Postgrad Med J (1992) **68**, 697 - 701

Leading Article

Endothelial regulation of vascular tone

Patrick Vallance

Department of Pharmacology & Clinical Pharmacology, St George's Hospital Medical School, London SW17 0RE, UK

A monolayer of endothelial cells coats the intimal surface of the entire vascular tree. These highly specialized cells detect signals in the lumen of the vessel and transduce them into messages understood by subjacent smooth muscle or passing blood cells. There have been many recent advances in the understanding of endothelial cell biology, and it is the role and clinical significance of endothelial cells as regulators of vascular smooth muscle tone that is the subject of this article.

Detection of signals in the lumen is the first step in endothelial regulation of blood vessel tone, and the cells are equipped to respond to both chemical and physical stimuli. The endothelial cell surface expresses receptors for circulating hormones including catecholamines, angiotensin and vasopressin, and local autacoids, including bradykinin, serotonin and acetylcholine.[1] Furthermore, the cell membrane responds directly to physical stimuli, admitting calcium through non-specific cation channels when subjected to stretch,[2] and allowing potassium efflux when shear stress is increased.[3] The result of these various stimuli is to alter the concentration of free ionized calcium within the endothelial cell and this in turn controls mediator synthesis and release.[4] Many endothelium-derived mediators may modify vascular tone, but the most striking example of the importance of endothelial cells in local cardiovascular control, was first provided by Furchgott & Zawadzki who demonstrated the phenomenon of endothelium-dependent relaxation.[5] In a seminal paper these authors described how the vasodilator actions of acetylcholine are mediated indirectly through release of a labile mediator from the endothelium. Initially called endothelium-derived relaxing factor (EDRF), the mediator of endothelium-dependent relaxation is now known to be a simple gas, nitric oxide (NO).[6,7] Once synthesized by the enzyme NO synthase from the semi-essential amino acid L-arginine,[8] NO diffuses from endothelium to the underlying smooth muscle where it activates guanylate cyclase to cause a rise in intracellular cyclic GMP[9] and relaxation of the vessel. The NO synthase present constitutively in healthy endothelial cells is calcium-dependent and responds rapidly to changes in the concentration of intracellular free calcium. The increase in calcium which follows stimulation of the acetylcholine receptor leads to activation of NO synthase. The NO generated can only act locally, since it has a chemical half-life of a few seconds in biological solutions[6] and is rapidly inactivated upon contact with haemoglobin.[10]

With the advent of specific inhibitors of NO synthesis, the role of endothelium-derived NO in cardiovascular control in animals and humans is now becoming clear. Using guanidino-substituted analogues of L-arginine (for example, N^G-monomethyl-L-arginine; L-NMMA) which compete with the natural substrate and inhibit NO synthesis,[11] it has been demonstrated that release of NO accounts for the vascular relaxant actions of acetylcholine, bradykinin and substance P in a variety of vascular beds.[12] Furthermore, certain agents usually thought of as vasoconstrictors, including noradrenaline and serotonin, also stimulate NO synthesis, with the NO released blunting the direct constrictor action on vascular smooth muscle. In the case of serotonin, this indirect effect can override the constriction such that, in vessels with healthy endothelium, serotonin may cause vasodilatation, whereas when endothelial integrity is breached, it is a potent vasoconstrictor.[13]

In addition to agonist-stimulated release of NO, in some vessels there is continuous, apparently unstimulated release of NO. Local infusion of L-NMMA to inhibit NO synthesis in the human forearm leads to a substantial fall in resting blood flow indicating that resistance vessels are in a constant state of active NO-mediated vasodilatation *in vivo*.[14] Consistent with this observation in humans *in vivo*, inhibition of basal NO synthesis leads to vasoconstriction of isolated blood vessels *in vitro*[11] and increased blood pressure of experimental animals *in vivo*.[15,16] The mechanisms underlying basal synthesis of NO are not yet clear. Many

Correspondence: P. Vallance, B.Sc., M.D., M.R.C.P.
Accepted: 6 April 1992

veins differ from resistance vessels and arteries and do *not* release NO continuously[17,18] under basal conditions, although will do so when stimulated by agonists.

