Original Article

Chronic Hyperinsulinemia Enhances Adrenergic Vasoconstriction and Decreases Calcitonin Gene–Related Peptide–Containing Nerve–Mediated Vasodilation in Pithed Rats

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The present study investigated the influence of chronic hyperinsulinemia on vascular responsiveness induced by adrenergic nerves and calcitonin gene–related peptide–containing (CGRPergic) nerves in pithed rats with insulin resistance. Male Wistar rats (6 weeks old) received 15% fructose solution in drinking fluid for 10 weeks (fructose-drinking rats: FDR), which resulted in significant increases in plasma levels of insulin, total cholesterol and triglyceride, and systolic blood pressure, as compared with control rats. Pithed FDR showed greater adrenergic nerve–mediated pressor response to spinal cord stimulation (SCS) at the lower thoracic vertebra (Th 9–12) and pressor response to exogenous noradrenaline than control rats. In pithed FDR with blood pressure artificially increased by continuous infusion of methoxamine and blockade of autonomic ganglia by hexamethonium, CGRPergic nerve–mediated depressor responses to SCS were significantly smaller than those in control rats, but depressor responses to other vasodilators such as acetylcholine, CGRP and sodium nitroprusside were similar to those in control rats. These results suggest that chronic hyperinsulinemia in FDR facilitates adrenergic nerve–mediated vasoconstriction, which is associated with attenuated CGRPergic nerve–mediated vasodilation. (*Hypertens Res* 2006; 29: 361–368)

***Key Words*: hyperinsulinemia, spinal cord stimulation, calcitonin gene–related peptide–nerve depressor, adrenergic pressor, pithed rat**

Introduction

Patients with type-2 diabetes (non-insulin dependent diabetes mellitus) are at high risk for complications of hypertension, and it has been suggested that there is a relationship between insulin levels and blood pressure (BP) (*1*). Additionally, several studies have reported that insulin resistance and/or hyperinsulinemia may contribute to the pathogenesis of hypertension (*2–5*). In fact, insulin increases sympathetic activity (*6*) and renal sodium reuptake (*3*) and promotes proliferation of vascular smooth muscle cells (*7*), which could increase BP. Moreover, hyperinsulinemia has been shown to contribute to an increase in sympathetic activity (*5*, *8*), and a close association between insulin resistance and hyperinsulinemia has been suggested in essential hypertension (*9*, *10*). This relationship implies that insulin resistance and hyperinsulinemia contribute to the pathogenesis of hyperten-

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sion, and furthermore, that insulin might play an important role in malfunction of the cardiovascular system. On the other hand, some investigations have shown that insulin acts as an endogenous vasodilator (*11*, *12*). Therefore, it has been hypothesized that insulin resistance might induce hypertension due to decreased insulin–induced vasodilation and the imbalance between its pressor and depressor effects (*9*). However, the association of insulin and BP has remained controversial. Previously, we reported that in pithed rats without a central vasoreflex, acute insulin infusion augments adrenergic nerve–mediated vasoconstriction, which is partially associated with inhibition of calcitonin gene–related peptide (CGRP)–containing (CGRPergic) nerve function as well as endothelium function (*13*). This finding implies that hyperinsulinemia may elevate BP by augmenting the sympathetic adrenergic activity.

Long-term diet feeding of fructose has been reported to induce hyperinsulinemia and insulin resistance in rats (*14*). Notably, hyperinsulinemia is more markedly induced by fructose drinking than by fructose diet feeding (*15*). Thus, in this study, to clarify whether insulin resistance and/or hyperinsulinemia is associated with the development of hypertension, we investigated the influence of chronic hyperinsulinemia on vascular responsiveness mediated by adrenergic nerves and CGRPergic nerves in pithed rats given a 15% fructose solution for 10 weeks as their drinking fluid.

Methods

Animals

Male Wistar rats weighing 315–420 g were used in this study. The animals were given food and water (control) or 15% fructose solution (fructose-drinking rats: FDR) ad libitum. They were housed in the Animal Research Center of Okayama University at a controlled ambient temperature of 22±2°C with 50±10% relative humidity and with a 12-h light/12-h dark cycle (lights on at 8:00 AM). The study was performed in accordance with the Guide for Animal Experimentation of the Faculty of Pharmaceutical Science, Okayama University.

