Endothelial vasomotor regulation in health and disease

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The purpose of this review is to provide the anaesthetists with a comprehensive update on the endothelial-cell control of local blood flow. This single cell layer was originally thought to represent only a passive barrier. It is now evident that it plays an active role in a broad variety of biological functions. Since the discovery of the endothelial-derived relaxing factor (EDRF), it has been the subject of a considerable amount of research. It is established that EDRF is secreted continuously at a basal state and that many physical stimuli as well as vasoactive substances can modulate its secretion. Evidence presented indicates that the endogenous vasodilatation produced by EDRF is similar to that of the exogenous nitrovasodilator nitroglycerine and nitroprusside (i.e., nitric oxide). Aside from EDRF, the endothelium produces other vasodilating as well as vasoconstricting factors. A review of the physiology of the endothelium regarding the local control of blood flow is provided along with its influence upon several pathophysiological states. Also included is an overview of the influence of anaesthetic agents on endothelial function. These findings linking vasomotor control to endothelial function will help to explain pathophysiological process and may lead to new therapeutic modalities.

Le but de cette revue de la littérature est de présenter une synthèse sur le rôle de l'endothélium dans la régulation du tonus vasculaire. Cette couche monocellulaire fut longtemps considérée comme une simple barrière diffusionnelle. Il est de plus en plus évident qu'elle joue un rôle très actif dans plusieurs fonctions biologiques. Depuis sa découverte, le facteur relaxant endothélial (EDRF), n'a cessé d'attirer l'attention des chercheurs. Présentement, on sait que l'EDRF est sécrété continuellement à l'état basal et que plusieurs stimuli physiques ainsi

que des substances vasoactives peuvent moduler sa sécrétion. L'action de l'EDRF procède par la synthèse du même intermédiaire des nitrovasodilatateurs (i.e., l'oxyde nitrique). L'endothélium vasculaire ne sécrète pas uniquement l'EDRF. Il peut aussi synthétiser d'autres facteurs relaxants (prostacycline, facteur hyperpolarisant) ainsi que des facteurs vasoconstricteurs. L'interaction des agents anesthésiques sur la fonction endothéliale est discutée. Ces nouvelles découvertes sur le mécanisme du contrôle local vasomoteur reliées à l'endothélium permettront de mieux comprendre les processus pathophysiologiques et possiblement nous aideront à développer de nouvelles modalités thérapeutiques permettant d'escompter des bénéfices cliniques.

Key words

ENDOTHELIUM; EDRF; NITRIC OXIDE.

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The purpose of this review is to provide anaesthetists with a comprehensive update on the endothelial-cell control of local blood flow in health and disease. It has become apparent in the last decade that vascular endothelium plays a major role in the coordination and control of the tone of blood vessels throughout the arterial and venous systems. Not only is it relevant in vasomotor tone regulation but also in modulating the inflammatory response, haemostasis, vascular cell growth and transcellular metabolism.¹⁻¹³ Certain pathophysiological conditions such as atheroma, diabetes, hypertension, immunological diseases, vasospasm following balloon catheter angioplasty, and accelerated arteriosclerosis in heart transplantation are associated with impaired endothelial function.^{1,2,4,5,7-10,14}

The vascular endothelium constitutes a single layer of thin squamous cells lining the intimal surface of the circulatory system. In humans, the endothelium has a surface area similar to that of six tennis courts and weighs 1 to 1.5 kg. This layer of cells, originally thought to represent only a passive diffusion barrier between the circulating blood and the interstitial tissue, constitutes a very active tissue with a broad variety of biological actions (Table I). It is implicated in the production of antithrombotic substances as well as in promoting thrombosis and in activating the coagulation system. Also, the vascular endothelium participates in the uptake and metabolism of circulating vasoactive compounds. Through this metabolic activity, the vascular endothelium has an important regulatory effect on the regional blood concen-

TABLE I Biologically active substances produced or metabolized by the vascular endothelium

<i>Antithrombotic substance</i>
Alpha 2-macroglobulin
Antithrombin III
Heparin sulphate
Plasminogen activator
Prostacyclin
Protein C
<i>Metabolized substances</i>
Adenine nucleotides
Adenosine
Angiotensin I and II
Bradykinins
Leukotrienes
Norepinephrine
Prostaglandins
Serotonin
Substance P
<i>Substances promoting thrombosis or activating the coagulation system</i>
Fibrinogen
Platelet aggregating factor
Thromboplastin
Thromboxane A ₂
von Willebrand factor
<i>Secreted substances</i>
Endothelial-derived constricting factors
Endothelial-derived hyperpolarizing factor
Endothelial-derived relaxing factor
Prostacyclin

GLOSSARY

Acetylcholine (ACh)
 Adenosine diphosphate (ADP)
 Adenosine triphosphate (ATP)
 Atrial natriuretic peptide (ANP)
 Calcitonin gene-related peptides (CGRP)
 Coronary artery disease (CAD)
 Cyclic adenosine monophosphate (cAMP)
 Cyclic guanosine monophosphate (cGMP)
 Diethyldithiocarbamate (DETCA)
 Endothelin (ET)
 Endothelium-derived hyperpolarizing factor (EDHF)
 Endothelium-derived relaxing factor (EDRF)
 Endothelium-derived contracting factors (EDCF)
 Hydroxy-L-arginine (L-HO Arg)
 L-N-mono-methyl-arginine (L-NMMA)
 Mean alveolar concentration (MAC)
 Messenger ribonucleic acid (mRNA)
 Nitric oxide (NO)
 Prostacyclin (PGI 2)
 Serotonin (5-HT)
 Substance P (SP)
 Vasoactive intestinal peptide (VIP)

trations of these circulating vasoactive substances. This constitutes one mode of action for the regulation of the local blood flow by the vascular endothelium. The endothelium is able to modulate blood flow further by the secreting vasoactive substances such as: (1) prostacyclin, (2) a non-prostanoid endothelium-derived relaxing factor (EDRF), (3) an endothelium-derived hyperpolarizing factor (EDHF), and (4) at least two endothelium-derived contracting factors (EDCF).

Historical perspective of endothelium-derived relaxing factor(s) (EDRF)

The discovery of a relaxing substance came from the work of Furchgott and Zawadzki.¹⁵ They showed that acetylcholine (ACh) evoked relaxation of isolated preparations of arteries of rabbits which had been pre-contracted by the addition of an exogenous constricting agent (norepinephrine) was dependent on the presence of endothelial cells. In subsequent studies, they extended this finding to all mammals.^{9,10} The mechanism of release of this endothelium-derived factor(s) is associated with ACh stimulation of a muscarinic receptor on the endothelial cell (Figure 1).

