

Endothelium-Derived Relaxing Factor Modulates Endothelin Action in Afferent Arterioles

Sadayoshi Ito, Luis A. Juncos, Noboru Nushiro, Cynthia S. Johnson, and Oscar A. Carretero

Endothelin is a potent vasoconstrictor, whereas endothelium-derived relaxing factor (EDRF) is a potent vasodilator. Both are produced by the endothelium. Although they have been studied extensively in large vessels, little is known about their actions in renal microvessels. Using microdissected rabbit afferent arterioles, we studied the vascular response to synthetic endothelin and its interaction with EDRF and the effect of endothelin on renin release. Afferent arterioles were either microperfused in vitro at 60 mm Hg to measure luminal diameter or incubated without microperfusion to assess renin release. When added to the bath, 10^{-10} or 10^{-9} M endothelin decreased the diameter by $32 \pm 8\%$ ($n=7$, $p<0.01$) or $76 \pm 7\%$ ($p<0.0001$), respectively. Pretreatment with *N*^ω-nitro L-arginine, which inhibits synthesis of EDRF, decreased basal diameter by $15 \pm 1\%$ ($p<0.001$) and augmented endothelin-induced constriction; decrease in diameter with 10^{-10} M endothelin was $78 \pm 10\%$ ($n=4$, $p<0.01$ versus nontreated). In afferent arterioles precontracted by endothelin, acetylcholine at concentrations of 10^{-8} to 10^{-5} M increased the diameter in a dose-dependent manner. Basal renin release was 0.62 ± 0.15 ng angiotensin I/hr/afferent arterioles/hr ($n=13$) and was not affected by endothelin (10^{-10} to 10^{-6} M). Increase in renin release by isoproterenol was the same in afferent arterioles pretreated with vehicle or endothelin (10^{-7} M; Δ , 0.49 ± 0.21 versus 0.42 ± 0.19 ; $n=13$). In summary, endothelin constricts afferent arterioles but, at the same doses, does not inhibit renin release, and afferent arterioles, small resistant vessels, produces EDRF, which in turn participates in the control of basal tone and opposes vasoconstrictor action of endothelin. (*Hypertension* 1991;17:1052–1056)

The endothelium produces vasodilators, collectively called endothelium-derived relaxing factors (EDRFs),¹ as well as vasoconstrictors, one of which has recently been identified and named endothelin.² Although both endothelin and EDRF have been studied extensively in large vessels, little is known about their actions on the glomerular afferent arterioles or about possible interactions between them.

In addition to its potent vasoconstrictor action, endothelin has been demonstrated to decrease renin release from rat renal cortical slices,³ isolated glomeruli,⁴ and dispersed rat renal cortical cells.⁵ Because endothelin causes the increase in intracellular calcium that is proposed to mediate both vasocon-

striction and inhibition of renin release,⁶ it has been postulated that vasoconstrictor and renin-inhibitory actions of endothelin may share a common mechanism. However, these two actions have never been studied directly in comparable afferent arteriole preparations from the same strain (and species).

In the present study, we microdissected rabbit afferent arterioles and either microperfused them in vitro to study vascular response or incubated them to assess renin release. We studied whether 1) endothelin constricts the afferent arterioles; 2) inhibiting synthesis of nitric oxide (an EDRF) affects endothelin-induced constriction; 3) endothelin-induced constriction is reversed by acetylcholine, an EDRF-dependent vasodilator; and 4) endothelin inhibits basal or isoproterenol-stimulated renin release.

Methods

Minimal essential medium (MEM) was obtained from GIBCO, Grand Island, N.Y.; bovine serum albumin (BSA), *N*^ω-nitro L-arginine (*N*-Arg), acetylcholine, and isoproterenol were obtained from Sigma Chemical Co., St. Louis; and synthetic human endo-

From the Hypertension and Vascular Research Division and the Division of Biostatistics and Research Epidemiology, Henry Ford Hospital, Detroit, Mich.

