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# Impaired Vagus Nerve-Mediated Control of Insulin Secretion in Wistar Fatty Rats

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**It has been reported that hyperglycemia in the portal venous blood suppresses afferent activity of the hepatic branch of the vagus nerve, which in turn accelerates efferent activity of the pancreatic branch of the vagus nerve to stimulate insulin secretion. The present study examined this neural control mechanism in genetically obese diabetic male Wistar fatty (*fa/fa*) rats. Adult (aged 12 to 14 weeks) Wistar fatty rats were obese, hyperinsulinemic, and hyperglycemic. Young (aged 5 to 6 weeks) Wistar fatty rats were slightly obese and hyperinsulinemic, but were euglycemic compared with the lean littermates. In both adult and young lean littermates, the plasma insulin response after an intragastric glucose load (1 g/kg) was diminished by intracerebroventricular (ICV) atropine methylbromide (methylatropine 10 nmol) pretreatment, and a transient increase in plasma insulin was observed after selective hepatic vagotomy, as reported in normal rats. In contrast, in both adult and young Wistar fatty rats, the plasma insulin response after an intragastric glucose load was not diminished by ICV methylatropine pretreatment, and plasma insulin decreased slightly after selective hepatic vagotomy. Further, afferent discharges of the hepatic vagal branch decreased and efferent discharges of the celiac/pancreatic vagal branch increased when 10 mg glucose was infused into the portal vein in the 9-week-old lean littermates, as reported in normal rats. In 7-week-old Wistar fatty rats, afferent discharges of the hepatic vagal branch decreased but efferent discharges of the celiac/pancreatic vagal branch did not increase after intraportal glucose infusion. It is concluded that the vagus nerve–mediated regulation of insulin secretion is impaired from an early stage of life in Wistar fatty rats. Efferent discharges of the vagus nerve to the pancreas seem not to be suppressed by afferent discharges from the hepatic vagus branch, which may lead to insufficient insulin secretion in response to nutrient ingestion followed by a delayed peak. These abnormalities may thus lead to the insulin resistance and fasting hyperinsulinemia that characterize the Wistar fatty rat model.**


HYPERINSULINEMIA is a common feature of obesity in humans[1] and animals.[2] Since animals with experimental lesions of the ventromedial hypothalamus develop both obesity and hyperinsulinemia, the nervous system has been proposed to contribute to the evolution of hyperinsulinemia in obesity.[2] Consistent with this hypothesis, electrical stimulation of the vagus nerve to the pancreas in vivo potentiates glucose-induced insulin release to a greater extent in genetically obese Zucker fatty (*fa/fa*) rats than in lean littermates.[3] Furthermore, the arginine-induced hypersecretion of insulin in Zucker fatty rats is abolished by atropine pretreatment.[4] These observations also suggest a contribution of the nervous system to hyperinsulinemia in genetically obese rats.

In normal rats, efferent impulses of the celiac/pancreatic vagus nerve increase when glucose is infused into the portal vein.[5] Recently, the hepatic/portal glucose sensor–mediated response of the vagus nerve was also suggested to be important for insulin secretion after glucose ingestion, the so-called incretin effect, in rats. Plasma insulin responses following an intragastric glucose load were diminished by prior selective hepatic vagotomy[6,7] or intracerebroventricular (ICV) injection of atropine methylbromide (methylatropine).[8] When glucose is injected into the portal vein, afferent impulses of the hepatic vagal branch decrease.[5] Therefore, it is postulated that the efferent vagal system, which stimulates insulin secretion, receives tonic inhibition by afferent discharges from the hepatic vagal branch when glucose concentrations are low in the portal blood. Acute sectioning of the hepatic vagus nerve causes an increase in plasma insulin,[9] and this phenomenon is abolished by ICV methylatropine pretreatment.[8] Thus, ICV injection of

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methylatropine is a useful tool for investigation of the contribution of the vagus nerve to insulin secretion.

Wistar fatty (*fa/fa*) rats, which were established by introducing the obesity (*fa*) gene of Zucker fatty rats into the Wistar Kyoto strain,[10] are less sensitive to insulin and less tolerant of glucose than Zucker fatty rats.[11] These rats exhibit obesity, hyperinsulinemia, and hyperglycemia as early as 8 weeks of age in males, and are thus a model of obese non–insulin-dependent, diabetes mellitus (NIDDM). The present study was conducted to examine the neural control of insulin secretion through the reflex arc of the vagal hepatic afferent/pancreatic efferent nerves in Wistar fatty rats.

