Original Article

Effects of Endothelium-Derived Hyperpolarizing Factor and Nitric Oxide on Endothelial Function in Femoral Resistance Arteries of Spontaneously Hypertensive Rats

Yoshitomo MORI[1)], Mitsumasa OHYANAGI[1)], Satsuki KOIDA[1)], Atsunori UEDA[1)], Kai ISHIKO[1)], and Tadaaki IWASAKI[1)]

In hypertension, endothelium-dependent relaxation is attenuated and this attenuation contributes to the increased peripheral resistance. However, the role of endothelium-derived hyperpolarizing factor (EDHF) in the arteries of hypertensive rats remains unclear. Therefore, the aim of this study was to evaluate the role of EDHF in the femoral resistance arteries of hypertensive rats. The femoral resistance arteries were isolated from 5-, 15- and 25-week-old spontaneously hypertensive rats (SHR) and age-matched Wistar Kyoto rats (WKY). Changes in internal diameter were examined with videomicroscopy. EDHF-mediated dilatation was determined by differences between the degree of acetylcholine (ACh)-induced dilatation in the presence of N^G-monomethy-L-arginine (L-NMMA) plus a prostaglandin I_2 inhibitor (indomethacin) and the degree of such dilatation in the presence of L-NMMA, indomethacin and KCl. Charybdotoxin (CTx) and apamin (a Ca^{2+}-activated K^+ channel [K_{Ca}] inhibitor)–sensitive EDHF dilatation was also compared between in 5-, 15- and 25-week-old SHR and WKY. ACh-induced vasodilatation was not different between 5-week-old SHR and WKY. There was no difference between NO- and EDHF-mediated vasodilatation in 5-week-old rats. ACh-induced vasodilatation was weaker in 15-week-old SHR than in WKY. NO-mediated vasodilatation did not differ between the two groups. EDHF-mediated dilatation was attenuated in SHR but not in WKY. ACh-induced dilatation was weaker in 25-week-old SHR than in WKY. NO- and EDHF-mediated vasodilatation were attenuated in SHR but not WKY. EDHF-mediated vasodilatation was attenuated before the loss of NO-mediated vasodilatation in the femoral resistance arteries of SHR. The attenuation of this vasodilatation was mediated by the CTx plus apamin–sensitive EDHF. (*Hypertens Res* 2006; 29: 187–195)

***Key Words*: endothelium-derived hyperpolarizing factor, hypertension, resistance artery**

Introduction

Vascular tone is a key determinant of local organ blood flow and peripheral resistance. Three different endothelium-derived vasodilators, nitric oxide (NO), prostacyclin, and the endothelium-derived hyperpolarizing factor (EDHF), play an important role in the control of local vascular tone (*1*). Whereas in the large conduit vessels, such as the aorta, endothelium-dependent responses are selectively mediated by NO, EDHF is the predominant endothelium-dependent vasodilator in resistance vessels (*2*). Since the small resistance arteries play an important role in the regulation of vascular resistance, the role of EDHF in the resistance arteries is important in cardiovascular diseases such as hypertension, diabetes mellitus and congestive heart failure (*3*, *4*).

From the [1)]Department of Internal Medicine, Division of Coronary Heart Disease, Hyogo College of Medicine, Nishinomiya, Japan.

Address for Reprints: Mitsumasa Ohyanagi, M.D., Ph.D., Department of Internal Medicine, Division of Coronary Heart Disease, Hyogo College of Medicine, 1–1, Mukogawa-cho, Nishinomiya 663–8501, Japan. E-mail: ohyanagi@hyo-med.ac.jp

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In hypertension, endothelium-dependent relaxation is attenuated and this attenuation contributes to the increased peripheral resistance. Endothelial dysfunction in hypertension has been linked to a decrease in NO (*5*, *6*). A recent study indicated that EDHF might compensate for the loss of NO and preserve the endothelium-dependent relaxation in the mesenteric arteries of hypertensive rats in which hypertension is induced by a high-salt diet (*7*). Moreover, another study showed increased EDHF responses in renal arteries from young spontaneously hypertensive rats (SHR) (*8*). In contrast, other studies have demonstrated the attenuation of EDHF-mediated relaxation in the mesenteric arteries of hypertensive rats (*9*, *10*). This is partly because the exact nature of EDHF remains to be identified. The EDHF candidates include epoxyeicosatrienoic acids (EETs) (*11*, *12*), K^+ (*13*), gap junctions (*14*), and hydrogen peroxide (*15*, *16*). Thus, more than one EDHF might exist, and the contribution of each EDHF to endothelium-dependent relaxation might vary, depending on the species tested and the vessels used. Some studies have shown that EDHF does not contribute to endothelium-dependent smooth muscle cell relaxation in the rat femoral arteries (*17*, *18*). However, one study showed that the charybdotoxin (CTx) plus apamin–sensitive EDHF predominates in the hindlimb vascular beds of rats (*19*). Therefore, the role of EDHF in the femoral arteries of hypertensive rats remains unclear. Moreover, changes in EDHF in the small resistance arteries or arterioles, which play a major role in regulating peripheral resistance, have not been evaluated.

