

**Catheterization.** The rats were anesthetized with pentobarbital sodium (50 mg/kg body wt ip). Polyethylene catheters (sp 8; Natsume, Tokyo, Japan) were inserted into the portal vein through the splenic vein 9 days before experiments, and silicon rubber catheters (FT-025; Bio-Medica, Osaka, Japan) were inserted into the left jugular vein and left femoral vein 5 days before experiments, and all lines of the catheters were led out through subcutaneous tissue to an IVH kit (Bio-Cannula; Bio-Medica).

**Hepatic vagotomy.** The abdominal cavity of the rat was opened with a midline incision. When the hepatic vagotomy was performed, special care was taken to ensure that no nervous tissue remained between the liver and esophagus from the esophagus plexus to the cardia of the stomach. This denervation was performed with the aid of a microscope to facilitate visualization of all nerve fibers. After all experiments, we opened rats’ abdominal cavity and ensured the hepatic vagotomy under a microscope.

**Chemical vagotomy.** Atropine is a peripherally acting muscarinic blocker. According to the method described previously (3), atropine (Sigma Chemical, St. Louis, MO), which was dissolved in saline, was administered intravenously at 0.5 mg/kg body wt 10 min before glucose administration. The control group was given only saline.

**Chemical sympathectomy.** 6-Hydroxydopamine (6-OHDA) is known to selectively destroy sympathetic nerve terminals. According to the method described previously (30), 6-OHDA (Sigma Chemical), which was dissolved in ice-cold 0.9% saline containing ascorbic acid (57 mM; Merck, Darmstadt, Germany), was administered intravenously at a dose of 0.19 mmol/kg body wt 48 h before the tests. The control group was given only the vehicle (0.9% saline containing 57 mM ascorbic acid).

**Experimental Procedures**

Each of the 14 rats received intraportal (n = 7) or intrajugular (n = 7) glucose, mannose, fructose, or galactose infusion for 60 min; another group of 14 rats received intraportal (n = 7) or intrajugular (n = 7) glucose infusion for 1.5 min in small amounts (total 30 μmol/kg body wt). Each of 6 rats received intraportal infusion of mannitol, 3-O-methylglucose (3-OMG), or 2-deoxy glucose (2-DG) for 60 min. The hepatic vagotomized (Hx) rats (n = 7), sham-operated (Sham) rats (n = 7), rats treated with atropine (Atropine, n = 6), and control (Control) rats (n = 6), rats treated with 6-OHDA (6-OHDA, n = 6) and control (Vehicle) rats (n = 6) received intraportal glucose infusion for 60 min. Blood samples were drawn from the femoral vein at −6, 0, 3, 6, 9, 15, 30, 45, and 60 min during the infusion study. After 18 h of fasting, all rats received intraportal or intrajugular infusions of physiological saline for 1 h to attain a stable state; then one group received infusion of 20% glucose (70 μmol·kg body wt−1·min−1) for 60 min or 1.5 min, and the other groups received intraportal infusions with the same concentrations of various sugars for 60 min.

**Assays**

Plasma glucose was determined by the glucose dehydrogenase method using Accu Check (Roche Diagnostics, Mainz-Hechtsheim, Germany), and plasma insulin was measured with a commercially available enzyme-linked immunosorbent assay kit (Morinaga, Yokohama, Japan).

**Calculation and Statistical Analysis**

All data are represented as means ± SE. The statistical significance of differences was examined by analysis of variance followed by Student’s t-test for individual comparison of mean values.

**RESULTS**

**Changes in Plasma Glucose and Insulin Levels During Intraportal or Intrajugular Glucose Infusion**

The changes in plasma glucose and insulin levels in the femoral vein during intraportal and intrajugular glucose infusion at the rate of 70 μmol·kg body wt−1·min−1 are shown in Fig. 1. During intraportal or intrajugular infusion of glucose, the plasma glucose concentration at 3 min was unchanged (5.0 ± 0.2 vs. 4.8 ± 0.2 mmol/l, respectively), however, the plasma insulin level during intraportal glucose infusion was significantly higher than that recorded during
intrajugular infusion (401.3 ± 7.4 vs. 291.2 ± 32.2 pmol/l, respectively, \( P < 0.01 \)). At 6 min, the plasma insulin level dropped rapidly during intraportal infusion, whereas the insulin levels gradually increased during intrajugular infusion.

