Brief Review

Mediators Produced by the Endothelial Cell

RYSZARD J. GRYGLEWSKI, REGINA M. BOTTING, AND JOHN R. VANE

SUMMARY This review discusses the role of three mediators, synthesized by vascular endothelial cells, that help to keep the surface of the normal endothelium nonthrombogenic. The first is prostacyclin, a product of arachidonic acid metabolism discovered in 1976. This labile prostanoid, with a half-life of approximately 3 minutes, relaxes vascular smooth muscle and inhibits the aggregation of blood platelets. Prostacyclin and its analogues are currently being tested clinically for use in cardiovascular diseases such as primary pulmonary hypertension. The second mediator discussed is endothelium-derived relaxing factor (EDRF), discovered in 1980, which also relaxes smooth muscle and inhibits the aggregation and adhesion of platelets. Substances that stimulate the release of EDRF include acetylcholine, bradykinin, and adenosine 5'-diphosphate. EDRF is even more labile than prostacyclin, with a half-life of about 6 seconds, and it has recently been identified as nitric oxide. Prostacyclin and EDRF are released together following stimulation of endothelial receptors and synergize to inhibit platelet aggregation. 13-Hydroxy-9,11-octadecadienoic acid, a third suggested mediator, is not released but acts from inside the cell to make the endothelial surface nonadhesive for circulating blood cells. It is proposed that these three mediators form the endothelial defense mechanism against blood-borne cells and chemicals and that breakdown of this barrier results in diseases such as hypertension and atherosclerosis. (Hypertension 12: 530-548, 1988)

KEY WORDS • prostacyclin • endothelium-derived relaxing factor • thromboresistance • 13-hydroxy-9,11-octadecadienoic acid • endothelin • 6-keto-prostaglandin E₁

EVER since William Harvey described the circulation of the blood, physiologists have realized that, "in sound and living vessels the blood remains fluid, but it coagulates in dead ones" (Ernst Brücke, 1857).¹ The vascular endothelium, which envelops the circulating blood in a continuous monolayer, is mainly responsible for this function as well as for the maintenance of the integrity and patency of vessels. These properties of endothelial cells have been linked with their capacity to synthesize a large number of active substances such as prostacyclin,² 13-hydroxy-9,11-octadecadienoic acid (13-HODE),³ 15-hydroxy-5,8,11,13-eicosatetraenoic acid (15-HETE),⁴ endothelium-derived relaxing factor (EDRF),⁵ von Willebrand factor,⁶ tissue-type plasminogen activator (t-PA),⁷ t-PA inhibitors,⁸ growth-promoting factors,⁹ platelet-activating factor (1-alkyl-2-acethyl-sn-glycero-3-phosphocholine),¹⁰ mucopolysaccharides,¹¹ type IV collagen,¹² a wide range of antioxidant enzymes,¹³ and others. An altered ability of the endothelial cells to make some of these factors (such as prostacyclin and EDRF) has been associated with cardiovascular diseases, including hypertension and atherosclerosis. A potent vasoconstrictor peptide, endothelin, has also recently been discovered, characterized, and synthesized.¹⁴

The production schedule of this complex biochemical factory is modulated by the interaction of the endothelium with blood cells or with plasma constituents in the vessel lumen and by the metabolic and functional state of the basement membrane and smooth muscle cells outside the endothelial lining. For instance, procedures that stimulate the generation of prostacyclin include the transfer of prostaglandin endoperoxides from platelets,¹⁵⁻¹⁶ or contact with activated leukocytes,¹⁷ or an increase in plasma levels of vasoactive peptides,¹⁸ or damage to or distention of the arterial wall. Some metabolic functions of the endothelium (especially seen in the pulmonary endothelium), such as conversion of angiotensin I to angiotensin II or inactivation of bradykinin and nucleotides, or taking up and metabolizing 5-hydroxytryptamine, catecholamines, and acetylcholine,¹⁹⁻²⁰ have also been associated with the concomitant secretion of prostacyclin¹⁸,²¹ or EDRF²¹⁻²³ (or both). Finally, oxygen tension in tissues⁵ has a strong influence on the biological functions of the endothelium; for example, hypoxia...
increases the levels of messenger RNA for endothelin production.\textsuperscript{14}

Such a complexity of systems makes it difficult to relate the importance of each of them to endothelial regulatory function. Three components will be reviewed here: prostacyclin, lipoxygenase products, and EDRF. All these products are more or less labile, and their generation, inactivation, and mechanism of action seem to be connected with the biological activities of oxygen free radicals.