Nitric oxide is by no means the only vasoactive mediator produced by endothelial cells. Vasodilator and vasoconstrictor prostanoids are synthesized and released,[19] a labile vasorelaxant endothelium-derived hyperpolarizing factor has been proposed,[20] endothelial cells express renin, angiotensin I and angiotensin converting enzyme,[21] constrictor superoxide anions are generated,[22] and a 21 amino acid peptide, endothelin, that is synthesized within endothelial cells,[23] is the most potent vasoconstrictor yet discovered. Despite this bewildering array of endothelial mediators, it appears that, in most instances, basal release of NO is the predominant endothelium-derived influence on vascular tone: removal of the endothelium usually leads to vasoconstriction, an effect mimicked by NO synthase inhibitors.[11] Indeed, a picture is emerging of the endothelium as the major basal dilator influence on blood vessels, continuously adjusting dilator tone through the release of NO from the luminal side of the vessel, with the sympathetic nervous system as the major basal constrictor influence, continuously adjusting constrictor tone through the release of noradrenaline, ATP and neuropeptide Y onto the adventitial side of the vessel. Together, the sympathetic nerves and endothelium provide a balanced system for rapid, short-lived alterations in vascular tone in response to systemic or local stimuli.

Where do the other endothelium-derived mediators fit into this reductionist view of vascular control? Endothelin, which has a long duration of action, might provide a slowly adapting background constrictor influence responding to, and enhancing the action of low background levels of circulating vasoconstrictor hormones.[24] The tissue renin-angiotensin system appears to make some contribution to vessel tone and provides a link between endothelium and nerves, with locally generated angiotensin II diffusing from endothelium through the vessel wall to increase noradrenaline release from neurones.[25,26] Prostaglandins may be particularly important in the control of vascular tone in the kidney[27] and certain placental and neonatal vessels. Synthesis and release of the unstable prostaglandin endoperoxide PGH_2 may account for the activity of the elusive short acting 'endothelium-derived constricting factor' (EDCF),[28] but its role in the control of human vasculature is entirely unknown. Superoxide anions have direct constrictor actions in some vessels. However, this free radical species also destroys NO and in many instances its actions on vascular tone are secondary to its inhibitory effects on basal NO-mediated relaxation.

With such potential to alter vascular tone it is inevitable that imbalance of endothelial mediator synthesis has been implicated in the pathogenesis of a variety of cardiovascular diseases. There is evidence for morphological and functional abnormalities of the vascular endothelium in hypertension,[29] diabetes[30] and atheroma.[31] Reduced endothelium-dependent relaxation has been demonstrated in patients with these conditions,[32–35] indicating reduced synthesis, release or effect of NO in response to agonists. The recent demonstration that naturally occurring methylated arginines (including L-NMMA) may act as endogenous inhibitors of NO synthase[36] provides a potential mechanism for reduced synthesis of NO in disease. Accumulation of endogenous methylated arginines occurs in at least one form of secondary hypertension; excretion of asymmetric dimethylarginine is attenuated in renal failure and the plasma concentrations of this endogenous compound rise to levels sufficient to inhibit NO synthesis.[36]

Overproduction of NO might also contribute to cardiovascular disease. Increased synthesis of NO contributes to the hypotension and hypo-reactivity to vasoconstrictors seen in animal models of endotoxic shock.[37–39] However, in this unusual situation, the endothelium is not the only vascular source of NO. After exposure to bacterial endotoxin, or certain inflammatory cytokines, a second type of NO synthase is expressed in endothelium and vascular smooth muscle.[40,41] This inducible NO synthase is calcium-independent, produces large amounts of NO over prolonged periods and leads to profound vasodilatation[38] and vascular damage.[42]

Much interest has focused on the role of NO in cardiovascular pathology, but abnormalities of other endothelium-derived mediators also occur. Increased circulating concentrations of endothelin have been found in patients with myocardial infarction,[43] renal failure,[44] hypertension,[45] and diabetes.[46] However, results have been inconsistent[47] and interpretation of the findings is not straightforward since the circulating concentrations of endothelin are too low to alter vascular tone and presumably represent 'spill-over' from altered production at some local site. An endothelin-secreting tumour malignant haemangioendothelioma) has been reported and in this situation, the circulating endothelin may well contribute to the raised blood pressure.[48] The precise physiological and pathophysiological significance of endothelin will soon become apparent with the advent of inhibitors of endothelin synthesis and specific endothelin receptor antagonists.[49]