The aim of this study was to determine whether there are alterations in EDHF in the small resistance femoral arteries of hypertensive rats and to evaluate the role of EDHF in these arteries.

Methods

SHR (aged 5, 15, and 25 weeks) and age-matched Wistar Kyoto rats (WKY) were the progeny of animals provided by Kouzo Okamoto (Kinki University, Osaka-Sayama, Japan). They received humane care and were maintained in accordance with the guidelines for animal experimentation of Hyogo College of Medicine. The systolic blood pressure (SBP) of rats was measured by the tail-cuff method using an electro-sphygmomanometer (PE-300; Tokai Irika, Tokyo, Japan).

Isolation of Blood Vessels

Microvessel isolation was performed as previously reported (*20*). The small branch of the femoral arteries (inner diameter: 51±3 to 91±4 μm) was removed and immediately placed in dissecting solution (in mmol/l: NaCl 145, KCl 4.7, $CaCl_2{\cdot}2H_2O$ 2.0, $MgSO_4{\cdot}7H_2O$ 1.2, D-glucose 5.0, pyruvate 2.0, MOPS (3-[*N*-morpholino]propanesulfonic acid) 2.0, EDTA 0.02, NaH_2PO_4 1.2, and 1% w/v bovine serum albumin). The artery was separated from the surrounding connective tissue under a microscope using ultrafine scissors and forceps and then placed in a variable temperature observation bath.

The artery was cannulated at both ends with pipettes and ligated with 11-0 nylon thread. The pipettes were pre-filled with Krebs solution (in mmol/l: NaCl 118.5, KCl 4.7, $CaCl_2{\cdot}2H_2O$ 2.55, $MgSO_4{\cdot}7H_2O$ 1.19, KH_2PO_4 1.19, $NaHCO_3$ 19.9, and dextrose 11.6) and were connected to a pressure servo-syringe (Living Systems Instrumentation, Burlington, USA) using silicone tubes.

The bath was perfused with Krebs solution at a rate of 4 ml/min from a reservoir, and the pH, P_{O_2}, and P_{CO_2} of the solution were adjusted with nitrogen gas and carbon dioxide gas to 7.35–7.4, 70–90 mmHg, and 35–40 mmHg, respectively. The bath was placed on a stage equipped with an inverted microscope (Diaphot-TMD; Nikon, Tokyo, Japan), and the artery was viewed on a TV monitor using a microscope CCD camera (C2400; Hamamatsu Photonix, Hamamatsu, Japan). The arterial internal diameter was measured by connecting an Argus 10 Image Processor (Hamamatsu Photonix) to the monitor. The temperature of the Krebs solution in the bath was gradually increased from room temperature to 37°C over more than 1 h. After the arterial internal diameter was stable at 60 mmHg, the experimental protocols were initiated.

Protocols

Acetylcholine (ACh)-induced vasodilatation was assessed prior to addition of N^G-monomethyl-L-arginine (L-NMMA), an NO synthesis inhibitor, or indomethacin, a cyclooxygenase inhibitor, and any vessel that did not respond was discarded. We did not use any agents to induce constriction before ACh-induced dilatation.

To examine whether NO was involved in ACh-induced vasodilatation, L-NMMA was added to the Krebs solution at 10^{-4} mol/l and was allowed to circulate for 30 min. To examine whether prostaglandin I_2 (PGI_2) was involved in ACh-induced vasodilatation, indomethacin was added to the bath at 10^{-5} mol/l. NO-mediated dilatation was determined by measuring the portion of ACh-induced dilatation that was abolished by L-NMMA, while PGI_2-mediated dilatation was determined by measuring the portion of dilatation abolished by indomethacin. EDHF-mediated dilatation was determined by the difference between the amount of ACh-induced dilatation in the presence of L-NMMA plus indomethacin and the amount of this dilatation in the presence of KCl (40 mmol/l) (*21*).

These NO-mediated, PGI_2-mediated, and EDHF-mediated dilatations were compared between SHR (aged 5, 15, and 25 weeks) ($n=6$ per group) and age-matched WKY ($n=6$ per group).

