Hyper-Response of Insulin Release to Arginine in Streptozotocin-Induced Diabetic Rats with Hepatic Vagotomy

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Abstract

Vagal glucose receptor and arginine sensor have been found to exist in the liver of normal rats. The present study was designed to examine whether these sensory receptor systems function in streptozotocin-induced mildly diabetic rats. One week after streptozotocin injection (35 mg/kg body wt. iv), hepatic vagotomy and installation of a jugular vein catheter were performed. Three days after the surgery, intraperitoneal glucose (1 g/kg body wt.) or arginine (1 g/kg body wt.) stimulation was done with the rat in an unanesthetized and unrestrained state. Hepatic vagotomy significantly reduced glucose-induced insulin release with an increase in plasma glucose in control and diabetic rats. The rate of reduction of plasma insulin release was similar in the two groups. Hepatic vagotomy also significantly enhanced arginine-induced insulin release in both groups, but in control rats there was no change in the response of plasma glucose, while in diabetic rats there was a significant reduction. The rate of enhancement of plasma insulin release by hepatic vagotomy was significantly greater in diabetic than in control rats. The results indicated that streptozotocin-induced mildly diabetic rats have hyperfunction of the arginine sensor and normofunction of the glucose receptor and modulate insulin release through these receptor systems in the liver.

There is convincing evidence of a variety of hepatic sensory receptors (Lautt, 1980; Sawchenko, 1979). Glucose is the most potent stimulator of insulin release. Russek (Russek, 1963), on the basis of his behavioral studies, suggested the existence of

a neural glucoreceptor in the liver. Nijima (Nijima, 1969), with an isolated perfused liver vagus preparation, recorded afferent discharges and found they were inversely related to the glucose concentration in the perfusion fluid. Schmitt (Schmitt, 1973) reported the effects of portal glucose injection on certain hypothalamic neurons. Nijima (Nijima, 1980) further observed that the injection of glucose into the portal vein increased the efferent activity of the pancreatic vagus nerve in the guinea pig.

Received March 22, 1990

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Recently, Lee and Miller (Lee and Miller, 1985) reported that the exaggeration of insulin release after glucose stimulation is mediated by the hepatic branch of the vagus nerve. These results indicate that the glucose receptor system in the liver modulates insulin release through the afferent vagus in the physiological condition.

Arginine has been recognized as the most potent amino acid for the stimulation of insulin and glucagon release (Floyd, 1966). We have recently found that insulin and glucagon release by intraperitoneal arginine was enhanced after selective sectioning of the hepatic branch of the vagus nerve in the physiological condition (Tanaka *et al.*, 1986a), and intraportal injection of arginine increased the afferent discharge rate of the hepatic branch of the vagus nerve with a slight decrease in the efferent discharge rate of the pancreatic vagal branch and an increase in the efferent discharge rate of the pancreatic splanchnic branch (Tanaka *et al.*, 1986b). Based on these findings, we have hypothesized that the arginine sensor system, similarly to the glucose receptor system in the liver, modulates insulin and glucagon release through the afferent vagus.

It is very interesting to investigate whether these receptor systems function under pathological conditions. In the present study, we investigated whether, through afferent information from the hepatic vagus nerve, these systems take part in the insulin release from the pancreas after glucose or arginine stimulation in mildly diabetic animals with a reserve of insulin release.

Materials and Methods

Animals

A total of 126 female Sprague-Dawley rats, weighing 240–260 g, were used for the present study. All animals received a pelleted laboratory chow diet (Oriental Yeast Co., Tokyo, Japan)

and had free access to tap water. They were housed in an environment with a constant temperature ($22 \pm 2^\circ\text{C}$) and a 12-h light-dark cycle. Body weight was measured weekly.

Experimental procedures

1) Induction of diabetes

Rats received an intravenous injection of streptozotocin (STZ) (35 mg/kg) (Upjohn Co., Kalamazoo, Mich, USA) freshly dissolved in 0.5 ml of citrate buffer. Seven days later, the diabetic rats with fed blood glucose levels between 150 and 200 mg/dl were used for the experiments.

