JCI The Journal of Clinical Investigation

Endothelium-dependent hyperpolarization caused by bradykinin in human coronary arteries.

M Nakashima, … , A A Taylor, P M Vanhoutte

J Clin Invest. 1993;92(6):2867-2871. https://doi.org/10.1172/JCI116907.

Research Article

The present study was designed to determine whether bradykinin induces endothelium-dependent hyperpolarization of vascular smooth muscle in human coronary arteries, and if so, to define the contribution of this hyperpolarization to endothelium-dependent relaxations. The membrane potential of arterial smooth muscle cells (measured by glass microelectrodes) and changes in isometric force were recorded in tissues from six patients undergoing heart transplantation. In the presence of indomethacin and NG-nitro-L-arginine (NLA), the membrane potential was -48.3 +/- 0.6 and -46.9 +/- 0.6 mV, in preparations with and without endothelium, respectively, and was not affected by treatment with perindoprilat, an angiotensin-converting enzyme inhibitor. In the presence of both indomethacin and NLA, bradykinin evoked transient and concentration-dependent hyperpolarizations only in tissues with endothelium, which were augmented by perindoprilat and mimicked by the calcium ionophore A23187. Glibenclamide did not inhibit membrane hyperpolarization to bradykinin. In rings contracted with prostaglandin F2 alpha, the cumulative addition of bradykinin caused a concentration-dependent relaxation during contractions evoked by prostaglandin F2 alpha, which was not abolished by NLA and indomethacin. The present findings demonstrate the occurrence of endothelium-dependent hyperpolarization, and its contribution to endothelium-dependent relaxations, in the human coronary artery.

Endothelium-dependent Hyperpolarization Caused by Bradykinin in Human Coronary Arteries

Mikio Nakashima, Jean-Vivien Mombouli, Addison A. Taylor, and Paul M. Vanhoutte
Center for Experimental Therapeutics, Baylor College of Medicine, Houston, Texas 77030

Abstract

The present study was designed to determine whether bradykinin induces endothelium-dependent hyperpolarization of vascular smooth muscle in human coronary arteries, and if so, to define the contribution of this hyperpolarization to endothelium-dependent relaxations. The membrane potential of arterial smooth muscle cells (measured by glass microelectrodes) and changes in isometric force were recorded in tissues from six patients undergoing heart transplantation. In the presence of indomethacin and N^G-nitro-L-arginine (NLA), the membrane potential was −48.3±0.6 and −46.9±0.6 mV, in preparations with and without endothelium, respectively, and was not affected by treatment with perindoprilat, an angiotensin-converting enzyme inhibitor. In the presence of both indomethacin and NLA, bradykinin evoked transient and concentration-dependent hyperpolarizations only in tissues with endothelium, which were augmented by perindoprilat and mimicked by the calcium ionophore A23187. Glibenclamide did not inhibit membrane hyperpolarization to bradykinin. In rings contracted with prostaglandin $F_{2\alpha}$, the cumulative addition of bradykinin caused a concentration-dependent relaxation during contractions evoked by prostaglandin $F_{2\alpha}$, which was not abolished by NLA and indomethacin. The present findings demonstrate the occurrence of endothelium-dependent hyperpolarization, and its contribution to endothelium-dependent relaxations, in the human coronary artery. (*J. Clin. Invest.* 1993. 92:2867–2871.)
Key words: relaxation • endothelium-derived hyperpolarizing factor • endothelium-derived relaxing factor • potassium channels • angiotensin-converting enzyme inhibitor

Introduction

Furchgott and Zawadzki (1) demonstrated the obligatory role of the endothelium in the relaxation of isolated arteries to acetylcholine, an observation that has been extended to numerous neurohumoral mediators in a variety of blood vessels from different animal species (see references 2 and 3). Endothelium-dependent relaxations have been demonstrated in human coronary arteries (4, 5). Endothelium-derived relaxing factor (EDRF,[1] identified as nitric oxide [6–8]) plays a major role in endothelium-dependent relaxations. In addition, in animal blood vessels, endothelium-derived hyperpolarizing factor (EDHF) (9–12), an unidentified diffusible substance (11, 13, 14) distinct from nitric oxide (10, 15, 16), contributes to endothelium-dependent relaxations by opening K^+ channels in the underlying vascular smooth muscle (17–21). The endothelium-dependent relaxations of isolated human coronary arteries are, in part, resistant to inhibitors of cyclooxygenase and nitric oxide synthase, which implies that an endothelial mediator other than prostacyclin and nitric oxide is involved (22). The present study was designed to determine whether or not endothelium-dependent hyperpolarization occurs in the human coronary artery, and if so, whether or not it contributes to endothelium-dependent relaxations.