Abnormalities of endothelial prostaglandin synthesis are seen in models of hypertension[50] and diabetes.[51] Increased generation of the constrictor PGH_2 could contribute to altered vascular re-

activity[51] and might also be associated with enhanced superoxide generation. Diminished destruction of superoxide anion due to alterations in activity of endothelial superoxide dismutase has been implicated in the pathogenesis of atheroma, hypertension and diabetes.[52]

If endothelial mediators are so intimately involved in the physiological and pathophysiological regulation of vascular tone, which are the opportunities for therapeutic advance? In conditions of increased vascular tone, NO donors or endothelin antagonists are obvious possibilities. In fact NO donors have been used in clinical practice for over 100 years: glyceryl trinitrate and other organic nitrates are metabolized to NO within the vessel wall,[53] whereas sodium nitroprusside and some newer nitrovasodilators liberate NO spontaneously in solution.[53] These agents all mimic the endogenous mediator and appear to be most potent at sites where continuous endothelial production of NO is low–veins, certain large conduit arteries, and vessels with endothelial damage. The reason for this profile of action of the nitrovasodilators is becoming clearer; low endogenous production of NO leads to up-regulation of guanylate cyclase in the vascular smooth muscle with consequent supersensitivity to NO.[54] In addition to its vasodilator actions, NO has anti-aggregatory and anti-adhesive effects on platelets,[12] and these observations have led to interest in the possibility that nitrovasodilators may have anti-platelet properties *in vivo*, although the evidence for a clinically useful effect is scant. Whether newer NO donors will offer a significant clinical advantage over existing drugs remains to be determined.

Endothelin is synthesized from an inactive precursor 'big endothelin' and drugs which interfere with this synthetic process–endothelin converting enzyme inhibitors[55]–or antagonists of endothelin receptors[49] might provide novel therapies to reduce vascular tone or prevent vasospasm. Certainly these agents will be useful tools to determine the roles of endothelin. Drugs to manipulate superoxide are also in the pipeline. Human recombinant superoxide dismutase, which destroys superoxide and thereby prolongs the half-life of NO, is already available for experimental use. However, this molecule does not enter cells and it is the advent of smaller molecules with superoxide dismutase activity which may provide more realistic therapeutic opportunities.

Research into NO donors, endothelin inhibitors, or superoxide manipulators are all targeted towards conditions associated with increased vascular tone, and novel treatments for hypertension, atheroma, vasospasm, re-stenosis after angioplasty, or diabetic vascular disease are bound to emerge. However, in some instances vascular tone is low and it may then be appropriate to manipulate the balance of local mediators in favour of vasoconstriction. In patients with septic shock, inhibition of NO synthesis with L-NMMA leads to a rise in vascular resistance and blood pressure with apparent haemodynamic stabilization.[56] This observation gives insight into the mechanisms of vasodilatation in septic shock in humans, and points the way for potential new therapies based on the L-arginine: NO pathway.

Within 12 years of the demonstration of EDRF by Furchgott and Zawadzki, fundamental research into endothelial biology has led directly to new therapies. It is perhaps ironic that the major action of NO synthase inhibitors, when used to raise blood pressure in septic shock, may be to attenuate pathological NO synthesis in smooth muscle rather than endothelium. Nevertheless this is one example of how the intense interest in the regulation of vascular tone by endothelial cells will advance understanding of disease and lead to new therapies.