Fructose-Induced Hyperinsulinemia in Wistar Rats

At 6 weeks of age, the animals were divided into two groups: one group receiving normal chow and water and another group receiving normal chow and 15% fructose solution for 10 weeks to establish stable hyperinsulinemia according to the report of Suzuki *et al.* (*14*). Body weight and food and fluid intakes were measured at 1-week intervals between 6 and 16 weeks of age.

Biochemical Analysis

At 6, 14 and 16 weeks of age, under light anesthesia with ether, blood samples were obtained from the heart after a 12-h fast. The plasma level of glucose was measured using a glucose analyzer (ADVANTAGE; Boehringer Mannheim, Tokyo, Japan) and triglyceride and total cholesterol were enzymatically measured using a commercially available kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Plasma insulin was measured by a double-antibody method with an ELISA insulin kit (Morinaga Biochemistry Co., Kanagawa, Japan).

Systolic BP and Heart Rate Measurements

The BP and heart rate (HR) of conscious rats were measured using a tail-cuff plethysmograph (TK-370C, UNICOM, Tokyo, Japan) between 9:00 and 12:00 AM. Both values were taken as the average of five readings.

Pithing and Measurement

At 16 weeks of age, the animals were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). Polyethylene catheters (PE-10) were positioned in the right and left jugular veins for administration of drugs, and a bilateral vagotomy was performed at the midcervical level. A polyethylene catheter (PE-50) was inserted into the left carotid artery and connected to a pressure transducer (model DX-100; Nihon Kohden, Tokyo, Japan). The BP (systolic and mean BP) was recorded on a polygraph (model RM-6000; Nihon Kohden). The HR triggered by arterial pulses was measured using a cardiotachometer (model AT-600G; Nihon Kohden) and was recorded on the polygraph.

After the trachea was cannulated, the animals were pithed by inserting a stainless-steel rod (1.4 mm in diameter) through the right orbit and the foramen magnum and down into the spinal cord to the level of the sacral end, and then the tip of the rod was raised to the thoracolumbar vertebrae (Th 9–12) according to the method described previously (*16*, *17*). Artificial respiration (4.5 ml/breaths/kg, 70 breaths/min) with room air was immediately started using an artificial respirator (model 683; Harvard Apparatus, South Natic, USA). The pithing rod served as the stimulating electrode, which was insulated except for 5 mm of the tip. The level of spinal cord stimulation (SCS) was determined by varying the depth of the insertion of the rod. The position of the rod within the vertebral canal was determined from the length of the rod. A stainless-steel needle was inserted subcutaneously into the dorsum, parallel to the vertebral column, to serve as an indifferent electrode. After the animals were pithed, *d*-tubocurarine (1 mg/kg, i.v.) was injected to prevent skeletal muscle contraction during SCS. The rectal temperature was maintained at approximately 37°C by means of a heater mat (model KN-475; Natsume, Tokyo, Japan).

Table 1. Changes in Body Weight, Food and Fluid Intake, Plasma Insulin, Plasma Total Cholesterol, Plasma Triglyceride and Blood Glucose Level in 15% Fructose–Drinking Rats (FDR; $n=12$) and Water-Drinking Rats (Control Rats; $n=9$) between 6 and 16 Weeks of Age

