Original Article

Vitamin C Improves Attenuated Angiotensin II-Induced Endothelium-Dependent Vasodilation in Human Forearm Vessels

Yoshitaka HIROOKA*, Kenichi ESHIMA*, Soko SETOGUCHI, Takuya KISHI, Kensuke EGASHIRA, and Akira TAKESHITA

Endothelial dysfunction might be related to an increase in superoxide anion production in patients with hypertension, hypercholesterolemia, diabetes mellitus, and heart failure. Studies in animal models indicate that angiotensin II increases superoxide anion production by vascular tissues. We examined whether angiotensin II attenuates endothelium-dependent vasodilation via an increase in superoxide anion production in human forearm vessels *in vivo*. Forearm blood flow was measured in 23 healthy young men. We examined forearm vasodilator responses to an intra-arterial infusion of acetylcholine (4, 8, and 16 $\mu\text{g}/\text{min}$) and sodium nitroprusside (0.8, 1.6, and 3.2 $\mu\text{g}/\text{min}$) before and during an intra-arterial infusion of angiotensin II ($n=8$), angiotensin II plus vitamin C ($n=8$), and vitamin C alone ($n=4$). Angiotensin II attenuated the forearm vasodilatory response to acetylcholine ($p<0.05$), and this attenuated response was abolished by vitamin C. Angiotensin II did not alter the forearm vasodilatory response to sodium nitroprusside, and vitamin C infusion did not affect the forearm vasodilatory response to either acetylcholine or sodium nitroprusside. The forearm vasodilator response to acetylcholine did not change during infusion of norepinephrine ($n=3$), which reduced forearm blood flow to a degree similar to that by angiotensin II infusion. These results suggest that angiotensin II attenuates endothelium-dependent forearm vasodilation, and vitamin C improves this impairment. Thus, angiotensin II likely attenuates endothelium-dependent vasodilation via an increase of superoxide anion production in the human forearm *in vivo*. (*Hypertens Res* 2003; 26: 953–959)

Key Words: antioxidants, endothelium, nitric oxide, angiotensin II, free radicals

Introduction

Previous reports suggest that patients with hypertension have abnormal endothelium-dependent vasodilator function (1–4). Recent studies indicate that increased production of vascular reactive oxygen species, particularly the production of superoxide anions, contributes to the functional abnormalities in hypertension (5–7). Enhanced superoxide production dimin-

ishes nitric oxide (NO) bioavailability by inactivating NO (6–8). In addition, patients with essential hypertension show increased production of superoxide anions and hydrogen peroxide by circulating leukocytes as well as decreased NO and antioxidant levels (9, 10). The increased superoxide anions might contribute to the impaired bioactivity of endothelium-derived NO in patients with hypertension (6, 7).

Recent studies have reported that endothelial dysfunction in patients with essential hypertension can be improved by

From the Department of Cardiovascular Medicine, Kyushu University Graduate School of Medical Sciences, Fukuoka, Japan.

* These authors contributed equally to this work.

This study was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan and the Japan Society for the Promotion of Science, and by a Grant for the Study of Clinical Vascular Function from Kimura Memorial Heart Foundation.

Address for Reprints: Yoshitaka Hirooka, M.D., Ph.D., Department of Cardiovascular Medicine, Kyushu University Graduate School of Medical Sciences, 3–1–1 Maidashi, Higashi-ku, Fukuoka 812–8582, Japan. E-mail: hyoshi@cardiol.med.kyushu-u.ac.jp

Received May 27, 2003; Accepted in revised form August 19, 2003.

Table 1. Baseline Hemodynamic Values

Parameter	Protocol 1 (n = 8)	Protocol 2 (n = 8)	Protocol 3 (n = 4)	Protocol 4 (n = 3)
Mean blood pressure (mmHg)	82 ± 3	80 ± 1	82 ± 2	81 ± 2
Heart rate (bpm)	68 ± 2	67 ± 3	65 ± 3	66 ± 3
FBF (ml/min/100 ml)	3.3 ± 0.4	3.6 ± 0.6	3.7 ± 1.1	4.1 ± 0.2
FVR (unit)	26.7 ± 2.9	26.6 ± 4.5	25.3 ± 4.4	19.0 ± 1.0

FBF, forearm blood flow; FVR, forearm vascular resistance. The protocols were as described in the Methods section. Values are the mean ± SEM.

treatment with an angiotensin converting enzyme inhibitor or an angiotensin II type-1 receptor antagonist (3, 11, 12). In an animal model of angiotensin II-induced hypertension, angiotensin II increased vascular superoxide production, which in turn reduced the biologic activity of endothelium-derived NO (13). This loss of NO bioactivity dramatically altered vascular reactivity, suggesting that this mechanism contributes to angiotensin II-induced blood pressure elevation (14). It is not known, however, whether angiotensin II increases superoxide production and impairs endothelium-dependent vasodilation in human forearm resistant vessels *in vivo*.

