Original Article

Acute Hyperglycemia and Hyperinsulinemia Enhance Adrenergic Vasoconstriction and Decrease Calcitonin Gene-Related Peptide-Containing Nerve-Mediated Vasodilation in Pithed Rats

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Recent clinical studies have demonstrated that transient postprandial hyperglycemia and hyperinsulinemia may contribute to the development of hypertension. Therefore, we investigated the influence of acute hyperglycemia and/or hyperinsulinemia induced by glucose or insulin infusion on neuronal and humoral control of vascular tone in rats. Euglycemic male Wistar rats were pithed under anesthesia and arterial blood pressure was measured. Changes in vascular responses to spinal cord stimulation (SCS) and intravenous bolus injections of noradrenaline, angiotensin II, calcitonin gene-related peptide (CGRP), acetylcholine and sodium nitroprusside (SNP) were studied by infusing various concentrations of glucose or insulin. Continuous glucose infusion, which increased both blood glucose and serum insulin levels, significantly augmented adrenergic nerve-mediated pressor responses to SCS without affecting pressor responses to injection of noradrenaline or angiotensin II. In pithed rats with artificially increased blood pressure and blockade of autonomic outflow, glucose infusion attenuated CGRPergic nerve-depressor responses to SCS without affecting depressor responses to injection of CGRP, acetylcholine or SNP. In pithed rats treated with octreotide, which increased blood glucose without increasing serum insulin levels, glucose infusion caused only significant augmentation of adrenergic nerve-mediated pressor responses. Combined infusion of insulin and glucose, which resulted in increased serum insulin levels with euglycemia, significantly augmented adrenergic nerve-mediated pressor responses and attenuated CGRPergic nerve-mediated depressor responses. The present results suggest that acute hyperglycemia and hyperinsulinemia increase adrenergic nerve-mediated vasoconstriction, which in turn blunts CGRPergic nerve function, and that the increase in plasma insulin concentration associated with hyperglycemia may be responsible for the alteration of neuronal vascular regulation. (*Hypertens Res* 2008; 31: 1033–1044)

Key Words: hyperglycemia, hyperinsulinemia, spinal cord stimulation, calcitonin gene-related peptide-containing nerve-mediated depressor, adrenergic nerve-mediated pressor

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Introduction

Epidemiological studies have revealed that approximately 40% of all patients with type 2 diabetes mellitus have complicating hypertension (1–3), suggesting that there is a relationship between hypertension and diabetes mellitus. Recent clinical studies have demonstrated that treatment with an α -glucosidase inhibitor in patients with impaired glucose tolerance, who have postprandial hyperglycemia, resulted in a significant reduction of hypertension (4, 5). Furthermore, a significant relationship between blood pressure level and plasma insulin and glucose response after a glucose load has been reported (6, 7), leading to the hypothesis that transient postprandial hyperglycemia and hyperinsulinemia may contribute to the development of hypertension.

It is widely accepted that resistance blood vessels play an important role in the maintenance of blood pressure and regulation of tissue blood flow. The tone of resistance arteries is mainly maintained by sympathetic adrenergic vasoconstrictor nerves. However, the vascular tone is controlled by not only sympathetic adrenergic nerves but also calcitonin gene-related peptide (CGRP)-containing (CGRPergic) vasodilator nerves (8). Our previous reports provided evidence that CGRPergic nerves suppress adrenergic nerve-mediated vasoconstriction *via* CGRP release, and, conversely, adrenergic nerves presynaptically inhibit the neurogenic release of CGRP from the nerves to decrease CGRPergic nerve function (9). Thus, both adrenergic and CGRPergic nerves regulate the tone of resistance arteries by reciprocal interaction. Also, angiotensin II, which has been shown to be associated with the pathogenesis of vascular complications of diabetes (10), regulates vascular tone as a humoral factor (11). Additionally, endothelial cells control the vascular tone by releasing relaxing and contracting factors (12).

Many studies have shown that an altered vascular responsiveness characterized by increased reactivity to vasoconstrictors and impaired vasodilation exists in the diabetic state (13–17). We have demonstrated that chronic hyperinsulinemia in fructose-drinking rats, an experimental model for insulin resistance and type 2 diabetes, facilitates adrenergic nerve-mediated vasoconstriction, which is associated with attenuated CGRPergic nerve-mediated vasodilation (18). However, whether transient postprandial hyperglycemia and hyperinsulinemia affect the control mechanism of vascular tone remains unknown.