References

1. Furchgott, R.F. The role of endothelium in the responses of vascular smooth muscle to drugs. *Ann Rev Pharmacol Toxicol* 1984, **24:** 175–197.
2. Lansman, J.B., Hallam, T.J. & Rink, T.J. Single stretch-activated ion channels in vascular endothelial cells as mechanotransducers? *Nature* 1987, **325:** 811–813.
3. Olesen, S., Clapham, D.E. & Davies, P.F. Haemodynamic shear stress activates a K^+ current in vascular endothelial cells. *Nature* 1988, **331:** 168–170.
4. Adams, D.J., Barakeh, J., Laskey, R. & Van Breemen, C. Ion channels and regulation of intracellular calcium in vascular endothelial cells. *FASEB J* 1989, **3:** 2389–2400.
5. Furchgott, R.F. & Zawadzki, J.V. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 1980, **288:** 373–376.
6. Palmer, R.M.J., Ferrige, A.G. & Moncada, S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* 1987, **327:** 524–526.
7. Ignarro, L.J., Buga, G.M., Wood, S.K., Byrns, R.E. & Chaudhuri, G. Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proc Natl Acad Sci USA* 1987, **84:** 9265–9269.
8. Palmer, R.M.J., Ashton, D.S. & Moncada, S. Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature* 1988, **333:** 664–666.
9. Gruetter, C.A., Gruetter, D.Y., Lyon, J.E., Kadowitz, P.J. & Ignarro, L.J. Relationship between cyclic guanosine 3′:5′-monophosphate formation and relaxation of coronary arterial smooth muscle by glyceryl trinitrate, nitroprusside, nitrite and nitric oxide: effects of methylene blue and methemoglobin. *J Pharmacol Exp Ther* 1981, **219:** 181–186.
10. Edwards, D.H., Griffith, T.M., Ryley, H.C. & Henderson, A.H. Haptoglobin–haemoglobin complex in human plasma inhibits endothelium dependent relaxation: evidence that endothelium derived relaxing factor acts as a local autocoid. *Cardiavasc Res* 1986, **20:** 549–556.