Methods

Solutions and drugs. The tissues were incubated in modified Krebs-Ringer bicarbonate solution with the following composition (mM): 118.3, NaCl 4.7, KCl 2.5, $CaCl_2$ 1.2, $MgSO_4$ 1.2, KH_2PO_4 25.0, $NaHCO_3$ 0.026 edetate calcium disodium (CaEDTA), and 11.1 glucose (control solution). High potassium solution (K^+ 60 mM) was prepared by isotonic substitution of NaCl by KCl. Solutions were aerated with a 95% O_2 and 5% CO_2 gas mixture. The following drugs were used: bradykinin, the calcium ionophore A23187, glibenclamide, indomethacin, prostaglandin $F_{2\alpha}$, (Sigma Immunochemicals, St. Louis, MO), lemakalim (Beecham, Brentford, United Kingdom), N^G-nitro L-arginine (Aldrich Chemical Co., Milwaukee, WI), and perindoprilat (Servier, Neuilly, France). Stock solutions were prepared in distilled water except for glibenclamide (prepared in ethanol), A23187 and lemakalim (dissolved in dimethylsulfoxide), and indomethacin (dissolved in water and an equal molar concentration of Na_2CO_3 with sonication). Preliminary experiments indicated that none of the solvents used produced detectable changes in membrane potential or isometric force at the concentrations used (data not shown).

Coronary arteries. Epicardial coronary arteries were obtained from the excised hearts of six male cardiac transplant patients (15 mo–68 yr old). The hearts were classified as surgical specimens, and their use was exempted by the institutional review board from required patient consent. Drug treatment during the 2 wk before the transplant operations included furosemide (six patients), digoxin (four patients), captopril (three patients), and dobutamine/dopamine (three patients). All patients were treated with immunosuppressive drugs (methylprednisolone, cyclosporine, and azathioprine) several hours before the operation. The coronary arteries were isolated 3–5 min after the removal of the heart from the chest and placed in cold (4°C) control solution. Approximately 15 min later, the surrounding tissues were removed under a binocular microscope, and only arteries with a luminal opening devoid of visible atherosclerotic lesions were used. The blood vessels for electrophysiological studies (external diameter = 1.0–1.5 mm) and for organ chamber experiments (external diameter = ~ 2 mm) were cut into rings (3–4 mm in length). In some rings, the endothelium was removed mechanically by inserting the tips of a watchmaker's forceps or an insect pin into the lumen and gently rolling the preparation back and forth over tissue paper saturated with cold control solution (19). The functional removal of endothelium was assessed by the disappearance of endothelium-dependent relaxations induced by the calcium ionophore, A23187 (see reference 4).

Address correspondence to Paul M. Vanhoutte, M.D., Ph.D., I.R.I.S., 6 place des Pleiades, 92415 Courbevioe, France.
Received for publication 12 February 1993 and in revised form 25 June 1993.

1. *Abbreviations used in this paper:* EDHF, endothelium-derived hyperpolarizing factor; EDRF, endothelium-derived relaxing factor.

J. Clin. Invest.

0021-9738/93/12/2867/05 $2.00
Volume 92, December 1993, 2867–2871

Electrophysiological studies. Rings of coronary artery (3–4 mm long) were opened longitudinally and pinned down on the bottom of an organ chamber (volume = 1.5 ml) with the endothelial side upward. The tissues were superfused with control solution (37°C) at constant flow (3 ml/min). After 90 min of incubation, glass capillary microelectrodes filled with 3 M KCl (tip resistance = 50–80 MΩ) were inserted into the arterial smooth muscle from the intimal side. All experiments were performed in the presence of indomethacin (10^{-5} M) and N^G-nitro-L-arginine (10^{-4} M) to prevent the formation of vasoactive prostanoids and nitric oxide, respectively (23, 24). The electrical signal was amplified by means of a recording amplifier (World Precision Instruments, New Haven, CT). The membrane potential was monitored continuously on an oscilloscope (model 5223; Tektronix Inc., Beaverton, OR) and recorded on a pen recorder (model TA550; Gould Inc., Recording Systems Division, Cleveland, OH). The following criteria were used to assess the validity of a successful impalement: a sudden negative shift in voltage followed by (*a*) a stable negative voltage for more than 2 min and (*b*) an instantaneous return to the previous voltage level on dislodgement of the microelectrode. At least four impalements of the same artery were made to assess the variability of the electrophysiological parameters, in the presence and in the absence of endothelium, respectively.