Prostacyclin

The discoveries of thromboxane A\textsubscript{2} (made by platelets) and of prostacyclin (made by the vessel wall) have led to many important new concepts in vascular pathophysiology.\textsuperscript{24}

Prostacyclin is a dienoic bicyclic eicosanoid that derives from the membrane-bound fatty acid, all-cis 5,8,11,14-eicosatetraenoic acid (arachidonic acid, AA). The chemical instability of prostacyclin at physiological pH (1/2 \sim 3 minutes) is a result of the cleavage of its furan ring with subsequent formation of 6-keto-prostaglandin F\textsubscript{1a} (6-keto-PGF\textsubscript{1a}). Unlike prostacyclin, 6-keto-PGF\textsubscript{1a} and the products of its enzymic metabolism, such as 6,15-diketo-PGF\textsubscript{1a}, 13,14-dihydro-6-keto-PGF\textsubscript{1a}, and dinor-6-keto-PGF\textsubscript{1a}, are chemically stable and have little or none of the biological actions of prostacyclin.\textsuperscript{25, 26}

An exception is 6-keto-PGE\textsubscript{1}, which is a stable compound exhibiting many of the properties of prostacyclin,\textsuperscript{27, 28} although usually it is less potent. However, in causing renin release\textsuperscript{29} or fibrinolysis,\textsuperscript{30} it is more active. The possible importance of 6-keto-PGE\textsubscript{1} stems from the claim that, in vitro, prostacyclin is transformed to 6-keto-PGE\textsubscript{1} by the action of 9-hydroxydehydrogenase\textsuperscript{27} from broken cell preparations, such as those of kidneys,\textsuperscript{6-9} platelets,\textsuperscript{17} and blood cells.\textsuperscript{30, 31}

6-Keto-PGF\textsubscript{1a} is not a substrate for the enzyme in homogenates of dog brain, cultured endothelial cells, or fibroblasts.\textsuperscript{11, 12} There is some dispute as to whether whole platelets can generate 6-keto-PGE\textsubscript{1} from prostacyclin.\textsuperscript{32} Formation of 6-keto-PGE\textsubscript{1} has also been observed in isolated organs, such as the rabbit liver\textsuperscript{33} and rat lungs perfused with salt solutions.\textsuperscript{33}

There are difficulties in accepting that 6-keto-PGE\textsubscript{1} is an important member of the endogenous prostaglandin family. First, it has not always been rigorously identified. Second, most of the work has been in vitro, and third, its plasma levels did not increase after the intravenous infusion of prostacyclin.\textsuperscript{34}

Bioavailability of Prostacyclin

The biosynthesis of prostacyclin by endothelial cells is initiated either through a transmembrane transference of prostaglandin endoperoxides from platelets\textsuperscript{15, 16} or by intracellular generation from AA, which is liberated from endothelial phospholipids by an activated phospholipase (Figure 1). For instance, release of prostacyclin from porcine endothelial cells by bradykinin is accompanied by activation of both phospholipase A\textsubscript{2} and phospholipase C.\textsuperscript{35, 36} The latter leads to rapid production of inositol trisphosphate with a subsequent mobilization of cytosolic Ca\textsuperscript{2+} and liberation of AA. In addition, the stimulation of prostacyclin formation by adenosine 5'-triphosphate (ATP) is secondary to ATP-induced inositol phospholipid metabolism in adrenal medullary endothelial cells.\textsuperscript{37}

A heme-containing oxygenase (cyclooxygenase) that requires no external source of electrons both cyclizes AA and adds the 15-hydroperoxy group to form PGG\textsubscript{2}. The hydroperoxy group of PGG\textsubscript{2} is reduced to the hydroxy group of PGH\textsubscript{2} by a peroxidase that utilizes a wide variety of compounds to provide the requisite pair of electrons. Both enzymes are contained in a single 71-kilodalton protein.\textsuperscript{38} Cyclooxygenase is inhibited by aspirin and aspirinlike drugs,\textsuperscript{39, 40} and its action is modulated by lipid hydroperoxides, including PGG\textsubscript{2}.\textsuperscript{41}

PGG\textsubscript{2} peroxidase (sometimes called PGH\textsubscript{2} synthase), which is a part of the cyclooxygenase complex, can adjust the overall yield of the enzyme. This and other peroxidases maintain the concentrations of lipid peroxides that determine the activity of both cyclooxygenase\textsuperscript{41} and prostacyclin synthase.