The duration of action of this vasodilating factor has

FIGURE 1 Mechanism of endothelium-dependent vasodilatation. Vasoactive substances and changes in flow stimulate endothelial cells to release EDRF (endothelium-derived relaxing factor), a powerful vasodilator substance. The EDRF induces relaxation of vascular smooth muscle cells through elevation of cyclic GMP (cGMP). Prostacyclin (PGI₂) is another endothelium-derived vasodilator acting through an increased formation of cyclic AMP (cAMP). The endothelial cell also produces a hyperpolarizing factor (EDHF) which causes relaxation of vascular smooth muscle cells through activation of sodium-potassium pumps. EDRF and PGI₂ are also released into the lumen, where they inhibit platelet aggregation synergistically. Acetylcholine (ACh); bradykinin (Bk); substance P (SP); calcitonin gene-related peptide (CGRP); noradrenaline (NA); serotonin (5HT); adenosine diphosphate (ADP); L-arginine (L-Arg); arachidonic acid (AA); atrial natriuretic factor (ANF); muscarinic receptor (M); serotonergic receptor (S₁); purinergic receptor (P_{2x} and P_{2y}); adrenergic receptor (α); and thrombin receptor (T) (adapted from Vrints and Herman⁴ with permission).

been determined by bioassay. Griffith *et al.*¹⁶ determined that EDRF had a biological half-life of 6.3 sec. Subsequent bioassay studies, in which the release of EDRF from cultured endothelial cells was monitored, showed that EDRF is rapidly inactivated by oxygenated buffer solutions, methylene blue, haemoglobin, free iron, and superoxide radicals, while anoxia prevents the synthesis of EDRF.^{4,16,17} To deplete the cytosol of superoxide dismutase (SOD) of bovine coronary arteries by the use of diethyldithiocarbamate (DETCA) inhibited ACh stimulated EDRF-dependent relaxation as well as inhibiting the effect of the nitrovasodilators (nitroglycerine and nitroprusside).¹⁸⁻²⁰ The lack of SOD activity increased the concentration level of superoxide anions thus inhibiting EDRF and nitrovasodilator action while the addition of SOD to the isolated arteries restored the effect of ACh and also that of nitrovasodilators. Therefore, the control of cellular superoxide anion levels by superoxide dismutase appears necessary for the action of EDRF and for the response of the nitrovasodilators on vascular smooth muscle. Indeed, although nitrovasodilators and organic nitrates differ in

various aspects, their common principle is the capacity to release nitric oxide (NO) from their molecule.

The similarities in the mechanisms of smooth muscle relaxation caused by the endothelium-dependent vasodilators and nitrovasodilators (nitroglycerine and nitroprusside) which were known to release NO suggested that EDRF may be the same intermediary. Formal demonstration that EDRF is NO came from experiments done by Ignarro *et al.*⁸ who showed that EDRF and an aqueous solution of NO had striking pharmacological similarities and that, under spectrophotometric analysis, EDRF and NO produced an identical spectral shift in the absorbance of haemoglobin. Using chemiluminescence, Palmer *et al.*^{21,22} demonstrated that the NO, liberated by bradykinin-stimulated endothelial cells, could be accounted for quantitatively and that the precursor substance for NO synthesis was L-arginine. Nitric oxide is synthesized by oxidation of the guanidino-nitrogen of arginine. The cytosolic enzyme which is necessary is activated by interaction of calcium and calmodulin and requires nicotinamide adenine dinucleotide phosphate as a cofactor

FIGURE 2 Substrate and cofactor requirements for nitric oxide (NO) synthase activation.

(Figure 2).²³ The mechanism of release of free NO has been questioned. It may not be released directly but via a more stable compound such as S-nitrosocysteine which, subsequently, at the level of the cell membrane, dissociates with the formation of NO.²⁴ Zembowicz *et al.*²⁵ have presented evidence that not only NO is released but also another more stable and more potent vasodilator is formed from hydroxy-L-arginine (L-HOArg) by cultured endothelial cells. This novel vasodilator appears to be derived from a chemical reaction between NO and L-HOArg which may indicate that a second endothelial-derived relaxing factor is produced by endothelial cells.

Biochemical action of EDRF

Nitric oxide is a simple gas which is highly reactive. Endothelial-derived relaxing factor, produced from the endothelial cell, will rapidly penetrate through the smooth muscle cell membrane and bind to the haeme portion of soluble guanylate cyclase. It will activate guanosine cyclase to increase the intracellular concentration of cyclic guanosine monophosphate (cGMP).²⁶⁻²⁹ The EDRF-induced increase of cGMP reduces the intracellular calcium level in vascular smooth muscle by several possible mechanisms. One of these is the inhibition of hydrolysis of phosphatidylinositol which, secondarily, mobilizes calcium from the sarcoplasmic reticulum. In addition, elevated concentration of cGMP can increase

calcium uptake by the sarcoplasmic reticulum, decrease the sensitivity to myosin light chain for calcium, and interfere with receptor-operated calcium channels. Thus, the overall effect is decreased intracellular calcium. Aside from increasing intracellular cGMP, EDRF can induce transient hyperpolarization of vascular smooth muscle by activating the sodium-potassium pump.³⁰ These effects on the smooth muscle cell membrane may only partially explain the endothelium-dependent relaxations evoked by locally released factors or physical stimuli.

Physiological stimulants of EDRF release

Basal state release of EDRF

In a basal, non-stimulated state, EDRF is produced and released continuously. Continuous release of EDRF may be due to viscous drag induced by shear stress and by the pulsatile nature of the arterial flow (Figure 3). The flow-induced shear stress, acting on the endothelial cell, is probably the most important stimulus for endothelial-dependent control of vascular tone.³¹⁻³⁷ This endothelium-dependent reaction plays an important role in modulating systemic and pulmonary vascular resistance. Basal EDRF activity is present in all arterial preparations tested. Chu *et al.*³⁸ demonstrated that NO modulated epicardial coronary basal vasomotor tone in awake dogs. After infusing an

FIGURE 3 Schematic representation of EDRF-release under basal state (a) and with the increase in flow and/or shear stress (b). Increase flow leads to endothelial liberation of EDRF which diffuse through the smooth muscle membrane to increase cyclic guanosine monophosphate (cGMP). Smooth muscle (S. M.), endothelial cell (E. C.), diameter (Diam.).

inhibitor of NO synthesis, L-N-mono-methyl-arginine (L-NMMA), they noticed a gradual decrease in the arterial diameter, increase in systemic blood pressure which was reversible only after reinfusion with L-arginine. Following prolonged administration of L-NMMA persistent hypertension with decreased vascular compliance was seen in all vascular beds.³⁹ The inability of the vascular system to readjust to baseline values suggested that the basal release of EDRF was of major importance for local vascular control. Several studies have established that coronary

arteries seem more reactive than peripheral arteries to endothelial-derived vasoactive substances. This may be related to a greater release of vasoactive substances or to a greater effector response.^{4,5,16,38,40,41} This flow-dependent control seems to be of particular importance in the coronary circulation where moment to moment variability and an extremely pulsatile flow pattern is present. Aside from this basal release, a variety of physiological stimuli and vasoactive substances stimulate the endothelium to release EDRF (Table II).