Organ chamber experiments. The rings were suspended between two stirrups in organ chambers (20 ml) filled with control solution (gassed with 95% O_2–5% CO_2, pH 7.4, maintained at 37°C). The experiments were performed in the presence of indomethacin (10^{-5} M). One of the stirrups was anchored inside the organ chamber, and the other was connected to a force transducer (model FTC103; Grass Instrument Co., Cleveland, OH) to record changes in isometric tension. The rings were stretched to the optimal point of their length–active tension relationship (range = 4–6 g), as determined by the contractile response to 60 mM K^+ at progressive levels of stretch.

Statistical analysis. The results are expressed as mean±SEM.; *n* represents the number of cells examined in electrophysiological study. A two-tailed Student's *t* test (for paired observations) was used to evaluate the statistical significance of differences between means. $P < 0.05$ was considered to be statistically significant.

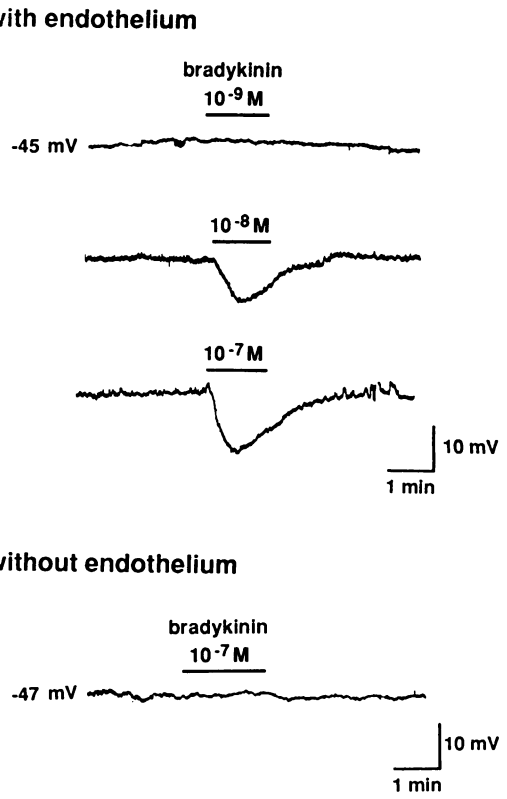

Figure 1. Cell membrane potential of vascular smooth muscle cells in human coronary artery incubated with indomethacin (10^{-5} M) and N^G-nitro-L-arginine (10^{-4} M). The tissues were obtained from the heart of a 15-mo-old (congenital heart disease). Changes in resting membrane potential were recorded in the absence (*bottom*) and presence (*top*) of endothelium.

Results

Electrophysiological studies. In the six coronary arteries studied for electrophysiological experiments, the membrane of the smooth muscle cells was electrically quiescent and did not exhibit rhythmic fluctuations in membrane potential. The resting membrane potentials averaged −48.7±0.6 mV (n = 28) and −46.9±0.6 mV (n = 21) in preparations with and without endothelium, respectively (Table I). Perindoprilat (10^{-6} M; inhibitor of converting enzyme) did not alter the resting membrane potential significantly (Table I).