To evaluate whether CTx (a large and intermediate conductance Ca^{2+}-activated K^+ channel [K_{Ca}] inhibitor) plus apamin (a small-conductance K_{Ca} channel inhibitor)–sensitive EDHF is involved in ACh-induced vasodilatation in the presence of

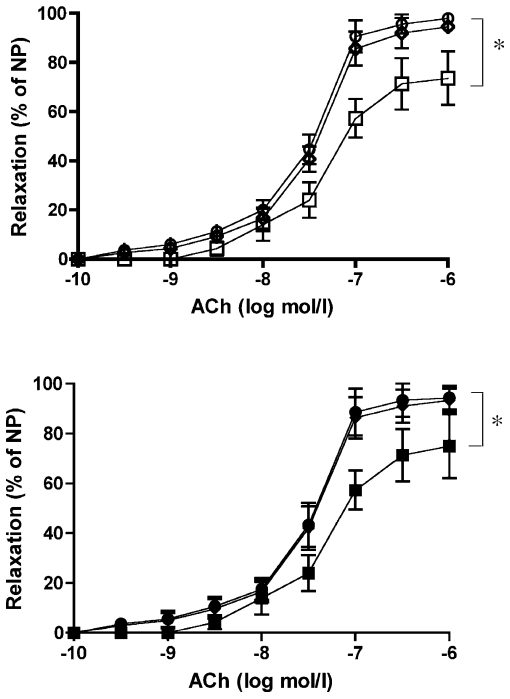

Fig. 1. *Concentration-response curves showing the response of the resistance femoral arteries to acetylcholine (ACh) in the absence or presence of L-NMMA and indomethacin. Upper: 5-week-old WKY. Open circles, control arteries (in the absence of any drugs); open squares, in the presence of L-NMMA; open triangles, in the presence of indomethacin. Lower: 5-week-old SHR. Closed circles, control arteries (in the absence of any drugs); closed squares, in the presence of L-NMMA; closed triangles, in the presence of indomethacin. There were no significant differences in ACh-induced vasodilatation between SHR and WKY. The effect of L-NMMA on this dilatation was not different between the two groups. Indomethacin did not have a significant effect on ACh-induced dilatation.* *p < *0.05* vs. *L-NMMA.*

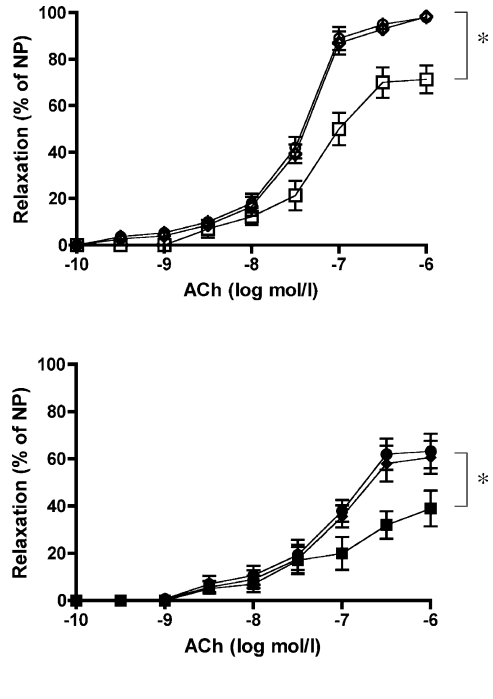

Fig. 2. *Concentration-response curves showing the response of the resistance femoral arteries to acetylcholine (ACh) in the absence or presence of L-NMMA and indomethacin. Upper: 15-week-old WKY. Lower: 15-week-old SHR. Symbols are the same as in Fig. 1. ACh-induced vasodilatation in SHR was weaker than that in WKY. The effect of L-NMMA on this dilatation was not different between the two groups. Indomethacin did not have a significant effect on ACh-induced dilatation.* *p < *0.05* vs. *L-NMMA.*

L-NMMA plus indomethacin, experiments were also conducted in the presence of CTx at 10^{-8} mol/l plus apamin at 10^{-7} mol/l (*22*, *23*).

The EDHF candidates include EETs (*11*, *12*), which are metabolites of cytochrome P-450 monooxygenase; gap junctions (*14*); and hydrogen peroxide (H_2O_2) (*15*, *16*). To identify these candidates of EDHF during ACh-induced vasodilatation of the femoral arteries from 15-week-old WKY (n=4 per group), 17-octadecynoic acid (17ODYA) (*23*, *24*), a cytochrome P450 monooxygenase inhibitor (10^{-5} mol/l), 18β-glycyrrhetinic acid (18βGA) (*25*), a gap junction inhibitor (4×10^{-5} mol/l), or catalase, an enzyme that selectively dismutates H_2O_2 into water and oxygen (1,250 U/ml) (*15*), was added separately to the bath in the presence of L-NMMA and indomethacin and circulated for 30 min.

To confirm whether ACh-induced dilatation of the vessels from 15-week-old WKY (n=3) was dependent on the endothelium, endothelium was denuded by injection of 5 ml of air through the vessel (*26*). Denudation was confirmed by the elimination of the vasodilator response to ACh. At the end of each study protocol, the passive diameter was assessed using sodium nitroprusside (10^{-4} mol/l). The percent of dilatation was calculated relative to the maximal dilation of the vessel in the presence of nitroprusside. L-NMMA was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Other agents were obtained from Sigma Chemical Co. (St. Louis, USA).

