Original Article

Long-Term Inhibition of Angiotensin Prevents Reduction of Periarterial Innervation of Calcitonin Gene-Related Peptide (CGRP)-Containing Nerves in Spontaneously Hypertensive Rats

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The aim of this study was to investigate age-related changes in the density of calcitonin gene-related peptide (CGRP)-containing nerve fibers in spontaneously hypertensive rats (SHR) and the effects of long-term inhibition of the renin-angiotensin system on these changes. The density of immunocytochemically stained nerve fibers in the mesenteric artery was quantified by computer-assisted image processing. An age-related decrease in the density of CGRP-like immunoreactive (LI)-containing nerve fibers but not neuropeptide Y (NPY)-LI-containing sympathetic nerve fibers was found in the mesenteric artery of SHR but not Wistar Kyoto rats (WKY). The density of NPY-LI-containing sympathetic nerve fibers was significantly greater in SHR than in WKY. SHR were treated for 7 weeks with angiotensin converting enzyme inhibitor (0.005% temocapril), angiotensin II type-1 (AT₁) receptor antagonist (0.025% losartan) or vasodilator (0.01% hydralazine) in their drinking water. Each drug treatment significantly lowered the systolic blood pressure measured by tail-cuff method. Long-term treatment of SHR with temocapril and losartan significantly increased the density of CGRP-LI-containing nerve fibers in mesenteric arteries. However, the density after hydralazine treatment was similar to the level in non-treated SHR. The density of NPY-LI-containing nerve fibers was not increased by any of the drug treatments. These results suggest that long-term inhibition of the renin-angiotensin system in SHR prevents remodeling of CGRPergic nerve fibers and prevents the reduction of CGRPergic nerve function. (*Hypertens Res* 2005; 28: 465–474)

Key Words: angiotensin converting enzyme inhibitor, angiotensin II type-1 receptor antagonist, calcitonin gene-related peptide (CGRP)-containing vasodilator nerve innervation, neuropeptide Y (NPY)-containing sympathetic nerve innervation, spontaneously hypertensive rat

Introduction

Non-adrenergic non-cholinergic (NANC) vasodilator nerves

innervate the resistance arteries and contribute to the regulation of vascular tone together with sympathetic adrenergic nerves (*1*). In previous reports, non-adrenergic non-cholinergic vasodilator nerves have been shown to densely inner-

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Fig. 1. Representative confocal laser micrographs of a whole-mount specimen (A and C) and cross section (B and D) showing perivascular nerve fibers containing calcitonin gene-related peptide (CGRP) (A and B)- and neuropeptide-Y (NPY) (C and D)-like immunoreactivity (LI) in mesenteric arteries of the SHR.

Table 1. Age-Related Changes in Systolic Blood Pressure (mmHg) of SHR and WKY

	4 weeks old (n=6)	8 weeks old (n=6)	15 weeks old (n=6)	22 weeks old (n=5)
SHR	113.0±7.5	172.0±4.9*	197.3±1.5*	201.9±3.6*
WKY	98.7±8.3	100.0±4.7	107.0±3.2	127.3±6.9

Values are mean±SEM * $p < 0.01$ vs. WKY. SHR, spontaneously hypertensive rat; WKY, Wistar Kyoto rat.

vate the rat mesenteric resistance artery, and calcitonin gene-related peptide (CGRP), a potent vasodilator neuropeptide, has been shown to act as a vasodilator neurotransmitter of NANC nerves (1–4). CGRP is distributed throughout the central and peripheral nervous systems and is located in areas involved in cardiovascular function (5). The dorsal root ganglia (DRG) is a prominent site of CGRP synthesis and contains the cell bodies of primary afferent neurons that extend CGRP-containing nerves (CGRPergic nerves) to peripheral sites such as the blood vessels and the central spinal cord (6). 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 (2, 4). Thus, we have

proposed that CGRPergic vasodilator nerves along with sympathetic vasoconstrictor nerves regulate the tone of the mesenteric resistance artery.