Bradykinin caused transient hyperpolarization of cell membranes in tissues with, but not in those without endothelium (in coronary arteries from five out of six patients) (Fig. 1). The response of human coronary arteries to bradykinin declined with repeated application. Reproducibility of membrane hyperpolarization induced by a given concentration of bradykinin (e.g., 10^{-8} M for 1 min) could be achieved by allowing a 30-min interval between exposure of the tissues to the peptide (data not shown). Incubation with perindoprilat (10^{-6} M) potentiated the hyperpolarization to bradykinin (at 10^{-8}

Table I. Resting Membrane Potential of Human Coronary Arteries

Case	Age	Clinical condition	Site of tissue	With endothelium		Without endothelium
				Control	Perindoprilat*	Control
A	64	Ischemic heart disease	RCA	−48.8±1.1 (8)	—	−48.3±0.3 (4)
B	10	Dilated cardiomyopathy	LCX	−50.9±0.9 (6)	−49.5±0.3 (8)	−49.6±1.0 (5)
C	68	Ischemic cardiomyopathy	LCX	−44.2±0.8 (5)	−43.6±0.8 (8)	−44.0±0.7 (5)
D	49	Alcoholic cardiomyopathy	RCA, LAD, LCX	−48.0±0.8 (5)	−47.7±0.7 (17)	−46.8±0.9 (4)
E	1	Congenital heart disease	LAD	−48.4±0.7 (5)	−49.6±1.2 (7)	−47.5±0.6 (4)
F	62	Idiopathic cardiomyopathy	LCX	−49.0±1.0 (4)	−50.8±2.8 (6)	—

Values are means±SEM. Numbers of cells in parentheses. RCA, right coronary artery; LCX, left circumflex; LAD, left anterior discending coronary artery. * Incubated with perindoprilat (10^{-6} M) >30 min before the measurements.

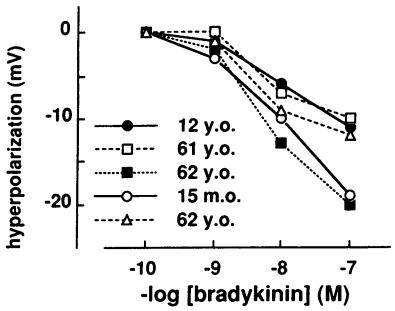

Figure 2. Concentration-hyperpolarization curves to bradykinin in human coronary arteries. The preparations were incubated with perindoprilat (10^{-6} M), indomethacin (10^{-5} M), and N^G-nitro-L-arginine (10^{-4} M). *y.o.*, years old; *m.o.*, months old.

M, −2.5±1.8 and −9.0±2.1 mV, in the absence and presence of perindoprilat, respectively; $n = 4$, four individuals, $P < 0.05$). In the presence of perindoprilat, the concentration-hyperpolarization curves to bradykinin (10^{-10}–10^{-7} M) were comparable in the coronary arteries of five out of six patients (Fig. 2).

In tissues from three patients (cases D, E, and F), membrane hyperpolarizations were observed in response to the activator of ATP-sensitive potassium channels lemakalim (10^{-6} M; Fig. 3 *A*), both in tissues with and without endothelium. Glibenclamide (3×10^{-6} M, an inhibitor of ATP-sensitive K^+ channels) abolished the membrane hyperpolarizations in response to lemakalim (Fig. 3 *A*). The membrane hyperpolarization induced by bradykinin was not inhibited by glibenclamide (Fig. 3 *B*). The calcium ionophore A23187 (10^{-7}–10^{-6} M) caused concentration-dependent membrane hyperpolarizations in tissues with endothelium, which were not attenuated by glibenclamide (Fig. 3 *C*).

Organ chamber experiments. In rings of coronary arteries of three of the patients (cases *B, F,* and *G*), the cumulative addition of bradykinin caused a concentration-dependent relaxation during contractions evoked by prostaglandin $F_{2\alpha}$ (8×10^{-6} M; Fig. 4). In rings incubated with perindoprilat, N^G-nitro-L-arginine did not abolish the relaxation to bradykinin (Fig. 4). Relaxations were not observed in rings without endothelium.