The elevation of glucose levels and the rapid insulin response were assessed by intraportal and intrajugular infusion of small amounts (total 30 \( \mu \)mol/kg body wt) of glucose infusion (Fig. 2). Plasma glucose levels did not increase during intraportal or intrajugular infusion. A remarkable insulin response was observed during intraportal infusion but not during intrajugular infusion.

**Changes in Plasma Glucose and Insulin Levels During Infusion of Various Sugars**

To assess whether the hepatoporal sensor is glucose specific, various monosugars were infused into the portal vein.

![Fig. 2. Changes in plasma glucose levels (A) and insulin levels (B) during intraportal or intrajugular glucose infusion for 1.5 min as small amount (total 30 \( \mu \)mol/kg body wt). Data represent means ± SE for 7 rats per group. *\( P < 0.05 \) vs. intrajugular infusion.](image1)

![Fig. 3. Changes in plasma glucose levels (A) and insulin levels (B) during intraportal or intrajugular fructose infusion. Data represent means ± SE for 7 rats per group. *\( P < 0.05 \) vs. intrajugular infusion.](image2)

The changes in plasma glucose and insulin levels during intraportal or intrajugular infusion of fructose are shown in Fig. 3. Plasma glucose levels increased slightly, but no significant differences were seen between intraportal and intrajugular infusions. In contrast, the plasma insulin concentration at 3 min after intraportal fructose infusion was significantly higher than that seen after intrajugular infusion (357.4 ± 42.2 vs. 257.3 ± 13.1 pmol/l, respectively, \( P < 0.05 \)). Glucose, mannose, or fructose infusion into the portal or jugular vein increased insulin levels 3 min after infusion (the rapid-phase insulin response), but the levels after portal infusion were higher than those seen after jugular infusion. In contrast, galactose infusion into the portal or jugular vein did not stimulate insulin secretion (401.3 ± 17.4, 387.7 ± 33.7, 351.4 ± 11.6, and 235.4 ± 12.6 pmol/l, respectively; Fig. 4A).

To address the possibility that the rapid phase of insulin response could be induced by osmolar changes or mediated by GLUT2 or phosphorylation by glucokinase, the nonmetaboliz-
able sugars 3-OMG, 2-DG, and mannitol, were infused into the portal vein (Fig. 4B). No insulin response was seen at 3 min to these nonmetabolizable sugars.

**Effect of Hepatic Vagotomy or Chemical Vagotomy on Insulin Response During Intraportal Glucose Infusion**

To determine the relationship between the hepatoportal glucose sensor and the vagus afferent tract in the insulin response, intraportal glucose infusion was conducted in Hx rats (Fig. 5). The levels of plasma glucose and insulin at 3 min were not significantly different between Hx and Sham rats (plasma glucose $5.4 \pm 0.1$ vs. $5.2 \pm 0.2$ mmol/l, plasma insulin $524.2 \pm 39.6$ vs. $511.3 \pm 49.6$ pmol/l, respectively). However, the insulin level at 6 min dropped rapidly in Sham rats but not in Hx rats ($359.0 \pm 26.9$ vs. $550.2 \pm 40.6$ pmol/l, respectively, $P < 0.05$). Subsequently, the insulin levels in Hx rats remained higher than those in Sham rats from 6 min to 30 min during intraportal glucose infusion.

Hepatic vagotomy had no effect on the insulin response at 3 min during intraportal glucose infusion, which suggested that activation of hepatic afferent vagus nerves did not contribute to stimulating the rapid-phase insulin response. To determine whether effenter vagus nerves had any effect on the insulin response, intraportal glucose infusion was examined in Atropine and saline-treated (Control) rats (Fig. 6). There were no significant differences in the levels of plasma glucose at 3 min ($6.0 \pm 0.4$ vs. $5.2 \pm 0.2$ mmol/l, respectively); however, the levels of plasma insulin were significantly lower in Atropine rats than in Control rats ($249.1 \pm 14.3$ vs. $391.4 \pm 15.4$ pmol/l, respectively, $P < 0.0001$), and the insulin responses during intraportal glucose infusion were diminished in Atropine rats at all time points. Eventually, the levels of plasma glucose in
Atropine rats were higher than those in Control rats at 30 min during intraportal glucose infusion (8.9 ± 0.4 vs. 7.5 ± 0.3 mmol/l, respectively, \( P < 0.05 \)).