Statistics

Results were expressed as the mean±SEM. Statistically significant differences in diameter between SHR and WKY were assessed by unpaired Student's *t*-test. Comparisons among the ACh-induced dilatation curves were performed with ANOVA, followed by Scheffe's multiple comparison test. Values of $p<0.05$ were considered to be statistically significant.

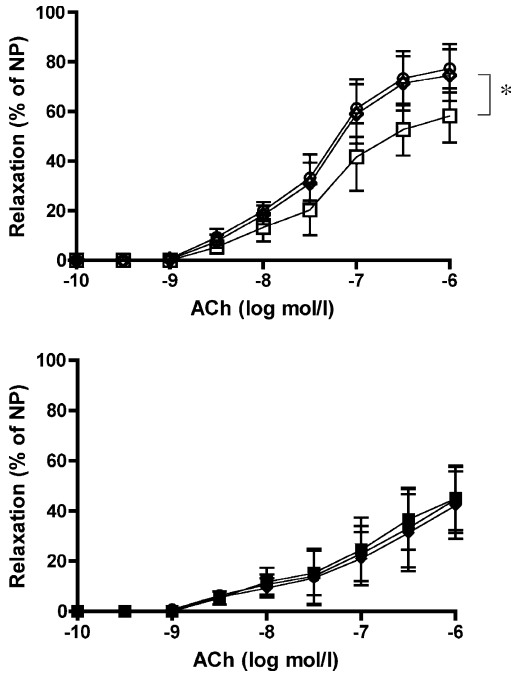

Fig. 3. *Concentration-response curves showing the response of the resistance femoral arteries to acetylcholine (ACh) in the absence or the presence of L-NMMA and indomethacin. Upper: 25-week-old WKY. Lower: 25-week-old SHR. Symbols are the same as in Fig. 1. ACh-induced vasodilatation in SHR was weaker than that in WKY. L-NMMA attenuated this dilatation in WKY but not SHR. Indomethacin did not have a significant effect on ACh-induced dilatation.* *p < *0.05* vs. *L-NMMA.*

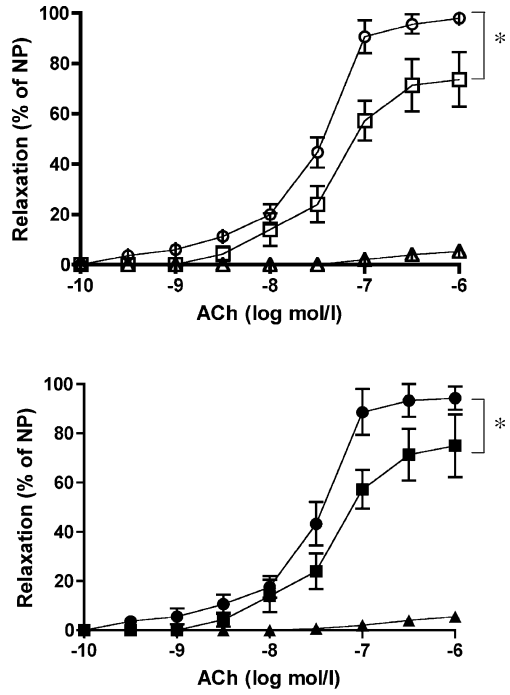

Fig. 4. *Concentration-response curves showing the response of the resistance femoral arteries to acetylcholine (ACh) in the absence or the presence of L-NMMA plus indomethacin and the presence of L-NMMA, indomethacin and KCl. Upper: 5-week-old WKY. Open circles, control arteries (in the absence of any drugs); open squares, in the presence of L-NMMA plus indomethacin; open triangles, in the presence of L-NMMA, indomethacin and KCl. Lower: 5-week-old SHR. Open circles, control arteries (in the absence of any drugs); open squares, in the presence of L-NMMA plus indomethacin; open triangles, in the presence of L-NMMA, indomethacin and KCl. There were no significant differences in ACh-induced vasodilatation between SHR and WKY. The effect of L-NMMA plus indomethacin on this dilatation was not different between the two groups. KCl abolished ACh-induced dilatation in both groups.* *p < *0.05* vs. *L-NMMA plus indomethacin.*

Results

The SBP values of SHR aged 5, 15, and 25 weeks were 136±3, 198±10, and 21±8 mmHg, respectively, and those of age-matched WKY were 115±8, 125±9, and 131±8 mmHg, respectively. The wall thickness/lumen diameter ratio in the resistance artery (internal diameter: 5-week-old animals, 51±3 μm; 15-week-old, 79±4 μm; 25-week-old, 91±4 μm) did not differ significantly between SHR and WKY at 5 or 15 weeks of age (5-week-old SHR, 0.12±0.02; 5-week-old WKY, 0.12±0.02; 15-week-old SHR, 0.17±0.03; 15-week-old WKY, 0.13±0.02). However, the ratio in SHR (0.23±0.05) at 25 weeks of age was significantly ($p<0.001$) greater than that in age-matched WKY (0.17±0.02) or in SHR at 5 or 15 weeks of age.