Supported by grant HL-28982 from the National Institutes of Health. L.A.J. was the recipient of research fellowship grant D11031 from the American Heart Association, Michigan Affiliate.

Address for correspondence: Sadayoshi Ito, MD, PhD, Hypertension and Vascular Research Division, Henry Ford Hospital, 2799 W. Grand Boulevard, Detroit, MI 48202.

thelin (endothelin-1) was obtained from Peninsula Labs, Inc., Belmont, Calif.

Effect of Endothelin on Microperfused Afferent Arterioles

Methods of microdissection and perfusion of afferent arterioles have been described elsewhere.⁷ Briefly, a single superficial arteriole with its glomerulus intact was microdissected from the kidney of each New Zealand White rabbit (weight, 1.0–1.2 kg) that had been fed standard rabbit chow (Ralston Purina, St. Louis) and transferred to a temperature-regulated chamber mounted on an inverted microscope. The arteriole was microperfused using methods similar to those described by Osgood et al.⁸ Perfusion pressure was measured by the Landis technique, using a fine pipette (tip diameter, 2 μm) introduced into the arteriole through the opening of the perfusion pipette. The arteriole was perfused with MEM containing 5% BSA (MEM–5% BSA, oxygenated with 95% O_2 –5% CO_2 to pH 7.4), and perfusion pressure was maintained at 60 mm Hg throughout the experiment.

The bath was identical to the arteriolar perfusate and exchanged continuously. Microdissection and cannulation of the afferent arterioles were completed within 90 minutes at 8°C, after which the temperature of the bath was gradually increased to 37°C for the remainder of the experiment. Once it had stabilized, a 30-minute equilibration period was allowed before any measurements were taken. Images of afferent arterioles were displayed at magnifications up to $\times 1,980$ and recorded with a video system consisting of a camera adaptor with a $\times 3.3$ photoeyepiece, black-and-white, charge-coupled device camera (Dage-MTI, Michigan, Ind.), monitor (Javelin Electronics, Torrance, Calif.), and video recorder (Sony). The diameter at the most constricted point was measured with an image-analysis system (Fryer, Carpentersville, Ill.).

Response to endothelin. After a 30-minute equilibration period, the bath was continuously exchanged with medium containing endothelin at a concentration of 10^{-11} M, observing the afferent arterioles for 20 minutes. After washing arterioles with plain MEM–5% BSA for 30 minutes, 10^{-10} and 10^{-9} M endothelin were tested as described with a 30-minute washout interval between tests.

Endothelin was dissolved in saline at a concentration of 10^{-4} M and stored at -70°C ; this stock solution was diluted with MEM–5% BSA before use.

Inhibition of EDRF synthesis. After the equilibration period, *N*-Arg, a compound that inhibits synthesis of nitric oxide (an EDRF),⁹ was added to both the bath and the arteriolar perfusate at a concentration of 10^{-4} M. Fifteen minutes later, three concentrations of endothelin were tested in the presence of *N*-Arg. We confirmed that pretreatment with *N*-Arg blocked the action of acetylcholine (10^{-5} M) in afferent arterioles precontracted with norepinephrine (5×10^{-7} M).

Acetylcholine. Afferent arterioles were exposed to 5×10^{-10} M endothelin for 20 minutes, during which constriction stabilized. Then, the bath was exchanged by medium containing both 5×10^{-10} M endothelin and increasing doses of acetylcholine (10^{-8} , 10^{-7} , 10^{-6} , and 10^{-5} M), and the arterioles were observed for 20 minutes at each dose.

Effect of Endothelin on Renin Release

Methods of microdissection and incubation of arterioles were described previously.¹⁰ In each rabbit, approximately 20 afferent arterioles with glomerulus and macula densa removed were microdissected from the outer cortex and divided into control and experimental groups. Arterioles in each group were transferred to a ladle with a nylon mesh bottom and preincubated in a small plastic microtube containing 1 ml oxygenated MEM with 0.1% BSA (MEM–0.1% BSA). After preincubation, arterioles were incubated in 100 μl of MEM–0.1% BSA for two consecutive 20-minute periods by transferring the ladle from one tube to the other. Renin released into the incubation medium was measured by radioimmunoassay of angiotensin I (Ang I), and the hourly renin release rate from a single arteriole was calculated and expressed in nanograms of Ang I per hour per arteriole per hour.