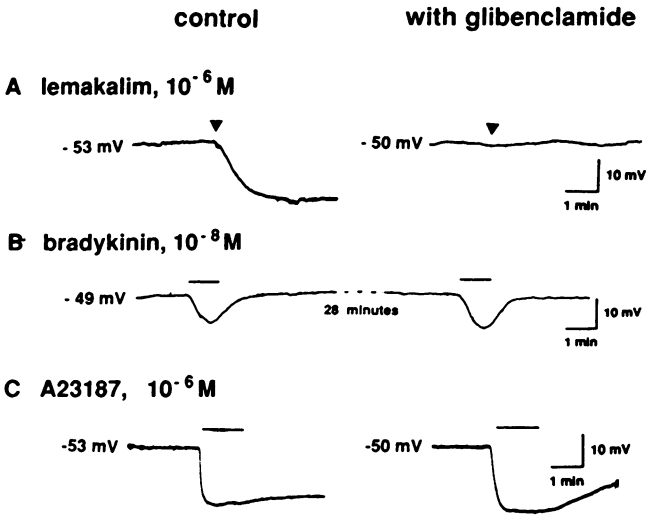

Figure 3. Effects of glibenclamide on membrane potential changes induced by lemakalim (*A*), bradykinin (*B*), and A23187 (*C*) in preparations from human coronary arteries (*A* and *C*, 49 yr old, alcoholic cardiomyopathy; *B*, 62 yr old, idiopathic cardiomyopathy). The tissues were incubated with perindoprilat (10^{-6} M), indomethacin (10^{-5} M), and N^G-nitro-L-arginine (10^{-4} M). The traces in *B* were recorded from the same cell. Traces in *A* and *C* were obtained in different cells.

Discussion

The aim of the present study was to investigate the membrane potential characteristics of vascular smooth muscle cells in the human coronary artery, to determine whether these parameters are influenced by the endothelium, and to assess whether or not electrophysiological changes in the vascular smooth muscle contribute at least in part to endothelium-dependent relaxations.

The present findings demonstrate that bradykinin causes endothelium-dependent hyperpolarization of vascular smooth muscle cells in the human coronary artery. A similar response has been documented repeatedly in animal blood vessels with bradykinin and other endothelial stimulants (10, 25, 26), and has been attributed to the release of a diffusible substance, EDHF (9, 11, 14). In the uterine artery of the guinea pig (27) and in small mesenteric arteries of the rat (20), nitric oxide can cause membrane hyperpolarization. By contrast, nitric oxide evokes relaxations, but does not hyperpolarize smooth muscle cells in the arteries of several species (8, 9, 15, 16, 18). Prostacyclin may also produce hyperpolarization of smooth muscle cell membranes in certain blood vessels (28). In the present experiments with human coronary arteries, the release of either EDRF (nitric oxide) or prostaglandins induced by bradykinin (5, 22, 29) cannot account for the observed hyperpolarization, which was not affected by combined inhibition of cyclooxygenase and nitric oxide synthase (23, 24). A similar conclusion has been reached in coronary arteries of various species (19, 30, 31). The effect of bradykinin is concentration dependent, and is mimicked by the calcium ionophore A23187 in tissues with endothelium, in agreement with earlier observations in animal arteries (19, 32). This indicates that, as the activation of nitric oxide synthase and cyclooxygenase, the process leading to the release of EDHF depends on intracellular calcium mobilization (33). Likewise, the production of EDHF is calcium-dependent and inhibited by calmodulin antagonists in canine and porcine coronary arteries (34, 35). As in animal coronary arteries, the endothelium-dependent hyperpolarization evoked by bradykinin is potentiated by the converting enzyme inhibitor, perindoprilat (36).

In human coronary arteries, unlike the rabbit cerebral artery (18, 37), endothelium-dependent hyperpolarizations were not inhibited by glibenclamide at a concentration that abolished the hyperpolarization to lemakalim. These observations suggest that the hyperpolarization to lemakalim. These observations suggest that the hyperpolarizations of human coronary smooth muscle induced by bradykinin and A23187 do not result from the opening of ATP-sensitive K^+ channels. A similar conclusion has been reached in coronary arteries of other species (19, 31, 35). In addition, these findings confirm that the hyperpolarization is not mediated by EDRF, since the hyperpolarization caused by exogenous nitric oxide in rat mesenteric arteries is prevented by glibenclamide (20). The types of potassium channels involved in endothelium-dependent hyperpolarizations differ among blood vessels and/or species (18, 37).