## MATERIALS AND METHODS

### *Animals*

Wistar fatty rats were provided by the Research and Development Division of Takeda Chemical Industries (Osaka, Japan)[11] and maintained at the Laboratory Animal Center of Yamagata University School of Medicine (Yamagata, Japan). Rats had free access to standard rat chow and tap water under a 12-hour light/dark cycle. Male rats were used for experiments after a 24-hour fast. For observation of insulin secretion in vivo, we used 12- to 14-week-old Wistar fatty rats weighing 480 to 530 g and lean littermates weighing 300 to 330 g as adult rats, and 5- to 6-week-old Wistar fatty rats weighing 100 to 170 g and lean littermates weighing 90 to 130 g as young rats. For electrophysiological observations, we used 7-week-old Wistar fatty rats and 9-week-old lean littermates weighing 200 to 250 g as suitable for the size of the experimental apparatus.

### *Blood Sampling and Assays*

After intraperitoneal pentobarbital anesthesia (50 mg/kg), a small Silastic catheter (OD 0.64 mm; Dow-Corning Asia, Kanagawa, Japan) was placed into the right jugular vein for collection of blood samples. Blood (0.5 mL) was drawn slowly over several seconds and replaced with the same volume of a 50% red blood cell suspension obtained from fasted donor rats and rinsed with 0.9% saline. Blood was collected in tubes containing aprotinin (Bayer, Leverkusen, Germany; 500 KIU/mL final concentration) and disodium EDTA (1 mmol/L). The separated plasma was stored at −20°C. The glucose level was measured by a glucose oxidase method (Glu/CII; Wako Pure Chemical, Osaka, Japan). Insulin levels were measured by radioimmunoassay using a double-antibody technique with rat insulin as the standard. Intraassay and interassay coefficients of variation were 8.1% and 17.0%, respectively.

### *ICV Injection*

A midline incision was made over the parietal cranium in anesthetized rats, and the overlying muscle tissue was reflected to fully expose the occipital bone. A small hole was drilled carefully in the skull with a dental drill 2 mm posterior to the bregma according to the atlas of Albe-Fessard et al.[12] For ICV injections, a 30-gauge stainless steel tube was sunk through the hole to a depth of 5 mm below the skull surface with the aid of a stereotaxic apparatus (Narishige Scientific Apparatus Laboratory, Tokyo, Japan). The tube was fixed with dental cement (Livcarbo; G-C Dental Industrial, Tokyo, Japan), and its placement in the third cerebral ventricle was confirmed after each experiment. Fifteen minutes after catheterization, 10 nmol atropine methylbromide (methylatropine; Sigma, St. Louis, MO) dissolved in 2 μL 0.9% saline or vehicle alone was injected ICV with a Hamilton microsyringe. Thirty minutes later, 1 g/kg glucose (40% glucose in water) was administered into the stomach orally through a polyethylene tube.

### *Selective Hepatic Vagotomy*

Selective hepatic vagotomy was performed according to the method of Prechtl and Powley.[13] In brief, after pentobarbital anesthesia and basal blood sampling, the hepatic branch of the anterior vagal trunk was sectioned below the diaphragm.

### *Vagus Nerve Activity*

After intraperitoneal anesthesia with 1 g/kg urethane and endotracheal intubation, the portal vein was cannulated for glucose infusion. Afferent discharges were recorded from fine filaments dissected from the peripheral cut end of the hepatic branch of the vagus nerve under a dissection microscope.[5] Efferent discharges were recorded from the fine filaments dissected from the central cut end of the vagus nerve branch innervating the pancreas (celiac/pancreatic branch). A dissected nerve filament was placed on a pair of silver-wire electrodes and immersed in a mixture of liquid paraffin and vaseline. Afferent or efferent nerve activity was amplified in a condenser-coupled differential amplifier, monitored by an oscilloscope, and stored on magnetic tape. All nerve activity was analyzed after conversion of the raw data to standard pulses by a window discriminator to separate discharges from background noise. A spike counter with a reset time of 5 seconds was used to interpret the data on the discharge rate. The effect of intraportal glucose injection (10 mg/200 μL over 1 minute) on nerve activity was determined by comparing the mean number of impulses per 5 seconds in 10 successive 5-second intervals in each rat.

### *Statistical Analysis*

Results are expressed as the mean ± SEM. Statistical analysis was performed with ANOVA or paired *t* test, as appropriate. A $P$ value less than .05 was considered statistically significant.

## RESULTS

### *Effect of ICV Injection of Methylatropine on Plasma Glucose and Insulin Responses to Intragastric Glucose in Adult Wistar Fatty Rats*

Adult Wistar fatty rats were hyperinsulinemic (742 ± 57 *v* 203 ± 24 pmol/L, $P < .01$) and hyperglycemic (8.9 ± 0.3 *v* 4.4 ± 0.2 mmol/L, $P < .01$) compared with the lean littermates (Fig 1). In ICV saline-injected rats, 30 minutes after an intragastric 1 g/kg glucose load, plasma insulin levels increased to 677 ± 175 pmol/L in adult lean littermates and to 998 ± 255 pmol/L in adult Wistar fatty rats. Plasma glucose increased to 7.8 ± 0.8 mmol/L in adult lean littermates and to 20.8 ± 1.5 mmol/L in adult Wistar fatty rats, respectively. The increase in plasma insulin following the intragastric glucose load was smaller in adult Wistar fatty rats versus the lean littermates ($P < .05$). In contrast, the increase in plasma glucose was greater in adult Wistar fatty rats versus lean littermates ($P < .01$).