TABLE II Stimulants of EDRF release

<i>Physiological</i>
Change in flow or shear stress
Change in oxygen tension
<i>Vasoactive substances</i>
Acetylcholine
Adenosine diphosphate
Adenosine triphosphate
Bradykinin
Calcitonin gene-related peptide
Calcium ionophore (A23187)
Cholecystokinin
Histamine
Noradrenaline
Serotonin
Substance P
Thrombin
Vasoactive intestinal peptide

Acetylcholine release of EDRF

As mentioned previously, acetylcholine is the classic means to induce EDRF release in isolated arteries. Acetylcholine stimulates muscarinic receptors on the endothelial cell of the coronary and peripheral arteries.^{15,42-46} There is some inconsistency regarding the action of ACh on endothelial cell release of EDRF. Knight *et al.*,⁴⁷ in intact sedated baboons, demonstrated that intracoronary injection of ACh in high doses caused coronary constriction with decreased coronary blood flow and contractility without any systemic haemodynamic changes. In contrast, low doses of ACh caused only coronary vasodilatation. These ACh responses were unaffected by α and β adrenoceptor blockade but were abolished by atropine. Acetylcholine administered in the baboons' iliac artery, at any dose, caused vasodilatation. This variability of ACh effects on different arterial beds may be explained by differences in species, doses or possibly, at high doses, the liberation of endothelial constricting factor(s).

The physiological role of these endothelial cells muscarinic receptors is still unclear. The ACh molecules released at the nerve ending are rapidly destroyed and are unlikely to be present in any measurable concentration in the circulating blood. To advocate abluminal (directed away from the lumen) release of ACh from neural tissue implies that ACh diffuses some distance to stimulate the endothelial cell. Diffusion of ACh through the subendothelial space appears to be inefficient. Furthermore, the inability to identify muscarinic cholinceptors on the surface of endothelial cells autoradiographically is puzzling.⁴⁸ Recently, evidence has been accumulated indicating that some endothelial cells contain ATP, serotonin and substance P (SP) and may possess enzymatic activity of choline acetyl transferase and, hence, are able to synthesize acetylcholine.⁴⁹⁻⁵² The questions that may be

asked are whether the endothelial cell can synthesize ACh and/or whether it can release vasoactive substances? If certain endothelial cells are able to release vasoactive substances and can synthesize ACh then, under appropriate conditions, these "special" endothelial cells might liberate substances which can interact with neighbouring endothelial cells to release EDRF.⁴

Pressure effects on the release of EDRF

With the augmentation of flow through large arteries, the increase in transmural pressure leads to a proportionate increase in EDRF (Figure 3). This response improves organ perfusion. Holtz *et al.*^{37,53} demonstrated in conscious dogs that an increase in coronary blood flow was matched by an increase in coronary artery diameter but this vasodilatation was not seen in endothelium-denuded coronary arteries.^{1,2,4} Endothelial-derived relaxing factor is not only a vasodilator but may also act as a second messenger to modify the production of vasoactive substances in other cells. Exposing slices of canine kidney to EDRF released from isolated blood vessels reduced renin production.⁵⁴ The anatomical proximity of the juxtaglomerular cells and the endothelial cells of the preglomerular arterioles may modulate renin release to EDRF in response to changes in perfusion pressure. It appears therefore that arterial flow variation is continuously monitored by the endothelium. Hence endothelial cells may act as local baroreceptors and modulate organ perfusion. The exact flow-sensing mechanism is not presently known, but Olesen *et al.*⁵⁵ demonstrated a flow-dependent vasodilatation linked to activation of potassium channels leading to hyperpolarization of smooth muscle cells. Recently Cooke *et al.*⁵⁶ reported that increased flow activated an endothelial potassium channel which was able to release an endogenous nitrovasodilator (NO). The mechanical deformation of the endothelial membrane may represent an important flow-sensing mechanism for endothelial cell control of arterial diameter.

Effects of hypoxia and anoxia on EDRF release

A continuous supply of oxygen is of vital importance for all living cells. This goal is achieved by several independent mechanisms to maintain an adequate supply of oxygenated blood for a broad range of metabolic demands. One of these mechanisms might be the direct effect of oxygen on endothelial cells. Release of EDRF is observed in rabbit aorta and femoral arteries subjected to hypoxia.⁵⁷ Interestingly the presence of calcium in the incubating medium is essential for EDRF synthesis and/or release under hypoxic conditions.⁵⁷ As mentioned before, the presence of haemoglobin or higher concentrations of superoxide-free radicals was able to attenuate substantially the hypoxic release of EDRF.^{58,59}

Endothelial cells seem to behave as functional oxygen sensors in the vascular wall. Vasomotion in response to local changes in PO_2 may be, at least in part, secondary to oxygen-induced changes in the release of EDRF. But EDRF is not the only factor that may be released. When the PO_2 falls to a critical level, endothelial cells cease to release EDRF and synthesis of some endothelium-dependent vasoconstricting factors (EDCF) may be activated. Recently, Kourembanas *et al.*⁶⁰ showed that hypoxia (PO_2 30 mmHg), in cultured human endothelium, was able to induce the endothelin gene and secretion of endothelin, while the reinstitution of a normal PO_2 was able to reverse this reaction. Thus, the low oxygen tension which may occur in the microcirculation might act as a continuous physiological stimulus for the release of EDRF and/or endothelin, thus contributing to the local regulation of vasoconstriction and vasodilatation.⁴

Anoxia, in canine femoral arteries, abolished ACh-induced relaxation and, at the same time, enhanced constrictor response to norepinephrine.¹⁰ Other stimulants of EDRF release, for instance, calcium ionophore A23187 (non-receptor dependent generator of EDRF) and bradykinin (receptor-dependent generator of EDRF), were also inhibited during anoxia. This suggests that EDRF production may be interrupted during anaerobic conditions. Similar results have been obtained with inhibitors of the mitochondrial electron transfer chain.⁵⁷

Vasoactive substances

Purinergetic vasodilatation via endothelium

While the potent actions of purines on blood vessels have been known for many years, it is only recently that the various mechanisms involved have been understood. Adenosine triphosphate and ADP receptors (purinoceptors) are present on the vascular endothelial and smooth muscle cells. The endothelial cell purinoceptor (P_{2y} subclass) produces vasodilatation via EDRF release similar to ACh stimulation.^{50,52} Contrary to endothelial cells, smooth muscle cells have two subclasses of purinoceptor population (P_{2x} and P_{2y}). Stimulation of P_{2x} leads to vasoconstriction while P_{2y} leads to vasodilatation. The overall smooth muscle cell response depends on the predominance of one subclass of receptor and the local production of EDRF.