Acetylcholine-Induced Dilatation

There were no significant differences in ACh-induced vasodilatation between 5-week-old SHR and age-matched WKY. The effect of L-NMMA on this dilatation was not different between 5-week-old SHR and WKY (Fig. 1).

ACh-induced vasodilatation was weaker in 15-week-old SHR than in age-matched WKY. L-NMMA attenuated this dilatation to a similar degree in both groups (Fig. 2).

ACh-induced vasodilatation was weaker in 25-week-old SHR than in age-matched WKY. L-NMMA attenuated ACh-induced vasodilatation in WKY but not in SHR (Fig. 3).

Indomethacin had no significant effects on ACh-induced dilatation in 5-, 15-, or 25-week-old SHR and WKY (Figs. 1–3).

The effect of L-NMMA plus indomethacin on ACh-induced dilatation was not different between 5-week-old SHR and WKY. The effect of KCl on this dilatation in the presence of L-NMMA plus indomethacin was not different between 5-

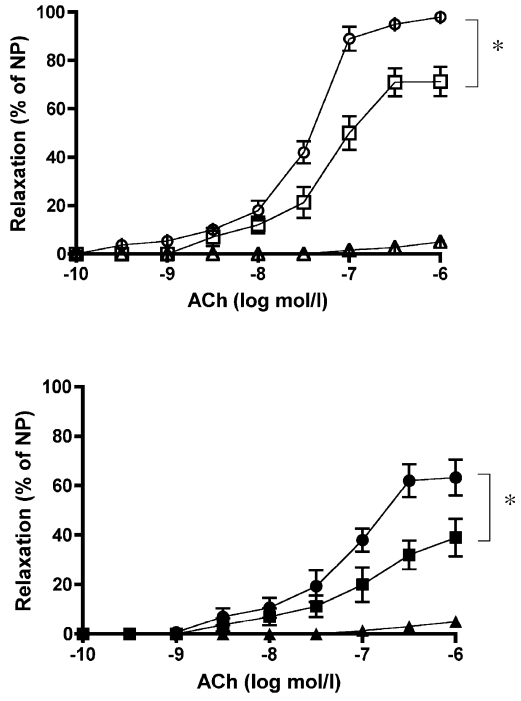

Fig. 5. *Concentration-response curves showing the response of the resistance femoral arteries to acetylcholine (ACh) in the absence or presence of L-NMMA plus indomethacin and the presence of L-NMMA, indomethacin and KCl. Upper: 15-week-old WKY. Lower: 15-week-old SHR. Symbols are the same as in Fig. 4. The effect of L-NMMA plus indomethacin on this dilatation was not different between the two groups. KCl abolished ACh-induced dilatation in both groups. EDHF-mediated dilatation was weaker in SHR than in WKY.* *p < *0.05* vs. *L-NMMA plus indomethacin.*

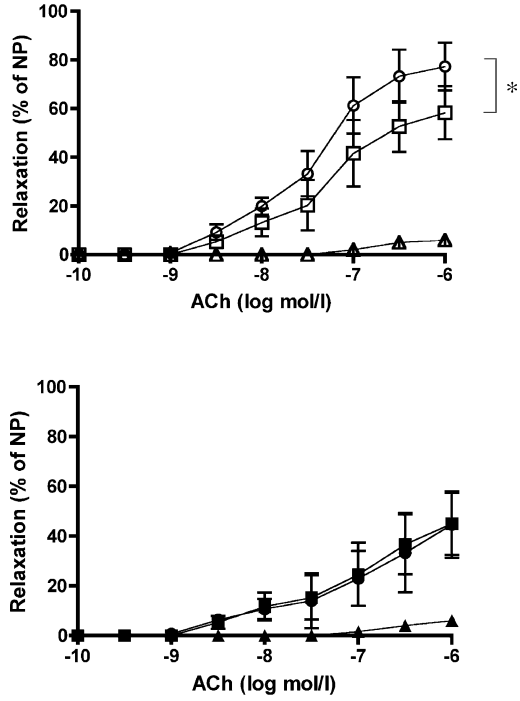

Fig. 6. *Concentration-response curves showing the response of the resistance femoral arteries to acetylcholine (ACh) in the absence or the presence of L-NMMA plus indomethacin and the presence of L-NMMA, indomethacin and KCl. Upper: 25-week-old WKY. Lower: 25-week-old SHR. Symbols are the same as in Fig. 4. L-NMMA plus indomethacin attenuated the dilatation in WKY but not in SHR. KCl abolished the ACh-induced dilatation in both groups. EDHF-mediated dilatation was weaker in SHR than in WKY.* *p < *0.05* vs. *L-NMMA plus indomethacin.*

week-old SHR and WKY. There were no significant differences in EDHF-mediated ACh-induced vasodilatation between SHR and WKY (Fig. 4).