The persistence in the present experiments of endothelium-

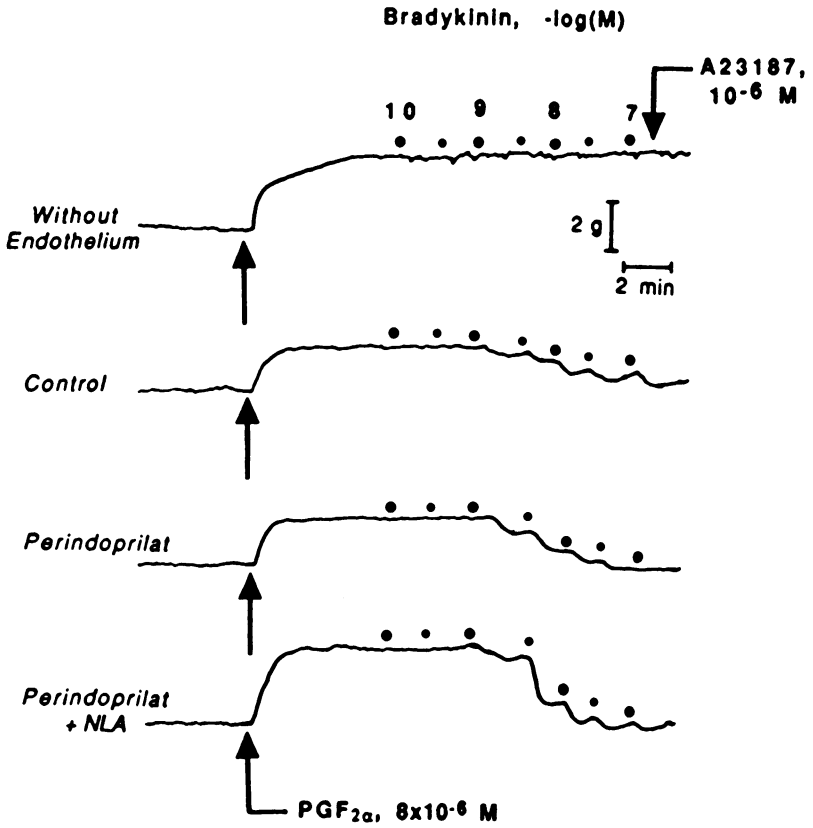

Figure 4. Relaxations to bradykinin in human coronary artery (patient with congenital dilated cardiomyopathy). The recordings of isometric force were obtained from experiments performed in parallel rings of the same coronary artery. Indomethacin (10^{-5} M) was present throughout. Perindoprilat (10^{-6} M) and N^G-nitro-L-arginine (10^{-4} M) were present 40 min before addition of prostaglandin $F_{2\alpha}$ (as indicated by arrows) and bradykinin (as indicated by dots, 10^{-10}–10^{-7} M, shown as -log). The addition of A23187 confirmed the successful removal of the endothelium.

dependent relaxations to bradykinin in the presence of N^G-nitro-L-arginine is consistent with the observation in epicardial human coronary arteries that the relaxation to substance P is not abolished by inhibitors of nitric oxide synthase (22). Taken into conjunction with the electrophysiological studies, it suggests that hyperpolarization may, at least in part, contribute to endothelium-dependent relaxations of the human coronary artery.

In conclusion, the present study demonstrates that endothelium-dependent hyperpolarization occurs in human coronary arteries in response to bradykinin and the calcium ionophore A23187. This effect does not result from opening of ATP-sensitive potassium channels. Since this endothelium-dependent hyperpolarization was present in coronary arteries from patients with different cardiac diseases, it may be a mechanism of importance for the regulation of the degree of contraction of vascular smooth muscle in healthy coronary arteries.

Acknowledgments

This study was supported in part by grant HL-90338 from the National Institutes of Health, and an unrestricted research award from the Bristol-Myers Squibb Research Institute.

References

1. Furchgott, R. F., and J. V. Zawadzki. 1980. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature (Lond.)*. 288:373–376.

2. Furchgott, R. F., and P. M. Vanhoutte. 1989. Endothelium-derived relaxing and contracting factors. *FASEB (Fed. Am. Soc. Exp. Biol.) J.* 3:2007–2018.

3. Lüscher, T. F., and P. M. Vanhoutte. 1990. The Endothelium: Modulator of Cardiovascular function. CRC Press, Inc., Boca Raton, FL. pp. 1–215.

4. Förstermann, U., A. Mugge, and J. C. Frolich. 1986. Endothelium-dependent relaxation of human epicardial coronary arteries: frequent lack of effect of acetylcholine. *Eur. J. Pharmacol.* 128:277–281.