In adult Wistar fatty rats, basal plasma insulin concentrations were lower in ICV methylatropine pretreatment versus ICV saline pretreatment (509 ± 43 *v* 742 ± 57 pmol/L, $P < .05$), whereas no detectable change was observed in the adult lean littermates (Fig 1). There was no difference in the basal plasma glucose concentration with ICV saline and methylatropine pretreatment, in either adult Wistar fatty rats or the lean littermates. In adult lean littermates after an intragastric glucose load, plasma insulin increased by 482 ± 107 pmol/L (from 203 ± 24 to 685 ± 105) at 10 minutes in the ICV saline-pretreated group, but only by 183 ± 44 pmol/L (from 215 ± 21 to 398 ± 40) at 10 minutes in the ICV methylatropine pre-

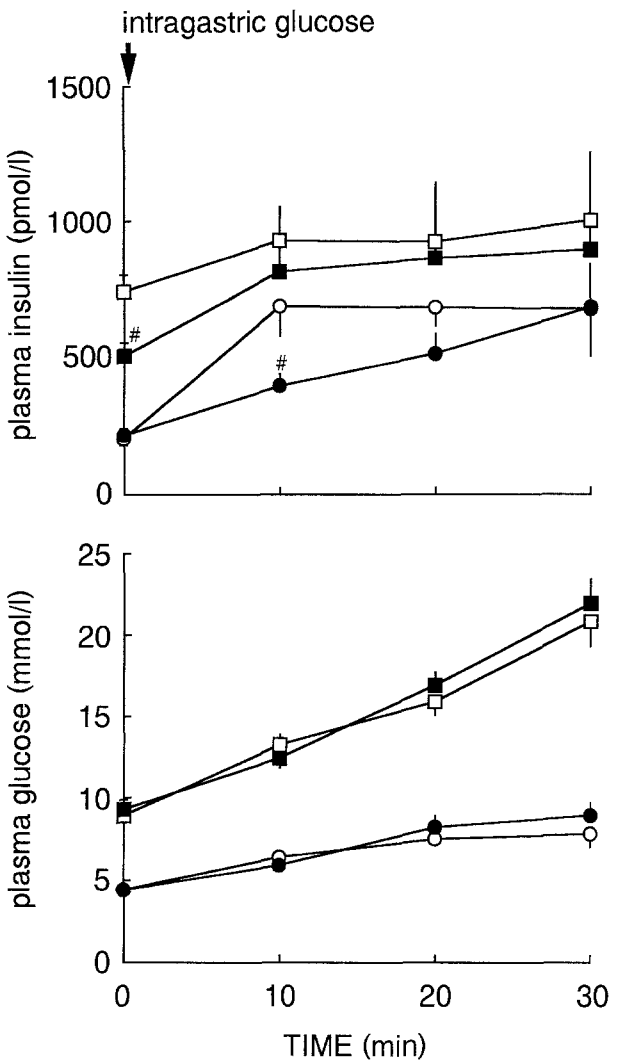


**Fig 1. Effect of ICV injection of methylatropine on plasma glucose and insulin response to intragastric glucose in adult Wistar fatty rats (□, ■) and lean littermates (○, ●). Saline 2 μL (□, n = 7; ○, n = 7) or methylatropine 10 nmol/2 μL (■, n = 7; ●, n = 7) were administered as a single ICV injection 30 minutes before 1 g/kg glucose ingestion. *$P < .05$ *v* ICV saline.**

treated group ($P < .05$). However, no significant difference was observed in the plasma insulin response to the intragastric glucose load for ICV saline versus methylatropine pretreatment in adult Wistar fatty rats, which increased by 183 ± 84 pmol/L (from 742 ± 57 to 926 ± 126) and by 305 ± 125 pmol/L (from 509 ± 43 to 814 ± 161) at 10 minutes, respectively. Plasma glucose concentrations following the intragastric glucose load were not different between the two groups (Fig 1).

