Age-Related Decrease of Calcitonin Gene-Related Peptide–Containing Vasodilator Innervation in the Mesenteric Resistance Vessel of the Spontaneously Hypertensive Rat

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We previously demonstrated that the mesenteric resistance blood vessels have nonadrenergic, noncholinergic vasodilator innervation in which calcitonin gene-related peptide (CGRP) is a possible neurotransmitter. The role of CGR-containing vasodilator nerves in hypertension was investigated in perfused mesenteric vascular beds isolated from spontaneously hypertensive rats (SHR). The adrenergic vasoconstrictor responses to perivascular nerve stimulation in both SHR (8-, 15-, and 30-week-old) and age-matched Wistar-Kyoto (WKY) rat preparations increased with aging, but the response was greater in SHR than in WKY rats at all ages. The preparation isolated from SHR and WKY rats was precontracted by continuous perfusion of Krebs’ solution containing \(7 \times 10^{-6} \, \text{M} \) methoxamine plus \(5 \times 10^{-6} \, \text{M} \) guanethidine. In both SHR and WKY rats, perivascular nerve stimulation (1–8 Hz) produced frequency-dependent vasodilation, which was blocked by \(1 \times 10^{-7} \, \text{M} \) tetrodotoxin, pretreatment with \(5 \times 10^{-7} \, \text{M} \) capsaicin, and denervation by cold storage (4°C for 72 hours). The vasodilation induced by perivascular nerve stimulation in SHR greatly decreased with age, whereas a slight decrease in the response with age was found in WKY rats. The neurogenic vasodilation in the young SHR preparation was similar in magnitude to the vasodilation in age-matched WKY rats, whereas the vasodilation in 15- and 30-week-old SHR was significantly smaller than that in age-matched WKY rats. In both SHR and WKY rats, perfusion of rat CGRP (\(1 \times 10^{-10} \, \text{to} \, 3 \times 10^{-8} \, \text{M} \)) produced marked vasodilation in a concentration-dependent manner. The CGRP-induced vasodilation in SHR increased with age, whereas an age-related decrease in vasodilation was found in WKY rats. Perivascular nerve stimulation (4 and 8 Hz) of the perfused mesenteric vascular bed evoked an increased release of CGRP-like immunoreactive substance in the perfusate, which was significantly less in 15-week-old SHR than in age-matched WKY rats. Immunohistochemical studies showed an age-related decrease in CGRP-like immunoreactive fibers in SHR but not in WKY rats. These results suggest that CGRP-containing vasodilator innervation is greatly decreased when SHR develop and maintain hypertension. It is also suggested that the decreased vasodilator mechanism by CGRP-containing nerves contributes to the development and maintenance of hypertension. (Circulation Research 1990;67:733–743)

Although the etiology of essential hypertension remains unresolved, elevated total peripheral vascular resistance maintains elevated blood pressure in chronic hypertension.1–3 It is generally accepted that peripheral vascular resistance is mainly controlled by sympathetic, adrenergic nerves.

In hypertension, the function of the control systems regulating peripheral resistance is considered to be impaired.4 In fact, enhanced activity of sympathetic vasoconstrictor nerves has been shown to be an important factor for increased tone of peripheral resistance vessels in spontaneously hypertensive rats (SHR),3,5 which are the best animal models for human essential hypertension.6,7

Recently, we have demonstrated that the mesenteric resistance blood vessel of the rat has nonadrenergic, noncholinergic vasodilator innervation and suggested that tone of the peripheral resistance vascular bed is controlled not only by sympathetic, adrenergic nerves but also by nonadrenergic, non-
cholinergic vasodilator nerves. Additionally, we have shown that the neurogenic vasodilator response was completely inhibited by pretreatment with capsaicin, a peptidergic and sensory neurotoxin. Capsaicin has been shown to diminish calcitonin gene-related peptide (CGRP)-like immunoreactive (CGRP-LI) fibers in the mesenteric artery. Exogenous CGRP, a potent vasodilator neuropeptide with 37 amino acid residues, produces marked vasodilation of the mesenteric artery, which mimics the neurogenic vasodilation. Thus, CGRP acts as a novel vasodilator neurotransmitter in nonadrenergic, noncholinergic vasodilator nerves.