Effect on basal renin release. Afferent arterioles were incubated in MEM–0.1% BSA for the first period and then transferred to a microtube containing endothelin for the second period. Three concentrations— 10^{-10} , 10^{-9} , and 10^{-8} M—were studied in six rabbits; 10^{-7} and 10^{-6} M concentrations were studied in seven other rabbits.

Effect on isoproterenol-stimulated renin release. Endothelin at a concentration of 10^{-7} M or its vehicle (saline) was added to the medium from preincubation to the end of incubations; isoproterenol at a concentration of 10^{-5} M was added during the second incubation period. The isoproterenol-induced increases in renin release in vehicle- and endothelin-treated arterioles were compared.

Statistics

Values are expressed as mean \pm SEM. All statistical analyses were performed using absolute values. Repeated-measures analysis of variance was used to examine whether control values changed over time. Paired *t* tests were used to examine whether changes in diameter induced by endothelin or *N*-Arg were significant, whether changes in renin release caused by endothelin were significant when compared with time controls, and whether changes in renin release caused by isoproterenol differed between nontreated and endothelin-treated groups. An independent two-sample *t* test was used to examine whether endothelin-induced changes in diameter differed between nontreated and *N*-Arg-treated groups. A pooled regression analysis followed by a one-sample *t* test on the slope of the dose–response curve were used to

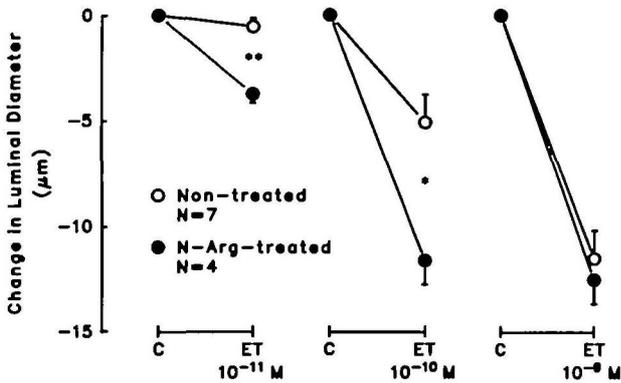


FIGURE 1. Plots of change in luminal diameter induced by endothelin (ET) in microperfused afferent arterioles treated with either vehicle or N^ω-nitro L-arginine (N-Arg), which inhibits synthesis of nitric oxide (an endothelium-derived relaxing factor). **p*<0.01 and ***p*<0.001 compared with nontreated group. ET at a concentration of 10⁻⁹ M caused a maximal vasoconstriction in both groups.

examine whether changes in diameter induced by acetylcholine were significant.

Results

Response to Endothelin

Basal luminal diameters were 15.8±1.1, 15.4±1.0, and 15.1±0.9 µm before application of 10⁻¹¹, 10⁻¹⁰, or

10⁻⁹ M endothelin, respectively, indicating stable basal diameter. Endothelin decreased the diameter in a dose-dependent manner (Figure 1). The decreases (Δ) at concentrations of 10⁻¹¹, 10⁻¹⁰, and 10⁻⁹ M were 0.6±0.4 (*n*=7, *p*=NS), 5.1±1.3 (*p*<0.01), and 11.5±1.3 µm (*p*<0.0001), respectively, representing 3±1.8%, 32±8%, and 76±7% decreases from the control diameter. With 10⁻¹⁰ M endothelin, full response was attained between 10 and 15 minutes after application compared with ~1 minute with 10⁻⁹ M endothelin.