Vitamin C is the primary water-soluble antioxidant in human plasma, and is capable of scavenging oxygen-derived free radicals and sparing other endogenous antioxidants from consumption (15–17). Previous studies have demonstrated that acute intra-arterial administration of vitamin C improves endothelium-derived NO-mediated vasodilation in forearm resistant arteries of patients with hypertension (18), hypercholesterolemia (19), or diabetes mellitus (20), and in all conditions associated with increased production of reactive oxygen species.

The aim of this study was to determine whether angiotensin II decreases endothelium-dependent vasodilation in the human forearm *in vivo*, and if so, whether the decrease occurs *via* an increase of superoxide production. We used vitamin C as an antioxidant to reduce the effects of superoxide production (18–22).

Methods

Subjects

The subjects were 23 healthy young men (mean ± SE: 22.5 ± 0.5 years old). Their mean body mass index was 20.1 ± 0.4 kg/m². None of the subjects had evidence of hypertension, hypercholesterolemia or hyperglycemia, and none had a smoking habit. The study protocol was fully explained to each subject prior to obtaining written informed consent. The study was approved by the Ethics Committee for Human Research at our institute.

General Procedures

The study was performed in an air-conditioned room at 25 to

26°C with each subject in the supine position and in a post-absorptive state. Under local anesthesia, the left brachial artery was cannulated with a 20-gauge intravascular catheter that served as a port for drug infusion and for arterial pressure measurement. Forearm blood flow (FBF) was determined using a mercury-in-silastic strain-gauge plethysmograph with a venous-occlusion technique as described previously (23–28). Forearm vascular resistance (FVR) was calculated by dividing the mean arterial pressure (diastolic pressure plus one third of the pulse pressure in mmHg) by the FBF. Plasma angiotensin II concentration was measured by radioimmunoassay.

Protocols

After placement of the cannula and strain-gauge plethysmograph, at least 15 min was allowed for subjects to become accustomed to the study conditions before the experiments were begun. We measured forearm vasodilator responses to acetylcholine (4, 8, and 16 µg/min) and sodium nitroprusside (0.8, 1.6, and 3.2 µg/min), which were infused intra-arterially over 2 min at each dose, as previously described (23–25). The order of acetylcholine and sodium nitroprusside administration was alternated, with infusion of the second drug beginning after FBF returned to baseline levels. These procedures were repeated after intra-arterial infusion of angiotensin II (5 ng/min) (Protocol 1; n = 8), angiotensin II (5 ng/min) plus vitamin C (20 mg/min) (Protocol 2; n = 8), or vitamin C (20 mg/min) (Protocol 3; n = 4) for 20 min. Infusion of angiotensin II was continued during the infusion of acetylcholine or sodium nitroprusside. We chose 5 ng/min as the dose of angiotensin II, because, in our preliminary trial, this dose was shown to cause a small reduction of FBF. In addition, we measured plasma angiotensin II concentration before and at the end of infusion of angiotensin II using plasma obtained from the blood sample from the antecubital vein in 13 subjects. The concentration was increased from 8 ± 2 pg/ml to 70 ± 6 pg/ml, and the latter concentration has previously been observed in some patients with heart failure. The dose of vitamin C used was similar to that reported to improve impaired endothelial function when infused into the brachial artery (18–22). As a control experiment, we infused norepinephrine (400 ng/min) (27) instead of angiotensin II, and examined the forearm vasodilatory response to acetyl-

choline and sodium nitroprusside (Protocol 4; $n = 3$). Heparinized saline (0.2 ml/min) was used as a vehicle for each protocol.