5. Bossaller, C., G. B. Habib, H. Yamamoto, C. Williams, S. Wells, and P. D. Henry. 1987. Impaired muscarinic endothelium-dependent relaxation and cyclic guanosine 5'-monophosphate formation in atherosclerotic human coronary artery and rabbit aorta. *J. Clin. Invest.* 79:170–174.

6. Furchgott, R. F. 1988. Studies on relaxation of rabbit aorta by sodium nitrate: the basis for the proposal that the acid-activatable inhibitory factor from bovine retractor penis is inorganic nitrate and the endothelium-derived relaxing factor is nitric oxide. *In* Mechanism of Vasodilation. Raven Press Ltd., New York. pp. 401–404.

7. Ignarro, L. J., R. E. Byrns, G. M. Buga, and K. S. Wood. 1987. Endothelium-derived relaxing factor from pulmonary artery and vein possesses pharmacologic and chemical properties identical to those of nitric oxide radical. *Circ. Res.* 61:866–879.

8. Moncada, S., R. M. J. Palmer, and E. A. Higgs. 1991. Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol. Rev.* 43:109–142.

9. Beny, J. L., and P. C. Brunet. 1988. Electrophysiological and mechanical effects of substance P and acetylcholine on rabbit aorta. *J. Physiol. (Lond.)*. 398:277–289.

10. Chen, G., H. Suzuki, and A. H. Weston. 1988. Acetylcholine releases endothelium-derived hyperpolarizing factor and EDRF from rat blood vessels. *Br. J. Pharmacol.* 95:1165–1174.

11. Feletou, M., and P. M. Vanhoutte. 1988. Endothelium-dependent hyperpolarization of canine coronary smooth muscle. *Br. J. Pharmacol.* 93:515–524.

12. Taylor, S. G., J. S. Southerton, A. H. Weston, and J. R. J. Baker. 1988. Endothelium-dependent effects of acetylcholine in rat aorta: a comparison with sodium nitroprusside and cromakalim. *Br. J. Pharmacol.* 94:853–863.

13. Kauser, K., W. J. Stekiel, G. Rubanyi, and D. R. Harder. 1989. Mechanism of action of EDRF on pressurized arteries: effect on K^+ conductance. *Circ. Res.* 65:199–204.

14. Chen, G., Y. Yamamoto, K. Miwa, and H. Suzuki. 1991. Hyperpolarization of arterial smooth muscle induced by endothelial humoral substances. *Am. J. Physiol.* 260:H1888–H1892.

15. Huang, A. H., R. Busse, and E. Bassenge. 1988. Endothelium-dependent hyperpolarization of smooth muscle cells in rabbit femoral arteries is not mediated by EDRF (nitric oxide). *Naunyn-Schmiedeberg's Arch. Pharmacol.* 338:438–442.

16. Komori, K., R. R. Lorenz, and P. M. Vanhoutte. 1988. Nitric oxide, ACh,

and electrical and mechanical properties of canine arterial smooth muscle. *Am. J. Physiol.* 255:H207–H212.

17. Chen, G., H. Hashitani, and H. Suzuki. 1989. Endothelium-dependent relaxation and hyperpolarization of canine coronary artery smooth muscles in relation to the electrogenic Na-K pump. *Br. J. Pharmacol.* 98:950–956.

18. Brayden, J. E. 1990. Membrane hyperpolarization is a mechanism of endothelium-dependent cerebral vasodilation. *Am. J. Physiol.* 259:H668–H673.

19. Nagao, T., and P. M. Vanhoutte. 1992. Hyperpolarization as a mechanism for endothelium-dependent relaxations in the porcine coronary artery. *J. Physiol. (Lond.).* 445:355–367.

20. Garland, C. J., and G. A. McPherson. 1992. Evidence that nitric oxide does not mediate the hyperpolarization and relaxation to acetylcholine in the rat small mesenteric artery. *Br. J. Pharmacol.* 103:1184–1190.

21. Fujii, K., M. Tominaga, S. Ohmori, K. Kobayashi, T. Koga, Y. Takata, and M. Fujishima. 1992. Decreased endothelium-dependent hyperpolarization to acetylcholine in smooth muscle of the mesenteric artery of spontaneously hypertensive rats. *Circ. Res.* 70:660–669.