Release of purine transmitter substance is not only derived from aggregating platelets but also from specialized non-sympathetic purinergetic neurons and from endothelial cells. Several studies have demonstrated the existence of non-sympathetic purinergetic innervation causing vasodilatation of the rabbit portal vein⁶¹ and of vessels of skeletal muscle,^{62,63} and vasoconstriction of intrapulmonary arteries.⁶⁴ The possibility that there are some intrinsic purinergetic cardiac neurones for vasodilatation of the coronary vessels has been raised.⁶⁵

FIGURE 4 Effect of histamine on endothelial and a smooth muscle cell response to stimulation. Histaminergic receptors (H-1 and H-2), calcium (Ca), endothelial cell (E.C.) and smooth muscle cell (S.M.C.).

Vascular response to histamine

Anaesthetists are wary of drugs which have the propensity for histamine release because of the severe haemodynamic effects. The profound depression of blood pressure produced by intravenous histamine administration is due to a decrease in vascular tone, a direct action on cardiac muscle and an increase in capillary permeability. These effects are the results of a complex interaction with both types of histaminic receptors (H-1 and H-2) located on the endothelial (H-1) and smooth muscles (H-1 and H-2) cells leading to heterogenous responses from different vascular beds and in different species (Figure 4).^{43,66,67}

The vasoconstricting effect of stimulation of the H-1 histaminergic receptors located on vascular smooth muscle is tempered by the effect of stimulation of endothelial histaminergic receptors releasing EDRF. Again the overall vascular response of histamine probably depends on the sensitivity and reactivity of both histaminergic receptor populations. Under pathological states (coronary atherosclerosis) uncoupling of these opposing forces will lead to a predominant vasoconstriction or vasospasm. Administration of histamine in atherosclerotic rabbit coronary arteries leads to vasoconstriction and myocardial ischaemia⁶⁸ while a similar administration of histamine in patients with coronary disease (CAD) resulted in vasospasm.⁶⁹

Vascular response to kallikrein

The intravenous administration of bradykinin produces hypotension by decreasing the peripheral resistance and increasing the vascular permeability at the level of the microcirculation. Because of its low plasma concentration, under physiological conditions, bradykinin contributes little to the maintenance of vascular tone.

FIGURE 5 Effect of bradykinin on endothelial, subendothelial tissues and smooth muscle cell. Endothelial cell (E.C.), smooth muscle cell (S.M.C.), prostaglandins (PGI₂, PGE₂ and PGF_{2α}).

The cardiovascular actions of bradykinin are related to the following properties: (1) stimulation of a bradykinin receptor (B-2) on the endothelial cell, (2) stimulation of production of different prostaglandins from endothelial cells and from subendothelial structures, and (3) stimulation of a different subclass of bradykinin receptor located on the smooth muscle cells. The arterial vasodilatation produced via B-2 endothelial receptor stimulation is mediated by EDRF, PGI₂ and PGE₂ secretion, whereas, in veins, bradykinin preferentially liberates PGF_{2α} (vasoconstrictor) instead of PGE₂.⁷⁰ This may explain the venous contraction observed following bradykinin exposure. With regard to smooth muscle cells, activation of the bradykinin receptors causes contraction. Using denuded arterial rings, Cheng *et al.*⁷¹ and Förstermann *et al.*⁷² demonstrated a predominant vasodilatation response to bradykinin even in the absence of endothelial cells. Pre-treating intact porcine coronary arteries with the following agents: NO inhibitor (L-NMMA), soluble guanylate cyclase inhibitor (Methylene blue) and G_i protein inhibitor (Pertussis toxin) still induced vasodilatation to bradykinin stimulation.⁷³ These findings lend support to the hypothesis that the vasodilating prostaglandins and/or other endothelial-derived vasodilator(s) may play a determinant role in arterial vascular tone (Figure 5).

Platelet aggregation and vasomotor tone

Endothelial cells are involved in the balance between coagulation, thrombus formation, and fibrinolysis. Plasminogen activator and anti-thrombin III released from the endothelium promote fibrinolysis and the presence of heparin sulphate at the cell membrane suppresses thrombosis. The inhibitory effect on platelet aggregation and thrombus formation of PGI₂ and EDRF is directly related

to endothelial-released factors.⁷⁴ Prostacyclin and EDRF can potentiate each other at subthreshold concentrations and prevent platelets from aggregating. It is important to mention that aggregating platelets release sufficient mediators to cause endothelial-dependent relaxation in normal human arteries.⁷⁵ Thus the released EDRF and PGI₂ provide a negative feedback to aggregating platelets, increasing blood flow (by vasodilatation) and thus diluting platelet mediators. Also, activated platelets participate in endothelial cell synthesis of PGI₂ via the release of endoperoxides and arachidonate precursors for production of PGI₂.⁷⁶ This phenomenon is known as transcellular metabolism. In these reactions one cell uses precursors and intermediates from another for the production of a new metabolite(s) with entirely different functions. Furthermore, biochemical and functional data indicate that in aspirin-treated endothelial cells where no PGI₂ production is inhibited and where no measurable EDRF, there is an alternative mechanism for platelet inactivation. This new mechanism involves the degradation of ADP by an endothelial membrane-bound enzyme (endothelial membrane ecto-adenosine diphosphatases) which consequently inhibits platelet proaggregatory activity.⁷⁶ The summation of these endothelial-cell mechanisms is responsible for maintaining the haemostatic system under control.

Serotonin released from aggregating platelets exhibits a complicated pattern of actions in the coronary arterial bed. It causes both dilatation and constriction of large and small coronary arteries.^{40,77-80} The vasodilatation is endothelium-dependent and originates from stimulation of serotonergic (S-1) receptors on the luminal side of endothelial cell membrane with liberation of EDRF. If these vessels are denuded of their endothelium then constriction is observed which is mediated by direct activation of

FIGURE 6 Illustration of platelet-vessel wall interaction and the effects of atherosclerotic coronary arteries causing impaired endothelium-dependent dilatation. A decrease in PGI₂ and EDRF production and/or inactivation is unable to inhibit platelet aggregation while platelet-derived products are still able to have full effect on smooth muscle cell. As a result, vasoconstrictor response to serotonin (5-HT) and thromboxane A₂ (TxA₂) will predominate. Arachidonic acid (AA), serotonin receptor (S), purine receptor (P), thrombin receptor (T), alpha-adrenergic receptor ($\alpha_{1,2}$), platelet-derived growth factor (PDGF), prostacyclin (PGI₂), adapted from Vrints and Herman⁴ with permission.

serotonergic (S-2) receptors on smooth muscle cell. Under normal physiological conditions, the S-1 receptor mediates a dominant endothelium-dependent dilatation. Under pathophysiological conditions (atheroma, atherosclerosis, thrombosis, etc.) there may be a shift in the vasomotor balance towards constriction when the intima is damaged and platelets come in contact with the subendothelial structures and release their content. Aggregating platelets are capable of releasing enough serotonin locally to evoke either constrictor or dilator responses.^{80,81} In addition, the responsiveness of the blood vessel wall to serotonin can be amplified in the presence of hypoxia,⁵¹ while an exaggerated response to serotonin is seen in hypertensive or atherosclerotic vessels (Figure 6).^{80,82,83}

Another vasoactive compound released from aggregating platelets is *thromboxane A₂*. Thromboxane causes direct vasoconstriction but has no endothelial-mediated effects.