Statistical Analysis

Basal values obtained from each protocol were examined using an unpaired Student's *t*-test and the basal values before and after drug infusion were compared using a paired Student's *t*-test. Responses to graded doses of acetylcholine or sodium nitroprusside before and after each drug administration were examined using one-way analysis of variance for repeated measures. Two-way analysis of variance was used to compare responses of FBF between the groups and dosages of the drugs. Bonferroni's correction for multiple comparison was used when necessary. All values were expressed as the mean $\pm$ SEM. Values of *p* less than 0.05 were considered to be statistically significant.

Results

Baseline Levels of Hemodynamics

Table 1 shows the baseline hemodynamic values. The mean blood pressure, heart rate, FBF, and FVR were similar during each protocol. The changes in FBF evoked by the infusion of angiotensin II, angiotensin II plus vitamin C, vitamin C alone, or norepinephrine were -1.2 ± 0.3 , -1.0 ± 0.3 , 0 ± 0.3 , or -1.2 ± 0.3 ml/min/100 ml, respectively. The decrease in FBF was significant ($p < 0.05$) after the infusion of angiotensin II, angiotensin II plus vitamin C, or norepinephrine, but the decreases were similar. Infusion of vitamin C alone did not alter FBF. Blood pressure and heart rate did not change during any of the protocols, and did not differ among the protocols.

Effect of Angiotensin II on Endothelium-Dependent and Endothelium-Independent Vasodilation

The forearm vasodilatory response evoked by acetylcholine was attenuated by angiotensin II ($p < 0.05$; Fig. 1A). The forearm vasodilatory response to sodium nitroprusside was not altered by angiotensin II (Fig. 1B).

Effect of Angiotensin II on Endothelium-Dependent and Endothelium-Independent Vasodilation with Co-Infusion of Vitamin C

Angiotensin II co-infusion with vitamin C did not attenuate the forearm vasodilatory response evoked by acetylcholine (Fig. 2A). In addition, co-infusion of vitamin C did not influence the forearm vasodilatory responses to sodium nitroprusside (Fig. 2B).

Effect of Vitamin C on Endothelium-Dependent and Endothelium-Independent Vasodilation

Forearm vasodilatory responses to acetylcholine and sodium nitroprusside were not affected by vitamin C (Fig. 3).

Effect of Norepinephrine on Endothelium-Dependent and Endothelium-Independent Vasodilation

Neither the forearm vasodilatory response evoked by acetylcholine (Fig. 4A) nor that evoked by sodium nitroprusside (Fig. 4B) was affected by norepinephrine.

Discussion

In the present study, angiotensin II attenuated the endothelium-dependent vasodilation evoked by acetylcholine. This attenuation was recovered by the co-infusion of vitamin C. These findings suggest that angiotensin II may increase superoxide production in the forearms of normal human subjects. The effect of vitamin C as an oxygen free radical scavenger was specific, as co-infusion of vitamin C did not alter the endothelium-independent vasodilation evoked by sodium nitroprusside. In addition, the infusion of vitamin C alone did not alter forearm vasodilator responses to acetylcholine. Furthermore, acetylcholine- and sodium nitroprusside-induced forearm vasodilation was not altered by norepinephrine. Taken together, these findings suggest that angiotensin II may increase superoxide production within the human forearm vascular bed *in vivo*. Such increased superoxide production would impair endothelial function, but the antioxidant vitamin C may improve the endothelial dysfunction by scavenging oxygen free radicals.

It has been well established that endothelial function is impaired in patients with hypertension as well as in hypertensive animals (1–4, 6, 7). Several mechanisms are thought to be involved in endothelial dysfunction in hypertension, including an increase in oxidative stress (6, 7). Endothelial vasodilator function is influenced by a balance between the production of NO and superoxide anions (6, 7, 29). Recent studies in animals suggest that an imbalance occurs due to decreased bioavailability of NO from either decreased production of NO by endothelial cells or increased degradation of NO by oxygen-derived free radicals (5, 30–32). NO has a very short half-life (from 0.5 to 5 s) and is rapidly broken down by superoxide anions under physiologic conditions (33). Potential sources of vascular superoxide production include NAD(P)H-dependent oxidases (34, 35), xanthine oxidase (36), and NO synthases (37, 38). There is accumulating evidence of the importance of NAD(P)H oxidase, particularly in relation with angiotensin II (13, 14, 38). NAD(P)H oxidase is present in vascular smooth muscle cells and in endothelial cells and is the principal source of superoxide production. Angiotensin II-induced hypertension is associated with increased vascular superoxide production in animal

Fig. 1. (A) Response of FBF to acetylcholine (ACh) before (white circles) and after (black circles) the infusion of angiotensin II ($n = 8$). (B) Response of FBF to sodium nitroprusside (SNP) before (white circles) and after (black circles) the infusion of angiotensin II ($n = 8$). * Indicates $p < 0.05$. Data are shown as the mean $\pm$ SEM.