Impairment of the control systems regulating peripheral resistance has been considered to lead to the development of hypertension, and increased total peripheral vascular resistance has been shown to contribute to the maintenance of elevated blood pressure (7, 8). Studies of spontaneously hypertensive rats (SHR) have shown that the enhanced activity of sympathetic vasoconstrictor nerves contributes to the increased tone of peripheral resistance arteries (8, 9). Previous studies have demonstrated that both the vasodilation mediated by CGRPergic nerves and the release of CGRP from the nerves in the mesenteric artery of SHR are reduced compared to those in normotensive Wistar Kyoto rats

CGRP-LI containing nerves

Fig. 2. Representative confocal laser micrographs showing age-related changes in the density of calcitonin gene-related peptide (CGRP)-like immunoreactive (LI) nerve fibers in mesenteric arteries of 4 (A, E)-, 8 (B, F)-, 15 (C, G)- and 22 (D, H)-week-old WKY (A–D) and SHR (E–H). The horizontal bar in the right lower corner of each panel indicates 100 μ m.

(WKY), and that these differences decrease with age. In recent studies, we have shown that the levels of CGRP mRNA in DRG of 15-week-old SHR were lower than those in WKY, and this phenomenon was also age-related (10). Therefore, we proposed that malfunction of CGRPergic vasodilator nerves regulating peripheral vascular resistance plays an important role in the development and maintenance of hypertension in SHR (7, 10, 11). However, the mechanisms underlying the reduced function of CGRPergic vasodilator nerves in SHR remain unresolved. Previous pharmacological studies showed that angiotensin II inhibits neurotransmission of CGRPergic nerves in SHR (12), and that chronic treatment of SHR with angiotensin converting enzyme (ACE) inhibitors augments the vasodilator responses mediated by CGRPergic nerves (13, 14). Additionally, long-term treatment of SHR with an ACE inhibitor or angiotensin II type-1 receptor (AT_1R) antagonists increases the levels of CGRP mRNA in DRG (15). Therefore, the renin-angiotensin system is involved in the reduction of CGRPergic nerve function in SHR.

The present study was, therefore, undertaken to investigate age-related changes in the innervation of periarterial CGRPergic nerves in SHR and age-matched WKY by using

immunohistochemical techniques. To assess the possible involvement of the renin-angiotensin system in the changes, long-term treatment of SHR with an ACE inhibitor (temocapril) or an AT_1R antagonist (losartan) was carried out in this study. Since CGRPergic vasodilator nerves interact reciprocally with adrenergic vasoconstrictor nerves in the rat mesenteric artery (4), it is expected that changes in CGRPergic innervation influence vascular adrenergic innervation. It is known that neuropeptide Y (NPY) is contained in postganglionic sympathetic nerve fibers together with norepinephrine (NE) (3). NPY has been shown to be associated with sympathetic vasoconstriction (16). In the present study, we also assessed age-related changes in the innervation of sympathetic NPY-containing nerves in SHR.

Methods

Animals

Male SHR at 4, 8, 15 and 22 weeks of age and age-matched WKY (purchased from Shimizu Experimental Animals, Shizuoka, Japan) were used in this study. The animals were given food and water *ad libitum*. They were housed in the

NPY-LI containing nerves

Fig. 3. Representative confocal laser micrographs showing age-related changes in the density of neuropeptide-Y (NPY)-like immunoreactive (LI) nerve fibers in mesenteric arteries of 4 (A, E)-, 8 (B, F)-, 15 (C, G)- and 22 (D, H)-week-old WKY (A–D) and SHR (E–H). The horizontal bar in the right lower corner of each panel indicates 100 μ m.

Animal Research Center of Okayama University at a controlled ambient temperature of 22°C with 50±10% relative humidity and with a 12-h light/12-h dark cycle (lights on at 8:00 AM). This study was performed in accordance with the Guide for Animal Experimentation of the Faculty of Pharmaceutical Science, Okayama University.

Immunocytochemical Studies

The animals were deeply anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and the superior mesenteric artery was cannulated and flushed gently with Krebs-Ringer bicarbonate solution (Krebs solution) to eliminate blood from the vascular bed. The modified Krebs solution had the following composition (mmol/l): NaCl 119.0, KCl 4.7, CaCl₂ 2.4, MgSO₄ 1.2, NaHCO₃ 25.0, KH₂PO₄ 1.2, disodium EDTA 0.03 and dextrose 11.1 (pH 7.4). Then, the third order branches of the mesenteric arteries were isolated, dissected out and placed in Zamboni's fixative containing 2% paraformaldehyde and 15% picric acid in 0.15 mol/l phosphate buffer for 48 h. After fixation, the arteries were rinsed repeatedly with phosphate-buffered saline (PBS), immersed in PBS containing 0.5% TritonX-100 overnight, and then incubated with PBS containing