2) Surgery

Hepatic vagotomy was accomplished as previously described (Tanaka *et al.*, 1986a). Under hexobarbital anesthesia (50 mg/kg), the hepatic branch of the vagus nerve, which clearly branches off the main vagal trunk a few millimeters proximal to the cardia, was exposed and completely sectioned at the proximal end under a binocular microscope. Sham vagotomy was achieved by the same surgical procedure except sectioning of the vagus nerve. Immediately after the hepatic and sham vagotomies, a polyethylene-10 catheter (Clay Adams Co., Parsippany, NJ, USA) was installed as a jugular vein catheter, the open end of which was exposed to the posterior neck through the subcutaneous tissue (Shah *et al.*, 1977).

3) Experiments

Three days after the surgery, two experiments were performed with the rats in an unanesthetized and unrestrained state after 18 h of overnight food deprivation.

Exp. 1 Intraperitoneal glucose stimulation (1 g/kg): Warmed (37°C) glucose solution (1 g/kg body wt., 20%, w/v) was injected intraperitoneally. Five-tenths of a milliliter of blood was withdrawn from the jugular vein catheter before and 5, 10, 15, 30, 60 min after the injection to measure plasma glucose and insulin, and was poured into chilled tubes each containing 4 mg EDTA.

Exp. 2 Intraperitoneal arginine stimulation (1 g/kg): Warmed arginine solution (1 g/kg body wt. 25%, w/v; pH 7.4) was injected intraperitoneally. Five-tenths of a milliliter of blood was withdrawn from the jugular vein catheter before and 5, 10, 15, 30, 60 min after the injection to measure plasma glucose and insulin, and

Table 1. Body weight, fed plasma glucose and pancreatic insulin content

	Body weight (g)		Fed plasma glucose	Insulin content
	at vagotomy	at experiment	(mg/dl)	(ng/g)
Control rats				
sham vagotomy	252.7±2.2	253.0±2.0	118.8±4.3	69.6±1.9
hepatic vagotomy	251.0±1.9	251.1±3.1	116.3±5.2	71.0±2.1
Diabetic rats				
sham vagotomy	248.5±2.0	249.2±1.9	166.0±4.0*	19.5±1.4*
hepatic vagotomy	248.3±1.5	248.8±1.8	167.8±4.0*	18.6±1.4*

Values are means±SEM.

* P<0.01 compared to control rats.

was poured into chilled tubes each containing 4 mg EDTA.

Immediately after each blood sampling, an equivalent amount of heparinized blood was replaced to minimize the effect of blood volume depletion (Berthoud *et al.*, 1986). The plasma was separated immediately and stored at -80°C until the assay.

4) Pancreatic insulin content

After the experiments, the pancreas was removed from each rat and weighed. Pancreatic insulin content was measured in a specimen after acid-alcohol extraction (Becker and Sha, 1969).

5) Verification of hepatic vagotomy

At the end of the experiments, the accomplishment of hepatic vagotomy was checked in all the rats with hepatic vagotomy by means of a binocular microscope.

Assay

Glucose was measured by means of a Beckman glucose analyzer II employing the glucose oxidase method. Immunoreactive insulin was measured by the modified double antibody method of Hales and Randle (Hales and Randle, 1963) with rat insulin as a standard (insulin assay kit, Amersham, Japan).

Statistical analysis

The data are depicted in the figures and tables as the mean±SEM. The analysis of variance was employed to compare four groups. When the F values proved to be significant, Student's *t*-test was used to identify significant

Fig. 1. Plasma glucose and insulin after i.p. glucose (1 g/kg) in control rats with sham vagotomy (○) and hepatic vagotomy (●). * P<0.05, ** P<0.01 compared to values at corresponding times in sham-vagotomized rats. Each point is mean±SEM., n=6.

differences between the groups. This test was also employed to compare two groups. The level of significance was $p < 0.05$.