The release of *platelet activating factor* (PAF) derived from platelets, neutrophils, mast cells and endothelial cells shows an endothelium-dependent vasodilatation only at higher concentrations. Platelet activating factor has a complex vasomotor effect because it is able to stimulate not only EDRF but also effect thromboxane and *leukotriene* release. For instance, intracoronary administration

of PAF initially causes vasodilatation that is not inhibited by indomethacin while the secondary vasoconstricting response which is dependent on thromboxane and *leukotrienes* release is indomethacin-sensitive. Thrombin, which is formed during the coagulation cascade, also evokes an endothelium-dependent relaxation (EDRF release) in various vessels including the coronary arteries and is capable of stimulating endothelin production.^{84,85}

The ADP and ATP release from platelets has been discussed previously. In summary, platelet-derived vasoactive substances have opposing effects depending on the integrity of the endothelium. Under normal conditions, relaxation predominates over constriction but in denuded vessels an exaggerated vasoconstricting response (vasospasm) may be provoked when platelet-derived factors are released locally. Two other mechanisms were described by which the endothelial cells are able to maintain platelets from further aggregation: (1) secreted products of platelets (endoperoxides and arachidonate products) are used by the transcellular metabolism of endothelial cells to produce PGI₂ and (2) ADP derived from platelets are metabolized by an endothelial ADP-ase.

Peptides and vascular response

Mammalian atrial myocytes contain a group of peptides

that possess natriuretic, aldosterone-inhibiting and vaso-relaxant properties.^{86,87} These, the *atrial natriuretic peptides* (ANP), are important for central blood volume control in humans. The ANPs are rapidly released in response to changes in atrial pressure. In the human brachial artery, infusion of synthetic ANP causes a dose-dependent vasodilatation that is rapid in onset (30 sec) and maximal in two to three minutes. After cessation of the infusion, arterial dilatation persists for another 30 to 60 min.⁸⁸ The rapid onset of vasodilatation suggests that the decrease in blood pressure during the systemic infusion of ANP is primarily due to vasodilatation rather than to volume loss through natriuresis. The plasma concentration of ANP capable of producing 50% vasodilatation in the previous study is well within the normal range measured in normal individuals.

The vasodilatation of ANP is heterogeneous being present in aortic, pulmonary, carotid and renal arteries but absent in coronary, mesenteric and femoral arteries.⁸⁸ The mechanism is an increased level of cGMP in both the endothelial and the smooth muscle cells. The production of cGMP by ANP proceeds via particulate guanosine cyclase as opposed to soluble guanosine cyclase⁸⁷ (Figure 1). While ANP secretion increases in response to atrial stretching, EDRF is able to modulate in a negative fashion the release of ANP. Removal of the atrial endocardium is responsible for increased release of ANP. This is directly related to a decrease in basal EDRF production either from the endocardium or from endothelial cells of coronary arteries since inhibitors of EDRF synthesis (haemoglobin, methylene blue and superoxide radicals) yield similar results.⁸⁵ This indicates that released EDRF (second messenger) is able to influence atrial myocytes in the production and release of ANP.

The vasodilating effect of ANP is endothelium independent which is in contrast to other peptides, such as SP, calcitonin gene-related peptides (CGRP) and vasoactive intestinal peptide (VIP) all of which induce endothelium-mediated dilatation (Figure 1). High vasodilator potency has been demonstrated for each of these peptides,^{4,89-93} and intracoronary application increases coronary flow. For SP, intracoronary administration yielded different results in systemic and coronary beds. Initially, coronary vasodilatation prevailed with a gradual vasoconstriction that predominated during steady state conditions.⁹³ The cause of the secondary vasoconstriction is not known. It may be related to liberation of a vasoconstricting substance from the endothelial cell. Although these vasoactive peptides circulate in measurable concentrations and affect vasomotor activity, they might also be secreted from the recently identified pericoronary nerves.⁹² Their overall contribution to the control of vascular tone in health and disease remains to be evaluated.

Prostacyclin

Prostacyclin (PGI₂) is a major prostaglandin produced by the endothelial cell. It has two major actions, firstly as a vasodilator and secondly by activating platelet adenylate cyclase and increasing platelet cAMP level, it inhibits platelet aggregation. Prostacyclin has a very short half-life of only one circulation time. Like EDRF, PGI₂ synthesis from endothelial cells is dependent on physical factors (pulsatile flow and shear stress) as well as endogenous mediators (bradykinin, thrombin, serotonin, platelet-derived growth factor, interleukin-1 and adenine nucleotides) and some drugs (calcium antagonist, captopril, dipyridamole, nitrates, diuretics and streptokinase) can stimulate its production.⁹⁴ It has mainly a local effect. The endothelial production of PGI₂ decreases with age and in association with certain disease states, for instance, diabetes mellitus and atherosclerosis.⁹⁴

Endothelium-derived hyperpolarizing factor (EDHF) and endothelium-derived contracting factors (EDCF)

Endothelial cells secrete two other factors that influence vascular smooth muscle tone. Several workers have identified an *endothelium-derived hyperpolarizing factor* (EDHF) following ACh stimulation. Endothelium-derived hyperpolarizing factor is able to cause transient changes in the membrane potential of smooth muscle cells. The contribution of membrane hyperpolarization to endothelium-dependent relaxations induced by ACh was investigated in the femoral vein of the rat using a microelectrode technique.⁹⁵ Acetylcholine caused endothelium-dependent relaxations and hyperpolarization in tissue contracted by norepinephrine. The relaxation to prolonged administration of ACh was sustained mainly by the release of endogenous nitrovasodilator while the hyperpolarization slowly declined with time. It appears that ACh proceeds via activation of M-1 muscarinic receptors to effect EDRF release while M-2 muscarinic receptors leads to EDHF release.^{46,96,97} The chemical nature of EDHF is presently unknown.