\begin{center}
\textbf{FIGURE 1.} Metabolism of arachidonic acid (AA).
\end{center}

Prostacyclin and other prostaglandins are formed from AA via cyclic endoperoxide intermediates. Prostacyclin breaks down chemically to the stable 6-keto-PGF\textsubscript{1a}. Glucocorticosteroids induce synthesis of the protein lipocortin, which inhibits phospholipase A\textsubscript{2} and prevents liberation of AA from phospholipids. Aspirinlike drugs block cyclooxygenase and prevent prostacyclin generation. Hydroperoxyeicosatetraenoic acids (HPETEs) and hydroxyeicosatetraenoic acids (HETEs) are generated from AA by a number of lipoxygenase enzymes. One of these, 5-HPETE, is the precursor of the potent chemotactic and bronchoconstrictor leukotrienes (LTA\textsubscript{4}, LTB\textsubscript{4}, LTC\textsubscript{4}, and LTD\textsubscript{4}). TXB\textsubscript{2} = thromboxane B\textsubscript{2}.\textsuperscript{42}
The activity of cyclooxygenase is stimulated by lipid hydroperoxides at low concentrations (10^{-10}-10^{-9} M) and inhibited by higher concentrations (>10^{-6} M; Figure 2).

Salicylate, aspirin, and indomethacin have been reported to inhibit 12-hydroperoxyeicosatetraenoic acid (HPETE) peroxidase, but this has not been confirmed. Other cyclooxygenase inhibitors may also influence peroxidase activity in parallel with their main mechanism of action, perhaps leading to a further suppression of prostaglandin biosynthesis.

The reduction of hydroperoxy-lipids to hydroxy-lipids is associated with the generation of oxygen free radicals, most likely hydroxyl radicals. It may well be that inhibition of the isomerization of PGH_{2} to prostacyclin by lipid hydroperoxides and by other organic hydroperoxides is mediated by the oxygen free radicals that arise as a result of the decomposition of these hydroperoxides. Endothelial cells in culture spontaneously generate superoxide anion (O_{2}^{-}) as well as when they are treated with interleukin 1, γ-interferon, phorbol myristate acetate, calcium ionophore A23187, or menadione. This O_{2}^{-} may serve as an alternative source of hydroxyl radicals, which have been postulated to be endogenous inhibitors of prostacyclin synthase.

Any potential intracellular activity of oxygen free radicals is reduced by the antioxidant enzymes of endothelial cells such as superoxide dismutase.
vascular wall to synthesize prostacyclin and its metabolites by patients with severe atherosclerosis and metabolites of other nicotinic acid derivatives are mediated by synthase inhibitors, such as dazoxiben, also stimulate the formation of prostacyclin in vivo, since they enhance the transference of PGH2 from platelets to the vascular endothelium.

Pulsatile pressure also induces the release of prostacyclin in isolated arteries, leading to the suggestion that this is a stimulus for a continuous basal release. However, circulating concentrations of 6-keto-PGF1α are very low in humans, as are the normal urinary metabolites. One interpretation of this result is that the prostacyclin defense mechanism is only called into play at the site of injury to a vessel. Certainly, as with other prostaglandins, prostacyclin release is increased by damage to cells.

Inhibitors of Prostacyclin Release
Glucocorticosteroids and lipocortin, cyclooxygenase inhibitors, low density lipoproteins, loaded with lipid hydroperoxides, and, unexpectedly, vitamin K, all inhibit the biosynthesis of prostacyclin in endothelial cells. As mentioned earlier, vitamin K (menadione) stimulates the generation of O2•− in cultured endothelial cells. Low density lipoproteins contain oxidized lipids, that can generate oxygen free radicals, which may also inhibit the synthesis of prostacyclin by endothelial cells. It has been suggested that oxygen free radicals generated during the biosynthesis of prostaglandins are responsible for arteriolar damage following brachial injury. Exposure of cultured endothelial cells to ozone also reduced the synthesis of prostacyclin.

Like glucocorticosteroids and unlike 17β-estradiol, testosterone impedes the biosynthesis of prostacyclin in vascular cells. This might contribute to the higher risk of cardiovascular accidents seen in men than in women.