	6 weeks	8 weeks	10 weeks	12 weeks	14 weeks	16 weeks
Body weight						
Control	194.4±3.3	277.8±3.7	325.6±4.8	357.8±6.4	372.8±7.2	374.4±6.1
FDR	191.7±2.9	260.4±4.5*	303.3±7.0*	337.9±7.5	355.4±7.5*	367.1±8.2
Food intake (g/100 g BW/day)						
Control	10.3±0.4	7.4±0.1	7.1±0.1	5.5±0.1	5.5±0.0	4.9±0.2
FDR	6.5±0.7**	4.2±0.2**	4.0±0.2**	3.1±0.2**	3.0±0.1**	2.6±0.2**
Fluid intake (g/100 g BW/day)						
Control	16.5±1.4	12.9±1.0	11.6±1.2	10.4±0.9	10.8±0.7	9.6±1.3
FDR	24.1±0.7**	22.0±20.0*	20.7±1.9*	15.7±1.1*	17.5±1.3**	15.4±1.5*
Plasma insulin (ng/ml)						
Control	0.27±0.04	n.d.	n.d.	n.d.	0.58±0.17	0.61±0.06†
FDR	0.28±0.03	n.d.	n.d.	n.d.	0.98±0.15*,†	1.20±0.11*,†
Plasma total cholesterol (mg/dl)						
Control	41.8±1.9	n.d.	n.d.	n.d.	42.3±2.1	46.7±2.4
FDR	43.2±1.2	n.d.	n.d.	n.d.	57.5 ±3.6*,†	57.4±2.1*,†
Plasma triglyceride (mg/dl)						
Control	45.9±3.0	n.d.	n.d.	n.d.	46.4±2.5	51.2±1.8
FDR	48.2±4.0	n.d.	n.d.	n.d.	63.9±4.8*,†	73.9±5.4*,†
Plasma glucose (mg/dl)						
Control	105.5±6.8	n.d.	n.d.	n.d.	120.8±5.1	129.4±4.2
FDR	110.1±3.7	n.d.	n.d.	n.d.	117.5±4.3	115.4±5.5

Values are expressed as the mean±SEM. *$p<0.05$, **$p< 0.01$ *vs*. control rats. $^{\dagger}p<0.01$ *vs*. 6 week-old rats. n.d., not determined; BW, body weight.

Spinal Cord Stimulation

After allowing BP and HR to stabilize, electrical stimulation at 2, 4 and 8 Hz, which induced a sharp increase in BP without changing HR, was applied to verify the position of the rod in the spinal column. Rectangular pulses (1 ms in duration and 20 V) were given for 30 s at 5–10 min intervals with an electronic stimulator (model SEN-3201, isolator 20865; Nihon Kohden).

After finishing the pressor experiments, mean BP was increased and maintained at a level of approximately 100 mmHg by continuous infusion of the α_1-adrenoceptor agonist methoxamine (20 μg/kg/min, i.v.). The autonomic ganglionic blocker hexamethonium (2 mg/kg/min, i.v.) was also infused to block the autonomic outflow. The increased BP was allowed to stabilize, and then the spinal cord was electrically stimulated. Rectangular pulses (1 ms in duration and 20 V) at 2 and 4 Hz were given for 30 s with an electronic stimulator (model SEN-3201, isolator 20865; Nihon Kohden).

Experimental Protocols

To assess changes in vascular responsiveness in the chronic hyperinsulinemic state, pressor and depressor responses induced by SCS and various vasoactive agents were evaluated in pithed rats without a central vasoreflex. After the animals were pithed and both BP and HR stabilized, SCS (2, 4 and 8 Hz), intravenous injections of noradrenaline (NA; 125, 250 and 500 ng/kg, i.v.) and angiotensin II (Ang II; 40, 100 and 200 pmol/kg, i.v.) were applied.

After finishing pressor experiments, the mean BP of pithed rats was increased by continuous infusion of methoxamine (20 μg/k/min, i.v.) concomitant with infusion of hexamethonium (2 mg/kg/min, i.v.). After the elevated BP stabilized, SCS (2 and 4 Hz) and bolus injections of acetylcholine (ACh; 0.05 and 0.5 nmol/kg, i.v.), rat CGRP (0.05 and 0.1 nmol/kg, i.v.) and sodium nitroprusside (SNP; 0.5 and 5 μg/kg, i.v.) were applied.

Statistical Analysis

The experimental results are presented as the mean±SEM. Statistical analyses were performed using Student's unpaired *t*-test and one-way analysis of variance followed by the Tukey's test. A p value less than 0.05 was considered statistically significant.