Although the malfunction of adrenergic nerves regulating peripheral vascular resistance is involved in cardiovascular disease, such as hypertension, the role of CGRP-containing vasodilator nerves in the cardiovascular function is not known. Recently, we have found that the CGRP-containing vasodilator nerves inhibit adrenergic vasoconstriction in the rat mesenteric artery. A more recent report revealed that the CGRP concentration in plasma is decreased in SHR when compared with normotensive Wistar-Kyoto (WKY) rats, suggesting a reduced activity of CGRP-containing nerves. The present study was, therefore, undertaken to investigate the role of CGRP-containing nerves in hypertension. We report that the control system of CGRP-containing vasodilator nerves regulating peripheral vascular tone is greatly decreased when SHR develop and maintain hypertension.

Materials and Methods

Animals

Male SHR at 8, 15, and 30 weeks of age and age-matched WKY rats were used in this study. The rats were given food and water ad libitum. They were housed in the Experimental Animal Center of Miyazaki Medical College at a controlled ambient temperature of 22°C with 50±10% relative humidity and with a 12-hr light/dark cycle (light on at 7:30 AM).

Blood Pressure Measurement

The rats were anesthetized with an intraperitoneal injection of 50 mg/kg sodium pentobarbital. The left carotid artery was cannulated, and the arterial pressure was measured with a Statham pressure transducer (model P23ID, Gould, Cleveland) and recorded on a polygraph (model RM-6000, Nihon Kohden, Tokyo).

Perfusion of the Mesenteric Vascular Bed

Under pentobarbital anesthesia, the mesenteric vascular bed was isolated and prepared for perfusion as described previously. The superior mesenteric artery was cannulated and gently flushed with a modified (see below) Krebs-Ringer bicarbonate solution (Krebs’ solution) to eliminate blood from the vascular bed. After removal of the entire intestine and associated vascular bed, the mesenteric vascular bed was separated from the intestine by cutting close to the intestinal wall. Only four main arterial branches from the superior mesenteric trunk running to the terminal ileum were perfused. All other branches of the superior mesenteric artery were tied off. The preparation was perfused with Krebs’ solution at a constant flow rate of 5 ml/min with a peristaltic pump (model SJ-1215, ATTO Co., Tokyo). The preparation was also superfused with the same solution at a rate of 0.5 ml/min to prevent drying. Modified Krebs’ solution of the following composition was used (mM): NaCl 120.0, KCl 5.0, CaCl2 2.4, MgSO4 1.2, NaHCO3 25.0, 2NaEDTA 0.027, and dextrose 11.0 (pH 7.4). The Krebs’ solution was bubbled with a mixture of 95% O2-5% CO2 before passage through a warming coil maintained at 37°C. Changes in the perfusion pressure were measured with a pressure transducer (model MPU-0.5A, Nihon Kohden) and recorded on a polygraph (model RM-25, Nihon Kohden).

Perivascular Nerve Stimulation and Perfusion of CGRP

After allowing the basal perfusion pressure to stabilize, the preparation was subjected to perivascular nerve stimulation (PNS) at 1–12 Hz and then was contracted with methoxamine at a submaximal concentration of 7×10–6 M in the presence of 5×10–6 M guanethidine, which was added to block adrenergic neurotransmission. The increased perfusion pressure was allowed to stabilize, and the preparation was again subjected to PNS at 0.5, 1, 2, 4, and 8 Hz. PNS was applied for 30 seconds through bipolar platinum ring electrodes placed around the superior mesenteric artery. Rectangular pulses of 1 msec in duration and supramaximal voltage (50 V) were applied using an electronic stimulator (model SEN 3301, Nihon Kohden). A decrease in perfusion pressure was observed. After the decrease in perfusion pressure induced by PNS at 8 Hz had returned to the prestimulation level (for at least 30 minutes), the preparation was perfused with a final concentration of rat CGRP, which was achieved by dilution with Krebs’ solution containing methoxamine and guanethidine.