Several experimental studies⁴¹ have now demonstrated the existence of an *endothelial-derived constricting factor* (EDCF). Endothelium-derived constricting factor can be released from endothelium in response to changes of pressure, rapid stretching, anoxia, electrical stimulation, large doses or repeated smaller doses of ACh and finally to arachidonic acid. In normal endothelium and in cultured endothelial cells, basal release of EDCF has been reported.⁹⁸⁻¹⁰⁰ One of the EDCF substances has been identified as a 21-amino-acid peptide that has been named *endothelin*.¹⁰¹⁻¹⁰³ Endothelin exists as a family of isoforms, ET-1 to ET-3.¹⁰⁴ Endothelin-1 is the only endothelin to be synthesized by the endothelial cells. Unlike EDRF and EDHF, endothelin has a longer-lasting vasoconstrictor

effect in most but not all vascular beds. It is tempting to associate a physiological role for endothelin in the control of basal vascular tone and in pathophysiological states but this must await further investigations. Certain pathophysiological states such as cardiogenic shock¹⁰⁵ and uraemia¹⁰⁶ show elevated plasma endothelin levels while cyclosporin,¹⁰⁷ transforming growth factor B,¹⁰⁶ thrombin¹⁰¹ and platelets¹⁰⁸ stimulate pre-proendothelin mRNA synthesis and actual release of endothelin from cultured cells. Endothelium-derived NO can interfere with endothelin production. Endothelin production is increased in pre-treated porcine aorta by an inhibitor of EDRF synthesis when stimulated by thrombin. The opposite response was seen when superoxide dismutase was added to the system. Indeed any agent causing an increased level of cGMP will inhibit endothelin synthesis.¹⁰⁰ Physical stimuli, such as increased shear-stress, caused suppression of endothelin gene expression and production.¹¹⁰ The potent endothelin-derived vasoconstriction may be antagonized by calcium channel blockers and/or by the lack of calcium in the extracellular medium.¹⁰

Presently, most authors are in agreement that circulating levels of endothelin are unlikely to affect systemic vasoconstriction but they do not rule out local vascular effects of endothelin. The endothelial cell has endothelin receptor located on the luminal side. Activation of these endothelial receptors brings about increase production of EDRF and/or PGI₂. Indeed, endothelin-1 can stimulate endothelial cells to secrete different vasodilating substances depending on the origin of the vascular endothelial cells.¹⁰⁹ For instance, endothelial cells derived from porcine coronary artery vasodilate in response to ET-1 challenge by releasing PGI₂ while endothelial cells from rabbit abdominal aorta exposed to ET-1 responded by secreting NO. Compared to EDRF, endothelin has no platelet inhibitory activity at physiological concentrations but at high pharmacological doses, endothelin can stimulate prostaglandin synthesis that can indirectly affect platelet activity.¹¹⁰

The endothelium has been implicated in the production of two other vasoconstricting substances: (1) a cyclooxygenase product (i.e., a prostanoid or a superoxide anion)¹¹¹ and (2) a non-prostanoid substance released during hypoxia.⁴

EDRF and anaesthetic agents

Anaesthetic inhalational agents may cause some degree of arterial and venous vasodilatation whose cause is multifactorial. Investigating the interaction of inhalational agents on endothelial function especially in the coronary circulation is pertinent. Blaise *et al.*¹¹² demonstrated that isoflurane (2.3%, 1.5 MAC) attenuated the induced contraction of canine coronary preparation. This attenu-

ation was endothelium-dependent. In the absence of endothelial cells, isoflurane had no effect on the serotonin and PGF₂ α -contracted coronary preparation but had a modest α -adrenergic inhibitory effect. Bollen *et al.*¹¹³ found that halothane produced more relaxation than isoflurane in constricted segments of porcine coronary artery. Muldoon *et al.*¹¹⁴ questioned whether halothane altered endothelium-mediated vasodilatation of vascular smooth muscle in different preparations. Their results indicated that halothane (2%) attenuated the ACh and bradykinin endothelium-mediated vasodilatation in carotid and aortic preparations, while the femoral artery preparation demonstrated intensification of the vasoconstriction. They postulated that halothane interfered with either the production, release or peripheral action of EDRF. Stone and Johns¹¹⁵ studied the effects of incremental doses (0.5–5%) of halothane, enflurane and isoflurane on isolated aortic vascular rat preparations. They examined intact and denuded vessels which were pre-contracted by phenylephrine with and without the addition of indomethacin. Isoflurane and enflurane at low concentrations caused vasoconstriction while at higher concentrations vasodilatation was evident in vessels with intact endothelium. Overall, halothane was not different from controls in intact vessels. After discontinuation of all three agents there was a rebound vasoconstriction above the baseline value which was worse if the vessels had been pre-treated with indomethacin suggesting that cyclooxygenase inhibition by indomethacin prevented the synthesis of prostanoid relaxing factor(s). In denuded rings, a dose-dependent relaxation with all three inhalational agents was seen, i.e., direct α -adrenergic inhibition, and incubation with indomethacin and/or removing the inhalational agent produced no change from the precontracted value. The authors concluded that: (1) at low concentrations, enflurane and isoflurane inhibited EDRF production and/or promoted the release of an endothelial constricting factor, and (2) at higher concentrations, direct action on smooth muscle relaxation predominated (α -adrenergic antagonist), and (3) that indomethacin inhibited the production of a prostanoid vasodilating agent from the endothelium.

The *in vivo* and *in vitro* evaluation of isoflurane and halothane on the effects of serotonin (vasoconstriction) on porcine coronary arteries was assessed by Witzeling *et al.*¹¹⁶ They concluded that: (1) for all concentrations of either volatile agent, a reduction in the induced vasoconstriction was seen; (2) the vasodilatation in intact vessels was greater; (3) serotonin stimulated serotonergic receptors on both endothelial and smooth muscle cells with an overall effect of vasoconstriction and (4) *in vivo*, both isoflurane and halothane, 1.5%, dilated constricted epicardial vessels. Greenblatt *et al.*¹¹⁷ in a complex experimental design examined the effect of isoflurane (1 MAC) and

halothane (1 MAC) in indomethacin-treated animals that also received an inhibitor of EDRF synthesis (L-NMMA). Thus, they were able to separate the contribution from EDRF-dependent vasodilatation from the vasodilatation caused by inhalational agents and from that due to endothelial prostanoid synthesis. They extended their study to include vascular rings from major vascular beds (coronary, renal, carotid, etc.). They found that isoflurane, compared with halothane, demonstrated a greater dependency on released endothelial relaxing factors for its haemodynamic action. Furthermore, they showed that EDRF-mediated tone is more prominent in coronary, renal, hepatic, splanchnic and cutaneous circulation during isoflurane anaesthesia. Their data may help to explain the different peripheral vascular responses of isoflurane compared with halothane.