Therefore, in the present study, we studied the influence of the combination of acute hyperglycemia and hyperinsulinemia, which correspond to postprandial hyperglycemia and hyperinsulinemia, on neuronal and humoral control of vascular tone by investigating vascular responses to spinal cord stimulation (SCS) and *i.v.* bolus injections of noradrenaline, CGRP, angiotensin II, acetylcholine or sodium nitroprusside (SNP) using euglycemic pithed rats, which have no vasoreflex. Additionally, we examined the influence of acute hyper-

glycemia or hyperinsulinemia on vascular responsiveness in pithed rats. Here we report evidence that acute hyperglycemia associated with hyperinsulinemia exerts neuronal control of vascular tone by facilitating adrenergic nerve-mediated vasoconstriction and inhibiting CGRPergic nerve-mediated vasodilation without affecting humoral tone control by angiotensin II and acetylcholine.

Methods

Animals

A total of 69 male euglycemic Wistar rats, weighing 300–350 g, were used in this study. The animals were given food and water *ad libitum*. They were housed in the Animal Research Center of Okayama University at a controlled ambient temperature of $22 \pm 10^\circ\text{C}$ with $50 \pm 10\%$ relative humidity and with a 12-h light/12-h dark cycle (lights on at 08:00 h). This study was carried out in accordance with the Guidelines for Animal Experiments of the Okayama University Advanced Science Research Center, the Japanese Government Animal Protection and Management Law (No. 115), and the Japanese Government Notification on Feeding and Safekeeping of Animals (No. 6). Every effort was made to minimize the number of animals used and their suffering. All experiments conformed to international guidelines on the ethical use of animals.

Pithing and Measurement

The animals were anesthetized with sodium pentobarbital (50 mg/kg, *i.p.*). Polyethylene catheters (PE-10) were positioned in the 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 arterial and mean blood pressures were recorded on a polygraph (model RM-6000; Nihon Kohden). The heart rate triggered by arterial pulses was measured using a cardi tachometer (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 vertebra (Th 9–12) according to the method described previously by Taguchi *et al.* (19), Kawasaki *et al.* (20) and Takatori *et al.* (21). Artificial respiration (4.5 mL/breath/kg, 70 breaths/min) with room air was immediately started using a respirator (model 683; Harvard Apparatus, South Natick, USA). The pithing rod served as the stimulating electrode, which was insulated except for 5 mm at the tip. The level of SCS was determined by varying the depth of insertion of the rod. The position of

the rod within the vertebral canal was determined from the length of the exposed rod. A stainless-steel needle was inserted subcutaneously in the dorsum, parallel to the vertebral column, to serve as an indifferent electrode. After the animals were pithed, *d*-tubocurarine (1 mg/kg) was intravenously injected to prevent skeletal muscle contraction during electrical stimulation of the spinal cord. The rectal temperature was maintained at approximately 37°C using a heating mat (model KN-475; Natsume, Tokyo, Japan).

Biochemical Analysis

At 30 min after starting the infusion of saline, mannitol, glucose or insulin, blood samples were taken from the right jugular veins. The plasma levels of glucose were measured using a glucose analyzer (ADVANTAGE; Boehringer Mannheim, Tokyo, Japan). Plasma insulin was measured by a double-antibody method with an ELISA insulin kit (Morinaga Biochemistry Co., Kanagawa, Japan).

Spinal Cord Stimulation

At 30 min after starting the infusion of saline, mannitol, glucose or insulin, electrical stimulation of the spinal cord at 2, 4 and 8 Hz, all of which induced a sharp increase in blood pressure without changing the heart rate, was applied to verify the position (Th 9–12) of the rod in the spinal column. Rectangular pulses (1 ms in duration and 10 V) were delivered for 30 s at 5- to 10-min intervals with an electronic stimulator (model SEN-3201, isolator 20865; Nihon Kohden). After finishing the pressor experiments, mean blood pressure was increased and maintained at a level of approximately 100 mmHg by continuous intravenous infusion of the α_1 -adrenoceptor agonist methoxamine (80 nmol/kg/min). The autonomic ganglionic blocker hexamethonium (1.4 μ mol/kg/min) was also intravenously infused to block autonomic outflow. The increased blood pressure was allowed to stabilize for 30 min, and then the spinal cord was electrically stimulated. Rectangular pulses (1 ms in duration and 10 V) at 4 and 8 Hz were delivered for 30 s at 5- to 10-min intervals with an electronic stimulator (model SEN-3201, isolator 20865; Nihon Kohden).

