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Impaired forearm vasodilatation by acetylcholine in patients with hypertension

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Summary. The aim of this study was to examine if vasodilatory responses to acetylcholine or sodium nitroprusside are altered in subjects with essential hypertension. In patients with essential hypertension (mean BP; 121 ± 4 mmHg, $n = 14$) and age-matched control subjects (mean BP; 88 ± 3 mmHg, $n = 10$), the forearm vasodilatory responses to acetylcholine and sodium nitroprusside were examined. The brachial artery was cannulated with a cannula through which drugs were locally infused. During the drug infusions, forearm blood flow was continuously measured using plethysmography. Basal forearm vascular resistance was higher in patients with essential hypertension than in control subjects (27.5 ± 3.8 vs 13.8 ± 1.7 units, $P < 0.01$). The increases in forearm blood flow or decreases in forearm vascular resistance in response to the infusions of acetylcholine were smaller in patients with essential hypertension than in control subjects ($P < 0.01$). However, the increases in forearm blood flow or decreases in forearm vascular resistance in response to the infusions of sodium nitroprusside were similar for the 2 groups. These results may suggest that the endothelium-dependent vasodilatory response to acetylcholine in the forearm resistance arteries is impaired in patients with essential hypertension.

Key words: Hypertension–EDRF–Human–Forearm resistance vessels

Introduction

Since Furchgott and Zawadski first described an important role of the endothelium in vascular relaxation evoked by acetylcholine [1], many experiments carried out on vascular rings of animals have shown that vasorelaxation induced by a variety of agents is endothelium-dependent [2–4]. Moreover, experiments carried out on animals and humans in vivo have demonstrated that the vasodilatation induced in resistance vessels by acetylcholine is endothelium-dependent [5–10].

It has been shown that in hypertensive animals, endothelium-dependent vasodilatation was decreased not only in the large vessels but also in the small resistance vessels [11–22]. Furthermore, two recently published reports suggested that endothelium-dependent vasodilatation is decreased in the resistance vessels of hypertensive patients [23, 24]. The investigators assessed endothelium-dependent vasodilatation of human forearm vessels by infusing acetylcholine locally into a brachial artery and by measuring forearm blood flow (FBF) simultaneously. Since these two studies were done on western populations, we felt that it was important to do similar studies on another population. Accordingly, in the present study, using similar methods, we aimed to examine how endothelium-dependent vasodilatation is altered in the forearm resistance vessels of Japanese patients with essential hypertension.

Methods

Subjects

Fourteen patients with essential hypertension (11 males and 3 females) and ten normotensive healthy volunteers (all males) were studied. The ages of the patients with essential hypertension ranged from 42 to 68 years (mean; 58 ± 2 years) and those of normotensive subjects from 44 to 70 years (mean; 52 ± 3 years). There was no significant difference in age between the 2 groups. Since the subjects were either outpatients or inpatients, sodium intake was not controlled. Blood pressure was measured at least on 2 occasions. Hypertension was defined as systolic blood pressure in excess of 140 mmHg, diastolic blood pressure in excess of 90 mmHg,

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or both. The subjects underwent physical examination, blood cell counts, urinalysis, and measurements of serum electrolytes and creatinine. They also underwent electrocardiographic, chest roentgenographic, echocardiographic, and fundoscopic examinations. Blood cell count, urinalysis, serum electrolytes, and creatinine were normal in the 14 patients with essential hypertension. Nine hypertensive patients had a normal electrocardiogram, and the other five patients demonstrated left ventricular hypertrophy on electrocardiography. On chest roentgenography, six patients showed cardiomegaly (cardiothoracic ratio; 53 ± 0.3%). Wall hypertrophy was revealed on echocardiograms in four patients (posterior wall; 1.5 ± 0.1 cm and interventricular septum; 1.3 ± 0.1 cm). On the fundoscopic examination, three patients had grade II changes, one had grade III changes, and the others had grade I changes in the Keith-Wagener classification. The stage of hypertension according to the World Health Organization (WHO) criteria was I in seven patients and II in seven patients. No patients had been taking medications for at least 2 weeks prior to the study. Other medical problems included diabetes mellitus (two patients), and impaired glucose tolerance (two patients). The study protocol was explained and informed consent was obtained from each subject.