Pettis *et al.*¹¹⁸ evaluated the effect of nitrous oxide on pigs' coronary arteries. Nitrous oxide (70%) decreased coronary artery diameter from control (70% nitrogen). They could not attribute the vasoconstricting effect of nitrous oxide to increased norepinephrine level, or endothelial-derived endothelin secretion. Nitrous oxide vasoconstriction was antagonized by calcium channel blockers, nitroprusside and partly by phentolamine. Inhalation of nitric oxide in awake lambs resulted in reduction of the pulmonary artery pressures and resistances during infusion of a thromboxane analogue U46619 (pulmonary vasoconstrictor) and during neutralization of heparin anticoagulation by protamine sulphate (release of thromboxane during neutralization).¹¹⁹ Inhalation of NO did not have any systemic effects. This finding may have therapeutic importance in treating the catastrophic pulmonary hypertension secondary to neutralization of heparin by protamine following extracorporeal circulation.

Recently, the role of some anaesthetic induction agents on the endothelial function has been evaluated. Propofol produced an endothelium-independent vasodilatation and may have a calcium channel blocking action.¹²⁰ Ozhan *et al.*¹²¹ compared propofol with thiopentone contractile responses in isolated coronary arteries of pigs. Propofol had an intrinsic non-endothelium-dependent vasodilatation while thiopentone caused slight vasoconstriction. Neither induction agent altered endothelium-dependent relaxation. Etomidate produced a mixed endothelium-dependent and independent vasodilatation.¹²² In several species, high doses of fentanyl were devoid of major effects on the coronary vasculature.¹²³ Local anaesthetics inhibited endothelium-dependent vasodilatation of isolated rat thoracic aorta.¹²⁴ The local anaesthetics appeared to exert their inhibitory effects distal to receptor activation since the non-receptor activation by calcium ionophore A23187 was also inhibited. The endothelium-independent vasodilator sodium nitroprusside dilated the aortic rings pre-treated

TABLE III Pathophysiological state with impaired endothelial function

Subarachnoid haemorrhage
Following angioplasty
Arterial and venous coronary artery bypass
Hypercholesterolaemia and lipid disorders
Coronary artery disease
Hypertension
Diabetes mellitus
Septic shock

with local anaesthetics indicating that the lack of vasodilatation with methacholine or calcium ionophore was not due to a smooth muscle cell dysfunction. Thus, it appears that local anaesthetics interfere in some way with the activation of soluble guanylate cyclase.

Pathophysiology

Normal arteries provide a constant defence mechanism against intravascular thrombosis and vasoconstriction. The vasoconstricting substances that are liberated from aggregating platelets may be offset by several endothelial-dependent vasodilating mechanisms leading to: (1) an increased flow to wash away and dilute the liberated substances, (2) an increased endothelial cell metabolism of those vasoactive substances, and (3) an anti-aggregation process by EDRF and PGI₂. It appears then that the vascular endothelium is a key element in maintaining a constant balance in vasomotor regulation and haemostasis.

In diseased arteries, this protective function is severely compromised (see Table III).

Subarachnoid haemorrhage

The cerebral artery vascular tone instability which occurs following subarachnoid haemorrhage is possibly related to the haemoglobin scavenging of EDRF.¹²⁵⁻¹²⁸ The experimental introduction of blood or haemoglobin into the subarachnoid space leads to vasoconstriction that persists for up to a week,⁵ correlating quite well with the clinical time course. The possibility also exists that local hypoxia may favour EDCF release over EDRF especially if there is an anatomical disruption of flow.¹²⁹

Angioplasty

Localized coronary spasm following percutaneous transluminal coronary angioplasty (PTCA) is frequent. Damage and functional impairment of the endothelial cells by the catheter contribute to the local vascular instability. Exposing the subendothelial structure enhances platelet aggregation and coronary spasm. Also, during the regeneration process the new endothelial cells are morphologically and functionally different. They show decreased receptor-mediated EDRF responsiveness,¹³⁰⁻¹³² increased reac-

tivity to vasoconstrictor agents and increased production of an endothelial-derived vasoconstricting superoxide anion.¹⁰⁹ Over time, these cells regain full function if no untoward event disturbs their maturation.⁵ Cartier *et al.*¹³³ demonstrated that the regenerated endothelium regained the ability to produce the contracting factor(s) first before producing the relaxing factor(s). Although these experiments were performed on rats, there is a striking cross-species homogeneity in the endothelium-dependent physiology. These findings may provide an explanation for the propensity for vasospasm in arteries undergoing repair.

Arterial and venous coronary artery bypass

The internal mammary arteries and saphenous veins are used extensively for myocardial revascularization and long-term patency is thought to be higher for internal mammary artery than for saphenous grafts.¹³⁴ Their morphological and biological characteristics may partly explain their different long-term patency.¹³⁴⁻¹³⁶ Damage to the endothelial layer, during saphenous vein harvesting, may contribute to early development of thrombosis. Platelets and an increased platelet-vessel wall interaction are important in vein graft occlusion.¹³⁷ Lüscher *et al.*¹³⁵ and Yang¹³⁸ demonstrated, for the internal mammary artery graft, a greater endothelium-dependent relaxation and procyclin production after ACh, ADP, and thrombin challenge than for saphenous vein grafts. In the latter, they also established in saphenous veins that the effects of nitric oxide are reduced by endothelium-derived contracting factors originating from the cyclooxygenase pathway. Thus, reduced production of EDRF and prostacyclin, morphological differences, and greater endothelial damage in venous grafts would be in keeping with a decreased clinical longevity. Anti-platelet therapy following coronary artery bypass improves long-term venous graft patency.^{137,139,140} Routine probing of the internal mammary artery prior to coronary anastomosis resulted in endothelial damage and impaired vasodilatation by decreased production of EDRF and prostacyclin.¹⁴¹ Lastly, the gastroepiploic artery has been used as a coronary bypass conduit in a few clinical studies. O'Neil *et al.*¹³⁶ demonstrated a higher release of vasoactive substances *in vitro* with the gastroepiploic artery than in the internal mammary and saphenous venous grafts which might influence the long-term patency of the gastroepiploic graft.