normal goat serum (1:100) for 60 min. The arteries were then incubated with the primary antibodies: anti-CGRP (1: 300; raised in rabbits; Biogenesis, Ltd., Poole, UK) or anti-NPY (1: 300; raised in rabbits; Phoenix Pharmaceuticals, Inc., Belmont, USA) for 72 h at 4°C. After incubation, the arteries were washed with PBS and the site of the antigen-antibody reaction was revealed by incubation with fluorescein isothiocyanate (FITC)-labeled goat anti-rabbit IgG (diluted 1: 100) (ICN Pharmaceuticals, Inc., Aurora, USA) for 60 min. The arteries were thoroughly washed with PBS, mounted in glycerol/PBS 2:1 (v/v) and observed under a confocal laser scanning microscope (CLSM510; Carl Zeiss, Germany) in Okayama University Medical School Central Research Laboratory. In a preliminary study, we found that the mesenteric artery had denser innervation of CGRP-containing nerves than the renal artery and femoral artery, so we assessed periarterial innervation of the mesenteric artery in this study.

Immunocytochemical Analysis

The immunostaining density of CGRP-like immunoreactive (CGRP-LI) and NPY-like immunoreactive (NPY-LI) nerve fibers was analyzed with a computer using the program Sim-

Fig. 4. Bar graphs showing age-related changes in the density of calcitonin gene-related peptide (CGRP)-like immunoreactive (LI)- (A) and neuropeptide-Y (NPY)-LI- (B) containing nerve fibers in the mesenteric arteries of 4-, 8-, 15-, and 22-week-old SHR and WKY. The vertical scale indicates the fold increase over the value in the 15-week-old SHR. Each bar indicates the mean $\pm$ SEM of 5–6 experiments. * $p < 0.01$ vs. WKY. † $p < 0.01$ vs. 4 week-old SHR.

ple PCI (View Sonic, Complex Inc., Imaging Systems, Cranberry Township, USA). Since the fluorescence radiance was different depending on the day of the experiment, the mesenteric arteries from both SHR at 4, 8, 15 and 22 weeks of age and age-matched WKY were isolated, fixed and immunostained on the same day, and non-treated 15-week-old SHR were used as a control for the radiance in each experiment. The same procedure was carried out on the preparations from 7-week-drug-treated SHR and non-treated 15-week-old SHR. Readings were made using a point counting computer program and the background level was subtracted from the experimental value to yield the corrected radiance or density. To determine the number of CGRP-LI and NPY-LI fibers, 5 horizontal lines were drawn on the image of the blood vessel in the same region where the density was analyzed. Then, the number of fibers that crossed each line was counted and taken as the total number of fibers.

Long-Term Treatment with Antihypertensive Drugs

Male SHR at 8 weeks of age received 0.005% temocapril, 0.025% losartan or 0.01% hydralazine in their drinking water and normal rat chow. Each SHR received the respective treatment for a period of 7 weeks. Control SHR received normal rat chow and drinking water supplemented with 0.1% NaHCO₃ solution, which was used as a vehicle for temocapril, losartan and hydralazine.

Systolic Blood Pressure Measurement

The systolic blood pressure (SBP) of each animal was measured weekly by tail-cuff plethysmography (model TK-370C; UNICOM, Tokyo, Japan) throughout the treatment period.

Statistical Analysis

All data are expressed as the mean $\pm$ SEM. Analysis of variance (ANOVA) followed by Spearman's t -test and Tukey's test were used to determine statistical significance where appropriate. Correlation analysis was carried out by using a Pearson correlation test. A value of $p < 0.05$ was considered statistically significant.

Drugs

The following drugs were used: hydralazine hydrochloride (Sigma Chemical Co., St. Louis, USA), losartan (Merck & Co., Inc., Rahway, USA), and temocapril (Sankyo Co., Tokyo, Japan). Losartan, temocapril and hydralazine were dissolved in drinking water containing 0.1% NaHCO₃ at final concentrations of 0.025%, 0.005%, and 0.01%, respectively.

Results

Immunocytochemistry of Periarterial Nerve Innervation in the Mesenteric Artery

Figure 1 shows representative distributions of CGRP-LI and NPY-LI nerve fibers in the small mesenteric arteries of SHR. Both CGRP-LI and NPY-LI nerve fibers were found in the adventitial layer and surrounded the artery like a network. As shown in Fig. 1, visual observation indicated that the density of NPY-LI nerve fibers in the mesenteric artery was greater than that of CGRP-LI fibers.