**Effect of Chemical Sympathectomy on Insulin Response During Intraportal Glucose Infusion**

To examine whether sympathetic adrenergic nerve stimulation was associated with the hepatoporal glucose sensor, the insulin response was examined during intraportal glucose infusion in 6-OHDA and Vehicle rats (Fig. 7). There were no significant differences in levels of plasma glucose and insulin at 3 min between 6-OHDA and Vehicle rats (plasma glucose 5.7 ± 0.2 vs. 5.2 ± 0.2 mmol/l, plasma insulin 445.8 ± 46.4 vs. 511.3 ± 49.6 pmol/l, respectively). Moreover, no significant difference was seen at any time between the two groups.

**DISCUSSION**

In this study, we have demonstrated that the rapid phase of the insulin response occurs via elevation of the portal glucose concentration during intraportal glucose infusion. This response was also seen during intraportal infusion of mannose or fructose but not during intraportal infusion of galactose or nonmetabolizable sugars.

The elevation of the plasma glucose level in peripheral blood was less than 0.7 mmol/l at 3 min during 20% glucose infusion into the portal vein at the rate of 70 \( \mu \)mol·kg body wt\(^{-1}\)·min\(^{-1}\). It has been reported that insulin secretion could not be induced in rat islets if the elevation of plasma glucose from the basal level was less than 1.0 mmol/l (25). Therefore, the elevation of the plasma glucose level in our experimental condition was too small to directly stimulate insulin secretion from the endocrine pancreas. Although the plasma glucose
level increased very little during intraportal glucose infusion, a rapid rise in the plasma insulin level was observed at 3 min followed by a rapid drop at 6 min, and these phenomena were seen only during intraportal infusion. The rapid-phase insulin response was probably induced by the elevation of the portal glucose concentration, because it was not seen during intragastric glucose infusion, despite the fact that the peripheral plasma glucose level was not different from that seen during intraportal infusion of glucose. Furthermore, we also examined short-time glucose infusion (for 1.5 min; total 30 μmol/kg body wt), in which the amount of glucose could not lead to elevation of the peripheral glucose level, to clarify whether the rapid-phase insulin response at 3 min was truly induced by the elevation of glucose concentration in the portal vein. Previous observations suggested that activation of the portal glucose sensor was needed for establishing a portal-peripheral glucose gradient (16, 17, 23). The glucose concentration in the portal vein at 3 min was elevated more than 1.0 mmol/l compared with the basal level (we measured the glucose concentration directly in the portal vein during glucose infusion using the dual-catheter method; data not shown), and a relative portal-peripheral glucose gradient could be attained, which subsequently could activate the portal glucose sensor.

It has been reported in studies of humans and rats that the preabsorptive, or cephalic-phase, insulin response, which was induced by meal ingestion, might last for about 10 min before a significant increase of peripheral plasma glucose level occurred (4, 5). Ahren and Holst (1) suggested that the cephalic-phase insulin response was more closely correlated with glucose tolerance than was the 30-min insulin response to an oral glucose load. They also showed that the cephalic-phase insulin response was mediated by the autonomic nervous system, but they did not refer to the location of the glucose sensing site. We were able to detect the occurrence of a much faster phase (rapid phase) insulin response and showed the association with a potential glucose-sensing site in the hepatoportal region. This rapid-phase insulin response is probably associated with the cephalic insulin response and glucose homeostasis.