Measurements of forearm blood flow and arterial pressure

Studies were done with the subjects in the supine position. Forearm blood flow was measured using a mercury-in-silastic strain gauge plethysmograph and the venous occlusion technique [26, 27]. The strain gauge was placed approximately 5 cm below the antecubital crease. The cuff pressure for occluding the forearm veins was 40 mmHg. Circulation to the hand was arrested during determination of forearm blood flow by inflating a cuff around the wrist to a pressure which was above systolic pressure. Forearm blood flow was taken as the average of 8 flow measurements made at 15 s intervals. Recordings were obtained at a paper speed of 10 cm/min. In some subjects, blood flow increased severely at the highest dose of acetylcholine. In such cases, recordings were done at a paper speed of 30 cm/s to avoid calculation errors. At the end of the study, calibration of the plethysmograph was done by turning the screw of the plethysmograph once in order to shorten the silicone tube by 0.64 mm while recording the change in the mercury conductance. Calculation of forearm blood flow was performed independently by two of the authors who were not aware of which drug was being infused, and the averaged value was used for statistical analysis. Blood pressure was measured by a sphygmomanometer applied to the other arm. All blood pressure measurements were performed by the same physician in order to minimize observer variation. Forearm vascular resistance was calculated by dividing mean arterial pressure (diastolic pressure plus one-third of the pulse pressure in mmHg) by forearm blood flow (ml/min per 100 ml of forearm volume); these values are expressed as units throughout this report. Heart rate was determined by counting the radial pulse for 1 min.

Forearm vascular responses to drugs

The brachial artery was cannulated with a 20 gauge Intravascular Over-the-Needle Teflon Catheter (QUICKCATH, Travenol Laboratories, Inc., USA) for drug infusions. After the placement of a cannula and a strain gauge plethysmograph, at least 15 min were allowed for the subjects to become accustomed to the study conditions before beginning the experiments. The arterial line was kept open by infusion of heparinized saline before drug infusion.

We examined forearm vasodilator responses to intra-arterial acetylcholine and sodium nitroprusside (SNP) at graded doses. Acetylcholine (4, 8, 16 and 24 μg/min) and SNP (0.2, 0.4, 0.8, and 1.2 μg/min) were infused intra-arterially for 2 min at each dose. Drugs were administered in a fixed order, i.e., acetylcholine followed by SNP. The volumes of infusion were 0.1, 0.2, 0.4, and 0.6 ml/min for progressive doses of each drug. After completion of the studies with acetylcholine, we waited at least 30 min before beginning infusion of SNP, by which time forearm blood flow had returned to the baseline value. Forearm blood flow was measured continuously at 15 s intervals in the ipsilateral arm during drug infusion. Since forearm blood flow reached a steady state 1 min after starting infusion of each drug, we used the last 1-min measurements during drug infusion of each dose for analysis. Blood pressure was measured twice during the last 1 min of drug infusion of each dose.

Preparations of acetylcholine and sodium nitroprusside

Since acetylcholine is unstable in a solution, 100 mg acetylcholine (Daiichi Seiyaku, Japan) was lyophilized and stored in a vial (0.4 mg of acetylcholine per vial), and was dissolved in physiological saline (10 ml) immediately before use. Sodium nitroprusside (Wakou Junyaku Kogyo, Japan) was dissolved in physiological saline at a concentration of 2000 ng/ml. Special care was taken not to expose the nitroprusside to light.

Statistical analysis

Statistical analysis of age, resting blood pressure, heart rate, forearm blood flow, and forearm vascular resistance was performed using Student's *t*-test. Statistical analysis of mean blood pressure heart rate, forearm blood flow, and forearm vascular resistance in response to intraarterial acetylcholine or SNP was performed by the use of one-way analysis of variance (ANOVA). Two-way ANOVA was used to make comparisons of the forearm vascular responses to the drugs between the two groups. All values are expressed as mean ± standard error (S.E.) and $P < 0.05$ was considered to be statistically significant.