The effect of L-NMMA plus indomethacin on ACh-induced dilatation was not different between 15-week-old SHR and WKY. The effect of KCl on this dilatation in the presence of L-NMMA plus indomethacin was weaker in SHR than WKY. EDHF-mediated ACh-induced vasodilatation in 15-week-old SHR was weaker than that in age-matched WKY (Fig. 5).

The effect of L-NMMA plus indomethacin on ACh-induced dilatation was not different between 25-week-old SHR and age-matched WKY. The effect of KCl on this dilatation in the presence of L-NMMA plus indomethacin was weaker in SHR than WKY. EDHF-mediated ACh-induced vasodilatation in 25-week-old SHR was weaker than that in age-matched WKY (Fig. 6).

The effect of CTx plus apamin on ACh-induced dilatation in the presence of L-NMMA plus indomethacin was not different between 5-week-old SHR and age-matched WKY (Fig. 7).

CTx plus apamin did not produce a significant effect on ACh-induced dilatation in the presence of L-NMMA plus indomethacin in 15-week-old SHR, but it did have a significant effect in age-matched WKY (Fig. 8).

CTx plus apamin did not produce a significant effect on ACh-induced dilatation in the presence of L-NMMA plus indomethacin in 25-week-old SHR, but it did have a significant effect in age-matched WKY (Fig. 9).

17-Octadecynoic acid (a cytochrome P450 monooxygenase inhibitor), 18βGA (a gap junction inhibitor) or catalase (an enzyme that selectively dismutates H_2O_2 into water and oxygen) had no significant effects on ACh-induced dilatation in 15-week-old WKY (Fig. 10).

Endothelial disruption abolished the ACh-induced dilatation in WKY (Fig. 10).

Discussion

The present study demonstrated that 1) ACh-induced vasodi-

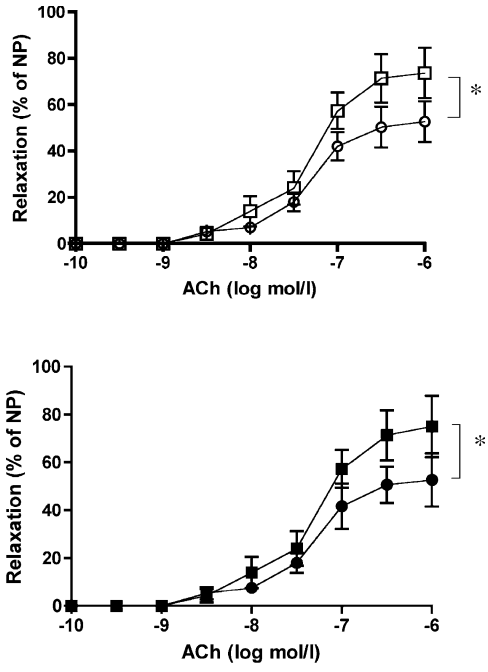

Fig. 7. *Concentration-response curves showing the response of the resistance femoral arteries to acetylcholine (ACh) in the presence of L-NMMA and indomethacin with or without CTx plus apamin. Upper: 5-week-old WKY. Open squares, in the presence of L-NMMA and indomethacin; open circles, in the presence of L-NMMA, indomethacin and CTx plus apamin. Lower: 5-week-old SHR. Closed squares, in the presence of L-NMMA and indomethacin; closed circles, in the presence of L-NMMA, indomethacin and CTx plus apamin. There were no significant differences in ACh-induced vasodilatation in the presence of L-NMMA and indomethacin between SHR and WKY. The effect of CTx plus apamin on this dilatation was not different between the two groups.* $*p < 0.05$ vs. *the presence of CTx plus apamin.*

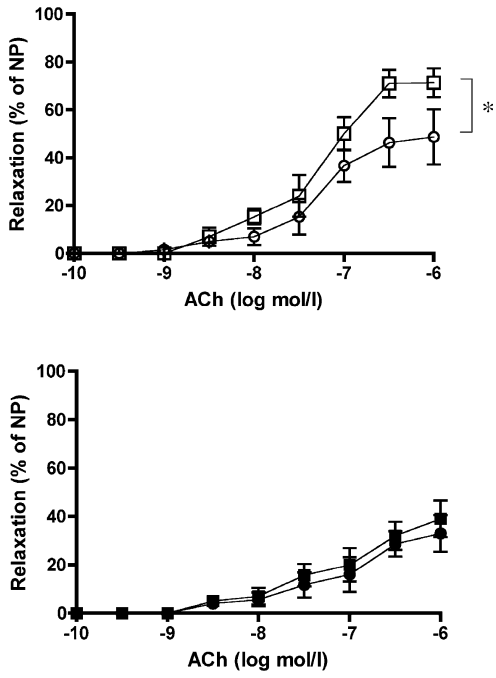

Fig. 8. *Concentration-response curves showing the response of the resistance femoral arteries to acetylcholine (ACh) in the presence of L-NMMA and indomethacin with or without CTx plus apamin. Upper: 15-week-old WKY. Lower: 15-week-old SHR. Symbols are the same as in Fig. 7. ACh-induced vasodilatation in the presence of L-NMMA and indomethacin was weaker in SHR than in WKY. CTx plus apamin attenuated this dilatation in WKY but not in SHR.* $*p < 0.05$ vs. *the presence of CTx plus apamin.*