Some vascular beds isolated from 15-week-old SHR and WKY rats were stored in Krebs’ solution at 4°C for 72 hours to achieve the degeneration of intramural nerves (cold storage denervation). In this preparation, to determine the intact responsiveness of smooth muscle, bolus infusion of 1 nmol acetylcholine or 1 nmol rat CGRP, which was diluted with Krebs’ solution containing methoxamine and guanethidine, was infused (model 975 infusion pump, Harvard Apparatus, South Natick, Mass.) directly into the perfusate proximal to the arterial cannula. The volumes of infusion were 100 μl for 10 seconds.

In some experiments, capsaicin (5×10–7 M) was perfused for 20 minutes, and the preparation was rinsed with capsaicin-free Krebs’ solution for 60 minutes.
At the end of each experiment, the preparation was perfused with 1×10^{-4} M papaverine to relax it completely.

**Release of CGRP-LI**

The mesenteric vascular beds isolated from 15-week-old SHR and age-matched WKY rats were perfused with Krebs' solution containing guanethidine (5×10^{-6} M) at a constant flow rate of 5 ml/min and superfused with Krebs' solution (0.5 ml/min). In experiments using Ca^{2+}-free Krebs' solution and tetrodotoxin, the preparation was perfused with Ca^{2+}-free Krebs' solution containing guanethidine or normal Krebs' solution containing tetrodotoxin and guanethidine 30 minutes before collecting the sample. The Ca^{2+}-free Krebs' solution was prepared by omitting CaCl_{2} and adding 0.1 mM EGTA. The perfusate was collected for 10 minutes before and after PNS (4 and 8 Hz for 30 seconds). Each sample was applied to a Sep-Pak C_{18} cartridge (Waters Associates, Milford, Mass.), and the absorbed peptide was eluted with 3 ml of 60% acetonitrile in 0.1% trifluoroacetic acid. The eluate was evaporated under a vacuum and stored at −80°C until a radioimmunoassay (RIA) for CGRP as described by Fujimori et al.16 The incubation buffer for the RIA was a 50 mM sodium phosphate buffer (pH 7.4) containing 0.1% bovine serum albumin, 0.1% Triton X-100, 80 mM NaCl, 25 mM 2NaEDTA, 0.05% NaN_{3}, and Trasylol 500 kallikrein inhibiting units/ml as described by Miyata et al.17 The samples were preincubated with rabbit anti-human CGRP-II serum (Peninsula Laboratories, Inc., Belmont, Calif.) at 4°C for 12 hours. Then, the reaction mixture was incubated with [2-{^{125}}I]iodohistidyl-	extsuperscript{10}CGRP (human) (Amersham Int., Buckinghamshire, UK) for an additional 24–36 hours at 4°C. The antibody-bound antigen was separated from free antigens by use of a second (anti-rabbit) antibody. The antibodies to CGRP cross-reacted 100% with rat- and human-CGRP, but not with substance P and vasoactive intestinal peptide. The lower detection limit was 1 fmol/tube for CGRP-LI.

In another series of experiments, the perfusate was subjected to an analysis with a reverse-phase high-performance liquid chromatography (HPLC). The mesenteric vascular bed isolated from 15-week-old SHR and WKY rats was perfused with Krebs' solution containing 5×10^{-6} M guanethidine. The PNS at 8 Hz was applied for 30 seconds, and the perfusate during and after PNS was collected for 10 minutes and was repeated three times at intervals of 60 minutes. The perfusate collected in each preparation was desalted by a Sep-pak C_{18} cartridge (Waters), applied to a Cosmosil ODS-SIL-300 column (4.6×300 mm) (Nakarai Chemicals, Kyoto, Japan), and eluted with a linear gradient of 10–60% acetonitrile in 0.1% trifluoroacetic acid using HPLC. CGRP in fractions collected every 1 minute was determined by RIA.