Various monosugars were infused at the same dosages as glucose to assess whether the rapid-phase insulin response mediated by the portal glucose sensor was glucose specific. The rapid-phase insulin response was also induced by mannose and fructose but not by galactose. Mannose was well metabolized in the pancreatic β-cells (40) and enhanced insulin secretion (2, 11, 28), whereas fructose could not pass the blood-brain barrier (19, 36, 38) and could not provoke insulin secretion in a perfused rat pancreas (11, 28). Accordingly, mannose and fructose may need to activate the portal glucose sensor to provoke the rapid-phase insulin response.

Galactose could change osmolarity in the portal vein in the same way as glucose does. Also, although the metabolism of galactose is different from that of glucose, galactose metabolites might interfere with the portal glucose-sensing system and stimulate insulin secretion. In this case, it is therefore not conclusive just to mention that the insulin response was not induced via osmolar changes; to justify this conclusion it must also be stated that galactose did not stimulate insulin secretion.

Changes in flux and osmolarity did not affect the rapid-phase insulin response, because it was not observed during intraportal infusion of mannitol. It has been reported that GLUT2 or glucokinase played important roles in the portal glucose-sensing system (7). It is well known that 2-DG and 3-OMG are transported into hepatocytes by GLUT2 (10) and that 2-DG, but not 3-OMG, is phosphorylated by glucokinase in hepatocytes (10, 44). Therefore, because nonmetabolizable monosugars did not induce the rapid-phase insulin response, transportation and phosphorylation of monosugars would not be crucial factors for the glucose sensing in the portal region.

The effect of the autonomic nervous system on the rapid-phase insulin response was examined, since many researchers have reported that vagus nerve stimulation would affect the portal glucose-sensing system. Because of the change in the firing rate of hepatic afferent nerves induced by intraportal glucose infusion, it was suggested that the increase in glucose clearance might be due to an activation of the autonomic nervous system (47, 48). In our study, a rapid-phase insulin response occurred, and no difference between Hx and Sham rats was seen in plasma glucose and insulin levels at 3 min after glucose infusion, however, a rapid drop of the insulin level at 6 min was seen in Sham rats but not in Hx rats. It was reported that the hepatic afferent vagus nerve’s discharge rate was decreased following intraportal glucose infusion and that the reduction in the discharge rate might decrease the activity of the adrenal nerve and increase the activity of the pancreatic vagus nerve, resulting in induction of insulin secretion by this nervous system (33). The drop in the plasma insulin level at 6 min disappeared after hepatic vagus denervation, which could suggest that the drop occurred as a result of hepatic vagus suppression. The high plasma level of insulin was maintained for up to 30 min in Hx rats compared with Sham rats. Previous reports demonstrated that elevation of plasma insulin levels in the portal vein induced hepatic insulin-sensitizing substance (HISS), which stimulates glucose uptake in peripheral tissues, and that the effect of HISS was abolished by hepatic vagotomy during portal glucose infusion (26, 27). Those authors also concluded that activation of the hepatic vagus nerve could be associated with peripheral insulin sensitivity (26). In the present study, we confirmed that the insulin level in the portal vein was about 200 pmol/l higher than that in the peripheral vein 3 min after intraportal glucose infusion (data not shown). Thus, the rapid-phase insulin response could act on the liver and induce immediate suppression of hepatic glucose output and subsequently be responsible for the upregulation of insulin sensitivity in peripheral tissues via HISS.

Although the hepatic vagus afferent nerve affected the rapid-phase insulin response, we considered whether it might not be necessary for this response system. It was reported that the reduction in hepatic vagus afferent activity was induced specifically by glucose (32); however, in our study intraportal mannose and fructose infusion could also induce the rapid-phase insulin response in normal rats. Furthermore, denervation of the hepatic vagus nerve did not affect the rapid elevation of plasma insulin at 3 min during intraportal glucose infusion. The 6-OHDA, which is toxic to adrenergic nerves (46), also did not affect the rapid-phase insulin response. These observations suggest that the crucial event in the rapid-phase insulin response is mediated by other nerve systems than the hepatic vagus and adrenergic nerves. A recent study showed that hypoglycemic detection at a portal region was mediated by spinal nerves but not by a vagal glucose-sensitive afferent nerve (15, 24). Furthermore, another report also suggested that an unknown neural mechanism might be located between the...
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