Experimental Protocols

To assess the underlying mechanisms involved in the vascular effect of acute hyperglycemia and/or hyperinsulinemia *in vivo*, the pressor and depressor responses to SCS and various vasoactive agents were evaluated in the pithed rats with glucose or insulin infusion. After the animals were pithed and both the blood pressure and heart rate were stabilized, continuous intravenous infusions of glucose (15%, 20% or 25%), 0.9% saline or 20% mannitol (0.1 mL/kg/min; $n=7$ –11/group) were carried out with an infusion pump (model 11;

Fig. 1. Blood glucose (A) and serum insulin (B) at 30 min after starting glucose, saline and mannitol infusion in pithed rats. Each bar and line represents the mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$ vs. saline. †† $p < 0.01$ vs. mannitol.

Harvard Apparatus) to produce experimentally both hyperglycemia and hyperinsulinemia. Saline and mannitol were used as a control for the effect of volume and osmolarity, respectively.

In another series of experiments, to investigate the effect of hyperinsulinemia, continuous infusions of insulin (25 or 40 nmol/L) and glucose (9% or 14%) were concomitantly carried out to maintain a normal glucose level.

To investigate the effect of hyperglycemia, a bolus injection of octreotide (50 or 150 μ g/kg) was carried out 10 min before starting the infusion of glucose; both concentrations of octreotide were previously shown to suppress endogenous insulin secretion concomitant with blood glucose elevation (22).

To confirm that a steady-state glucose level was reached at 30 min after starting the various infusions, SCS (2, 4 and 8 Hz) and intravenous injections of noradrenaline (0.75, 1.5 and 3 nmol/kg) and angiotensin II (50, 100 and 200 pmol/kg) were applied. Subsequently, the mean blood pressure of the pithed rats was increased by continuous concomitant infusion of methoxamine (80 nmol/kg/min) and hexamethonium (1.4 μ mol/kg/min). After the elevated blood pressure stabilized, SCS (4 and 8 Hz) and bolus injections of rat CGRP (0.1 and 0.2 nmol/kg), acetylcholine (0.05 and 0.5 nmol/kg) and SNP (1.7 and 17 nmol/kg) were applied. The order of application of the SCS and vasoactive agents was randomized in each pressor or depressor experiment. Vascular responses were expressed as the peak increase or decrease in mean blood pressure (Δ mmHg) from the baseline value before SCS or each agonist injection. During the glucose infusion, the blood glucose levels were monitored using a glucose analyzer (ADVANTAGE; Boehringer Mannheim, Tokyo, Japan).

Fig. 2. Typical records showing vasoconstrictor responses to spinal cord stimulation (SCS) (A, D and G: 2, 4 and 8 Hz; closed triangles) and bolus intravenous injections of noradrenaline (NA) (B, E and H: 0.75, 1.5 and 3 nmol/kg; closed circles) and angiotensin II (Ang II) (C, F and I: 50, 100 and 200 pmol/kg; closed squares) in pithed rats infused with saline (upper trace), mannitol (middle trace); and 20% glucose (lower trace). The numbers in the box represent the blood glucose level (mmol/L). BP, blood pressure.

Statistical Analysis

The experimental results are presented as the means $\pm$ SEM. Statistical analyses were performed using one or two-way analysis of variance (ANOVA) followed by Tukey's test, as appropriate. A *p* value less than 0.05 was considered statistically significant.