**Immunohistochemistry**

The isolated mesenteric arteries were fixed in paraformaldehyde and picric acid for 24–72 hours at 4°C.10 The tissues were dehydrated with ethanol, cleared with xylene, and rehydrated. Antibodies to rat CGRP (Cambridge Research Biochemicals, Cambridge, UK) were applied at a 1:1,600 dilution for 16–24 hours at 4°C. First, biotinylated anti-rabbit immunoglobulin G and then fluorescent isothiocyanate-labeled avidin were applied to the tissues. The tissues were mounted in buffered glycerol and examined under a fluorescence microscope (Carl Zeiss, Inc., Thornwood, N.Y.). Antibodies preincubated with rat CGRP (3×10^{-6} M) served as the control. The rat CGRP antibodies were found not to cross-react with substance P (1×10^{-5} M) or vasoactive intestinal peptide (1×10^{-3} M).

**Statistical Analysis**

The experimental results are presented as the mean±SEM. One-way analysis of variance followed by Dunnett's test was used to determine the significance of the difference between values of different ages. Unpaired Student's t test was used to determine the significance of difference between two means. A value of p<0.05 was considered statistically significant.

**Drugs**

The following drugs were used: acetylcholine HCl (Daichi Pharmaceutical, Tokyo), capsaicin (Sigma Chemical Co., St. Louis), guanethidine sulfate (Tokyo Kasei, Tokyo), methoxamine HCl (Nihon Shinyaku, Kyoto, Japan), rat CGRP (Peptide Institute, Osaka, Japan), substance P (Peptide Institute), tetrodotoxin (Sigma), and vasoactive intestinal peptide (Sigma). Capsaicin was dissolved in 50% ethanol and diluted with Krebs' solution (final alcohol concentration was 0.4 μg/ml). Both rat CGRP and acetylcholine were dissolved in distilled water and diluted with Krebs' solution containing 7×10^{-6} M methoxamine and 5×10^{-6} M guanethidine, when injected as a bolus.

**Results**

**Blood Pressure and Perfusion Pressure in SHR and WKY Rats**

As shown in Figure 1, the mean carotid arterial pressures in SHR at 15 (p<0.01 by Dunnett's test) and 30 (p<0.05 by Dunnett's test) weeks of age were significantly greater than that at 8 weeks of age; no significant difference was found in the WKY blood pressures at any ages. Mean carotid arterial pressures in SHR at 8, 15, and 30 weeks of age were significantly greater than those of age-matched WKY rats. However, there was no significant difference in the resting mean perfusion pressure between SHR and WKY rats at any age, except for at 30 weeks (perfusion pressure being greater in SHR than WKY rats [Figure 1]).
Adrenergic Vasoconstrictor Response in SHR and WKY Rats

As shown in Figure 2, PNS of the perfused mesenteric vascular bed from SHR at 15 weeks of age produced a frequency-dependent (1–12 Hz) increase in perfusion pressure due to vasoconstrictor response (Figures 2A and 2C). These pressor responses to PNS were abolished by guanethidine (Figures 2A and 2B), the neurotoxin tetrodotoxin (data not shown),8,14 and the α-adrenoceptor antagonist prazosin (data not shown)8,14 and by cold storage denervation (Figure 2B). These results indicate that vasoconstrictor responses to PNS were produced by an endogenous transmitter (norepinephrine) from arterial sympathetic, adrenergic nerves.

The pressor responses to PNS at 8 and 12 Hz were significantly greater in SHR than in WKY rats at all ages (Figure 3A). Furthermore, pressor responses to PNS were greater in both 15- and 30-week-old SHR and WKY rats than in 8-week-old SHR and WKY rats (Figures 3B and 4). Thus, the vasoconstriction

Figure 1. Graphs showing blood pressure of anesthetized spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats and perfusion pressure and pressor response to continuous perfusion of methoxamine in the perfused mesenteric vascular bed. *p<0.05, ***p<0.001 compared with WKY rats by unpaired Student's t test.