Drugs

The following drugs were used: ACh chloride (Daiichi Phar-

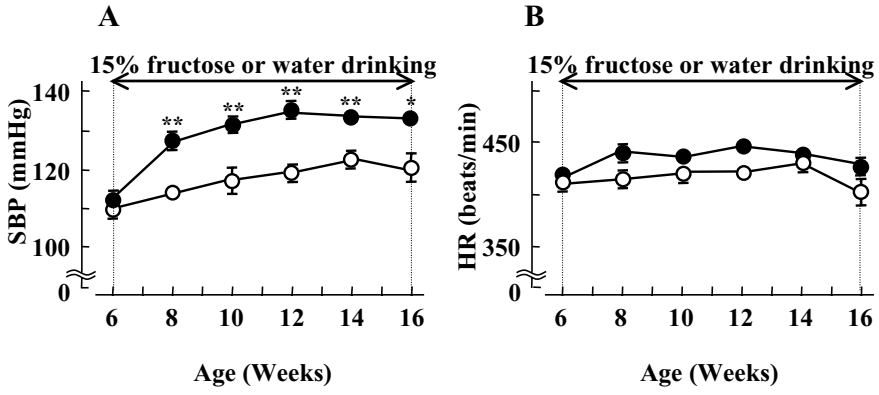

Fig. 1. *Changes in systolic blood pressure (SBP) (A) and heart rate (HR) (B) in 15% fructose–drinking rats (FDR;* n = *12) and water-drinking rats (control rats;* n = *9) from 6 to 16 weeks.* ¡ *, control rats;* ●*, FDR. HR and SBP were measured by the tail-cuff plethysmography method weekly. Each bar represents the mean±SEM.* *p < *0.05,* **p < *0.01* vs. *control rats.*

maceutical Co., Tokyo, Japan), Ang II (Peptide Institute, Osaka, Japan), D-fructose (Wako Pure Chemical Industries, Ltd.), hexamethonium bromide (Sigma Chemical Co., St. Louis, USA), methoxamine hydrochloride (Nihon Shinyaku Co., Kyoto, Japan), NA hydrochloride (Sankyo Co., Tokyo, Japan), rat α-CGRP (Peptide Institute) and *d*-tubocurarine (Sigma) and SNP (Sigma). All drugs were dissolved in 0.9% saline and infused at a rate of 0.2 ml/min using an infusion pump (model 11; Harvard Apparatus) or given as bolus doses (0.2 ml/kg).

Results

Changes in Body Weight and Food and Fluid Intake

The body weight of FDR at 8, 10 and 14 weeks of age showed a significant decrease when compared with that of control rats (Table 1). After starting 15% fructose solution as drinking fluid, food intake in FDR was markedly decreased compared to that in control rats, but fluid intake was significantly increased compared with that of control rats (Table 1).

Changes in Plasma Levels of Insulin, Triglyceride, Total Cholesterol and Glucose

Table 1 shows plasma concentrations of insulin, total cholesterol and triglyceride and blood glucose levels after 12-h fasting in FDR and control rats at 6, 14 and 16 weeks of age. At 14 and 16 weeks of age, plasma insulin, total cholesterol and triglyceride levels in FDR were significantly higher than those in control rats. However, no difference was found in blood glucose levels between FDR and control rats. Also, plasma levels of insulin, total cholesterol and triglyceride but not blood glucose in 16-week-old FDR were significantly higher than those in 6-week-old FDR (Table 1).

Changes in Systolic BP and HR

As shown in Fig. 1A, systolic BP in FDR was markedly elevated at 2 weeks after starting 15% fructose solution treatment and the elevation of systolic BP in FDR lasted until 16 weeks of age. Significant differences in systolic BP between FDR and control rats were found between 2 weeks and 10 weeks after fructose administration (Fig. 1A). However, there was no significant difference in HR between FDR and control rats (Fig. 1B).

Changes in Pressor Responses to SCS and Bolus Injections of NA and Ang II

The basal systolic BP (47.4±1.3 mmHg, $p<0.01$) and basal mean BP (35.4±1.3 mmHg, $p<0.01$), but not the HR (270.5±11.0 beats/min), at 30 min after pithing in FDR were significantly greater than those in pithed control rats (systolic BP, 37.5±2.8 mmHg; mean BP, 26.8±2.2 mmHg; HR, 272.1±9.8 beats/min).