Drugs

The following drugs were used: acetylcholine chloride (Daiichi-Sankyo Co., Tokyo, Japan), angiotensin II (Peptide Institute, Osaka, Japan), D-glucose (Nacalai Tesque Inc., Kyoto, Japan), D-mannitol (Nacalai Tesque Inc.), hexamethonium bromide (Sigma Chemical Co., St. Louis, USA), methoxamine hydrochloride (Nippon Shinyaku Co., Kyoto, Japan), noradrenaline hydrochloride (Daiichi-Sankyo Co., Tokyo, Japan), rat α -CGRP (Peptide Institute), *d*-tubocurarine

(Sigma) and SNP (Sigma). All drugs were dissolved in 0.9% saline and intravenously infused at a rate of 0.1 mL/min using an infusion pump (model 11; Harvard Apparatus), or given as bolus doses (0.2 mL/kg).

Results

Effect of Glucose Infusion on Blood Values

Figure 1 shows the blood glucose and plasma insulin concentrations at 30 min after continuous infusion of 15–25% glucose, saline or mannitol in pithed rats. As shown in Fig. 1A, glucose infusion increased blood glucose in a dose-dependent manner, while saline or mannitol infusion did not cause any changes in blood glucose. There was a significant difference between the effects of glucose infusion and saline or mannitol infusion (Fig. 1A).

Serum insulin levels after infusion of glucose at all doses

Fig. 3. Bar graphs showing the effects of saline, mannitol and glucose (15%, 20% and 25%) infusions on pressor responses to spinal cord stimulation (SCS) (A: 2, 4 and 8 Hz) and to bolus intravenous injection of noradrenaline (NA) (B: 0.75, 1.5 and 3 nmol/kg) and angiotensin II (Ang II) (C: 50, 100 and 200 pmol/kg) in pithed rats. The ordinate indicates the peak increase in mean blood pressure (MBP) from baseline (Δ mmHg). Each bar and line represents the mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$ vs. saline. † $p < 0.05$, †† $p < 0.01$ vs. mannitol. MBP, mean blood pressure.

were significantly higher than those after infusion of saline. The plasma concentration of insulin after 20% glucose infusion was greater than that after 15% or 25% glucose infusion (Fig. 1B).

Effect of Glucose Infusion on Pressor Responses to SCS and Bolus Injections of Noradrenaline or Angiotensin II

The basal mean blood pressure and heart rate at 30 min after pithing were 41.7 ± 1.3 mmHg ($n=35$) and 308.8 ± 8.2 beats/min ($n=33$), respectively.

The basal mean blood pressure and heart rate at 30 min after continuous infusion of saline, mannitol and 15%, 20% and 25% glucose in pithed rats were 39.6 ± 2.0 mmHg and 279.6 ± 11.6 beats/min, 45.0 ± 1.7 mmHg and 302.3 ± 28.3 beats/min, 41.2 ± 1.5 mmHg and 336.7 ± 10.3 beats/min, 44.1 ± 2.8 mmHg and 336.0 ± 11.2 beats/min, and 45.5 ± 3.3 mmHg and 314.7 ± 13.0 beats/min, respectively. Continuous infusion of glucose slightly increased basal blood pressure by 2–6 mmHg in pithed rats. However, there was no significant difference in basal mean blood pressure or heart rate between any dose of glucose infusion and saline or mannitol infusion.

The SCS at 2, 4 and 8 Hz in the pithed rats receiving continuous infusion of saline, mannitol or 15–25% glucose caused a frequency-dependent, sharp increase in blood pressure (Fig. 2A, D and G) without changing the heart rate (data

not shown). Also, bolus injections of noradrenaline (0.75, 1.5 and 3 nmol/kg) (Fig. 2B, E and H) or angiotensin II (50, 100 and 200 pmol/kg) (Fig. 2C, F and I) induced dose-dependent increases in blood pressure. The blood glucose level in each infusion group was almost constant throughout the experiment.

As shown in Fig. 3A, pressor responses to SCS at 2, 4 and 8 Hz with 20% and 25% glucose infusion but not with 15% glucose infusion were significantly greater than those with saline or mannitol infusion.

There was no significant difference in the pressor responses to bolus injections of noradrenaline (0.75, 1.5 and 3 nmol/kg) (Fig. 3B) and angiotensin II (50, 100 and 200 pmol/kg) (Fig. 3C) between any dose of glucose infusion and saline or mannitol infusion.