Figure 2. Typical recordings of the neurogenic vasodilation induced by perivascular nerve stimulation (▲ and ▼) and effects of various agents and treatments on perfused mesenteric vascular beds of spontaneously hypertensive rats (SHR) at 15 weeks of age. Panel A: Frequency-dependent decrease in perfusion pressure induced by perivascular nerve stimulation and its abolition by tetrodotoxin (1×10⁻⁷ M) in the contracted preparation. Panel B: No observed vasodilation induced by perivascular nerve stimulation but marked relaxation induced by bolus infusion (●) of acetylcholine (ACh) or rat calcitonin gene-related peptide (CGRP) in the contracted preparation, which was denervated by cold storage at 4°C for 72 hours, was demonstrated. PPV, papaverine. Panel C: Abolition of vasodilation in response to perivascular nerve stimulation after capsaicin (5×10⁻⁷ M) treatment for 20 minutes.
induced by adrenergic nerve stimulation in both SHR and WKY rats became greater with age.

Neurogenic Vasodilator Response in SHR and WKY Rats

To maintain active tone of the mesenteric vascular bed, the preparation was contracted by continuous perfusion of $7 \times 10^{-5}$ M methoxamine ($\alpha_1$-adrenergic receptor agonist) in the presence of $5 \times 10^{-6}$ M guanethidine (adrenergic neuron blocker), which was added to block adrenergic neurotransmission. As shown in Figure 1, there was no significant difference in the methoxamine-induced rises in mean perfusion pressure before PNS between SHR and WKY rats at all ages.

In the SHR preparation with active tone, PNS at 4 and 8 Hz produced a frequency-dependent decrease in perfusion pressure due to vasodilation, as shown in Figure 2A. The vasodilation was abolished by combined perfusion of $1 \times 10^{-7}$ M tetrodotoxin (Figure 2A) and by cold storage denervation (Figure 2B). Additionally, PNS at 1–12 Hz did not produce a vasodilator response when the preparation was pretreated with $5 \times 10^{-7}$ M capsaicin. Similar results were obtained in the WKY preparation.

Effect of Aging on Neurogenic Vasodilator Response

As shown in Figure 4A, PNS from 0.5 to 8 Hz, in the contracted preparation of 8-week-old SHR, produced frequency-dependent vasodilation. The PNS-induced (0.5–4 Hz) vasodilation in 8-week-old SHR preparations was similar in magnitude to the vasodilation observed in age-matched WKY preparations, and the vasodilation at 8 Hz was significantly greater in SHR than in WKY rats (Figure 5A). However, the PNS-induced vasodilator responses at all frequencies were markedly decreased in 15-week-old SHR preparations (Figure 4B) and in 30-week-old SHR preparations (Figure 4C). Significant differences were found between 8-week-old SHR and 15- and 30-week-old SHR (Figure 5B). In WKY rats, there was only a small reduction in the PNS-induced vasodilator response with age (Figure 5B). The PNS-induced vasodilator responses of 15- and 30-week-old SHR were significantly less than those of age-matched WKY preparations (Figure 5A).

Effect of Aging on CGRP-Induced Vasodilation

In the contracted mesenteric vascular bed of SHR and WKY rats, perfusion of rat CGRP produced potent vasodilation in a concentration-dependent manner. The CGRP-induced vasodilation was significantly greater in SHR at 15 and 30 weeks of age than in age-matched WKY rats; it was not different between 8-week-old SHR and WKY preparations (Figure 6A and Table 1). Both $pD_2$ values (negative logarithm of the concentration causing half maximum relaxation) and maximum response to rat CGRP.
were greater in the preparations of 15- and 30-week-old SHR than in the preparations of 8-week-old SHR; those of WKY preparations decreased at 15 and 30 weeks of age (Figures 4 and 6B, Table 1).