Results

Characteristics of STZ-induced Diabetic Animals

Table 1 shows the body weight, fed plasma glucose and pancreatic insulin content of the animals which were subjected to the various experiments. There was no effect of hepatic vagotomy on the body weight of either diabetic or control rats.

The average amount of plasma glucose fed to diabetic rats was 49.4 mg/dl higher than that fed to controls. Pancreatic insulin content in diabetic rats was significantly lower than that of control rats ($p < 0.01$). However, there was no significant difference between the amount of pancreatic insulin in hepatic vagotomized and sham vagotomized groups in both diabetic and control rats.

Intraperitoneal Glucose Stimulation

In control rats, plasma glucose was significantly higher at 5, 10 and 15 min,

Fig. 2. Plasma glucose and insulin after i.p. glucose (1 g/kg) in STZ-induced diabetic rats with sham vagotomy (○) and hepatic vagotomy (●). * $P < 0.05$, ** $P < 0.01$ compared to values at corresponding times in sham-vagotomized rats. Each point is mean $\pm$ SEM., $n = 6$.

Fig. 3. Plasma glucose and insulin after i.p. arginine (1 g/kg) in control rats with sham vagotomy (○) and hepatic vagotomy (●). * $P < 0.05$, ** $P < 0.01$ compared to values at corresponding times in sham-vagotomized rats. Each point is mean $\pm$ SEM., $n = 6$.

Table 2. Average peak plasma insulin and Integrated insulin release during glucose or arginine stimulation

	Peak insulin level (ng/ml)		Integrated insulin release (ng/ml·min)	
	Glucose i.p.	Arginine i.p.	Glucose i.p.	Arginine i.p.
Control rats				
sham vagotomy	8.25±0.65	6.79±0.59	293.9±11.1	142.8±11.5
hepatic vagotomy	5.56±0.47**	9.27±0.82*	189.9±16.8**	185.5± 8.1**
Diabetic rats				
sham vagotomy	3.08±0.38#	5.19±0.70	116.9± 7.7#	149.9± 8.5
hepatic vagotomy	1.70±0.15**#	16.12±2.5**#	83.2± 3.0**#	342.4±38.6**#

Values are means±SEM.

* P<0.05, ** P<0.01 compared to sham vagotomized rats.

P<0.01 compared to control rats.

and plasma insulin was significantly lower at 10, 15 and 30 min in the hepatic vagotomized group than in the sham vagotomized group after glucose stimulation (Fig. 1).

In diabetic rats, there was no significant difference between the basal plasma glucose or insulin in the hepatic vagotomized and sham vagotomized groups (Fig. 2). Plasma glucose levels after glucose stimulation were significantly higher at 5, 10, 15 min in the hepatic vagotomized group than in the sham vagotomized group, while plasma insulin levels were significantly lower at the same time periods in the hepatic vagotomized group.

The effects of hepatic vagotomy were compared in diabetic and control rats by comparing peak insulin level and the integrated insulin release during i.p. glucose stimulation in Table 2. Diabetic rats had a significantly lower peak insulin level than control rats in both the sham vagotomized and hepatic vagotomized groups. In diabetic rats, the average peak insulin level of the hepatic vagotomized group was 55.2% lower than that of the sham vagotomized group, while in control rats, it was 67.4% lower. The effect of hepatic vagotomy on the peak insulin level in both diabetic and control rats was similar (χ^2 -test, $p>0.1$).

Regarding the integrated insulin release

Fig. 4. Plasma glucose and insulin after i.p. arginine (1 g/kg) in STZ-induced diabetic rats with sham vagotomy (○) and hepatic vagotomy (●). * P<0.05, ** P<0.01 compared to values at corresponding times in sham-vagotomized rats. Each point is mean±SEM, n=6.

during i.p. glucose stimulation, diabetic rats had a significantly smaller increase than control rats in both the sham vagotomized and hepatic vagotomized groups ($p < 0.01$). However, the effect of hepatic vagotomy on the integrated insulin release was similar in both diabetic and control rats ($27.9 \pm 3.0\%$ vs. $35.2 \pm 1.7\%$, $p > 0.1$).