The capacity of vascular tissue to generate prostacyclin decreases in old and in atherosclerotic animals. Indeed, it has been postulated that there is a direct link between the ability of the vascular wall to synthesize prostacyclin and its susceptibility to thrombotic or atherosclerotic episodes. Paradoxically, excretion of prostacyclin metabolites by patients with severe atherosclerosis exceeded that by healthy volunteers, and synthesis of prostacyclin was actually increased in atherosclerotic rabbits. Vascular biosynthesis of prostacyclin does not seem to play a major role in the pathogenesis of hypertension. Hypertensive, salt-sensitive Dahl rats or patients with essential hypertension had abnormally high rates of synthesis, whereas spontaneously hypertensive rats either had low prostacyclin levels or showed no difference from normal. Prostacyclin synthesis can be low at the time immediately preceding development of hypertension, and this may contribute to its progress. The accelerated synthesis that parallels raised blood pressure may therefore be a compensatory adaptive mechanism in response to the hypertensive state.

Actions of Prostacyclin
The platelet-suppressant and vasodilator actions of prostacyclin are mediated by the stimulation of adenylyl cyclase in platelets and in vascular smooth muscle. In intact endothelial cells, prostacyclin also increases cyclic adenosine 3',5'-monophosphate (cAMP) levels, an effect that might influence the proliferation, permeability, and contractility of the endothelial lining.

The link between prostanooids and cyclic nucleotides in the vascular wall is extended by their activity on the enzymes that catalyze the metabolism of cholesteryl esters. Prostacyclin and 6-keto-PGE1 both increase cAMP levels in vascular smooth muscle, and, in parallel, these prostanooids enhance the activity of acid cholesteryl ester hydrolase, though they do not influence the activity of acyl-CoA/cholesterol O-acyl-transferase (EC 2.3.1.26). The latter, incidentally, is inhibited by PGE2. Thus, the combined actions of prostacyclin and PGE2 will trigger an outflow of free cholesterol from endothelial cells. This process is facilitated by the extracellular presence of a sterol carrier protein (e.g., high density lipoprotein apoproteins). It could well be that, in vivo, the prostacyclin and/or PGE2 generating system is responsible for clearing cholesteryl esters off the vascular wall, whereas an inhibition of cyclooxygenase and/or prostacyclin synthase by lipid hydroperoxides brings about an accumulation of cholesteryl esters in the vascular wall, formation of foam cells, and atherosclerosis.

Recently, Willis et al. found that prostacyclin and its analogues suppress the accumulation of cholesterol esters by macrophages and also suppress the release of growth factors from endothelial cells, macrophages, and platelets. The latter effect is seen at concentrations that are one tenth of those required to inhibit platelet aggregation. Indeed, prostacyclin inhibits release of platelet-derived growth factor from platelet α granules in preference to β-thromboglobulin and platelet factor 4. In addition, a decrease in the lipid content of vascular wall cells has been seen in humans after prostacyclin infusion. A
The fibrinolytic and cytoprotective properties of prostacyclin do not seem to be mediated by cAMP, and yet their impact on the therapeutic prospects of prostacyclin and its analogues is noteworthy. The first observation on the thrombolytic action of prostacyclin in dogs with pulmonary thromboembolism was followed by reports of shortening of the euglobulin clot lysis time in patients who had been treated with a continuous intravenous infusion of prostacyclin for arteriosclerosis obliterans or Raynaud’s phenomenon. The mechanism of the fibrinolytic activity of prostacyclin is not known, but it does not depend on the endothelium.

Although prostacyclin exhibits fibrinolytic action ex vivo, it shows no activity on euglobulin clots in vitro. Of 10 prostanooids investigated, only 6-keto-PGE\(_2\) had fibrinolytic activity in vitro. The thrombolytic action of prostacyclin may therefore be a result of inhibition of the binding of fibrinogen to platelets and the fibrinolytic action of a metabolite such as 6-keto-PGE\(_2\).

Even though the thrombolytic effect of prostacyclin is weaker and less general than that of streptokinase, prostacyclin potentiates streptokinase-induced thrombolysis in vivo. Indeed, human blood prostacyclin stimulates thrombolysis at concentrations that have no effect on hemostasis.

The vasodilator, platelet-suppressant, and thrombolytic properties of prostacyclin may all contribute to its beneficial therapeutic effect in patients with acute thrombotic episodes, such as retinal vein occlusion or sudden deafness of vascular origin. However, these properties of prostacyclin cannot account for its proven therapeutic efficacy in patients with chronic peripheral vascular disease associated with tissue ischemia, such as arteriosclerosis obliterans or Raynaud’s phenomenon.