Release of CGRP-LI

In the perfused mesenteric vascular beds isolated from 15-week-old SHR (mean carotid arterial pressure, 160±3.6 mm Hg; p<0.01 by Dunnett's test) and WKY (mean carotid arterial pressure, 98.2±6.7 mm Hg) rats, a small amount of CGRP-LI was detected in the perfusate before PNS, probably a spontaneous release of CGRP (Table 2). However, there was no significant difference in the spontaneous release of CGRP-LI between SHR and WKY rats. The PNS of the mesenteric vascular bed at 4 and 8 Hz evoked a frequency-dependent increase of CGRP-LI release, which was blocked by 1×10^{-7} M tetrodotoxin and the removal of calcium from the medium (data not shown). The release of CGRP-LI induced by PNS at 4 and 8 Hz was significantly less in SHR than in WKY rats (Table 2).

When characterized by reversed-phase HPLC, the released CGRP-LI during PNS eluted in a major peak corresponding to synthetic rat CGRP, indicating that CGRP-LI material detected by RIA originated in CGRP itself (data not shown).

Immunohistochemistry

As shown in Figures 7A and 7B, there were numerous CGRP-LI fibers around the mesenteric arteries from SHR and WKY rats at 8 weeks of age. A marked reduction in the density of CGRP-containing nerves was found in the arteries of aged SHR (30-week-old) compared with 8-week-old SHR (Figure 7D). However, slight or no decrease, and even an increase, in CGRP-containing fibers was found in the aged WKY rats (Figure 7C).

Discussion

The present study showed that perivascular nerve stimulation of the mesenteric vascular bed with active tone in both SHR and WKY rats produced a vasodilator response, which was abolished by tetrodotoxin, a neurotoxin, and by cold storage denervation. These results are in accord with previous findings. Thus, the PNS-induced vasodilation in SHR and WKY preparations is neurogenic in nature. In addition, the neurogenic vasodilator response was abolished by pretreatment with capsaicin, which markedly diminished CGRP-LI fibers in the mesentery. Inasmuch...
FIGURE 5. Graphs showing age-related changes in vasodilator response to perivascular nerve stimulation (PNS) of methoxamine-contracted, perfused mesenteric vascular beds of spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats. Panel A: Comparison between SHR and WKY responses (*p<0.05, **p<0.01 compared with WKY rats by unpaired Student’s t test). Panel B: Comparison of the response among different ages of SHR and WKY rats (*p<0.05, **p<0.01 compared with 8-week-old SHR and WKY rats by Dunnett’s test). Relaxation is expressed as a percentage of the maximum response induced by 1×10^{-4} M papaverine at the end of the experiment.

FIGURE 6. Graphs showing age-related changes in vasodilator response to exogenously applied rat calcitonin gene-related peptide (CGRP) in methoxamine-contracted, perfused mesenteric vascular beds from spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats. Panel A: Comparison between SHR and WKY responses (*p<0.05, **p<0.01 compared with WKY rats by unpaired Student’s t test). Panel B: Comparison of the response among different ages of SHR and WKY rats (*p<0.05, **p<0.01 compared with 8-week-old SHR and WKY rats by Dunnett’s test). Relaxation is expressed as a percentage of the maximum response induced by 1×10^{-4} M papaverine at the end of the experiment.
as capsaicin does not affect adrenergic and cholinergic nerves, the neurogenic response is likely to be mediated by nonadrenergic, noncholinergic vasodilator nerves. Capsaicin has been shown to deplete not only CGRP but also tachykinins such as substance P, neurokinin A, and neurokinin B. However, we have demonstrated that exogenously applied CGRP, but not tachykinins, causes vasodilation of the rat mesenteric vascular bed, which mimics the effect of PNS. Substance P, like acetylcholine, has been shown to produce endothelium-dependent vasodilation, perhaps through releasing an endothelium-derived relaxing factor. The long distance between the nerve terminals and the endothelial cells makes it unlikely that substance P and acetylcholine released from nerves will reach the endothelial cells in sufficient concentrations to induce vasodilation. Therefore, we proposed that CGRP is a potential neurotransmitter for the neurogenic vasodilation of mesenteric resistance blood vessels. Furthermore, this notion is supported by the present finding that perivascular nerve stimulation of the mesenteric vascular bed produced an increased release of CGRP-LI. Inasmuch as the release of CGRP-LI by PNS was abolished by tetrodotoxin and calcium removal from the medium, the PNS-evoked CGRP-LI release is neurogenic and Ca^2+ dependent. Taken together, it appears that the mesenteric resistance vessels in SHR and WKY rats have nonadrenergic, noncholinergic vasodilator innervation in which CGRP acts as a neurotransmitter.