11. Palmer, R.M.J., Rees, D.D., Ashton, D.S., & Moncada, S. L-arginine is the physiological precursor for the formation of nitric oxide in endothelium-dependent relaxation. *Biochem Biophys Res Commun* 1988, **153:** 1251–1256.
12. Moncada, S., Palmer, R.M.J. & Higgs, E.A. Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol Rev* 1991, **43:** 109–142.
13. Golino, P., Piscione, F., Willerson, J.T. *et al.* Divergent effects of serotonin on coronary-artery dimensions and blood flow in patients with coronary atherosclerosis and control patients. *N Engl J Med* 1991, **324:** 641–648.
14. Vallance, P., Collier, J. & Moncada, S. Effects of endothelium-derived nitric oxide on peripheral arteriolar tone in man. *Lancet* 1989, **ii:** 997–1000.
15. Rees, D.D., Palmer, R.M.J. & Moncada, S. Role of endothelium-derived nitric oxide in the regulation of blood pressure. *Proc Natl Acad Sci USA* 1989, **86:** 3375–3378.
16. Aisaka, J., Gross, S.S., Griffith, O.W. & Levi, R. NG monomethyl-L-arginine an inhibitor of endothelium-derived nitric oxide synthesis, is a potent pressor agent in the guinea pig: does nitric oxide regulate blood pressure *in vivo*? *Biochem Biophys Res Commun* 1989, **160:** 881–886.
17. Vallance, P., Collier, J. & Moncada, S. Nitric oxide synthesised from L-arginine mediates endothelium dependent dilatation in human veins *in vivo*. *Cardiovasc Res* 1989, **23:** 1053–1057.
18. Martin, G.R., Bolofo, M.L. & Giles, H. Inhibition of endothelium-dependent vasorelaxation by arginine analogues: a pharmacological analysis of agonist and tissue dependence. *Br J Pharmacol* 1992, **105:** 643–652.
19. Gryglewski, R.J., Botting, R.M. & Vane, J.R. Mediators produced by the endothelial cell. *Hypertension* 1988, **12:** 530–548.
20. Chen, G., Yamamoto, Y., Miwa, K. & Suzuki, H. Hyperpolarization of arterial smooth muscle induced by endothelial humoral substances. *Am J Physiol* 1991, **260:** H1888–H1892.
21. Dzau, V.J. Multiple pathways of angiotensin production in the blood vessel wall: Evidence, possibilites and hypotheses. *Hypertension* 1989, **7:** 933–936.
22. Katusic, Z.S. & Vanhoutte, P.M. Superoxide anion is an endothelium-derived contracting factor. *Am J Physiol* 1989, **257:** H33–H37.
23. Yanagisawa, M., Kurihara, H., Kimura, S. *et al.* A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* 1988, **332:** 411–415.
24. Dohi, Y., Hahn, A.W.A., Boulanger, C.M., Bühler, F.R. & Lüscher, T.F. Endothelin stimulated by angiotensin II augments contractility of spontaneously hypertensive rat resistance arteries. *Hypertension* 1992, **19:** 131–137.
25. Webb, D.J., Seidelin, P.H., Benjamin, N., Collier, J.C. & Struthers, A.D. Sympathetically mediated vasoconstriction is augmented by angiotensin II in man. *J Hypertens* 1988, **6:** S542–S543.
26. Taddei, S., Favilla, S., Duranti, P., Simonini, N. & Salvetti, A. Vascular renin-angiotensin system and neurotransmission in hypertensive persons. *Hypertension* 1991, **188:** 266–277.
27. Aiken, J.W. & Vane, J.R. Intra-renal prostaglandin release attenuates the renal vasoconstrictor activity of angiotensin. *J Pharmacol Exp Ther* 1973, **184:** 678–687.
28. Kato, T., Iwama, Y., Okamura, O., Hashimoto, H., Ito, T. & Satake, T. Prostaglandin H2 may be the endothelium-derived contracting factor released by acetylcholine in the aorta of the rat. *Hypertension* 1990, **15:** 475–481.
29. Lüscher, T.F., Vanhoutte, P.M., Boulanger, C., Dohi, Y. & Bühler, F.R. Endothelial dysfunction in hypertension. In: Rubanyi, G. (ed) *Cardiovascular Significance of Endothelium-Derived Vasoactive Factors.* Futura, New York, 1991, pp. 199–221.
30. Pieper, G.M. & Gross, G.J. Endothelial dysfunction in diabetes. In: Rubanyi, G. (ed) *Cardiovascular Significance of Endothelium-derived Vasoactive Factors.* Futura, New York, 1991, pp. 223–249.
31. Förstermann, U., Mügge, A., Alheid, U., Haverich, A. & Frölich, C. Atherosclerosis impairs endothelium-dependent vascular relaxation in atherosclerotic human coronary arteries. *Circ Res* 1986, **58:** 783–789.
32. Panza, J.A., Quyyumi, A.A., Brush, J.E. & Epstein, S.E. Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. *N Engl J Med* 1990, **323:** 22–27.
33. Linder, L., Kiowski, W., Bühler, F.R. & Lüscher, T.F. Indirect evidence for release of endothelium-derived relaxing factor in human forearm circulation *in vivo*. Blunted response in essential hypertension. *Circulation* 1990, **81:** 1762–1767.
34. Creager, M.A., Cooke, J.P., Mendelsohn, M.E. *et al.* Impaired vasodilation of forearm resistance vessels in hypercholesterolemic humans. *J Clin Invest* 1990, **86:** 228–234.
35. Saenz De Tajeda, I., Goldstein, I., Azadzoi, K., Krane, R.J. & Cohen, R.A. Impaired neurogenic and endothelium-mediated relaxation of penile smooth muscle from diabetic men with impotence. *N Engl J Med* 1989, **320:** 1025–1030.
36. Vallance, P., Leone, A., Calver, A., Collier, J. & Moncada, S. Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. *Lancet* 1992, **339:** 572–576.
37. Kilbourn, R.G., Jurban, A., Gross, S.S. *et al.* Reversal of endotoxin-mediated shock by N^G-methyl-L-arginine, an inhibitor of nitric oxide synthesis. *Biochem Biophys Res Commun* 1990, **172:** 1132–1138.
38. Rees, D.D., Cellek, S., Palmer, R.M.J. & Moncada, S. Dexamethasone prevents the induction by endotoxin of a nitric oxide synthase and the associated effects on vascular tone. An insight into endotoxin shock. *Biochem Biophys Res Commun* 1990, **173:** 541–547.
39. Joulou-Schaeffer, G., Gray, G.A., Fleming, I., Schott, C., Parratt, J.R. & Stoclet, J.-C. Loss of vascular responsiveness induced by endotoxin involves the L-arginine pathway. *Am J Physiol* 1990, **259:** H1038–H1043.
40. Radomski, M.W., Palmer, R.M.J. & Moncada, S. Glucocorticoids inhibit the expression of an inducible, but not the constitutive, nitric oxide synthase in vascular endothelial cells. *Proc Natl Acad Sci USA* 1990, **87:** 10043–10047.
41. Busse, R. & Mulsch, A. Induction of nitric oxide synthase by cytokines in vascular smooth muscle. *FEBS Lett* 1990, **275:** 87–90.
42. Palmer, R.M.J., Bridge, L., Foxwell, N.A. & Moncada, S. The role of nitric oxide in endothelial cell damage and its inhibition by glucocorticoids. *Br J Pharmacol* 1992, **105:** 11–12.
43. Miyauchi, T., Yanagisawa, M., Tomizawa, T. *et al.* Increased plasma concentrations of endothelin-1 and big endothelin in acute myocardial infarction. *Lancet* 1989, **ii:** 53–54.
44. Tomita, K., Ujie, K., Nakanishi, T. *et al.* Plasma endothelin levels in patients with acute renal failure. *N Engl J Med* 1990, **321:** 1127.
45. Kohno, M., Yasunari, K., Murakawa, K. *et al.* Plasma immunoreactive endothelin in essential hypertension. *Am J Med* 1990, **88:** 614–618.
46. Takahasi, K., Ghatei, M.A., Lam, H.C., O'Halloran, D.J. & Bloom, S.R. Elevated plasma endothelin levels in patients with diabetes mellitus. *Diabetologia* 1990, **33:** 306–310.
47. Lüscher, T.F., Boulanger, C.M., Dohi, Y. & Yang, Z. Endothelium-derived contracting factors. *Hypertension* 1992, **19:** 117–130.
48. Yokokawa, K., Tahara, H. & Kohno, M. *et al.* Hypertension associated with endothelin-secreting malignant hemangioendothelioma. *Ann Intern Med* 1991, **114:** 213–215.
49. Ihara, M., Fukuroda, T., Saeki, T. *et al.* An endothelin receptor (ET_A) antagonist isolated from streptomyces misakiensis. *Biochem Biophys Res Commun* 1991, **178:** 132–137.
50. Lüscher, T.F. & Vanhoutte, P.M. Endothelium-dependent contractions to acetylcholine in the aorta of the spontaneously hypertensive rat. *Hypertension* 1986, **8:** 344–348.