Results

Table 1 shows the hemodynamic data before and during graded infusions of acetylcholine and sodium nitroprusside. Resting mean blood pressure and forearm vascular resistance were higher ($P < 0.01$ for both), and resting forearm blood flow was smaller ($P < 0.05$) in the hypertensive patients than in the control subjects. Resting heart rate was similar between the two groups. Intra-arterial infusions of acetylcholine and SNP did not alter mean blood pressure or heart rate

Table 1. Responses of MBP, HR, FBF, and FVR to acetylcholine, and SNP

	ACh (μg/min)					SNP (μg/min)				
	Control	4	8	16	24	Control	0.2	0.4	0.8	1.2
Normotensives (n = 10)										
MBP (mmHg)	85±3	86±3	86±3	86±3	85±4	87±2	88±4[c]	87±3	87±2	89±4[c]
HR (bpm)	62±2	62±3	61±3	61±3	63±3	61±2	64±2[c]	61±2	62±2	63±2[c]
FBF (ml/min per 100 ml)	7.9±0.9	11.8±2.0	24.3±5.1	37.7±6.2	58.6±8.7	8.1±0.9	9.6±1.2[c]	12.1±1.8	17.7±2.4	20.4±2.9[c]
FVR (units)	12.5±1.6	9.9±2.0	5.6±1.3	3.0±0.6	2.0±0.5	11.2±1.1	9.2±1.3[c]	8.2±1.4	5.4±0.6	4.5±0.6[c]
Hypertensives (n = 14)										
MBP (mmHg)	119±4[b]	119±4	118±4	118±4	117±4	119±4[b]	119±4	120±4	119±4	121±4
HR (bpm)	68±3	68±3	69±3	69±3	69±3	70±4	70±4	69±4	69±4	70±4
FBF (ml/min per 100 ml)	5.2±0.5[a]	6.2±0.9	9.6±2.0	17.8±2.6	19.6±3.2	5.7±0.7[a]	6.4±0.8	8.3±0.9	11.2±1.4	13.3±1.7
FVR (units)	27.5±3.8[b]	25.1±3.9	21.8±4.8	9.2±1.7	8.2±1.4	26.6±4.0[b]	22.8±3.2	17.6±2.9	14.1±2.9	10.7±1.4

[a] $P < 0.05$ vs resting value in normotensive subject, [b] $P < 0.01$ vs resting value in normotensive subjects
[c] $n = 7$
ACh, Acetylcholine; *SNP*, sodium nitroprusside; *FBF*, forearm blood flow; *MBP*, mean blood pressure; *HR*, heart rate; *bpm*, beats per minute; *FVR*, forearm vascular resistance