*Effect of ICV Injection of Methylatropine on Plasma Glucose and Insulin Responses to Intragastric Glucose in Young Wistar Fatty Rats*

Young Wistar fatty rats were hyperinsulinemic (476 ± 55 *v* 77 ± 21 pmol/L, $P < .01$) but euglycemic (4.6 ± 0.4 *v* 4.7 ± 0.3 mmol/L, nonsignificant) compared with the lean littermates (Fig 2). Plasma insulin increased to 332 ± 73 pmol/L in young lean littermates and to 754 ± 128 pmol/L in ICV saline-pretreated young Wistar fatty rats at 20 minutes following the intragastric glucose load. Plasma glucose increased to 8.9 ± 0.9 mmol/L in young lean littermates and to 8.0 ± 0.7 mmol/L in ICV saline-pretreated young Wistar fatty rats at 30 minutes following the intragastric glucose load. The increase in plasma insulin and glucose was not different between the two groups.

In young lean littermates pretreated with ICV methylatropine, the plasma insulin response to the intragastric glucose load was 70 ± 21 pmol/L (from 96 ± 12 to 166 ± 20) at 20 minutes, which was smaller than the response of the ICV saline-pretreated group, 255 ± 61 pmol/L (from 77 ± 21 to 332 ± 73) at 20 minutes ($P < .05$). In young Wistar fatty rats, basal and post–intragastric glucose plasma insulin concentrations were decreased in ICV methylatropine-pretreated rats, but were not statistically different from the levels in vehicle-pretreated rats.

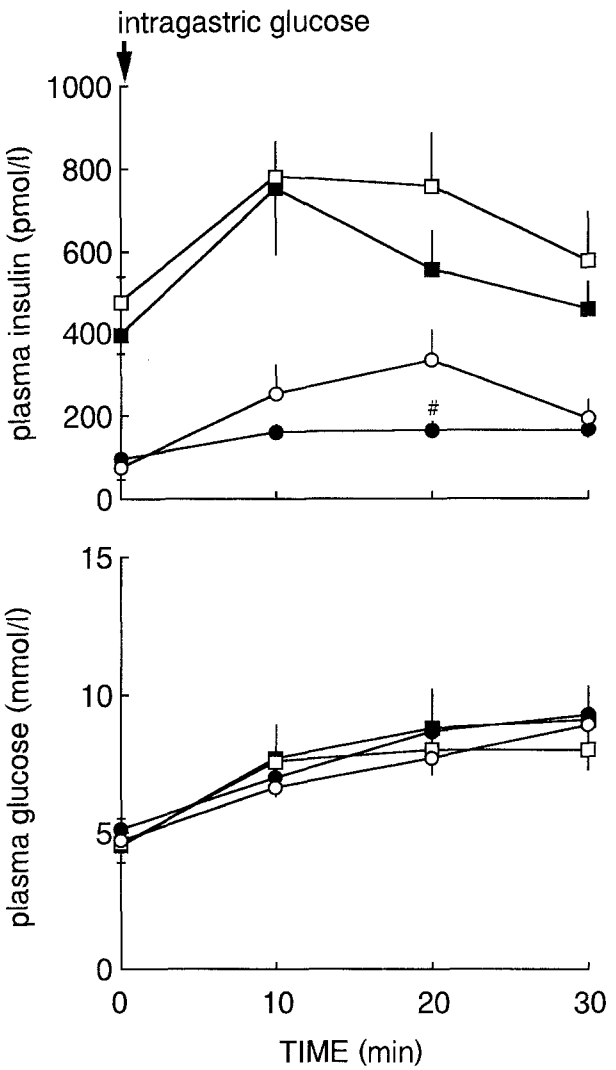


**Fig 2. Effect of ICV injection of methylatropine on plasma glucose and insulin response to intragastric glucose in young Wistar fatty rats (□, ■) and lean littermates (○, ●). Saline 2 μL (□, n = 7; ○, n = 6) or methylatropine 10 nmol/2 μL (■, n = 7; ●, n = 7) were administered as a single ICV injection 30 minutes before 1 g/kg glucose ingestion. *$P < .05$ *v* ICV saline.**