Age-Related Changes in the Density of CGRP-LI and NPY-LI Nerve Fibers in the Mesenteric Arteries of SHR and WKY

Table 1 shows the SBP in 4-, 8-, 15- and 22-week-old SHR and age-matched WKY. There was no significant difference in the SBP of WKY at any of these ages. The SBP in SHR at 8, 15 and 22 weeks of age was significantly greater than that in age-matched WKY. However, there was no significant difference between the SBP in SHR and WKY at 4 weeks of age.

Figures 2 and 3 show typical patterns of age-related changes in the density of CGRP-LI and NPY-LI nerve fibers in the small mesenteric arteries of SHR and WKY, respectively. To quantitatively evaluate age-related changes in the density of CGRP-LI- and NPY-LI nerve fibers in the mesenteric artery, an immunocytochemical technique was used in this study. A marked and significant reduction in the density of CGRP-LI nerve fibers was found in the arteries of aged

Fig. 5. Relationship between number of calcitonin gene-related peptide (CGRP)-like immunoreactive (LI) (A, C) and neuropeptide-Y (NPY)-LI nerve fibers (B, D) and density of CGRP-LI and NPY-LI nerves (%) in the mesenteric arteries of SHR at 8 and 15 weeks of age.

Fig. 6. Changes in systolic blood pressure (SBP) after 7-week treatment with various anti-hypertensive drugs in SHR. Each point indicates the mean $\pm$ SEM of 5 experiments. * $p < 0.01$ vs. non-treated SHR (Control).

SHR (22 weeks old) compared to the density in 4-, 8- or 15-week-old SHR. The densities of CGRP-LI nerve fibers in SHR at 4, 8 and 15 weeks of age were significantly greater than those in age-matched WKY, but no significant difference was found between 22-week-old SHR and WKY.

On the other hand, there was no age-related change in the density of NPY-LI nerve fibers in SHR, as shown in Fig. 4. Rather, a slight increase in NPY-LI nerve fibers was found in the aged SHR (Fig. 4). In WKY arteries, the densities of NPY-LI nerve fibers at 4, 8, 15 and 22 weeks of age were significantly lower than those in the arteries of SHR at 4, 8, 15

and 22 weeks of age (Fig. 4).

Correlations between the Numbers and Densities of Periarterial Nerve Fibers (CGRP-LI and NPY-LI Nerve Fibers)

Figure 5 shows the relationships between the numbers of CGRP-LI and NPY-LI nerve fibers, which were visually counted, and the densities of CGRP-LI and NPY-LI nerves (%), which were quantified by computer-assisted image processing, in non-treated control SHR at 8 and 15 weeks of age. There were significant positive correlations between the density and the numbers of CGRP-LI and NPY-LI nerve fibers in SHR at all of these ages. Similar results were obtained from WKY at 4 (CGRP-LI, $p < 0.01$, $r = 0.8060$; NPY-LI, $p < 0.01$, $r = 0.7399$), 8 (CGRP-LI, $p < 0.01$, $r = 0.9038$; NPY-LI, $p < 0.05$, $r = 0.8060$), 15 (CGRP-LI, $p < 0.01$, $r = 0.8965$; NPY-LI, $p < 0.01$, $r = 0.5838$) and 22 (CGRP-LI, $p < 0.01$, $r = 0.8368$; NPY-LI, $p < 0.01$, $r = 0.7756$) weeks of age and from SHR at 4 (CGRP-LI, $p < 0.01$, $r = 0.5375$; NPY-LI, $p < 0.05$, $r = 0.7373$), and 22 (CGRP, $p < 0.01$, $r = 0.7179$; NPY-LI, $p < 0.01$, $r = 0.3959$) weeks of age (data not shown).

Changes in SBP during Long-Term Treatment with Antihypertensive Drugs

As shown in Fig. 6, long-term treatment with losartan, temocapril or hydralazine significantly lowered the SBP in SHR, compared with that in non-treated control SHR.

Fig. 7. Effect of 7-week treatment with temocapril (B), losartan (C) or hydralazine (D) on the density of calcitonin gene-related peptide (CGRP)-like immunoreactive (LI)-containing nerve fibers in the mesenteric arteries of SHR. The horizontal bar in the left lower corner of each panel indicates 100 μ m. In E, the vertical scale indicates the fold increase over the value in non-treated SHR. Each bar indicates the mean $\pm$ SEM of 5 experiments. * $p < 0.01$ vs. non-treated SHR (Control, A).