Hypercholesterolaemia and lipid disorders

Low-density lipoprotein (LDL) impairs EDRF activity. This impairment is probably mediated by oxidatively modified LDL (lipid peroxides and oxidized LDL). Increased plasma LDL activates macrophages favouring formation and deposition of oxidized LDL in the vascular wall.^{5,142-144} Interestingly, when arterial segments are perfused with oxidized-LDL, an increase in the vasocon-

strictive response to α -agonist was noticed and this response was worsened if the endothelium was removed.¹⁴⁵ This indicates that the endothelium is still able to secrete EDRF since the response is worsened in denuded arterial rings. One may speculate that the secreted EDRF is either neutralized by oxidized-LDL and/or scavenged by subendothelial macrophages (Figure 6). The increased subendothelial distance seen in atheromatosis does not appear to be a limiting factor for the diffusion of NO.² The use of calcium antagonists was only able to block the oxidized LDL vasoconstriction response in denuded arterial rings.²

Dietary-induced hypercholesterolaemia has shown consistently, in coronary arteries, an impairment of endothelial-dependent relaxation with preservation of other non-endothelial-dependent vasomotor responses, i.e., α -adrenergic response.¹⁴⁶⁻¹⁵⁴ Indeed in a primate model, this endothelial impairment is reversible with dietary manipulation.⁸² Patients with known coronary artery disease on a dietary programme to modify their lipoprotein profiles revealed angiographically improved endothelial-dependent dilatation.^{148,155}

Coronary artery disease (CAD)

In the absence of CAD, intracoronary administration of ACh causes vasodilatation. In contrast, patients with CAD and after a heart transplant respond with a coronary constriction after ACh challenge.¹⁵⁶⁻¹⁵⁸ In the CAD patients, the important flow-dependent coronary dilatation is substantially impaired but can be restored. The use of nitrovasodilators of different classes is well established for therapy of angina pectoris, myocardial infarction and congestive heart failure. The modulation by the endothelium to exogenous administration of nitrates has recently been investigated. The relaxing effects of nitrovasodilators are reduced in the presence of intact endothelium compared with preparations without endothelium. This reduction in vasodilatation is directly related to endothelial release of EDRF. Restoration of full nitrovasodilatation was only possible if the intact arterial rings were pre-incubated with the EDRF inhibitor L-NMMA or when the arterial rings were denuded of their endothelium.^{159,160} This finding may have clinical implications in the use of nitrovasodilators to preferentially dilate those vascular segments with dysfunctional endothelial cells (unstable angina, myocardial infarction and post-CABG). Finally, nitrates have been shown to be inhibitors of endothelin production.¹⁶¹

Hypertension

In chronic hypertension, there are several reports of a loss or a change in endothelium-mediated functions in the peripheral arteries of humans¹⁴⁶ and animals.^{134,162} The following factors were implicated in this endothelial

dysfunction: (1) decreased release of EDRF and/or other factors (cyclooxygenase products), (2) decreased smooth muscle cell response and (3) increased release of endothelial-derived contracting factor(s).^{145,160,163} In the genetically spontaneous hypertensive rat, EDRF release by ACh was normal but there was a simultaneous release of an endothelium-derived contracting substance(s).¹⁶⁴ In hypertensive Dahl rats, EDRF release was decreased and/or there was decreased smooth muscle cell response after ACh stimulation.¹⁶⁵ Thus, the endothelium-dependent relaxation is impaired in the majority of hypertensive animal models. But the underlying mechanisms are different for each species. Different antihypertensive therapies, in normal rats, potentiated the endothelial relaxation to ACh challenge.¹⁶⁵ Also, some antihypertensive agents modulated the endothelium-dependent relaxations to agonist more than others. These properties may show some therapeutic benefits in cardiovascular diseases.

Diabetes mellitus

Cardiovascular disease is a complicating feature in human diabetes mellitus. Some manifestations of cardiovascular deterioration in diabetes have been suggested to be a consequence of both morphological and functional derangements of vascular smooth muscles. Conflicting results have been reported with regard to endothelium-dependent relaxation in diabetic animals. Models of induced diabetes (streptozotocin-induced) and spontaneously diabetic rats demonstrated impaired endothelium-dependent relaxation.^{167,168} While no difference in endothelium-dependent peak relaxation could be detected in diabetic vessels from control, the endothelial-dependent relaxation response in diabetic vessels was more transient, i.e., returned faster to their baseline values.¹⁶⁹ The addition of superoxide dismutase to the preparation was able to normalize the time course. The authors suggested that the subendothelial compartment of diabetic rats must have produced more oxygen-derived free radicals that could inactivate EDRF.

Another consequence of hyperglycaemia is the increased glycosylation of the amino groups of protein. One of the targeted areas is the subendothelial collagen. Because matrix collagen turnover is slow, advanced glycosylation end products accumulate in diabetes mellitus. Recently Bucala *et al.*¹⁷⁰ demonstrated that these end products of glycosylation quenched EDRF. These results implicate advanced glycosylation products as important modulators of nitric oxide activity and endothelium-dependent relaxation.

Septic shock

In animals, agonist-induced NO released from vascular endothelium leads to peripheral vasodilatation, a decrease in blood pressure and tachycardia, but these effects are

short-lived and vascular tone returns to normal once the agonist is terminated. This agonist is able to activate the calcium-dependent NO synthase enzyme only for the duration of exposure. Recently cultured endothelial cells from porcine aorta express a second, distinct, inducible calcium-independent NO synthase in response to bacterial lipopolysaccharide endotoxin.¹⁷¹ Once induced, NO synthesis is released continuously for several hours without further stimulation. This inducible NO synthase is reported to be present also in activated macrophage.¹⁷²

Not only may cultured endothelial cells but also vascular rings incubated with endotoxin or cytokines lead to induction of NO synthase with progressive relaxation and a decreased vasoconstrictor response.^{173,174} Only L-NMMA can reverse the effects of endotoxin-induced NO synthase, thus establishing NO as the mediator of vasodilatation. Therapeutic intervention with the use of NO inhibitor has already been applied in two patients with severe septic shock.¹⁷⁵ The patients selected were those who had not responded to conventional therapy and were thought unlikely to survive. Intravenous administration of L-NMMA had an immediate (<2) but short-lived (<15 min) increase in systemic blood pressure.

Interestingly, previous incubation with glucocorticoids inhibited NO synthase induction by endotoxins and the resulting vascular relaxation. If glucocorticoids are added to the preparation after the induction of NO synthase by endotoxins, it will have no effect upon the continuous synthesis of NO.¹⁷¹

Thus, endotoxins or cytokines induce a distinct calcium-independent NO synthase which may explain the observed vasodilatation in endotoxaemia. Furthermore, the glucocorticoid findings may help explain the discrepancy concerning their clinical benefits when used in septic shock.

Conclusion

The endothelial cell should be regarded as a highly active metabolic and endocrine organ interacting with the circulating cells. Many pathophysiological conditions (atheromatosis, hypertension, balloon angioplasty causing denudation, and endothelial impairment following heart transplantation) interfere in this well orchestrated vascular balance. New knowledge of the underlying mechanisms in the endothelial-dependent vasomotor control gives us a greater understanding of the many pathophysiological processes and may help us to develop new therapeutic modalities yielding greater clinical benefit.

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