Inhibition of EDRF Synthesis

Figure 2 shows an example of endothelin-induced vasoconstriction in nontreated (left) and N-Arg-treated afferent arterioles as well as the change in basal diameter induced by N-Arg (right). With N-Arg pretreatment, basal diameter decreased by 15±1% from 17.4±0.1 to 14.7±0.3 µm (*n*=4, *p*<0.001). Furthermore, N-Arg augmented the vasoconstrictor action of endothelin (Figure 1); after N-Arg pretreatment, 10⁻¹¹ and 10⁻¹⁰ M endothelin decreased diameters by 3.7±0.4 (26±3%) and 11.6±1.2 µm (78±10%), respectively, which was significantly larger than nontreated arteriole diameters. With 10⁻⁹ M endothelin, vasoconstriction reached a maximum in both N-Arg-treated and nontreated groups.

Effect of Acetylcholine

Endothelin at a concentration of 5×10⁻¹⁰ M reduced the diameter from 17.3±1.3 to 9.7±1.3 µm

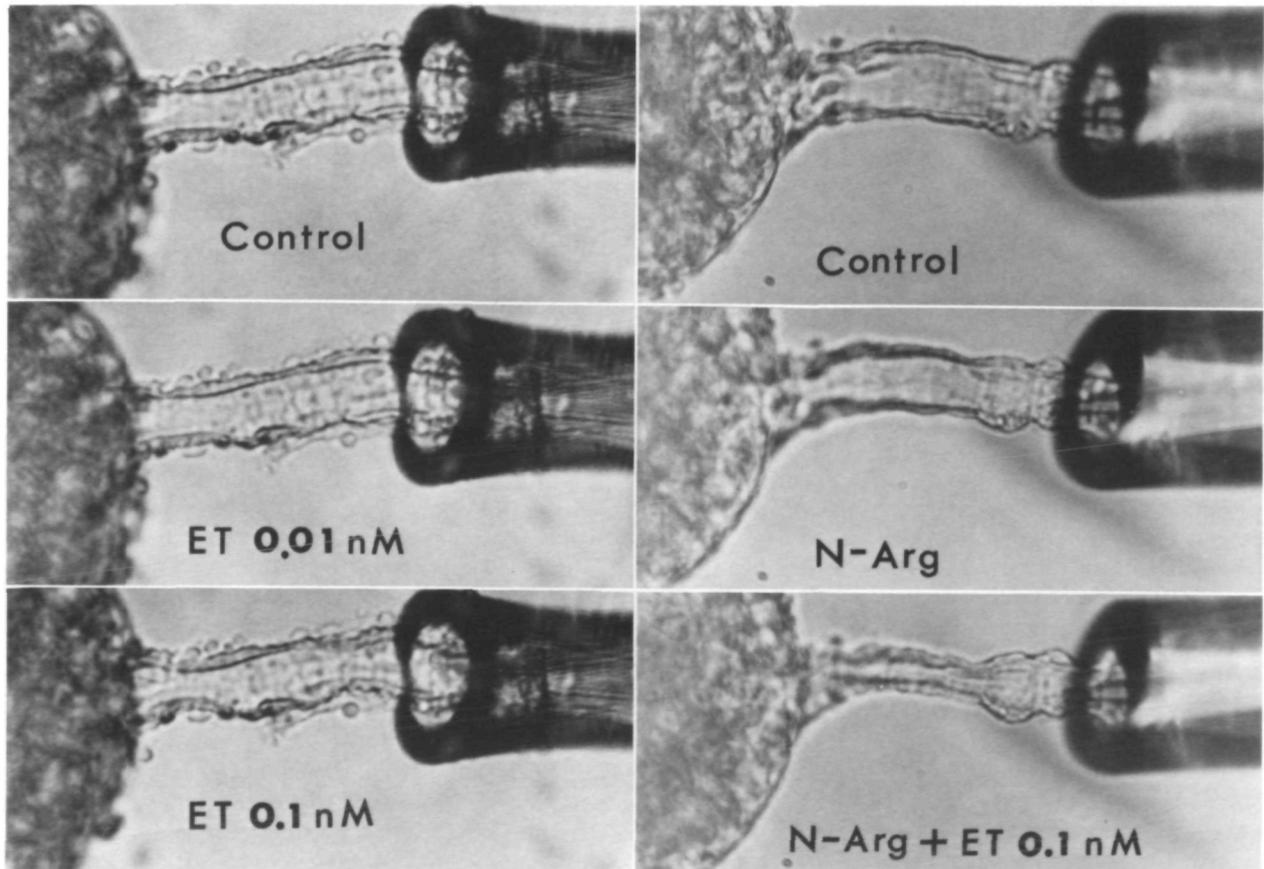


FIGURE 2. Photomicrographs of examples of endothelin (ET)-induced vasoconstriction in nontreated (left) and N^ω-nitro L-arginine (N-Arg)-treated afferent arterioles and change in basal diameter by N-Arg (right). Magnification, ×600.

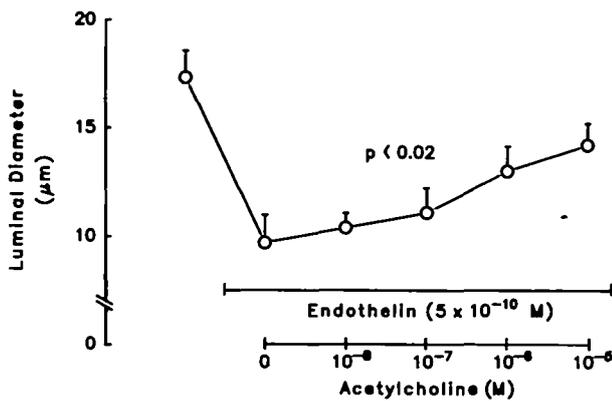


FIGURE 3. Plot of effect of increasing doses of acetylcholine on endothelin-induced vasoconstriction in microperfused afferent arterioles. Slope obtained with regression analysis was significantly different from zero ($p < 0.02$).