Effect of Glucose Infusion on Depressor Responses to SCS and Bolus Injections of CGRP, Acetylcholine or SNP

As shown in Fig. 4A, E and I, when the blood pressure of pithed rats was increased by infusion of methoxamine in the presence of hexamethonium, SCS at 4 and 8 Hz caused a frequency-dependent depressor response without changing the heart rate (data not shown). Bolus injection of CGRP at doses of 0.1 and 0.2 nmol/kg induced a dose-dependent and long-lasting fall in blood pressure similar to the pattern of response

Fig. 4. Typical records showing vasodilator responses to spinal cord stimulation (SCS) (A, E and I: 4 and 8 Hz; inverted closed triangles) and bolus intravenous injections of calcitonin gene-related peptide (CGRP) (D, H and L: 0.1 and 0.2 nmol/kg; closed circles), acetylcholine (ACh) (B, F and J: 0.05 and 0.5 nmol/kg; closed squares) and sodium nitroprusside (SNP) (C, G and K: 1.7 and 17 nmol/kg; closed diamonds) in the pithed rats infused with saline (upper trace), mannitol (middle trace) and 20% glucose (lower trace). The blood pressure was artificially increased by continuous infusion of methoxamine (80 nmol/kg/min) and in the presence of hexamethonium (1.4 μ mol/kg/min). The numbers in the box represent the blood glucose level (mmol/L). BP, blood pressure.

to SCS (Fig. 4D, H and L). Bolus injections of acetylcholine (0.05 and 0.5 nmol/kg) (Fig. 4B, F and J) or SNP (1.7 and 17 nmol/kg) (Fig. 4C, G and K) caused a sharp fall in blood pressure in a dose-dependent manner. The blood glucose level in each infusion group was almost constant throughout the experiment.

As shown in Fig. 5A, depressor responses to SCS (4 and 8 Hz) with 15% and 20%, but not 25% glucose infusion were smaller than those with saline or mannitol infusion. A significant difference ($p < 0.05$) was found in the group infused with 20% glucose compared with the group infused with saline or mannitol. However, depressor responses to bolus injections of CGRP (0.1 and 0.2 nmol/kg) (Fig. 5B), acetylcholine (Fig. 5C) and SNP (Fig. 5D) in the groups treated with glucose infusions were similar to those in the groups treated with saline or mannitol infusion.

Effect of Glucose Infusion after Pretreatment with Octreotide on Blood Values and Vascular Responses

Figure 6A and B show blood glucose and plasma insulin concentrations at 30 min after 20% glucose infusion in pithed rats treated with octreotide (50 or 150 μ g/kg). The treatment with octreotide at a dose of 50 μ g/kg increased both plasma insulin and blood glucose levels after 20% glucose infusion, while 20% glucose infusion after treatment with octreotide at a dose of 150 μ g/kg resulted in increased blood glucose, but serum insulin levels are similar to the level of saline infusion.

The basal mean blood pressure and heart rate at 30 min after continuous infusion of 20% glucose in pithed rats treated with octreotide at 50 and 150 μ g/kg were 40.9 ± 1.3 mmHg and 275.2 ± 12.8 beats/min and 39.7 ± 2.1 mmHg and

Fig. 5. Bar graphs showing the effects of saline, mannitol and glucose (15%, 20% and 25%) infusions on vasodilator responses to spinal cord stimulation (SCS) (A: 4 and 8 Hz) and bolus injections of calcitonin gene-related peptide (CGRP) (B: 0.1 and 0.2 nmol/kg), acetylcholine (ACh) (C: 0.05 and 0.5 nmol/kg) and sodium nitroprusside (SNP) (D: 1.7 and 17 nmol/kg) in pithed rats. The ordinate indicates the peak decrease in mean blood pressure (MBP) from baseline (Δ mmHg). Each bar and line represents the mean $\pm$ SEM. The blood pressure was artificially increased by continuous infusion of methoxamine (80 nmol/kg/min) in the presence of hexamethonium (1.4 μ mol/kg/min). * $p < 0.05$ vs. saline. † $p < 0.05$, vs. mannitol. MBP, mean blood pressure.

289.7 $\pm$ 24.5 beats/min, respectively. There was no significant difference in basal mean blood pressure or heart rate between any dose of octreotide and glucose infusion and saline or mannitol infusion.