The present study demonstrated that the neurogenic vasodilation mediated by CGRP-containing nerves in SHR significantly decreased with age, whereas the age-related decrease in the vasodilator response was small in WKY rats. It seems unlikely that the decreased neurogenic vasodilation in SHR results from the increased vasoconstrictor response to methoxamine that was used to induce active tone of the mesenteric artery, because there was no significant difference in the methoxamine-induced vasoconstriction between SHR and WKY preparations. In immunohistochemical studies, many CGRP-LI fibers were observed in the mesenteric artery of young WKY rats and especially in that of young SHR. This finding parallels the profound neurogenic vasodilation in young SHR and WKY rats. On the contrary, a marked reduction in CGRP-LI fibers was found in the arteries from aged SHR, but no decrease in CGRP-LI fibers was observed in the aged WKY rats. These results also parallel the marked reduction in neurogenic vasodilation in aged SHR; there was only a slight decrease in WKY rats. In addition, the present study showed a smaller release of CGRP-LI evoked by PNS in aged SHR than in aged WKY rats. These results strongly suggest that the decreased neurogenic vasodilation in adult SHR results from diminished population or neuronal activity of CGRP-containing vasodilator nerves. This notion is supported by recent findings of Xu et al. that plasma concentration of CGRP, which might be derived from the peripheral vascular system, was more significantly decreased in SHR (10–12-week-old) than in WKY rats.

### Table 1. Vasodilator Responses to Exogenously Applied Rat Calcitonin Gene-Related Peptide in Methoxamine-Contracted, Perfused Mesenteric Vascular Beds From Spontaneously Hypertensive and Wistar-Kyoto Rats

<table>
<thead>
<tr>
<th>Age</th>
<th>pD2</th>
<th>% Max</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WKY</td>
<td>SHR</td>
</tr>
<tr>
<td>8-week-old</td>
<td>9.37±0.09</td>
<td>9.35±0.05</td>
</tr>
<tr>
<td>15-week-old</td>
<td>9.04±0.06</td>
<td>9.41±0.09*</td>
</tr>
<tr>
<td>30-week-old</td>
<td>9.02±0.15</td>
<td>9.53±0.11†</td>
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Values are mean±SEM. pD2 negative logarithm of the molar concentration of 50% effective dose. % Max, maximum relaxation induced by rat calcitonin gene-related peptide expressed as percent of the maximum relaxation induced by 1×10^-4 M papaverine; WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats.

*p<0.01 compared with age-matched WKY rats by unpaired Student's t test.

tp<0.01 compared with 8-week-old SHR by Dunnett's t test.

tp<0.05 compared with age-matched WKY rats by unpaired Student's t test.

### Table 2. Release of Calcitonin Gene-Related Peptide–Like Immunoreactivity Induced by Perivascular Nerve Stimulation in the Perfused Mesenteric Vascula Bed Isolated From 15-Week-Old Spontaneously Hypertensive and Wistar-Kyoto Rats