($58 \pm 10\%$ of control values, $n=6$, $p < 0.02$). Acetylcholine increased the diameter significantly in a dose-dependent manner ($p < 0.02$), with the diameter reaching $14.2 \pm 1.0 \mu\text{m}$ ($86 \pm 12\%$ of control values) at 10^{-5} M (Figure 3).

Effect on Basal Renin Release

Table 1 shows the effect of endothelin on the basal renin release rate. Despite strong vasoconstrictor action in microperfused afferent arterioles, endothelin at concentrations of 10^{-10} to 10^{-6} M had no effect on basal renin release.

Effect on Isoproterenol-Stimulated Renin Release

Isoproterenol increased renin release from 0.61 ± 0.15 to 1.09 ± 0.28 ng Ang I/hr/afferent arterioles/hr ($n=13$, $p < 0.05$) in vehicle-treated arterioles and from 1.09 ± 0.37 to 1.52 ± 0.47 ng Ang I/hr/afferent arterioles/hr in endothelin-treated arterioles ($p < 0.05$); the increases (Δ) induced by isoproterenol did not differ (0.49 ± 0.21 and 0.42 ± 0.19 ng Ang I/hr/afferent arterioles/hr, respectively; $p > 0.8$).

TABLE 1. Effect of Endothelin on Basal Renin Release

Group	Rabbits studied (n)	Renin release rate (ng angiotensin I/hr/afferent arterioles/hr)	
		Control period	Experimental period
Time control	6	0.81 ± 0.29	1.19 ± 0.38
Endothelin (M)			
10^{-10}	6	1.04 ± 0.31	1.34 ± 0.32
10^{-9}	6	0.67 ± 0.22	0.78 ± 0.22
10^{-8}	6	1.10 ± 0.33	1.55 ± 0.43
Time control	7	0.45 ± 0.13	0.69 ± 0.18
Endothelin (M)			
10^{-7}	7	0.84 ± 0.57	0.99 ± 0.27
10^{-6}	7	0.75 ± 0.38	0.95 ± 0.35

Values are given as mean \pm SEM.