As shown in Fig. 7A and B, pressor responses to SCS (2, 4 and 8 Hz) but not noradrenaline injection (0.75, 1.5 and 3 nmol/kg) in rats given 20% glucose infusion after treatment with octreotide were significantly greater than those in rats given saline or mannitol infusion. However, 20% glucose infusion in pithed rats treated with octreotide did not significantly alter depressor responses to SCS (4 and 8 Hz) (Fig. 7C) and bolus injections of CGRP (0.1 and 0.2 nmol/kg) (Fig. 7D), compared with those in rats given saline or mannitol infusion.

Effect of Combined Insulin and Glucose Infusion on Blood Values and Vascular Responses

As shown in Fig. 6C and D, combined insulin (25 or 40 nmol/L) and glucose (9% or 14%) infusion significantly increased serum insulin levels in a dose-dependent manner, while the combination did not cause any changes in blood glucose levels.

The basal mean blood pressure and heart rate at 30 min

after combined insulin (25 or 40 nmol/L) and glucose (9% or 14%) infusion in pithed rats were 38.2 $\pm$ 3.1 mmHg and 294.3 $\pm$ 15.6 beats/min and 40.8 $\pm$ 3.7 mmHg and 299.7 $\pm$ 16.4 beats/min, respectively. There was no significant difference in basal mean blood pressure and heart rate between any dose of combined insulin and glucose infusion and saline or mannitol infusion.

As shown in Fig. 8A and B, pressor responses to SCS (2, 4 and 8 Hz) and bolus injections of noradrenaline (0.75, 1.5 and 3 nmol/kg) in animals receiving combined 40 nmol/L insulin and 14% glucose infusion but not in those receiving combined 25 nmol/L insulin and 9% glucose infusion were significantly greater than those in animals given saline or mannitol infusion. Depressor responses to SCS (4 and 8 Hz) (Fig. 8C) in rats receiving combined insulin (25 or 40 nmol/L) and glucose (9% or 14%) infusion were smaller than those by saline or mannitol infusion. A significant difference ($p < 0.05$) was found in the group infused with combined 40 nmol/L insulin and 14% glucose infusion compared with the group infused with saline or mannitol. However, there was no significant difference in the depressor responses to bolus injections of CGRP (0.1 and 0.2 nmol/kg) (Fig. 8D) between any dose of combined insulin and glucose infusion and saline or mannitol infusion.

Fig. 6. Bar graphs showing changes in the levels of blood glucose (A and C) and serum insulin (B and D). Upper and lower graphs show plasma levels at 30 min after starting infusions of 20% glucose in pithed rats treated with octreotide (50 or 150 µg/kg) and after starting combined infusion of insulin (25 or 40 nmol/L) and glucose (9% or 14%), respectively. Each bar and line represents the mean $\pm$ SEM. $^*p < 0.05$, $^{**}p < 0.01$ vs. saline. $^{\dagger}p < 0.01$ vs. mannitol.

Fig. 7. Bar graphs showing the effect of 20% glucose infusion on pressor and depressor responses to spinal cord stimulation (SCS) (A: 2, 4 and 8 Hz; C: 4 and 8 Hz) and to bolus intravenous injection of noradrenaline (NA) (B: 0.75, 1.5 and 3 nmol/kg) and calcitonin gene-related peptide (CGRP) (D: 0.1 and 0.2 nmol/kg) in pithed rats treated with octreotide (50 or 150 µg/kg). The ordinate indicates the peak increase or decrease in mean blood pressure (MBP) from baseline (Δ mmHg). Each bar and line represents the mean $\pm$ SEM. $^*p < 0.05$, vs. saline. $^{\dagger}p < 0.05$, vs. mannitol. MBP, mean blood pressure.