Fig. 2. (A) Response of FBF to acetylcholine (ACh) before (white squares) and after (black squares) co-infusion of angiotensin II and vitamin C ($n = 8$). (B) Response of FBF to sodium nitroprusside (SNP) before (white squares) and after (black squares) infusion of angiotensin II and vitamin C ($n = 8$). Data are shown as the mean $\pm$ SEM.

models (13, 14, 39). Infusion of angiotensin II in rats results in hypertension, which correlates with increased NAD(P)H oxidase-derived superoxide anion production. Infusion of norepinephrine fails to increase superoxide anion production, however, which is consistent with the results of a previous study (14). In the previous study, these alterations were corrected by pretreatment with losartan. Our study extends the observations of previous studies *in vitro* and in animal and human studies *in vivo*.

In humans, Berry *et al.* demonstrated that NAD(P)H oxidase is a source of basal superoxide anion production in human internal mammary arteries and saphenous veins *in vitro* (40). They also demonstrated that angiotensin II increases superoxide anions in human internal mammary arteries, but not in saphenous veins. They suggested that NAD(P)H oxidase and xanthine oxidase are sources of superoxide produc-

tion in human internal mammary arteries. In their study, superoxide anion production evoked by angiotensin II occurred within 30 min. It was also reported that angiotensin II-induced superoxide anion generation in human vascular endothelial cells occurs within 30 min *via* activation of membrane-bound NAD(P)H oxidase (41). We infused angiotensin II for 20 min prior to the infusion of acetylcholine or sodium nitroprusside, and co-infused angiotensin II with a graded dose of acetylcholine or sodium nitroprusside. Our results extend prior observations that the pressor effect of intra-brachial infusion of angiotensin II in humans is attenuated by co-infusion of vitamin C (42), although in that previous study the effect of norepinephrine was not examined as a control. In addition, this effect occurred even at 3 min after beginning each graded dose of angiotensin II infusion (42), suggesting that angiotensin II-induced superoxide production

Fig. 3. (A) Response of FBF to acetylcholine (ACh) before (white triangle) and after (black triangles) the infusion of Vitamin C ($n = 4$). (B) Response of FBF to sodium nitroprusside (SNP) before (white triangles) and after (black triangles) the infusion of Vitamin C ($n = 8$). Data are shown as the mean $\pm$ SEM.

Fig. 4. (A) Response of FBF to acetylcholine (ACh) before (white circles) and after (black circles) the infusion of norepinephrine ($n = 3$). (B) Response of FBF to sodium nitroprusside (SNP) before (white circles) and after (black circles) the infusion of norepinephrine ($n = 3$). Data are shown as the mean $\pm$ SEM.

occurs quickly. A recent study in spontaneously hypertensive rats supports this observation (31). We used norepinephrine as a control because it results in a reduction of FBF similar to that by angiotensin II, thereby controlling for the non-specific effects of decreased FBF. Therefore, we believe that infusion of angiotensin II into the brachial artery in our study was sufficient to produce superoxide anions in the forearm vasculature. Thus, we were able to observe the effect of angiotensin II on the attenuated acetylcholine-induced forearm vasodilation and its reversal by vitamin C.

Vitamin C is the primary water-soluble antioxidant in human plasma, and its scavenging of oxygen free radicals could spare glutathione from oxidation (15–17). The depletion of thiol leads to decreased synthesis of NO, possibly by altering NO synthase activity and/or the availability of essential cofactors for the enzyme, including NAD(P)H and tetrahydrobiopterin (43, 44). Furthermore, vitamin C im-

proves impaired endothelial function in subjects or patients with coronary risk factors (18–22). We did not directly measure superoxide anion production from the forearm vascular bed, because it is technically difficult *in vivo*. The impaired forearm endothelial vasodilation evoked by acetylcholine in hypertensive patients can be improved by vitamin C, and this effect can be reversed by the NO synthase inhibitor, *N*^G-monomethyl-L-arginine (L-NMMA) (18). These results suggest that NO inactivation by oxygen free radicals contributes to endothelial dysfunction in hypertensive patients. We did not use L-NMMA in our study, because it reduces baseline FBF and markedly inhibits acetylcholine-induced forearm vasodilation, thereby confounding interpretation of the data. In addition to vitamin C, an angiotensin II type-1 receptor blocker has been shown to improve endothelial function in patients with hypertension (12). Regarding the role of xanthine oxidase as a source of superoxide anions, oxypurinol