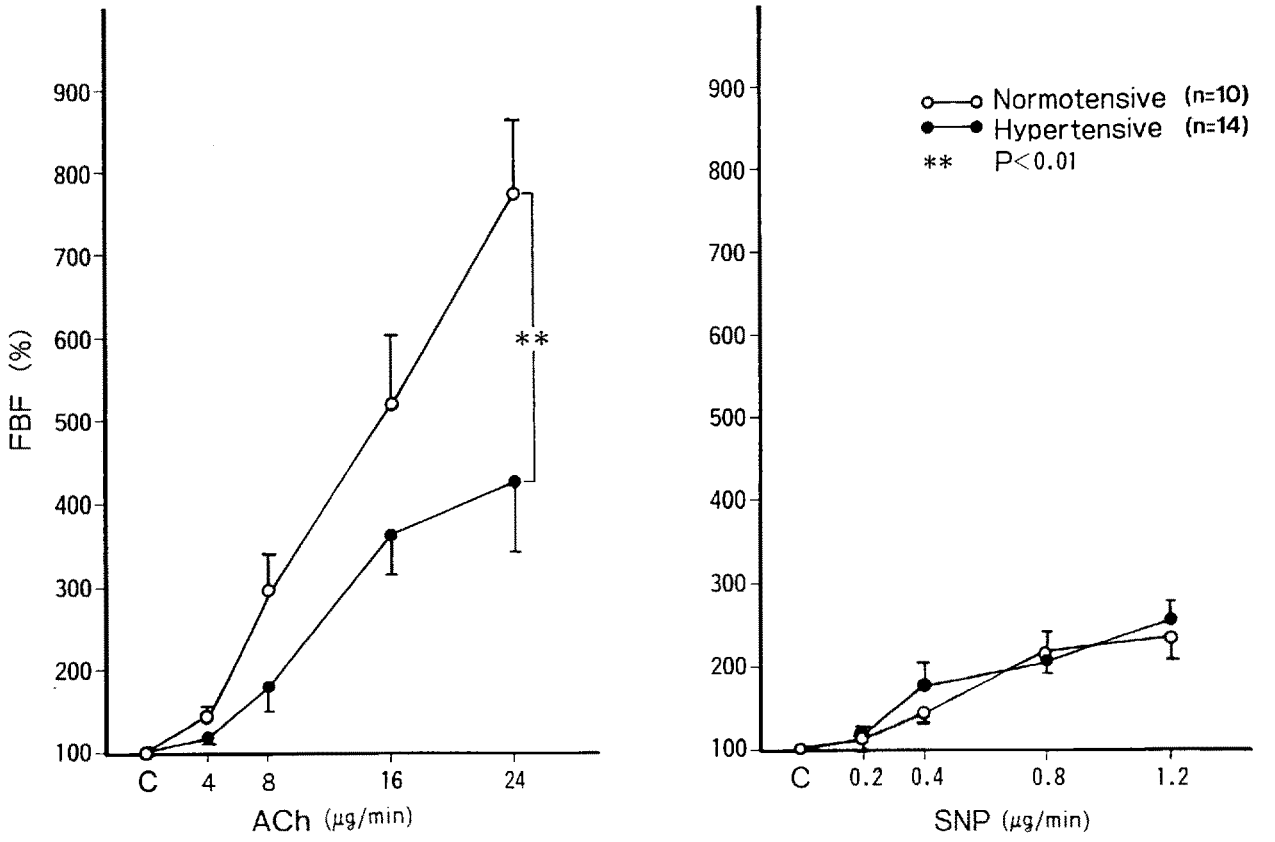

Fig. 1. Change in forearm blood flow in response to acetylcholine and sodium nitroprusside in normotensive and hypertensive subjects. Since resting blood flow was different between the two groups, forearm blood flow was normalized. The increases in forearm blood flow to acetylcholine infusion were much greater in normotensive subjects than in patients with essential hypertension ($P < 0.01$).

** $P < 0.01$ indicates statistical differences between the two groups. $P < 0.05$ at the doses of 8 and 24 μg/min acetylcholine. Not significant at the doses of 4 and 16 μg/min acetylcholine.
ACh, acetylcholine; *SNP*, sodium nitroprusside; *%FBF*, normalized forearm blood flow; *Normotensive*, normotensive subjects; *Hypertensive*, hypertensive subjects

in either the control subjects or in the hypertensive patients. Figure 1 shows the changes in normalized forearm blood flow in response to the drugs at graded doses. Acetylcholine and SNP increased forearm blood flow ($P < 0.01$) in a dose-dependent fashion in each group. The increases in forearm blood flow in response to SNP were similar between the groups. However, the increases in forearm blood flow to