Intraperitoneal Arginine Stimulation

In control rats, there was no difference between basal plasma glucose or insulin in the hepatic vagotomized and sham vagotomized groups (Fig. 3). Plasma glucose levels in the two groups after arginine stimulation were also showed not significantly different at any time. However, plasma insulin levels after arginine stimulation were significantly higher at 10 and 15 min in the hepatic vagotomized group.

In diabetic rats, there was no significant difference between basal plasma glucose and insulin in hepatic vagotomized and sham vagotomized groups (Fig. 4), although the plasma glucose level after arginine stimulation was significantly lower at 15 min in the hepatic vagotomized group. Plasma insulin levels after arginine stimulation were significantly higher at 10 and 15 min in the hepatic vagotomized group.

The effect of vagotomy in diabetic rats was also compared to that of control rats (Table 2). Diabetic rats showed significantly higher peak insulin level than control rats in hepatic vagotomized group, but no difference between diabetic rats and control rats was observed in the sham vagotomized group. In diabetic rats, the average peak insulin level of the hepatic vagotomized group was 310.6% higher than that of the sham vagotomized group, while in control rats it was 136.5% higher. The effect of hepatic vagotomy on peak insulin was significantly greater in diabetic rats than in control rats (χ^2 -test, $p < 0.01$).

Regarding the integrated insulin release during i.p. arginine stimulation, diabetic rats

showed no significant difference from control rats in the sham vagotomized group, but they showed a significant difference from control rats in the hepatic vagotomized group (Table 2). The effect of hepatic vagotomy on the integrated insulin release was also significantly greater in diabetic rats than in control rats ($123.6 \pm 17.0\%$ vs. $29.8 \pm 1.0\%$, $p < 0.01$).

Discussion

Electrophysiological studies provide considerable evidence that there is a glucoreceptor system in the liver which sends information concerning the glucose concentrations in the portal venous blood through vagal nerves to the brainstem center (Schmitt, 1973; Nijima, 1982; Oomura *et al.*, 1974). The injection of glucose into the portal vein caused a decrease in the afferent discharge rate in the hepatic branch of the vagus nerve and an increase in the efferent discharge rate in the pancreatic branch of the vagus nerve (Sakaguchi and Yamaguchi, 1979). These observations suggest that the glucoreceptor system in the liver reflexively modulates insulin release from the pancreas through the vagus nerve (Sakaguchi and Yamaguchi, 1981).

In the present study we found that hepatic vagotomy reduced i.p. glucose-induced insulin release, producing an increase in plasma glucose in normal rats. The results imply that glucose in the portal vein produces a decrease in the afferent activity of the hepatic branch of the vagus nerve and a reflex increase in the efferent activity of the pancreatic branch. The increase in the efferent activity enhanced insulin release, since stimulation of the pancreatic vagus stimulates insulin release (Nishi *et al.*, 1987). By sectioning the hepatic vagus, this enhancement was avoided, resulting in the reduction in insulin release.

As indicated in RESULTS, the damage of insulin release ability in STZ-induced diabetic rats in this study was very mild. These rats showed signs of mild glucose intolerance with no difference in fasting plasma glucose or body weight change. The ability to release insulin in response to glucose was reduced but that in response to arginine was normal, although pancreatic insulin content was decreased.