improves acetylcholine-induced forearm vasodilation in hypercholesterolemic, but not hypertensive patients (45). Thus, we speculate that NAD(P)H oxidase may be more important than xanthine oxidase as a source of superoxide anions in hypertensive patients, although we cannot exclude the possibility that xanthine oxidase activity plays a role in hypertension (46).

The basal level of FBF did not change during infusion of vitamin C despite the fact that the magnitude of the decrease in FBF evoked by angiotensin II did not differ between the angiotensin infusion group and the angiotensin II plus vitamin C infusion group. While we do not have a clear explanation for these results, previous studies have also demonstrated that vitamin C alone did not increase FBF in patients with endothelial dysfunction (18–22). In addition, we found it difficult to observe the differences in the change in basal level of FBF between angiotensin II infusion and co-infusion of vitamin C, probably because angiotensin II is a potent vasoconstrictor.

Because sodium nitroprusside-induced forearm vasodilation is mediated by NO, we considered it possible that this vasodilation might be attenuated by angiotensin II. Upon testing this relation, however, we found that angiotensin II had no effect on the sodium nitroprusside-induced forearm vasodilation. Although we do not have a clear explanation for this finding, one possibility is that angiotensin II or superoxide anions might influence endothelial NO synthase activity. Alternatively, because sodium nitroprusside induces forearm vasodilation more directly, it may have been more difficult to observe the effect of angiotensin II than the effect of acetylcholine.

We cannot exclude the possibility of a long-term effect of norepinephrine on endothelial function. We chose a dose of norepinephrine which caused a reduction of FBF similar to that by angiotensin II. Thus, we suggest that angiotensin II attenuates acetylcholine-induced forearm vasodilation, but that norepinephrine does not, provided that either drug is infused for only 20 min. If norepinephrine were to be infused for a much longer period, it might induce effects similar to those by angiotensin II. Interestingly, FBF responses to acetylcholine or isosorbide dinitrate have been shown to be impaired in patients with pheochromocytoma (47).

In conclusion, the results of the present study suggest that angiotensin II decreases endothelium-dependent vasodilation in the human forearm *in vivo*, and that vitamin C improves this attenuation, probably by scavenging superoxide anions produced by angiotensin II.