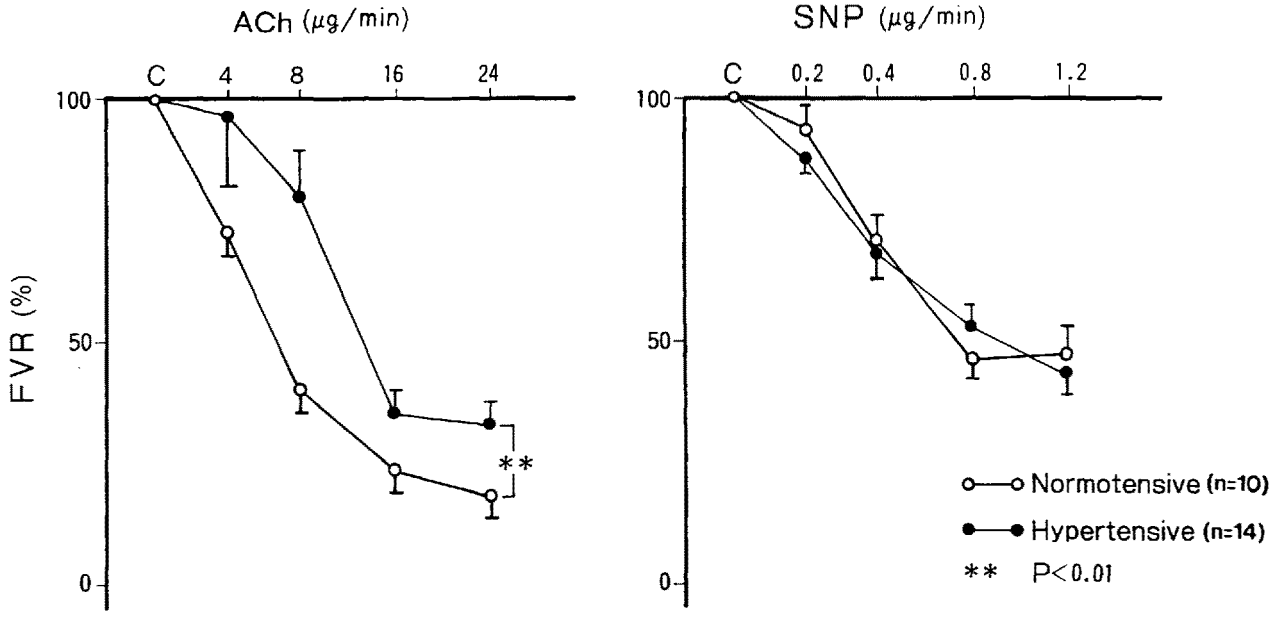

Fig. 2. Forearm vasodilatory responses to acetylcholine and sodium nitroprusside in normotensive and hypertensive subjects. Since resting vascular resistance was different, forearm vascular resistance was normalized. The vasodilatory responses to acetylcholine infusion were smaller in patients with hypertension than in normal subjects ($P < 0.01$) $P < 0.01$ indicates statistical differences between the two groups. $P < 0.01$ at the dose of 8 μg/min of acetylcholine. $P < 0.05$ at the dose of 24 μg/min of acetylcholine. Not significant at the doses of 4 and 16 μg/min of acetylcholine. *ACh*, acetylcholine; *SNP*, sodium nitroprusside; *%FVR*, normalized forearm vascular resistance; *Normotensive*, normotensive subjects; *Hypertensive*, hypertensive subjects

acetylcholine were attenuated in the hypertensive patients ($P < 0.01$). Since the basal forearm vascular resistance was different between the two groups, the changes in forearm vascular resistance in response to drugs were normalized and compared (Fig. 2). The decreases in forearm vascular resistance in response to SNP were not different between the two groups. However, the decreases in forearm vascular resistance in response to acetylcholine were attenuated in the hypertensive patients ($P < 0.01$).

Discussion

The major finding of this study was that forearm vasodilatation induced by intra-arterial infusion of acetylcholine was less in the hypertensive patients than in the control subjects, while forearm vasodilatation induced by intra-arterial infusion of SNP did not differ between the two groups. These results suggest that endothelium-dependent forearm vasodilatation is attenuated in the hypertensive subjects, while endothelium-independent vasodilatation is not altered.

In this study, we assumed that the vasodilatation of the forearm blood vessels induced by intra-arterial acetylcholine was mediated by the endothelium. This assumption was based on previous findings in animals. Studies performed in vascular rings of animals have clearly demonstrated that vasodilatation induced by acetylcholine is mediated by the endothelium [2–4]. Moreover, recent studies on animals have suggested that vasodilatation induced in resistance vessels by acetylcholine is dependent on the intact endothelium [5–9]. In particular, Furchgott et al. have shown that collagenase (an enzyme capable of removing endothelial cells) completely blocked the vasodilatation induced by acetylcholine in the perfused mesenteric arterial vasculatures of rabbits [7]. In rats, hemoglobin (an agent which can inactivate endothelium-derived relaxing factor (EDRF)) markedly attenuated vasodilatation induced by acetylcholine in the mesenteric circulation [7]. Further, Pohl et al. showed that gossypol, which abolishes EDRF production and/or its release, abolished acetylcholine-induced vasodilatation in autoperfused hindlimbs of rabbits [8]. Moreover Griffith et al., using a microradiographic technique, showed that hemoglobin abolished acetylcholine-induced vasodilatation in the arterioles of the rabbit ear [6]. Recently Vallance et al., using N^G-monomethyl-L-arginine (L-NMMA), a specific inhibitor of the synthesis of endothelium-derived nitric oxide, demonstrated that the vasodilatation induced by acetylcholine in human forearm resistance vessels is endothelium-dependent [10].

In previous experiments in hypertensive animals, it has been reported that endothelium-dependent

vasodilatation is impaired. Konishi and Su have reported that endothelium-dependent vasorelaxations induced by acetylcholine and calcium ionophore A23187 in rings of the thoracic aorta of genetically hypertensive rats were reduced compared to that in the thoracic aorta of normotensive rats [11]. Endothelium-dependent relaxation of the aorta induced by acetylcholine is also impaired in renal hypertension, salt-induced hypertension, coarctation, and DOCA-salt hypertension in rats [11, 18, 19]. It has been reported that endothelium-dependent vasodilatation is impaired in arterioles of hypertensive animals as well [16, 21, 22]. In particular, Tesfamariam and Halpern have found that acetylcholine-induced vasodilatation was smaller in mesenteric resistance vessels of stroke-prone spontaneously hypertensive rats than in those of normotensive Wistar-Kyoto rats [16]. Watt and Thurston have reported that acetylcholine-induced vasodilatation in mesenteric arteries of spontaneously hypertensive rats was smaller than that in Wistar-Kyoto rats [22]. In the present study, we have demonstrated that vasodilatation of forearm blood vessels induced by acetylcholine was impaired in humans with hypertension, whereas vasodilation induced by SNP was not. These results may suggest that endothelium-dependent vasodilatation is impaired in forearm blood vessels of hypertensive patients.