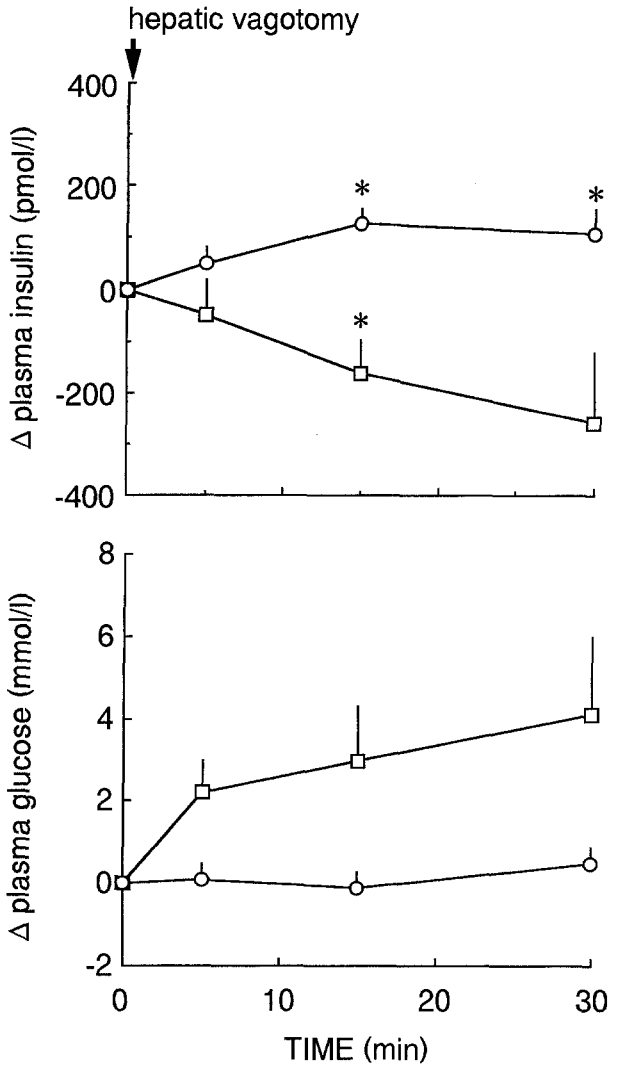


**Fig 3. Changes in plasma glucose and insulin concentrations from the basal level after cutting the hepatic vagus nerve in adult Wistar fatty rats (□, n = 7) and lean littermates (○, n = 9). Basal plasma glucose was 13.1 ± 1.4 in Wistar fatty rats and 5.4 ± 0.2 mmol/L in lean littermates. Basal plasma insulin was 1,118 ± 104 and 160 ± 23 pmol/L, respectively. *$P < .05$ *v* basal.**

The increase in plasma glucose after the intragastric glucose load was not different between the two groups (Fig 2).

*Changes in Plasma Glucose and Insulin Levels After Selective Hepatic Vagotomy in Adult Wistar Fatty Rats*

After sectioning of the hepatic vagus branch, basal plasma insulin concentrations increased by 128 ± 29 pmol/L (from 160 ± 23 to 288 ± 41) at 15 minutes ($P < .05$ *v* 0 minutes) in adult lean littermates but decreased by 163 ± 64 pmol/L (from 1,118 ± 104 to 860 ± 93) at 15 minutes ($P < .05$ *v* 0 minutes) in adult Wistar fatty rats (Fig 3). Plasma glucose concentrations did not change in adult lean littermates and increased insignificantly by 4.1 ± 1.9 mmol/L (from 13.1 ± 1.4 to 17.0 ± 2.6) at 30 minutes in adult Wistar fatty rats. However, a significant difference was observed in plasma glucose levels between these two groups after selective hepatic vagotomy ($P < .01$).

*Changes in Plasma Glucose and Insulin Levels After Selective Hepatic Vagotomy in Young Wistar Fatty Rats*

In young lean littermates, plasma insulin increased by 105 ± 29 pmol/L (from 82 ± 13 to 187 ± 36) at 15 minutes ($P < .05$ *v* 0 minutes; Fig 4) after selective vagotomy, as observed in adult lean littermates. However, in young Wistar fatty rats, in contrast to adult Wistar fatty rats, plasma insulin did not decrease significantly after selective vagotomy. Plasma glucose increased after selective hepatic vagotomy in both lean littermates by 3.2 ± 1.0 mmol/L (from 4.6 ± 0.3 to 7.8 ± 0.8) at 30 minutes ($P < .05$ *v* 0 minutes) and in young Wistar fatty rats by 2.3 ± 0.6 mmol/L (from 5.0 ± 0.4 to 7.3 ± 0.5) at 30 minutes ($P < .05$ *v* 0 minutes). There was no significant difference in plasma glucose levels after selective vagotomy between the two groups.