Fig. 8. Bar graphs showing the effect of combined infusion of insulin (25 or 40 nmol/L) and glucose (9% or 14%) on pressor and depressor responses to spinal cord stimulation (SCS) (A: 2, 4 and 8 Hz; C: 4 and 8 Hz) and to bolus intravenous injection of noradrenaline (NA) (B: 0.75, 1.5 and 3 nmol/kg) and calcitonin gene-related peptide (CGRP) (D: 0.1 and 0.2 nmol/kg) in pithed rats. The ordinate indicates the peak increase or decrease in mean blood pressure (MBP) from baseline (Δ mmHg). Each bar and line represents the mean $\pm$ SEM. * p < 0.05, ** p < 0.01 vs. saline. † p < 0.05, †† p < 0.01 vs. mannitol. MBP, mean blood pres-

Discussion

The present study demonstrated that, in euglycemic normal pithed rats, continuous glucose (15–25%) infusion increased the plasma levels of both glucose and insulin. It is likely that the elevated level of plasma insulin was due to insulin secretion from the pancreas caused by an increase in the blood glucose level, and that this condition of acute hyperglycemia and hyperinsulinemia induced by continuous glucose infusion is similar to the glucose and insulin response during oral glucose tolerance tests in obese Zucker rats, which are a prediabetic animal model with glucose intolerance (23). Additionally, in the present study, the basal blood pressure and heart rate of the pithed rats with glucose infusion were similar to those receiving saline and mannitol infusion. Therefore, it seems unlikely that acute hyperglycemia and hyperinsulinemia directly affect the basal blood pressure and heart rate in pithed rats, which have no vasoreflex.

The main finding of the present study is that SCS-induced pressor responses in an acute hyperglycemic and hyperinsulinemic state, which was produced by continuous glucose infusion, were significantly greater than those in an acute euglycemic and euinsulinemic state produced by continuous saline or mannitol infusion. The pressor response to SCS in the pithed rat has been shown to be mediated by activation of

sympathetic adrenergic nerves, since an adrenergic neuron blocker (guanethidine) and autonomic ganglionic blocker (hexamethonium) abolish the response (19, 21). In contrast, glucose infusion did not affect the pressor response to exogenously applied noradrenaline, which is mediated by postsynaptic α_1 -adrenoceptors. Therefore, it is very likely that the acute hyperglycemic and hyperinsulinemic state facilitates sympathetic neurotransmission, probably by increasing noradrenaline release from the sympathetic nerve terminals without affecting the sensitivity of α_1 -adrenoceptors on vascular smooth muscle cells. Additionally, the present study demonstrated that the acute hyperinsulinemia produced by continuous infusion of insulin resulted in augmentation of the pressor response to SCS in the euglycemic pithed rats. Thus, it appears that the augmented SCS-induced pressor response of pithed rats receiving glucose infusion mainly resulted from acute hyperinsulinemia, which increases sympathetic nerve activity.

Hoffman *et al.* (24) reported that acute hyperglycemia without hyperinsulinemia produces sympathetic neural activation in skeletal muscles of normal humans, suggesting that hyperglycemia alone has the ability to increase sympathetic nerve activity. Similarly, in the present study, acute hyperglycemia produced by glucose infusion in the presence of octreotide, which suppressed endogenous insulin secretion, significantly increased the pressor response to SCS without

Fig. 9. Schematic drawing of possible mechanisms underlying the alteration of adrenergic vasoconstriction and calcitonin gene-related peptide (CGRP)-containing nerve-mediated vasodilation under acute hyperglycemia and hyperinsulinemia corresponding to postprandial hyperglycemia and hyperinsulinemia in pithed rats. α_1 -R, α_1 -adrenoceptor; CGRP-R, CGRP-receptor; AT_1 -R, angiotensin II type 1 receptor; M-R, muscarinic receptor; NA, noradrenaline; Ang II, angiotensin II; ACh, acetylcholine; SNP, sodium nitroprusside.

affecting exogenously applied noradrenaline in the euinsulinemic pithed rats. Therefore, it seems likely that not only hyperinsulinemia but also hyperglycemia is responsible for the increased activity of sympathetic nerves.

Another important finding of the present study is that the acute hyperglycemic and hyperinsulinemic state produced by continuous glucose infusion resulted in a decrease in the depressor response to SCS in euglycemic pithed rats with artificially increased blood pressure and blockade of autonomic outflow. However, glucose infusion had no effect on the depressor response to exogenous CGRP injection. Previous reports have demonstrated that the depressor response to SCS in pithed rats with artificially elevated blood pressure and blockade of 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, which causes CGRP depletion in CGRPergic nerves (21, 25). Our previous report showed that acute hyperinsulinemia induced by exogenously applied insulin in euglycemic pithed rats resulted in marked reduction of the vasodilation mediated by CGRPergic nerve stimulation (21). Taken together, these findings suggest that the transmitter (CGRP) release from CGRPergic nerves is decreased in pithed rats in an acute hyperglycemic and hyperinsulinemic state.