Effects of Long-Term Treatment of SHR with Anti-hypertensive Drugs on the Density of Periarterial Nerve Fibers

Long-term treatment with losartan or temocapril caused a significant increase in the density of CGRP-LI nerve fibers compared to that in the non-treated control SHR (Fig. 7). However, hydralazine treatment had no effect on the density of CGRP-LI nerve fibers. Significant positive correlations between the density and the numbers of CGRP-LI were found in SHR treated with losartan ($p < 0.05$, $r = 0.4055$), temocapril ($p < 0.01$, $r = 0.6128$) or hydralazine ($p < 0.01$, $r = 0.8765$) (data not shown).

As shown in Fig. 8, the density of NPY-LI nerve fibers was slightly but not significantly decreased by long-term treatment of SHR with the antihypertensive drugs. Significant positive correlations between the density and the numbers of NPY-LI were found in SHR treated with losartan ($p < 0.01$, $r = 0.5487$), temocapril ($p < 0.01$, $r = 0.4763$) or hydralazine ($p < 0.01$, $r = 0.7911$) (data not shown).

Discussion

The main findings of this study were that CGRP-LI nerve fibers in the mesenteric arteries of SHR were significantly reduced with age, and that long-term treatment with an ACE inhibitor or an AT₁R antagonist prevented this reduction and restored the density of CGRP-LI nerves to levels similar to those in young SHR. It has been reported that the content of CGRP in the DRG of SHR was lower than that in age-matched WKY (17). The neurogenic release of CGRP-LI in the mesenteric artery of SHR at 15 weeks of age has been shown to be smaller than that in age-matched WKY (11). Additionally, the plasma concentration of CGRP is decreased in SHR compared with that in WKY, whereas the CGRP content in the abdominal aorta is higher in SHR than in WKY (18). Furthermore, it has been reported that CGRP mRNA levels in the DRG of SHR are significantly lower than those in WKY (15, 17). These findings led us to expect that the innervation of CGRP-LI nerve fibers would be decreased in SHR compared to WKY. However, the present study revealed a greater density of CGRP-LI fibers in the mesenteric arteries

Fig. 8. Effect of 7-week treatment with temocapril (B), losartan (C) or hydralazine (D) on the density of neuropeptide-Y (NPY)-like immunoreactive (LI)-containing nerve fibers in the mesenteric arteries of SHR. The horizontal bar in the left lower corner of each panel indicates 100 μm . In E, the vertical scale indicates the fold increase over the value in non-treated SHR. Each bar indicates the mean $\pm$ SEM of 5 experiments.

of 4-, 8- and 15-week-old SHR, but not 22-week-old SHR, than in those of age-matched WKY. It has been reported that SHR at all ages had larger NE contents in the mesenteric arteries than WKY (19). Vascular adrenergic nerves presynaptically inhibit the release of CGRP *via* the transmitter NE to decrease the function of CGRPergic nerves (2, 4, 20). Additionally, adrenergic nerve-mediated vasoconstriction has been shown to be markedly potentiated by the depletion of CGRPergic nerves with capsaicin (2). Taken together, these findings suggest that sympathetic adrenergic nerves interact reciprocally with CGRPergic nerves, and both types of nerves regulate the tone of blood vessels. Therefore, the present finding that young SHR had increased innervation of CGRP-LI nerves suggests that CGRPergic nerve innervation may be increased to counteract the increased activity of sympathetic nerves. Therefore, these findings also suggest that the age-related reduction in the density of CGRPergic nerves in SHR may have resulted in an age-related increase in the activity of sympathetic adrenergic nerves, since there was no age-related change in adrenergic innervation in SHR. Increased activity of adrenergic nerves and the renin-angiotensin system in SHR may cause a decrease in the plasma CGRP concentration (18), since CGRP is released from the vascular CGRPergic nerve endings.

In the present study, long-term treatment of SHR with an ACE inhibitor (temocapril) or an AT₁R antagonist (losartan) resulted in a significant increase in the density of CGRPergic nerve fibers, whereas vasodilator (hydralazine) treatment did not show such an effect. Since these antihypertensive drugs lowered the blood pressure of SHR, it is unlikely that lowered blood pressure was responsible for the increased density of CGRPergic nerve fibers. Previous *in vitro* studies have shown that long-term treatment with an ACE inhibitor such as captopril or temocapril prevented the reduction of CGRPergic nerve-mediated vasodilation in SHR mesenteric arteries (13, 14). However, no such effect was observed after treatment with either hydralazine, β -adrenoceptor antagonists (propranolol and pindolol), or calcium channel antagonists (nicardipine, amlodipine and pranidipine), despite a significant reduction in blood pressure by these agents (13, 14, 21). Direct application of an ACE inhibitor to the mesenteric arteries of non-treated SHR was without effect on the CGRPergic nerve-mediated vasodilation (14), suggesting that long-term application is essential for the improvement of CGRPergic nerve function. In addition to these findings, an *in vivo* study demonstrated that long-term treatment of SHR with the ACE inhibitor captopril prevented the reduction of depressor response induced by spinal cord stimulation, which is medi-