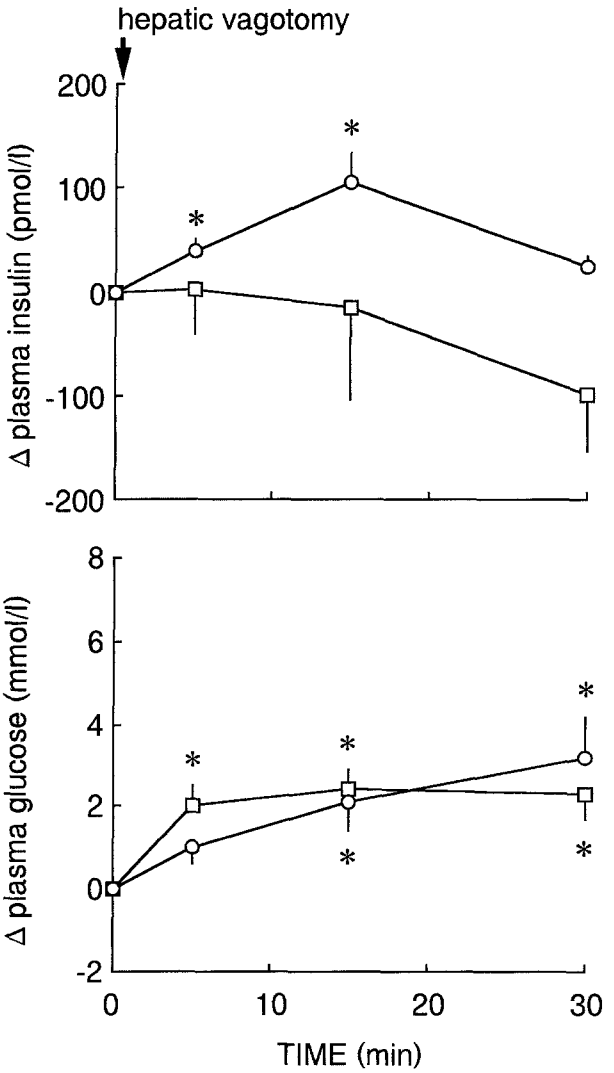


**Fig 4. Changes in plasma glucose and insulin concentrations from the basal level after cutting the hepatic vagus nerve in young Wistar fatty rats (□, n = 12) and lean littermates (○, n = 7). Basal plasma glucose was 5.0 ± 0.4 in Wistar fatty rats and 4.6 ± 0.3 mmol/L in lean littermates. Basal plasma insulin was 658 ± 101 and 82 ± 13 pmol/L, respectively. *$P < .05$ *v* basal.**

*Changes in the Firing Rate of the Afferent Hepatic Vagus Nerve and Efferent Celiac/Pancreatic Vagus Nerve Following Intraportal Glucose Infusion in Wistar Fatty Rats*

After 10 mg glucose injection into the portal vein, the rate of afferent discharges from the hepatic branch of the vagus nerve decreased in both Wistar fatty rats and the lean littermates (Fig 5). On the other hand, the rate of efferent discharges from the celiac/pancreatic branch of the vagus nerve increased in lean littermates, but not in Wistar fatty rats. A representative example from each group is shown in Fig 5. When the mean number of impulses per 5 seconds in 10 successive 5-second intervals was compared (Fig 6), afferent discharges from the hepatic branch of the vagus nerve decreased from 65.2 ± 3.8 impulses/5 s at 0 minutes to 51.0 ± 4.9 impulses/5 s at 30 minutes ($P < .05$) in lean littermates, and from 74.2 ± 12.1 impulses/5 s at 0 minutes to 58.6 ± 0.5 impulses/5 s at 30 minutes ($P < .05$) in Wistar fatty rats. Efferent discharges from the celiac/pancreatic branch of the vagus nerve increased from 65.8 ± 2.3 impulses/5 s at 0 minutes to 86.0 ± 7.6 impulses/5 s at 60 minutes ($P < .05$) in lean littermates but did not change in Wistar fatty rats in response to intraportal glucose injection. Thus, the difference in efferent discharges between the two groups became significant at 60 minutes ($P < .05$). This increase in efferent discharges from the celiac/pancreatic branch of the vagus nerve in response to intraportal glucose was not observed when the hepatic branch of the vagus nerve was dissected in lean littermates (data not shown).

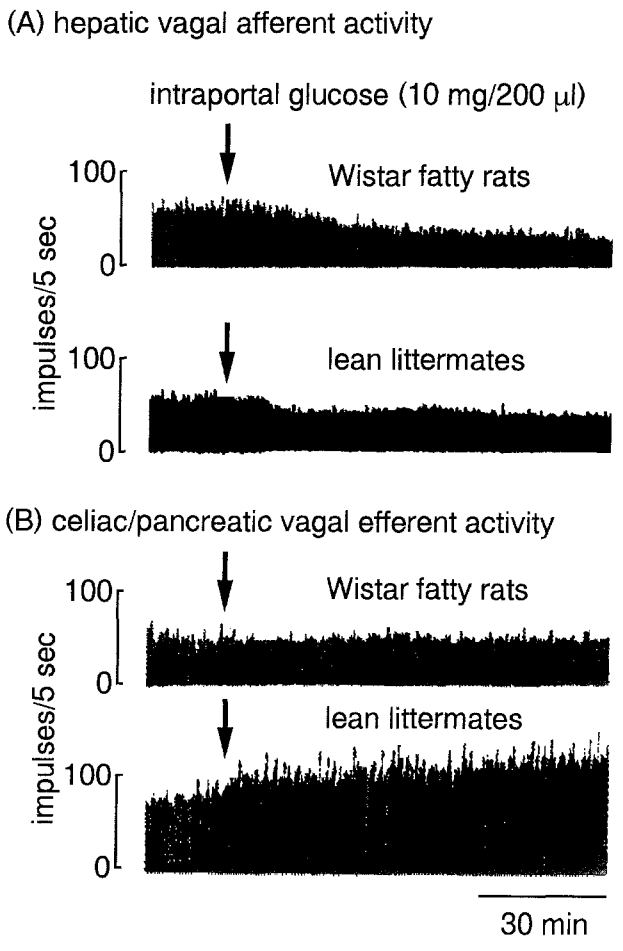


**Fig 5. Representative time course of impulses of the afferent hepatic vagus nerve and efferent celiac/pancreatic vagus nerve following intraportal glucose infusion in Wistar fatty rats (A) and lean littermates (B).**