Four patients with diabetes mellitus were included in this study. Two patients had overt diabetes mellitus (fasting blood glucose was 176 and 280 mg/dl) and two had an impaired glucose tolerance test (fasting blood glucose was 88 and 96 mg/dl). Since it has been shown in animals and humans that diabetes impairs endothelium-dependent vasorelaxation [28–30], it is possible that the presence of impaired endothelium-dependent vasodilatation in our subjects might have been due to the inclusion of diabetic patients. However, acetylcholine-induced forearm vasodilatation in non-diabetic hypertensive patients was still smaller than in control subjects (the maximal response was 474 ± 109% for non-diabetic hypertensive patients; $n = 10$, and 776 ± 94% for control subjects; $n = 10$, $P < 0.01$).

The increases in forearm blood flow in response to SNP were smaller than those in response to acetylcholine. We should consider the possibility that the doses of SNP used in this study might have been too low for a possible difference in response to SNP between the 2 groups to be detected. Therefore, we infused larger doses of SNP, i.e., 1.6, 2.4, and 3.2 μg in three subjects. The higher doses of SNP did not increase blood flow (FBF was 17.1 ± 1.9 ml/min per 100 ml during 1.6 μg/min of SNP and 17.2 ± 1.9 ml/min per 100 ml during 2.4 μg/min of SNP). Systemic effects of SNP become apparent with the dose of 3.2 μg/min and systolic blood pressure decreased by about 10 mmHg at this dose. Thus, our data indicate that endothelium-independent vasodilatation was similar between the two groups.

There are several possible mechanisms for impaired acetylcholine-induced vasodilatation in hypertension, including (1) a decreased release of endothelium-derived relaxing factor(s), (2) an impaired diffusion of this (these) factor(s) from the endothelium to the vascular smooth muscle cells, (3) a decreased responsiveness of the vascular smooth muscle to vasodilator substances, (4) a release of endothelium-derived constrictor factor or factors, and (5) altered muscarinic receptor function. In the present study, the vasodilatory responses to SNP were similar between the two groups and thus it appears unlikely that decreased responsiveness of the vascular smooth muscle cells was responsible for the impaired acetylcholine-induced vasodilatation of forearm blood vessels of hypertensive subjects. Thus, impaired vasodilatation induced by acetylcholine in forearm blood vessels of hypertensive patients is most likely due to abnormal endothelial function. In fact, previous studies have shown that endothelial cells of hypertensive blood vessels in animals appear voluminous, bulging into the lumen and varying in size and shape [31, 32]. The orientation of endothelial cells and of their nuclei is markedly distorted [32]. These findings of morphological abnormalities of the endothelial cells may explain the presence of functional abnormalities found in this study. However, there is a possibility that only muscarinic receptor function is altered in patients with essential hypertension.

It has been shown in spontaneously hypertensive rats that the impaired endothelium-dependent vasodilatation is not due to a reduced endothelial relaxing factor but to the liberation of a constricting factor [33]. Thus, one may argue that the impaired acetylcholine-induced forearm vasodilatation observed in this study is due to the releasing of a constricting factor. This possibility can not be excluded and further studies are needed to clarify this proposal.

We do not know whether impaired acetylcholine-induced forearm vasodilatation in hypertensive subjects is a primary abnormality or a result or cause of hypertension. It has been suggested that, in hypertensive animals, hypertensive treatments would restore endothelial function [15, 19, 20, 32]. Such findings suggest that impaired endothelium-dependent vasodilatation is a result of hypertension. Nevertheless, impaired endothelium-dependent vasodilatation may accelerate the existing vascular abnormality and cause a vicious cycle that will contribute to the final consequence of hypertension.

In summary, we have shown that vasodilatation of human forearm resistance vessels induced by acetylcholine was impaired in humans with hypertension. Our results may suggest that endothelium-dependent

vasodilatation of resistance vessels is impaired in humans with hypertension.

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