References

1. Linder L, Kiowski W, Bühler FR, *et al*: Indirect evidence for release of endothelium-derived relaxing factor in the human forearm circulation *in vivo*: blunted response in essential hypertension. *Circulation* 1990; **81**: 1762–1767.
2. Panza JA, Quyyumi AA, Brush JE Jr, *et al*: Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. *N Engl J Med* 1990; **323**: 22–27.
3. Hirooka Y, Imaizumi T, Masaki H, *et al*: Captopril improves impaired endothelium-dependent vasodilation in hypertensive patients. *Hypertension* 1992; **20**: 175–180.
4. Taddei S, Virdis A, Mattei P, *et al*: Vasodilation to acetylcholine in primary and secondary forms of human hypertension. *Hypertension* 1993; **21**: 929–933.
5. Nakazono K, Watanabe N, Matsuno K, *et al*: Does superoxide underlie the pathogenesis of hypertension? *Proc Natl Acad Sci USA* 1991; **88**: 10045–10048.
6. Zalba G, José GS, Moreno MU, *et al*: Oxidative stress in arterial hypertension: role of NAD(P)H oxidase. *Hypertension* 2001; **38**: 1395–1399.
7. Dhalla NS, Temsah RM, Netticadan T: Role of oxidative stress in cardiovascular diseases. *J Hypertens* 2000; **18**: 655–673.
8. Mugge A, Elwell JH, Peterson TE, *et al*: Release of intact endothelium-derived relaxing factor depends on endothelial superoxide dismutase activity. *Am J Physiol* 1991; **260**: C219–C225.
9. Prabha PS, Das UN, Koratkar R, Sagar PS, Ramesh G: Free radical generation, lipid peroxidation and essential fatty acids in uncontrolled essential hypertension. *Prostaglandins Leukot Essent Fatty Acids* 1990; **41**: 27–33.
10. Russo C, Olivieri O, Girelli D, *et al*: Anti-oxidant status and lipid peroxidation in patients with essential hypertension. *J Hypertens* 1998; **16**: 1267–1271.
11. On Y-K, Kim C-H, Oh B-H, Lee M-M, Park Y-B: Effects of angiotensin converting enzyme inhibitor and calcium antagonist on endothelial function in patients with essential hypertension. *Hypertens Res* 2002; **25**: 365–371.
12. Ghiadoni L, Virdis A, Magagna A, *et al*: Effect of the angiotensin II type 1 receptor blocker candesartan on endothelial function in patients with essential hypertension. *Hypertension* 2000; **35**: 501–506.
13. Rajagopalan S, Kurz S, Münzel T, *et al*: Angiotensin II-mediated hypertension in the rat increases vascular superoxide production via membrane NADH/NADPH oxidase activation. *J Clin Invest* 1996; **97**: 1916–1923.
14. Laursen JB, Rajagopalan S, Galis Z, *et al*: Role of superoxide in angiotensin II-induced but not catecholamine-induced hypertension. *Circulation* 1997; **95**: 588–593.
15. Bendich A, Machlin IJ, Scandurra O, *et al*: The antioxidant role of vitamin C. *Adv Free Radic Biol Med* 1986; **2**: 419–444.
16. Frei B, England L, Ames BN: Ascorbate is an outstanding antioxidant in human blood plasma. *Proc Natl Acad Sci USA* 1989; **86**: 6377–6381.
17. Retsky KL, Freeman MW, Frei B: Ascorbic acid oxidation products protect human low density lipoprotein against atherogenic modification. *J Biol Chem* 1993; **268**: 1304–1309.
18. Taddei S, Virdis A, Ghiadoni L, *et al*: Vitamin C improves endothelium-dependent vasodilation by restoring nitric oxide activity in essential hypertension. *Circulation* 1998; **97**: 2222–2229.
19. Ting HH, Timimi FK, Haley EA, *et al*: Vitamin C improves endothelium-dependent vasodilation in forearm resistance vessels of humans with hypercholesterolemia. *Cir-*