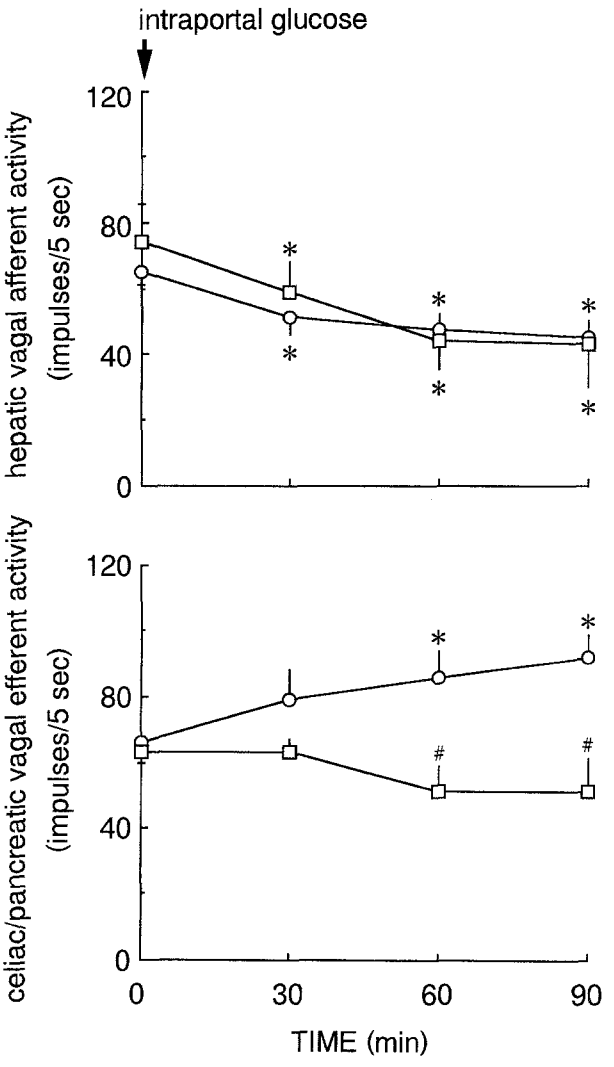


**Fig 6. Changes in impulses of the afferent hepatic vagus nerve and efferent celiac/pancreatic vagus nerve following intraportal glucose infusion in Wistar fatty rats (□, n = 5) and lean littermates (○, n = 5). *$P < .05$ *v* basal; #$P < .05$ *v* lean littermates.**

## DISCUSSION

The importance of the vagus nerve in stimulating insulin secretion after glucose ingestion has been observed even under anesthesia in the rat.[6-8] ICV injection of methylatropine prior to an intragastric glucose load is thought to block neural control of insulin secretion.[8] In the present study, pretreatment with ICV methylatropine diminished the insulin response to an intragastric glucose load in both young and adult lean littermates of Wistar fatty rats. This shows that neural control is important for insulin secretion soon after glucose ingestion in the lean littermates, as observed in normal rats.[6-8] In contrast, in both young nondiabetic fatty and adult diabetic Wistar fatty rats, ICV

methylatropine pretreatment did not affect the plasma insulin response to an intragastric glucose load. These findings suggest that the neural control of insulin secretion is impaired from an early stage of life in Wistar fatty rats, and therefore, the impaired neural control is not a consequence of the diabetic state that develops in these rats.

It is believed that the vagus nerve–mediated reflex regulation of insulin secretion is composed of the vagal hepatic afferent and vagal pancreatic efferent systems. When glucose concentrations are low in the portal vein, efferent fibers to the pancreas are tonically suppressed by afferent fibers arising from hepatic/portal glucose sensors.[5,9] When the plasma glucose concentration increases in the portal vein, hepatic vagal afferent activity decreases and efferent activity to the pancreas increases to stimulate insulin secretion. This concept is supported by a report that electrical stimulation of the cut end of the afferent hepatic vagal branch decreases plasma insulin concentrations.[9] The reflex center mediating this loop may exist in the brain stem and/or the hypothalamus. Methylatropine is known to pass through the blood-brain barrier less effectively than atropine,[14] and does not likely act on pancreatic β cells directly, because pretreatment with ICV or intraperitoneal methylatropine does not affect the plasma insulin response to an intravenous glucose load.[8] Therefore, ICV methylatropine most likely blocks muscarinic cholinergic receptors in the central nervous system near the third cerebral ventricle. The hypothalamus, located near the third ventricle, is known to influence insulin secretion and is thought to be the center of the autonomic nervous system.[15,16] Thus, the hypothalamus is likely the center of the vagus nerve–mediated control of insulin secretion.