Streptozotocin-treated rats are usually utilized as a model of insulin dependent diabetes mellitus (IDDM) (Like and Rossini, 1976; Lutt, 1980), but the condition of the STZ-induced diabetic rats in the present study is more similar to non insulin dependent diabetes (NIDDM) with a reserve of insulin release induced by using a small dose of streptozotocin (Lucke, 1984). Recently, a model of NIDDM has been obtained in adult rats following a neonatal streptozotocin injection (Portha *et al.*, 1974; Weir *et al.*, 1981; Bonner-Weir *et al.*, 1981). This model has characteristics of hypo-response of insulin release to glucose but hyper-response of insulin release to non-glucose secretagogues including arginine (Leahy *et al.*, 1984). The diabetic model used in the present study is therefore similar to this model.

The present study demonstrated that i.p. glucose-induced insulin release was reduced by hepatic vagotomy in diabetic rats. The effect was rather small compared to that in controls when the peak insulin level and integrated insulin release during i.p. glucose stimulation were assessed, and the difference between the STZ-induced diabetic and control rats was not significant. The results suggest that the glucose receptor system in the liver functions normally in STZ-induced diabetic rats with a reserve of insulin release.

We reported that intraperitoneal arginine administration increased plasma insulin and glucagon more in hepatic vagotomized than in sham vagotomized groups in normal rats

(Tanaka *et al.*, 1986a). The injection of L-arginine into the portal vein increased the firing rate of afferent discharges in the hepatic branch of the vagus nerve and decreased the efferent discharge rate in the pancreatic branch of the vagus nerve (Tanaka *et al.*, 1986b). These observations suggest the existence of an arginine sensor system in the liver that modulates insulin and glucagon release from the pancreas in normal rats. Stimulation of these arginine sensors inhibits the insulin and glucagon release through the afferent vagus.

By comparing the effects of hepatic vagotomy on the peak insulin level and the integrated insulin release during i.p. arginine stimulation in the present study of STZ-induced diabetic rats, it was demonstrated that the increase in insulin release caused by i.p. arginine was enhanced and the increase in glucose caused by i.p. arginine was suppressed in hepatic vagotomized rats. The results suggest that the arginine sensor system in the liver hyperfunctions in STZ-induced diabetic rats with a reserve of insulin release.

What role might these neural-sensor systems play in the physiological and the diabetic conditions? We speculate that, in the physiological condition, the autonomic center probably receives a constant afferent influx of glucose and arginine related signals, through the hepatic vagus nerve, which may, in turn, interact with vagal efferent mechanisms controlling the centrifugal influence on appropriate glucose or arginine induced insulin release, and thus provide a feedback control of glucose homeostasis.

Glucose is the most potent stimulator of insulin release among the major components of ingested carbohydrate food-stuffs. After ingestion, glucose itself directly stimulates insulin release, resulting in the prevention of hyperglycemia. However, an excessive supply of glucose would cause hyperglycemia. Liver glucose receptors sense the increase in portal glucose and send

signals to promote insulin release, and also act to maintain blood glucose homeostasis.

Arginine is also a potent stimulant and is ingested as a component of protein food-stuffs. After ingestion, arginine directly stimulates insulin release from the pancreas. However, excessive supply and direct stimulation by arginine would cause exaggerated insulin release. Liver arginine sensors sense the increase in portal arginine and send signals to prevent exaggerated insulin secretion, and maintain blood glucose homeostasis.

In a mildly diabetic condition with an increase in plasma glucose after a meal is consumed, the liver glucose receptor system normally acts to stimulate further insulin release to prevent hyperglycemia, similar to the results seen in the physiological condition in the present study. On the other hand, the function of the arginine sensor in preventing insulin secretion is increased, with a tendency to result in a hyperglycemic state.

The cause of the difference between the function of the glucose receptor and that of the arginine sensor may be explained by noting that unlike hypersensitivity to arginine, postprandial hyperglycemia inhibits hypersensitivity to glucose in mildly diabetic rats.

In conclusion, the functions of glucose receptor and arginine sensor systems are preserved in STZ-induced mildly diabetic rats with a reserve of insulin release and, furthermore, the arginine sensor system hyperfunctions in these rats, and this may worsen the diabetic state by strengthening the inhibition of arginine-induced insulin release through this system.

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