22. Chester, A. H., G. S. O'Neil, S. Tadjkarimi, R. M. Palmer, S. Moncada, and M. H. Yacoub. 1990. The role of nitric oxide in mediating endothelium dependent relaxations in the human epicardial coronary artery. *Int. J. Cardiol.* 29:305–309.

23. Mülsch, A., and R. Busse. 1990. N^G-Nitro-L-arginine (N^S-[imino(nitroamino)metyl]-L-ornithine) impairs endothelium-dependent dilatations by inhibiting cytosolic nitric oxide synthesis from L-arginine. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 341:143–147.

24. Moore, P. K., O. A. Al-Swayeh, N. W. S. Chong, R. A. Evans, and A. Gibson. 1990. L-N^G-nitro arginine (L-NOARG), a novel, L-arginine-reversible inhibitor of endothelium-dependent vasodilatation in vitro. *Br. J. Pharmacol.* 99:408–412.

25. Bolton, T. B., and L. H. Clapp. 1986. Endothelial-dependent relaxant actions of carbachol and substance P in arterial smooth muscle. *Br. J. Pharmacol.* 87:713–723.

26. Beny, J. L., P. C. Brunet, and H. Huggel. 1987. Interaction of bradykinin and des-Arg9-bradykinin with isolated pig coronary arteries: mechanical and electrophysiological events. *Regul. Pept.* 17:181–190.

27. Tare, M., H. C. Parkington, H. A. Coleman, T. O. Neild, and G. J. Dusting. 1990. Hyperpolarization and relaxation of arterial smooth muscle caused by nitric oxide derived from the endothelium. *Nature (Lond.).* 346:69–71.

28. Siegel, G., F. Schnalke, G. Stock, and J. Grote. 1989. Prostacyclin, endothelium-derived relaxing factor and vasodilatation. *Adv. Prostaglandin Thromboxane Leukotriene Res.* 19:267–270.

29. Förstermann, U., A. Mugge, U. Alheid, A. Haverich, and J. C. Frolich. 1988. Selective attenuation of endothelium-mediated vasodilation in atherosclerotic human coronary arteries. *Circ. Res.* 62:185–190.

30. Beny, J. L., and P. C. Brunet. 1988. Neither nitric oxide nor nitroglycerin accounts for all the characteristics of endothelially mediated vasodilatation of pig coronary arteries. *Blood Vessels.* 25:308–311.

31. Nakashima, M., T. Akata, and H. Kuriyama. 1992. Effects on the rabbit coronary artery of LP-805, a new type of releaser of endothelium-derived relaxing factor and a K^+ channel opener. *Circ. Res.* 71:859–869.

32. Chen, G., and H. Suzuki. 1990. Calcium dependency of the endothelium-dependent hyperpolarization in smooth muscle cells of the rabbit carotid artery. *J. Physiol. (Lond.).* 421:521–534.

33. Singer, H. A., and M. J. Peach. 1982. Calcium- and endothelium-mediated smooth muscle relaxation in rabbit aorta. *Hypertension (Dallas).* 4 (Suppl. II): III9–II25.

34. Nagao, T., S. Illiano, and P. M. Vanhoutte. 1992. Calmodulin antagonists inhibit endothelium-dependent hyperpolarization in the canine coronary artery. *Br. J. Pharmacol.* 107:382–386.

35. Illiano, S., T. Nagao, and P. M. Vanhoutte. 1992. Calmidazolium inhibits endothelium-dependent relaxations that are resistant to nitro-L-arginine in the canine coronary artery. *Br. J. Pharmacol.* 107:387–392.

36. Mombouli, J. V., S. Illiano, T. Nagao, T. Scott-Burden, and P. M. Vanhoutte. 1992. Potentiation of endothelium-dependent relaxations to bradykinin by angiotensin I converting enzyme inhibitors in canine coronary artery involves both endothelium-derived relaxing and hyperpolarizing factors. *Circ. Res.* 71:137–144.

37. Standen, N. B., J. M. Quayle, N. W. Davies, J. E. Brayden, Y. Huang, and M. T. Nelson. 1989. Hyperpolarizing vasodilators activate ATP-sensitive K^+ channels in arterial smooth muscle. *Science (Wash. DC).* 245:177–180.