latation in 5-week-old SHR was not different from that in age-matched WKY; 2) ACh-induced vasodilatation in 15-week-old SHR was attenuated compared with that in age-matched WKY and this dilatation mediated by EDHF, but not NO, was attenuated in SHR compared with age-matched WKY; 3) ACh-induced vasodilatation in 25-week-old SHR was also attenuated compared with that in age-matched WKY, and ACh-induced dilatation mediated by NO was attenuated in 25-week-old SHR compared with that in age-matched WKY.

In hypertension, endothelium-dependent relaxation is attenuated and this attenuation contributes to the increased peripheral resistance. Endothelial dysfunction in hypertension has been linked to a decrease in NO bioavailability, reflecting the impaired generation of NO and/or the enhanced scavenging and inactivation of NO by oxygen-derived free radicals (*5*, *6*). However, the role of NO in hypertension still remains controversial. Chang *et al*. showed that endothelium-dependent or -independent mesenteric vasodilatation is enhanced in 12- to 15-week-old SHR compared with WKY rats, supporting the concept that NO function is enhanced in the hypertensive state (*27*). In the present study, NO function in 15-week-old SHR was not different from that in age-matched WKY. The difference between our data and Chang's data may be due to the difference of vascular beds. Further studies are necessary to elucidate this point. However, it is clear that the responses mediated by NO decrease with age in SHR, beginning at an age of 25 weeks.

EDHF also plays an important role in the control of local vascular tone, especially in resistance arteries (*28*). Moreover, EDHF has been proposed to act as a backup system to maintain endothelial function in situations associated with a decreased bioactivity of NO (*7*). Our previous studies showed that EDHF also plays the role of a backup system to maintain endothelial function in the femoral arteries of rats with congestive heart failure (*26*). The compensatory role of EDHF in hypertension might be important. A recent study indicated that EDHF might compensate for the loss of NO and preserve the endothelium-dependent relaxation in mesenteric arteries

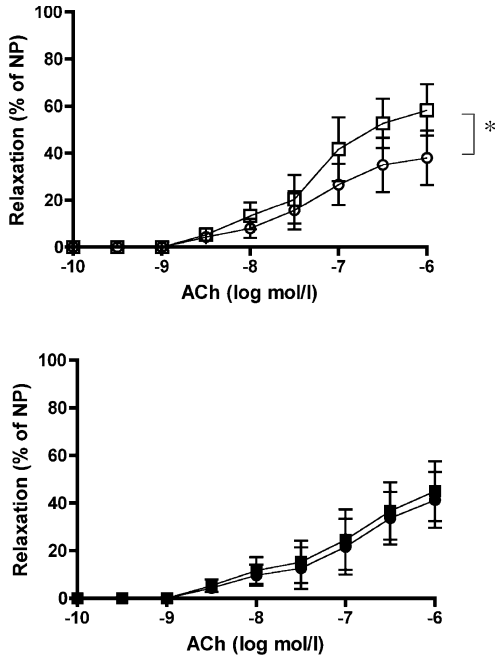

Fig. 9. *Concentration-response curves showing the response of the resistance femoral arteries to acetylcholine (ACh) in the presence of L-NMMA and indomethacin with or without CTx plus apamin. Upper: 25-week-old WKY. Lower: 25-week-old SHR. Symbols are the same as in Fig. 7. ACh-induced vasodilatation in the presence of L-NMMA and indomethacin was weaker in SHR than in WKY. CTx plus apamin attenuated this dilatation in WKY but not in SHR.* *p < *0.05* vs. *the presence of CTx plus apamin.*

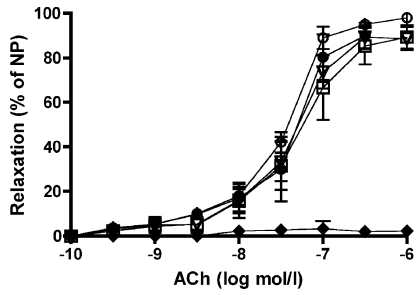

Fig. 10. *Concentration-response curves showing the response of the resistance femoral arteries to acetylcholine (ACh) in the absence or the presence of 17-octadecynoic acid (a cytochrome P450 monooxygenase inhibitor; 17ODYA), 18β-glycyrrhetinic acid (a gap junction inhibitor; 18βGA) or catalase (an enzyme that selectively dismutates H_2O_2 into water and oxygen) in 15-week-old WKY. 17ODYA, 18βGA or catalase had no significant effects on ACh-induced dilatation. Open circles, control arteries (in the absence of any drugs); open squares, in the presence of 17ODYA; closed triangles, in the presence of 18βGA; closed circles, in the presence of catalase. Endothelial disruption abolished the ACh-induced dilatation (closed diamonds).*