- ulation 1997; **95**: 2617–2622.
20. Ting HH, Timimi FK, Boles KS, et al: Vitamin C improves endothelium-dependent vasodilation in patients with non-insulin-dependent diabetes mellitus. *J Clin Invest* 1996; **97**: 22–28.
 21. Levine GN, Frei B, Koulouris SN, et al: Ascorbic acid reverses endothelial vasomotor dysfunction in patients with coronary artery disease. *Circulation* 1996; **93**: 1107–1113.
 22. Heitzer T, Just H, Münzel T: Antioxidant vitamin C improves endothelial dysfunction in chronic smokers. *Circulation* 1996; **94**: 6–9.
 23. Masumoto A, Hirooka Y, Shimokawa H, et al: Possible involvement of Rho-kinase in the pathogenesis of hypertension in humans. *Hypertension* 2001; **38**: 1307–1310.
 24. Masumoto A, Hirooka Y, Hironaga K, et al: Effect of pravastatin on endothelial function in patients with coronary artery disease (cholesterol-independent effect of pravastatin). *Am J Cardiol* 2001; **88**: 1291–1294.
 25. Setoguchi S, Hirooka Y, Eshima K, et al: Tetrahydrobiopterin improves impaired endothelium-dependent forearm vasodilation in patients with heart failure. *J Cardiovasc Pharmacol* 2002; **39**: 363–368.
 26. Tagawa T, Hirooka Y, Shimokawa H, et al: Long-term treatment with eicosapentaenoic acid improves exercise-induced vasodilation in patients with coronary artery disease. *Hypertens Res* 2002; **25**: 823–829.
 27. Takeshita A, Jingu S, Imaizumi T, et al: Augmented cardiopulmonary baroreflex control of forearm vascular resistance in young athletes. *Circ Res* 1986; **59**: 43–48.
 28. Higashi Y, Sasaki S, Nakagawa K, et al: Sodium chloride loading does not alter endothelium-dependent vasodilation of forearm vasculature in either salt-sensitive or salt-resistant patients with essential hypertension. *Hypertens Res* 2001; **24**: 711–716.
 29. Hilgers KF, Stumph C: Angiotensin II, the endothelium and superoxide anions. *J Hypertens* 2002; **20**: 1271–1273.
 30. Dobrian AD, Schriver SD, Prewitt RL: Role of angiotensin II and free radicals in blood pressure regulation in a rat model of renal hypertension. *Hypertension* 2001; **38**: 361–366.
 31. Brosnan MJ, Hamilton CA, Graham D, et al: Irbesartan lowers superoxide levels and increases nitric oxide bioavailability in blood vessels from spontaneously hypertensive stroke-prone rats. *J Hypertens* 2002; **20**: 1–6.
 32. Shastri S, Gopalakrishnan V, Poduri R, et al: Tempol selectively attenuates angiotensin II evoked vasoconstrictor responses in spontaneously hypertensive rats. *J Hypertens* 2002; **20**: 1381–1391.
 33. Rubanyi GM, Vanhoutte PM: Oxygen-derived free radicals, endothelium, and responsiveness of vascular smooth muscle. *Am J Physiol* 1986; **250**: H815–H821.
 34. Mohazzab KM, Kaminski PM, Wolin MS: NADH oxidoreductase is a major source of superoxide anion in bovine coronary artery endothelium. *Am J Physiol* 1994; **266**: H2568–H2572.
 35. Pagano P, Ito Y, Tornheim K, et al: An NADPH oxidase superoxide-generating system in the rabbit aorta. *Am J Physiol* 1995; **268**: H2274–H2280.
 36. Zweier JL, Broderick R, Kuppusamy P, et al: Determination of the mechanism of free radical generation in human aortic endothelial cells exposed to anoxia and re-oxygenation. *J Biol Chem* 1994; **269**: 24156–24162.
 37. Xia Y, Tsai AL, Berka V, et al: Superoxide generation from endothelial nitric-oxide synthase: a Ca^{2+} /calmodulin-dependent and tetrahydrobiopterin regulatory process. *J Biol Chem* 1998; **273**: 25804–25808.
 38. Vasquez-Vivar J, Kalyanaraman B, Martasek P, et al: Superoxide generation by endothelial nitric oxide synthase: the influence of cofactors. *Proc Natl Acad Sci USA* 1998; **95**: 9220–9225.
 39. Griendling KK, Ollerenshaw JD, Minieri CA, et al: Angiotensin II stimulates NADH and NADPH activity in cultured vascular smooth muscle cells. *Circ Res* 1994; **74**: 1141–1148.
 40. Berry C, Hamilton CA, Brosnan MJ, et al: Investigation into the sources of superoxide in human blood vessels: angiotensin II increases superoxide production in human internal mammary arteries. *Circulation* 2000; **101**: 2206–2212.
 41. Zhang H, Schmeißer A, Garlisch CD, et al: Angiotensin II-induced superoxide anion generation in human vascular endothelial cells: role of membrane-bound NADH/NADPH-oxidases. *Cardiovasc Res* 1999; **44**: 215–222.
 42. Dijkhorst LT, Stroes ESG, Koomans HA, et al: Acute simultaneous stimulation of nitric oxide and oxygen radicals by angiotensin II in humans *in vivo*. *J Cardiovasc Pharmacol* 1999; **33**: 420–424.
 43. Meister A: Glutathion-ascorbic acid antioxidant system in animals. *J Biol Chem* 1994; **269**: 9397–9400.
 44. Murphy ME, Piper H-M, Watanabe H, et al: Nitric oxide production by cultured aortic endothelial cells in response to thiol depletion and replenishment. *J Biol Chem* 1991; **266**: 19378–19383.
 45. Cardillo C, Kilcoyne CM, Cannon III RO, et al: Xanthine oxidase inhibition with oxypurinol improves endothelial vasodilator function in hypercholesterolemic but not in hypertensive patients. *Hypertension* 1997; **30**: 57–63.
 46. Suzuki H, DeLano FA, Parks DA, et al: Xanthine oxidase activity associated with arterial blood pressure in spontaneously hypertensive rats. *Proc Natl Acad Sci USA* 1998; **95**: 4754–4759.
 47. Higashi Y, Sasaki S, Nakagawa K, et al: Excess norepinephrine impairs both endothelium-dependent and -independent vasodilation in patients with pheochromocytoma. *Hypertension* 2002; **39**: 513–518.