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 GMP⁹ 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 solutions⁶ 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

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. Vaso-dilator 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 kidney²⁷ and certain placental and neonatal vessels. Synthesis and release of the unstable prostaglandin endoperoxide PGH₂ 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 diabetes³⁰ 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 synthase³⁶ 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 vasodilatation³⁸ 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 inconsistent⁴⁷ 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 hypertension⁵⁰ and diabetes.⁵¹ Increased generation of the constrictor PGH₂ could contribute to altered vascular re-

activity⁵¹ 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 inhibitors⁵⁵—or antagonists of endothelin receptors⁴⁹ might provide novel ther-

apies 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.

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