SCS at 2, 4 and 8 Hz in pithed control rats and FDR caused a frequency-dependent, sharp increase in BP (Fig. 2A, D) without changing HR (data not shown). Also, bolus injections of NA (125, 250 and 500 ng/kg) (Fig. 2B, E) and Ang II (40, 100 and 200 pmol/kg) (Fig. 2C, F) induced dose-dependent increases in BP. NA injection but not Ang II injection caused an increase in HR (data not shown).

In pithed FDR, pressor responses to SCS (2, 4 and 8 Hz) (Figs. 2D and 3A) and bolus injections of NA (125, 250 and 500 ng/kg) (Figs. 2E and 3B) and Ang II (200 pmol/kg) (Figs. 2F and 3C) were significantly greater than those in pithed control rats. In addition, in FDR, the SCS-induced pressor responses were much greater than the NA-induced pressor responses (Fig. 3). Pressor responses to Ang II injection at doses of 40 and 100 pmol/kg in pithed FDR tended to be greater than those in control rats, but no significant difference

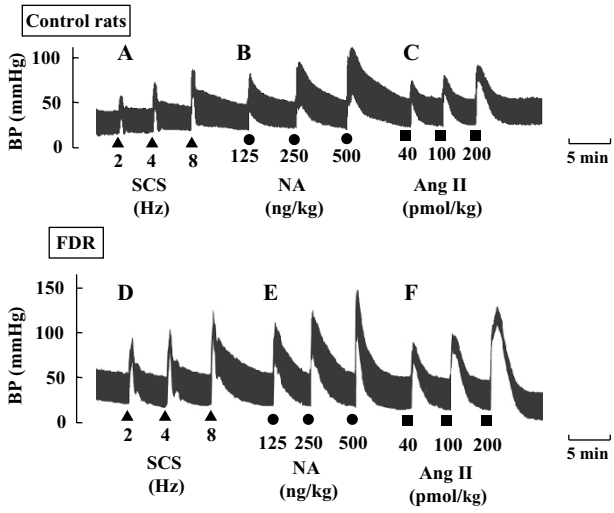

Fig. 2. *Typical records showing pressor responses induced by spinal cord stimulation (SCS) (A and D) and bolus injections of noradrenaline (NA; 125–500 ng/kg, i.v.) (B and E) and angiotensin II (Ang II; 40–200 pmol/kg, i.v.) (C and F) in pithed control rats (upper trace) and 15% fructose–drinking rats (FDR) (lower trace). BP, blood pressure.*

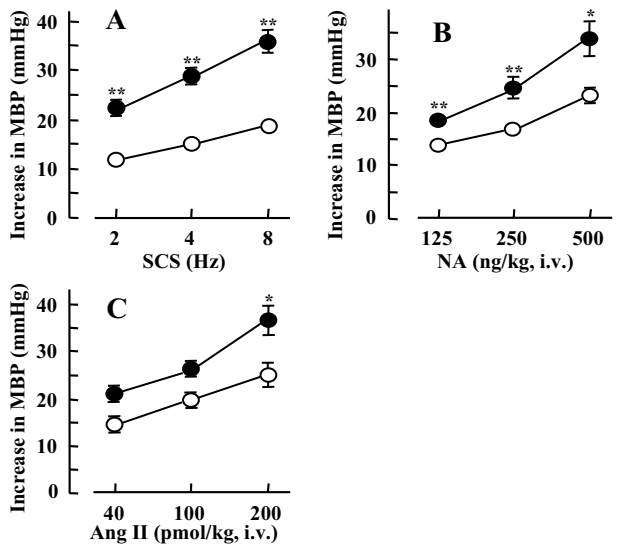

Fig. 3. *Pressor responses to spinal cord stimulation (SCS; 2, 4 and 8 Hz) (A) and to bolus injections of noradrenaline (NA; 125–500 ng/kg, i.v.) (B) and angiotensin II (Ang II; 40–200 pmol/kg, i.v.) (C) in pithed 15% fructose–drinking rats (FDR;* n = *12) and control rats (*n = *9).* ¡, *control rats;* ●, *FDR. Each bar indicates the mean±SEM.* *p < *0.05,* **p < *0.01* vs. *control rats. MBP, mean blood pressure.*

between pithed FDR and control rats was found (Figs. 2F and 3C).