<table>
<thead>
<tr>
<th>Condition</th>
<th>SHR (n=7) (fmol/ml)</th>
<th>WKY (n=5) (fmol/ml)</th>
</tr>
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<tbody>
<tr>
<td>Pre-PNS</td>
<td>0.259±0.077</td>
<td>0.195±0.030</td>
</tr>
<tr>
<td>PNS (4 Hz)</td>
<td>0.473±0.099</td>
<td>0.707±0.068</td>
</tr>
<tr>
<td>Net-PNS</td>
<td>0.214±0.040*</td>
<td>0.512±0.061</td>
</tr>
<tr>
<td>Pre-PNS</td>
<td>0.275±0.080</td>
<td>0.188±0.022</td>
</tr>
<tr>
<td>PNS (8 Hz)</td>
<td>0.790±0.064*</td>
<td>1.310±0.124</td>
</tr>
<tr>
<td>Net-PNS</td>
<td>0.515±0.073†</td>
<td>1.122±0.108</td>
</tr>
</tbody>
</table>

Values are mean±SEM. SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats; Pre-PNS, spontaneous release of calcitonin gene-related peptide-like immunoreactivity before perivascular nerve stimulation (PNS); Net-PNS, net release of calcitonin gene-related peptide-like immunoreactivity induced by PNS (PNS-induced release minus spontaneous release).

*p<0.01, tp<0.001 compared with WKY rats by unpaired Student's t test.
FIGURE 7. Calcitonin gene-related peptide–like immunoreactive (CGRP-LI) fibers in the mesenteric artery. Panels A and B: Abundance of CGRP-LI fibers in young spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats at 8 weeks of age. Panels C and D: Marked decrease in CGRP-LI fibers observed in adult SHR at 30 weeks of age, but not in the age-matched WKY rats. Bar, 50 μm.
neurogenic vasodilation in the adult SHR may be due to the diminished vasodilator response to vasodilator transmitter CGRP. However, the present study showed that the vasodilator effect of exogenously applied CGRP (rat CGRP) in the perfused mesenteric vascular bed was greater in aged SHR than in young SHR, whereas the vasodilator response to CGRP in WKY rats decreased with aging. Therefore, the slight decrease of PNS-induced vasodilation with age in WKY preparations is most likely due to the decreased response of vascular smooth muscles to the released CGRP. On the other hand, it appears that increased vasodilator response to CGRP in SHR results from increased sensitivity of receptors to CGRP, which is caused by the decreased release of CGRP from CGRP-containing nerves.

There is evidence that increased total peripheral vascular resistance in SHR might result from enhanced activity of sympathetic adrenergic nerves. However, adrenergic nerve activity has been observed to be greater in young, prehypertensive SHR, but it is not different between adult SHR and adult WKY rats. Therefore, it is proposed that adrenergic nerves may play an important role as an initiating factor in the hypertensive process. The present study demonstrated that the adrenergic nerve-mediated vasoconstrictor response was enhanced by aging in WKY rats and especially in SHR. On the other hand, the CGRP-containing nerve-mediated vasodilation was greatly decreased with age in SHR, but only slightly decreased with age in WKY rats. We have shown that adrenergic nerve-mediated vasoconstriction is markedly potentiated by denervation of CGRP-containing nerves with capsaicin. This indicates that CGRP-containing nerves inhibit adrenergic nerve-mediated vasoconstriction. It is presumable that the age-related increase in adrenergic response in SHR may result from the age-related decrease in activity of CGRP-containing nerves. However, adrenergic responses in young SHR were greater than those in young WKY rats, whereas CGRP-nerve-mediated vasodilations in young SHR and WKY rats were similar. This may have been due to the enhanced release of norepinephrine, an adrenergic transmitter, in young SHR. In adult SHR, it is likely that the decreased activity of CGRP-containing nerves results in not only reduced vasodilation but also the facilitation of adrenergic vasoconstriction and that these effects subsequently lead to elevated peripheral vascular resistance. In WKY rats, slight or unaltered activity of CGRP-containing nerves may counteract adrenergic vasoconstriction and maintain normal vascular resistance.

In conclusion, the present results suggest that neuroactivity or the population of CGRP-containing vasodilator nerves is decreased in aged SHR with established hypertension. It is also suggested that changes in activity of CGRP-containing vasodilator nerves may be an important factor in producing increased adrenergic vasmotor function, which may contribute to the elevated peripheral resistance of chronic hypertension.

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