51. Tesfarmarian, B., Jakubowski, J.A. & Cohen, R.A. Contraction of diabetic rabbit aorta caused by endothelium-derived PGH_2-TXA_2. *Am J Physiol* 1989, **257:** H1326–H1333.
52. Mügge, A., Elwell J.H., Peterson, T.E. & Harrison, D.G. Release of intact endothelium-derived relaxing factor depends on superoxide dismutase activity. *Am J Physiol* 1991, **260:** C219–C225.
53. Feelisch, M. The biochemical pathways of nitric oxide formation from nitrovasodilators: appropriate choice of exogenous NO donors and aspects of preparation and handling of aqueous NO solutions. *J Cardiovasc Pharmacol* 1991, **17** (Suppl 3): S25–S33.
54. Moncada, S., Rees, D.D., Schulz, R. & Palmer, R.M.J. Development and mechanism of a specific supersensitivity to nitrovasodilators after inhibition of vascular NO synthesis *in vivo*. *Proc Natl Acad Sci USA* 1991, **88:** 2166–2170.
55. Fukuroda, T., Noguchi, K., Tsuchida, S. *et al.* Inhibition of biological actions of big endothelin-1 by phosphoramidon. *Biochem Biophys Res Commun* 1990, **172:** 390–395.
56. Petros, A., Bennett, D. & Vallance, P. Effect of nitric oxide synthase inhibitors on hypotension in patients with septic shock. *Lancet* 1991, **338:** 1557–1558.