Changes in Depressor Responses to SCS and Bolus Injections of ACh, CGRP and SNP

As shown in Fig. 4A and F, pressor responses to SCS at 4 Hz in control rats and FDR were blocked by hexamethonium, an autonomic ganglion blocker, indicating that the pressor response is mediated by sympathetic nerves. When the BP was increased by infusion of methoxamine in the presence of hexamethonium, SCS at 4 and 8 Hz caused a frequency-dependent depressor response (Fig. 4B, G) without changing HR (data not shown). Bolus injections of CGRP at doses of 0.05 and 0.1 nmol/kg induced a dose-dependent, long-lasting fall in BP similar to the pattern of response to SCS (Fig. 4C, H). Bolus injections of ACh (0.05 and 0.5 nmol/kg) (Fig. 4D, I) and SNP (0.5 and 5 μg/kg) (Fig. 4E, J) caused a sharp fall in BP in a dose-dependent manner.

As shown in Figs. 4G and 5A, depressor responses to SCS (2 and 4 Hz) in pithed FDR were significantly smaller than those in pithed control rats. However, depressor responses to bolus injections of CGRP (Figs. 4H and 5B), ACh (Figs. 4I and 5C) and SNP (Figs. 4J and 5D) in pithed FDR were similar to those in pithed control rats.

Discussion

The present study demonstrated that rats given 15% fructose solution as their drinking fluid showed a marked increase in plasma insulin levels without a significant increase in blood glucose levels, suggesting that these animals had hyperinsulinemia due to insulin resistance. It is important to note that FDR with hyperinsulinemia showed hypertension. Thus, it appears that hypertension is closely associated with chronic hyperinsulinemia and insulin resistance.

The main finding of the present study is that the SCS-induced pressor response in the pithed FDR with a chronic hyperinsulinemic state was markedly greater than that in control pithed rats. Additionally, FDR showed a significant increase in pressor response to exogenously applied NA, which is mediated by the postsynaptic α_1-adrenoceptor. Furthermore, the augmented pressor response was more pronounced for SCS than for exogenously applied NA. In pithed rats, pressor responses to SCS have been shown to be mediated by activation of sympathetic adrenergic nerves, since an adrenergic neuron blocker (guanethidine) and autonomic ganglionic blocker (hexamethonium) abolished the response (*13*, *16*). Therefore, it is very likely that sympathetic nerve activity is augmented in the chronic hyperinsulinemic state. We have reported that acute hyperinsulinemia produced by continuous infusion of insulin resulted in potentiation of pressor responses to SCS and NA injection in the euglycemic pithed rat (*13*). Thus, it is probable that the augmented pressor response in FDR mainly results from chronic hyperinsulinemia, which would induce increased sympathetic nerve activity and vasoreactivity of the blood vessels.