of hypertensive rats in which hypertension was induced by a high-salt diet (*7*). Bussemaker *et al*. also demonstrated that EDHF-mediated responses were prominent in young SHR (*8*). However, our data show that the dilatation responses mediated by EDHF in the femoral arteries of SHR in the hypertensive stage are attenuated before the decrease in vascular responses mediated by NO. Our data also showed that this attenuation of EDHF-mediated dilatation is due to the CTx plus apamin–sensitive EDHF–mediated vasodilatation (Fig. 5). The difference between our present data and the findings of Sofola and Bussemaker may be due to the differences in the etiology of hypertension and in the vascular beds between the two studies. Other studies have reported that the responses mediated by EDHF in the mesenteric arteries of SHR were attenuated (*9*, *10*). These results are consistent with our present findings. EDHF plays a more important role in ACh-induced dilatation in the microvessels than does NO. Therefore, the activity of EDHF may decrease before the decrease in vascular responses mediated by NO in the microvessels in SHR. Although the SBP in 5-week-old SHR was higher than that in age-matched WKY, NO-mediated and EDHF-mediated dilatation were not different between the SHR and WKY groups. These data suggest that the cause of the attenuation of EDHF-mediated dilatation in 15- and 25-week-old SHR is hypertension. The mechanisms leading to the attenuation of EDHF-mediated relaxation in hypertension are poorly understood, but it has been reported that they are unrelated to enhanced vascular oxidative stress (*29*).

The exact nature of EDHF remains to be identified. The EDHF candidates include EETs (*11*, *12*), which are metabolites of cytochrome P-450 monooxygenase; K^+ (*13*); gap junctions (*14*); and hydrogen peroxide (*15*, *16*). Thus, more than one EDHF might exist, and the contribution of each EDHF to endothelium-dependent relaxation might vary, depending on the species tested and the vessels used. We attempted to identify the possible candidates (*e.g.*, gap junction, EETs, H_2O_2) for EDHF in rat femoral arteries; inhibition of these candidates had no significant effect. However, it is possible that there are other EDHF candidates in addition to the above three.

The femoral arteries supply the blood to the muscles of the lower extremities, which play an important role in regulation of the peripheral arterial resistance, while the mesenteric arteries supply the blood to the intestines. Therefore, the influence of EDHF may be different in these two vascular beds. Some studies have shown that EDHF does not contribute to endothelium-dependent smooth muscle cell (SMC) relaxation in the femoral arteries (*17*, *18*). Sandow *et al*. demonstrated that a lack of myoendothelial gap junctions (MEGJs) in the femoral arteries accounts for the absence of electrical coupling between endothelial cells and SMCs and the absence of EDHF in these vessels (*25*). In our study, gap

junction inhibitors had no effect on the ACh-induced dilatation in the rat femoral arteries. This is consistent with the findings of Sandow. However, our data also show that CTx plus apamin attenuated ACh-induced dilatation in rat femoral resistance arteries. Our data suggest that ACh-induced dilatation in response to EDHF occurs in these vessels. These data are consistent with a previous study by Parkington *et al*. that showed that the CTx plus apamin–sensitive EDHF was predominant in the hindlimb of rats (*19*). Moreover, in general, the hyperpolarizing mechanism of EDHF is considered to be mediated by K_{Ca} channels on vascular smooth muscle. Our data demonstrate that CTx plus apamin–sensitive EDHF–mediated dilatation is attenuated before the loss of NO-mediated vasodilatation in the femoral resistance arteries of SHR.

EDHF responses in some vessels are dependent on the activation of a cytochrome P450 epoxygenase and the generation of EETs (*11*, *12*). Previous studies of other vascular beds *in vivo* have implicated the EETs as EDHFs (*30*). In contrast, EETs do not play a significant role in vasodilatation in guinea pig coronary arteries (*31*, *32*). Our data showed that an inhibitor of EETs (17ODYA) had no effect in the femoral arteries of rats. This is consistent with the finding of Parkington that CTx plus apamin–sensitive EDHF was predominant in the hindlimb vascular bed of rats (*19*).

EDHF candidates also include hydrogen peroxide (*15*, *16*). Previous studies of other vascular beds *in vitro* have implicated hydrogen peroxide as an EDHF. However, in our study, an enzyme that selectivity dismutates H_2O_2 into water and oxygen, catalase, had no effect on ACh-induced dilatation in rat femoral arteries. The difference between our present data and those reported by others may be due to the differences of vascular beds used. Further studies are necessary to elucidate this point.

Conclusion

EDHF-mediated vasodilatation was attenuated before the loss of NO-mediated vasodilatation in the femoral resistance arteries of SHR, and this attenuation was mediated by the CTx plus apamin–sensitive EDHF.

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