ated by CGRPergic nerves (10). Recently, we reported evidence that expression of CGRP mRNA in the DRG, a prominent site of CGRP synthesis, is decreased in SHR (22), and this decrease is prevented by long-term treatment of SHR with an ACE inhibitor (temocapril) or AT₁R antagonists (losartan and olmesartan) (15). These repeated findings are in good accord with the present finding that the reduction of the density of CGRPergic nerve fibers in SHR was prevented by chronic inhibition of the renin-angiotensin system by an ACE inhibitor or AT₁R antagonist. Angiotensin II as well as angiotensin I and a renin substrate (*N*-acetyltetradecapeptide) have been reported to inhibit the neurotransmission of CGRPergic nerves in SHR but not WKY (12). Thus, it is very likely that long-term inhibition of the renin-angiotensin system by ACE inhibitors or AT₁R antagonists in SHR prevents the decrease of CGRPergic nerve function by increasing the innervation of CGRPergic nerve fibers. This suggests that circulating angiotensin II, in addition to the converted form in the vasculature, is responsible for the decrease in the innervation and function of CGRPergic nerves in SHR. In the DRG, stimulation of AT₁R has been shown to inhibit the synthesis of CGRP (23). Deng *et al.* (24) reported an increased level of angiotensin II in the hearts of 15-week-old SHR compared to those of age-matched WKY, suggesting that the angiotensin II level may be increased in the vasculature of SHR. Therefore, it is likely that increased circulatory or tissue production of angiotensin II is responsible for the reduced innervation of CGRPergic nerve fibers, that is remodeling, in SHR.

A previous study showed that the content of CGRP in the mesenteric arteries of SHR was greater than that in age-matched WKY and was further increased by long-term treatment with an AT₁R antagonist or ACE inhibitor but not with hydralazine (15). In a previous *in vitro* study, long-term treatment with an ACE inhibitor was shown to facilitate both the neurogenic release of CGRP and CGRPergic nerve-mediated vasodilation in the mesenteric arteries of SHR (13). Since angiotensin II presynaptically attenuates the neurotransmission of CGRPergic nerves in SHR (12), the inhibition of angiotensin II action may facilitate the neurotransmission. In addition, the increases in CGRP contents of the mesenteric arteries (22), synthesis of CGRP in the DRG (15) and innervation of CGRPergic nerve fibers, which result from chronic inhibition of the renin-angiotensin system, further facilitate the neurotransmission of CGRPergic nerves in SHR. Therefore, it appears that long-term inhibition of the renin-angiotensin system prevents or reverses the reduction of function and the remodeling of perivascular CGRPergic nerves in SHR. Since CGRP is the most potent vasodilator known (1, 25), it is possible that the increased release of CGRP from the nerves may induce a decrease in the vascular resistance and lead to a reduction in blood pressure.

The pattern of distribution of NPY is the same as that of sympathetic adrenergic nerves, and NPY coexists with NE in the nerves (26), suggesting that the level of innervation of NPY-LI fibers is the same as that of sympathetic adrenergic

nerves. In the present study, the innervation of NPY-LI nerve fibers in the mesenteric arteries of both SHR and WKY was greater (as seen by visual inspection) than that of CGRP-LI nerves, suggesting that the mesenteric artery is highly innervated by sympathetic NPY-LI nerves. The present findings showed that no significant changes in innervation of NPY-LI nerves occurred after long-term treatment of SHR with hypertensive drugs, while a rather small reduction in the density was observed after the drug treatment. Since the level of sympathetic innervation of the mesenteric artery was very high, even though the reduction of the innervation was small, it may have been sufficient to result in decreased adrenergic activity, and consequently in decreased vascular tone, leading to lowered blood pressure.

In summary, the present study suggests that CGRPergic nerve innervation in SHR decreases with age, leading to the remodeling of periarterial nerves. Our findings also suggest that long-term inhibition of the renin-angiotensin system in SHR prevents remodeling of CGRPergic nerve fibers and prevents the reduction of the function of CGRPergic nerves.

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