Discussion

The present study demonstrates that endothelin causes strong constriction of afferent arterioles. However, endothelin had no effect on renin release at concentrations as great as a thousandfold that needed for maximal vasoconstriction. Furthermore, our observations suggest that afferent arterioles produce EDRF, which in turn influences both the basal tone of the arterioles and the vasoconstrictor action of endothelin.

The effect of endothelin on the renal microvasculature was recently studied directly by Loutzenhiser et al¹¹ in the isolated, perfused hydronephrotic rat kidney. They showed that endothelin caused strong constriction of the arterioles, whereas it had less effect on the efferent arteriole. In the afferent arterioles, the threshold concentration was 0.01 nM and ED_{50} was in the 10^{-10} M range, showing a concentration-response relation similar to that of the present study. Consistent with previous observations both in vivo and in vitro,² the action of endothelin was substantially different from that of angiotensin II (Ang II) or norepinephrine in our preparation. Endothelin was more potent than Ang II (ED_{50} , 10^{-9} to 10^{-8} M) or norepinephrine (ED_{50} , 10^{-7} M), whereas endothelin-induced constriction was slower in onset (especially at a concentration of 10^{-10} M) and difficult to wash out (15–20 minutes for 10^{-10} M).

Endothelin has been demonstrated to decrease renin release in vitro.^{3–5} It has been postulated that the renin-inhibitory and vasoconstrictor actions of endothelin may share a common mechanism, which is mediated by an increase in intracellular calcium. However, to our knowledge, effects of endothelin on afferent arteriolar resistance and renin release have never been studied directly in comparable preparations. In the present study, although endothelin caused very strong vasoconstriction, it did not alter basal or isoproterenol-stimulated renin release, even at concentrations as great as thousandfold that required to cause maximal vasoconstriction. These observations may suggest that the mechanism of endothelin-induced afferent arteriole constriction is not directly linked to the inhibition of renin release.

It is not clear why endothelin inhibited renin release in other studies but not in the present study. It could be argued that the lack of endothelin effect on renin release results from the inability of our preparation to respond to inhibitors of renin release. However, this is unlikely because we have shown that both adenosine (or its analogue) and the presence of the attached macula densa inhibit renin release.¹² It may be that structures other than afferent arterioles (e.g., the macula densa and glomeruli) are necessary for endothelin to inhibit renin release. It is also possible that endothelin enhanced the production of prostaglandin I_2 ,¹³ thereby overcoming the renin-inhibitory action of endothelin.

In vivo experiments have shown that intravenous administration of N^G -monomethyl L-arginine

(LNMMA), another compound that inhibits nitric oxide synthesis, increased systemic as well as renal vascular resistance.¹⁴ In addition, low doses of endothelin caused vasodilation that was blocked by LNMMA,¹⁵ suggesting that endothelin may induce EDRF synthesis. However, these studies did not exclude the possibility that the changes observed were merely secondary to, for example, a possible increase in renin release.¹⁶ Because our preparation is free of systemic hemodynamic or hormonal influences, results from the present study strongly suggest that EDRF, which is produced locally in afferent arterioles, may be important in the regulation of both basal tone and vascular response to endothelin.

Kon et al¹⁷ recently reported that when acetylcholine, an EDRF-dependent vasodilator, was infused into the proximal portion of the main renal artery, renal blood flow and glomerular filtration rate increased, whereas infusion into the distal portion had either the opposite or no effect. This may indicate that the renal microvasculature downstream from the main renal artery produces little EDRF in response to acetylcholine. In contrast, results from both the present study and that by Edwards¹⁸ have demonstrated that acetylcholine dilates isolated afferent arterioles precontracted by either endothelin or norepinephrine, strongly suggesting that renal microvasculature can produce EDRF.