Selective hepatic vagotomy increased plasma insulin concentrations in adult lean littermates, as observed in normal rats.[8,9] In contrast, in diabetic adult Wistar fatty rats, plasma insulin did not increase but rather decreased upon hepatic vagotomy. This suggests that the vagus nerve–mediated regulation of insulin secretion is impaired in this model of NIDDM. It is not certain why plasma glucose did not change even when plasma insulin increased in adult lean littermates, as observed in normal rats.[8,9] Selective hepatic vagotomy, in addition to insulin, may promote some counterregulatory mechanism(s). However, glucagon is not a likely candidate, since plasma glucagon levels do not change after selective vagotomy in normal rats (H. Ohnuma, K. Yamatani, H. Manaka, unpublished observation, November 1995). Plasma insulin also increased in young lean littermates but remained at basal levels in young Wistar fatty rats after selective hepatic vagotomy. This again suggests that impairment of the vagus nerve–mediated regulation of insulin secretion occurs early in the life of Wistar fatty rats. Plasma glucose increased significantly in both young Wistar fatty rats and lean littermates after selective hepatic vagotomy. The reason is not certain, although the surgical procedure might be a stressor for these small rats, since the increment in plasma glucose was not different between the two groups.

When 10 mg glucose was injected into the portal vein, afferent discharges from the hepatic vagal branch decreased in both Wistar fatty rats and lean littermates, as observed in normal rats.[5] This suggests that the afferent vagal pathway from hepatic/portal glucose sensors is intact in Wistar fatty rats. In contrast, the rate of efferent discharges from the celiac/pancreatic vagal branch did not increase in Wistar fatty rats, whereas they increased in lean littermates, as observed in normal rats.[5] Therefore, impairment of the vagus nerve–mediated control of insulin secretion in Wistar fatty rats may occur due to an inability of the center of the vagus nerve in the hypothalamus to respond to suppressive hepatic vagal afferent activity. When efferent discharges from the vagus nerve to the pancreas are not controlled by suppressive afferent discharges from the hepatic vagal branch from an early stage of life in Wistar fatty rats, insulin secretion soon after ingestion is insufficient, followed by a delayed peak. Ultimately, this may lead to insulin resistance and fasting hyperinsulinemia.[1]

Basal plasma insulin concentrations before an intragastric glucose load are lower in ICV methylatropine-pretreated versus ICV saline-pretreated rats, particularly in adult Wistar fatty rats. Whereas it is obvious that the vagus nerve–mediated control of insulin secretion in Wistar fatty rats is different from that observed in lean littermates and normal rats, it is more difficult to explain this phenomenon.[8] However, it is interesting that the decline of plasma insulin levels after selective hepatic vagotomy in adult Wistar fatty rats may reflect the decline of plasma insulin observed in ICV methylatropine-treated fatty rats. One may predict that the plasma insulin response to an intragastric glucose load would increase when the tonic suppression is relieved by ICV methylatropine. However, this did not occur, because ICV methylatropine diminished the plasma insulin response to an intragastric glucose load in lean littermates. In normal rats, we have observed that both selective hepatic vagotomy[6,7] and ICV methylatropine[8] diminish the plasma insulin response to an intragastric glucose load. Therefore, the vagus nerve–mediated control may be more important for an appropriate early insulin secretion than for overall insulin secretion after glucose ingestion. In fact, plasma insulin concentrations at 30 minutes after an intragastric glucose load are not different between ICV methylatropine- and saline-injected lean littermates. This also suggests that the vagus nerve–mediated control is important for insulin secretion soon after glucose ingestion, whereas the overall insulin response is more dependent on other factors such as plasma glucose levels. The latter is concordant with the observations that selective hepatic vagotomy[6] or ICV methylatropine[8] do not affect the insulin response to intravenous glucose infusion.

In conclusion, these studies demonstrate that the suppressive hepatic vagal afferent activity at the center of the vagus nerve in the hypothalamus is impaired from an early stage of life in Wistar fatty rats. When efferent discharges from the vagus nerve to the pancreas are not controlled by suppressive afferent discharges from the hepatic vagal branch from an early stage of life in Wistar fatty rats, insulin secretion soon after ingestion is insufficient, followed by a delayed peak. This may lead to the insulin resistance and fasting hyperinsulinemia that characterize this model of NIDDM.

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