In a previous *in vitro* study, insulin has been shown to act on CGRP receptors and cause vasodilation in mesenteric resistance arteries of rats (26). Additionally, CGRPergic

nerve terminals have presynaptic CGRP receptors that inhibit neurotransmitter CGRP release *via* a negative feedback mechanism (27). Therefore, it appears possible that acute excess insulin stimulates presynaptic CGRP receptors to inhibit the neurotransmitter CGRP release from CGRPergic nerve terminals, resulting in the decrease in vasodilator response to SCS of the pithed rats in the acute hyperglycemic and hyperinsulinemic state.

The plasma glucose elevation induced by glucose infusion was dose-dependent. However, the effects of glucose infusion on the plasma insulin level and on the neurogenic pressor and depressor responses were not dose-dependent and showed a bell-shaped pattern, since 25% glucose infusion induced a smaller increase in plasma insulin, a smaller facilitatory effect on the adrenergic pressor response, and a smaller inhibitory effect on the CGRPergic depressor response than did 20% glucose infusion. These findings suggest that the alteration of the neurogenic vascular responses to glucose infusion depends on the plasma insulin level. This notion is supported by the present finding that the CGRPergic nerve-mediated depressor response to SCS was attenuated in the acute hyperinsulinemic state, but was unaffected by the acute hyperglycemic state.

Previous *in vitro* studies using rat mesenteric resistance arteries have demonstrated that CGRPergic vasodilator nerves functionally attenuate the adrenergic nerve-mediated vasoconstriction, suggesting that adrenergic and CGRPergic nerves regulate the tone of resistance arteries by reciprocal interactions (9, 28). Studies using an experimental hypertension model demonstrated that the density of CGRPergic vasodilator nerve innervation in the mesenteric resistance arteries of spontaneously hypertensive rats (SHR) decreases with ageing, suggesting that the remodeling of the CGRPergic innervation contributes to the development and maintenance of hypertension in SHR (20, 28, 29). In the present *in vivo* study, the SCS-induced pressor response in pithed rats without an autonomic ganglion blocker resulted from activation of both sympathetic nerves and CGRPergic nerves. Therefore, it seems likely that the increase in the pressor response to SCS observed in the acute hyperglycemic and hyperinsulinemic state resulted in part from the decreased vasodilation mediated by CGRPergic nerves.

The present study showed that the angiotensin II-induced pressor response in an acute hyperglycemic and hyperinsulinemic state produced by glucose infusion was similar to that in control rats receiving saline infusion. In contrast to this finding, the angiotensin II-induced contractile response in the normal rat under severe hyperglycemic conditions and in the streptozotocin-induced type 1 diabetic rat has been shown to be enhanced (30, 31). This result and our finding suggest that severe hyperglycemia but not moderate hyperglycemia may lead to the upregulation of angiotensin II type 1 receptors. This notion may be supported by the preliminary finding that severe hyperglycemia produced by 35% glucose infusion augmented the pressor response to angiotensin II.

In the present study, glucose infusion did not affect the depressor responses to bolus injection of acetylcholine, which causes endothelium-dependent vasodilation, or SNP (a nitric oxide donor). Many studies have demonstrated a reduction of endothelial function in type 1 and 2 diabetic models (13, 32, 33). Therefore, it is probable that the period of exposure to the hyperglycemic and hyperinsulinemic state in the present study was too short to reduce endothelial function.

In conclusion, the present findings suggest that acute hyperglycemia and hyperinsulinemia increase adrenergic nerve-mediated vasoconstriction, which is partly associated with the blunted CGRPergic nerve function, and that plasma insulin concentration associated with hyperglycemia may be responsible for the alteration of neuronal vascular regulation. Therefore, it is likely that transient postprandial hyperglycemia and hyperinsulinemia elicit abnormal neuronal regulation of vascular tone, which may partly contribute to the development of hypertension (Fig. 9).

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