In conclusion, the present study shows that although endothelin is a potent vasoconstrictor, it does not inhibit renin release in rabbit afferent arterioles, suggesting that different mechanisms are involved. It also provides evidence that afferent arterioles, small resistance vessels (~20 μm in diameter) may produce EDRF, which in turn plays an important role in the control of afferent arteriole resistance.

Acknowledgment

We gratefully acknowledge the excellent technical assistance of Chisa Yamaguchi, BS.

References

1. Vanhoutte PM, Rubanyi GM, Miller VM, Houston DS: Modulation of vascular smooth muscle contraction by the endothelium. *Annu Rev Physiol* 1986;48:307-320
2. Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, Yazaki Y, Goto K, Masaki T: A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* 1988;332:411-415
3. Matsumura Y, Nakase K, Ikegawa R, Hayashi K, Ohyama T, Morimoto S: The endothelium-derived vasoconstrictor peptide endothelin inhibits renin release in vitro. *Life Sci* 1989;44:149-157
4. Rakugi H, Nakamaru M, Saito H, Higaki J, Ogihara T: Endothelin inhibits renin release from isolated rat glomeruli. *Biochem Biophys Res Commun* 1988;155:1244-1247
5. Takagi M, Matsuoka H, Atarashi K, Yagi S: Endothelin. A new inhibitor of renin release. *Biochem Biophys Res Commun* 1988;157:1164-1168
6. Churchill PC: Second messengers in renin secretion. *Am J Physiol* 1985;249:F175-F184
7. Ito S, Carretero OA: An in vitro approach to the study of macula densa-mediated glomerular hemodynamics. *Kidney Int* 1985;28:1206-1210
8. Osgood RW, Patton M, Hanley MJ, Venkatachalam M, Reineck HJ, Stein JH: In vitro perfusion of the isolated dog glomerulus. *Am J Physiol* 1983;244:F349-F354
9. Moore PK, al-Swayeh OA, Chong NWS, Evans RA, Gibson A: L-N^G-Nitro-arginine (L-NOARG), a novel, L-arginine-reversible inhibitor of endothelium-dependent vasodilation in vitro. *Br J Pharmacol* 1990;99:408-412
10. Itoh S, Carretero OA, Murray RD: Renin release from isolated afferent arterioles. *Kidney Int* 1985;27:762-767
11. Loutzenhiser R, Epstein M, Hayashi K, Horton C: Direct visualization of effects of endothelin on the renal microvasculature. *Am J Physiol* 1990;258:F61-F68
12. Itoh S, Carretero OA, Murray RD: Possible role of adenosine in the macula densa mechanism of renin release in rabbits. *J Clin Invest* 1985;76:1412-1417
13. Rakugi H, Nakamaru M, Tabuchi Y, Nagano M, Mikami H, Ogihara T: Endothelin stimulates the release of prostacyclin from rat mesenteric arteries. *Biochem Biophys Res Commun* 1989;160:924-928
14. Tolins JP, Palmer RM, Moncada S, Raji L: Role of endothelium-derived relaxing factor in regulation of renal hemodynamic responses. *Am J Physiol* 1990;258:H655-H662
15. Whittle BJR, Lopez-Belmonte J, Rees DD: Modulation of the vasodepressor actions of acetylcholine, bradykinin, substance P and endothelin in the rat by a specific inhibitor of nitric oxide formation. *Br J Pharmacol* 1989;98:646-652
16. Vidal MJ, Romero JC, Vanhoutte PM: Endothelium-derived relaxing factor inhibits renin release. *Eur J Pharmacol* 1988;149:401-402
17. Kon V, Harris RC, Ichikawa I: A regulatory role for large vessels in organ circulation. *J Clin Invest* 1990;85:1728-1733
18. Edwards RM: Response of isolated renal arterioles to acetylcholine, dopamine and bradykinin. *Am J Physiol* 1985;248:F183-F189

KEY WORDS • vascular reactivity • renin • N^G-nitro L-arginine • acetylcholine • endothelin • arterioles • endothelium