Another important finding of the present study is that the

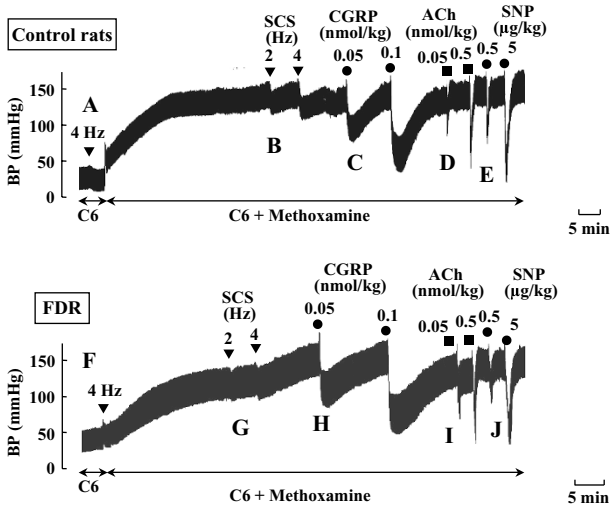

Fig. 4. *Typical records showing depressor responses to spinal cord stimulation (SCS) (B and G) and to bolus injections of acetylcholine (ACh; 0.05 and 0.5 nmol/kg, i.v.) (D and I), calcitonin gene–related peptide (CGRP; 0.05 and 0.1 nmol/kg, i.v.) (C and H) and sodium nitroprusside (SNP; 0.5 and 5 μg/kg, i.v.) (E and J) in pithed control rats (upper trace) and 15% fructose–drinking rats (FDR) (lower trace). The blood pressure (BP) was artificially increased by continuous infusion of methoxamine (20 μg/kg/ min, i.v.) in the presence of hexamethonium (C6; 2 mg/kg/min, i.v), which blocked the SCS (4 Hz)-induced pressor response (A and F).*

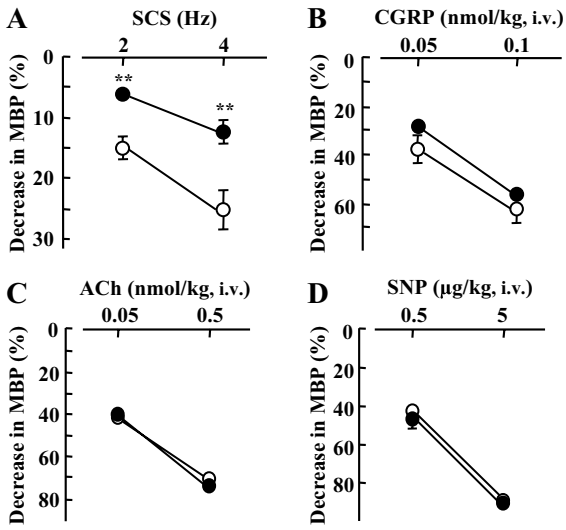

Fig. 5. *Depressor responses to spinal cord stimulation (SCS; 2 and 4 Hz) (A) and to bolus injections of calcitonin gene–related peptide (CGRP; 0.05 and 0.1 nmol/kg, i.v.) (B), acetylcholine (ACh; 0.05 and 0.5 nmol/kg, i.v.) (C) and sodium nitroprusside (SNP; 0.5 and 5 μg/kg, i.v.) (D) in pithed 15% fructose–drinking rats (FDR;* n = *12) and control rats (*n = *9) with artificially elevated mean blood pressure (MBP).* ¡, *control rats;* ●, *FDR. Each bar indicates the mean ± SEM. MBP was increased and maintained at approximately 100 mmHg by continuous infusion of methoxamine.* **p < *0.01* vs. *control rats.*

depressor response to SCS was strongly decreased in pithed FDR with artificially increased BP and blocked autonomic outflow. We have reported that the depressor response to SCS in pithed rats with artificially increased BP and blocked autonomic outflow is mediated by endogenous CGRP released from CGRPergic vasodilator nerves, since the response is abolished by CGRP(8–37), a CGRP receptor antagonist, and capsaicin, a CGRP depletor from CGRPergic nerves (*13*, *16*, *18*). Furthermore, our previous report showed that acute hyperinsulinemia induced by exogenously applied insulin in euglycemic pithed rats markedly inhibited the CGRPergic mediated depressor response to SCS (*13*). The present findings showed that the CGRPergic nerve–mediated vasodilator response in FDR was significantly smaller than that in the controls, while there was no change in the vasodilator response to exogenous CGRP injection. These results suggest that the transmitter (CGRP) release from CGRPergic nerves is decreased in FDR. In the preliminary study, the level of CGRPergic nerve–mediated vasodilation in the mesenteric vascular beds isolated from Otsuka Long-Evans Tokushima Fatty (OLETF) rats, which are hyperinsulinemic and are used as the animal model for human type-2 diabetes, was markedly lower than that in control Long-Evans Tokushima (LET) rats. Therefore, it appears that the chronic hyperinsulinemia in FDR suppresses the function of CGRPergic vasodilator nerves.

Previous *in vitro* studies using rat mesenteric resistance arteries demonstrated that CGRPergic vasodilator nerves functionally attenuate the adrenergic nerve–mediated vasoconstriction, since abolition of CGRPergic nerve function by capsaicin and CGRP(8–37) causes augmentation of adrenergic nerve–mediated vasoconstriction (*19*, *20*). Our previous reports suggested that CGRPergic nerves suppress sympathetic nerve–mediated vasoconstriction *via* CGRP release, and conversely, sympathetic nerves presynaptically inhibit the release of CGRP from the nerves to decrease CGRPergic nerve function (*19*, *20*). Thus, we have proposed that CGRPergic vasodilator nerves along with sympathetic vasoconstrictor nerves regulate the tone of the mesenteric resistance arteries. Recent studies of spontaneously hypertensive rats (SHR) have shown that malfunction of the CGRPergic vasodilator nerves regulating peripheral vascular resistance plays an important role in the development and maintenance of hypertension in SHR (*21*, *22*). The SCS-induced pressor response in pithed rats without an autonomic ganglion blocker, which results from activation of both sympathetic nerves and CGRPergic nerves, is also attenuated by the simultaneous activation of CGRPergic nerves. It is thus clear that the increased pressor response to SCS observed in FDR

results from the reduced vasodilation mediated by CGRPergic nerves.

CGRP, a vasodilator neurotransmitter, is synthesized at the dorsal root ganglia and released from perivascular nerve terminals to induce vasodilation. In streptozotocin-induced diabetic rats, the content of CGRP in the nodose ganglia has been reported to be unchanged as compared to that in normal control rats (*23*). In addition, there is no difference in the pattern of CGRP distribution between the ganglion cells of the normal pancreas and those of the type 1 diabetic pancreas (*24*). In a previous study using fructose-fed rats, an angiotensin type 1 receptor blocker (olmesartan) was reported to inhibit the hyperinsulinemia (*25*). The present study showed that the Ang II–induced pressor response in FDR was greater than that in control rats, suggesting that FDR had an increased sensitivity to angiotensin type 1 receptors, and that this may have contributed to the induction of hyperinsulinemia. Moreover, in a preliminary study, we found that long-term administration (4 weeks) of the insulin sensitizer, pioglytazone, to FDR did not result in an increase in plasma insulin, an increase in sympathetic nerve–mediated pressor responses, or a decrease in CGRPergic nerve–mediated depressor responses. In addition, the treatment of FDR with pioglytazone did not increase the systemic blood pressure. Taken together, the findings of the present study suggest that the CGRPergic nerve function is decreased in the chronic hyperinsulinemic state, *i.e.*, in insulin resistance, and thereby sympathetic nerve–mediated vasoconstriction is extremely augmented and high BP is developed.

On the other hand, the present study showed that the depressor responses to bolus injections of ACh, which induces endothelium-dependent vasodilation, or SNP (NO donor) in FDR were similar to those in control rats. There have been many studies about the relation between insulin resistance and the reduction of endothelium-dependent vasodilation induced by ACh (*26–29*). Recent studies have shown that insulin resistance and/or obesity is associated with a reduction of endothelial function (*30–32*). Furthermore, tumor necrosis factor-α (TNF-α), resistin and free fatty acid (FFA), which are secreted from large adiposities and cause worsening of insulin resistance and endothelium disorder, have been reported to be significantly increased in obese patients (*33–35*). These factors play major roles in diminishing the concentration of adiponectin, which is a hormone secreted by adipocytes that acts as an antidiabetic and antiatherogenic adipokine (*36*, *37*). However, in the present study, the mean body weight of FDR was smaller than that in control rats, and the mean blood glucose level of FDR was similar to that of the control rats throughout the experiment. We conjecture that these factors did not have a major impact on the endothelial function in FDR, and thus the endothelium-dependent vasodilation in response to intravenous injection of ACh in FDR appeared to be similar to the response in control rats.

In conclusion, the findings of the present study suggest that chronic hyperinsulinemia augments sympathetic vasoconstriction, which is mediated by suppression of CGRPergic vasodilator nerve function. It is also suggested that both augmented sympathetic vasoconstriction and decreased CGRPergic nerve–mediated vasodilation are responsible for hypertension in FDR. The present study provides a hyperinsulinemia model with hypertension and demonstrates a clear relationship between the development of insulin resistance and hypertension.

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