Clinicians began to discuss an ill-defined metabolic protection by prostacyclin against ischemic injury of affected limbs. Later, they borrowed the term cytoprotection from André Robert, who coined it to describe the ability of several prostaglandins (including prostacyclin) to protect the gastric mucosa from damage by noxious agents. Recently, the term cellular protection has been proposed for the protective effects of prostacyclin in vitro and histoprotection for the protection of tissues in vivo. Indeed, in vitro, prostacyclin protects platelets against deterioration, cardiac myocytes against hypoxic damage, glial cells and neurons against anoxic injury, and hepatocytes against chemical damage, whereas in vivo in humans, intravenous infusion of prostacyclin for several hours on each of 4 days promotes the healing of ischemic skin ulcers. Prostacyclin also protects against postischemic reperfusion damage to animal brains and hearts. Like its thrombolytic effect, the histoprotective action of prostacyclin is intensified by heparin in a way that is not understood.

Among the possible mechanisms underlying the histoprotective action of prostacyclin, the most interesting proposal is that prostacyclin in some way neutralizes the damaging oxygen free radicals generated in the ischemic area on reperfusion. These free radicals may derive from several sources. Certainly, in the heart, neutrophils that infiltrate the ischemic myocardium may generate oxygen free radicals as a result of activation of their nicotinamide adenine dinucleotide phosphate (NADPH)-dependent membrane oxidase or their metabolism of AA. The ischemic myocardium itself can produce oxygen free radicals, either as a result of interconversion of xanthine dehydrogenase to xanthine oxidase with a concomitant massive decomposition of ATP to hypoxanthine or as a result of the depletion of ubiquinone 10 (coenzyme \(Q_0\), \(CoQ_0\)) from cardiac mitochondria.

Prostacyclin, ubiquinone, or superoxide dismutase protect against hypoxic or ischemic injury to mitochondria, cells in culture, perfused isolated heart, or infarcted myocardium in vivo. The protective action of ubiquinone may be achieved through coupling the nucleotide dehydrogenases with the cytochrome oxidase system in the inner mitochondrial membrane or, simply, by quenching superoxide anions, whereas superoxide dismutase enzymically destroys superoxide anions. One suggestion is that prostacyclin or its stable metabolites (e.g., 6-keto-PGE\(_2\), stable metabolite X, but not 6-keto-PGF\(_{1α}\)) act as a replacement for ubiquinone in the \(CoQ_{10}\)-deficient ischemic cardiac mitochondria.

Prostacyclin was first used clinically in 1979 to improve the circulation in patients with severe peripheral vascular disease, and since then many possible therapeutic uses have been tested. Intravenous infusion for 6 hours a day for 4 or more days seems to be the optimal regimen in the treatment of vascular deficiencies brought about by atherosclerosis or as a result of interconversion of xanthine dehydrogenase or their metabolism of AA. The ischemic myocardium itself can produce oxygen free radicals, either as a result of interconversion of xanthine dehydrogenase to xanthine oxidase with a concomitant massive decomposition of ATP to hypoxanthine or as a result of the depletion of ubiquinone 10 (coenzyme \(Q_0\), \(CoQ_0\)) from cardiac mitochondria.

Another area of potential therapeutic and diagnostic importance is in primary pulmonary hypertension. Studies of both children and adults with primary pulmonary hypertension show a more than 20% fall in pulmonary vascular resistance in approximately 70% of patients treated with intravenous prostacyclin. Pulmonary hypertension that results from mitral stenosis, chronic lung disease, congestive heart failure, or adult
Lipoxygenase Products

Hydroperoxides and Hydroxy-lipids

At the time of the discovery of prostacyclin, it was not known that endothelial cells produce lipid hydroperoxides, so the concept of potent inhibitors of prostacyclin synthase,42. 47. 56 An early report suggested that rabbit arterial walls generate an unknown hydroxyeicosatetraenoic acid (HETE). A few years later, 15-HPETE and 15-HETE along with 11-HETE were isolated from cultured vascular cells. Intracellular mechanisms other than activation of lipoxygenases may give rise to hydroperoxides (HPETEs) and the derived hydroxylipids (HETEs). Formation of lipid hydroperoxides could be initiated by hydroxyl radicals that oxidize lipids, by a cooxidation process linked to cyclooxygenase, or by a microsomal cytochrome P-450 oxidase. 12(R)-HETE, 19(S)-HETE, 19(R)-HETE, and 20-HETE have been identified as some AA-derived products of the last mentioned process in the cornea and kidney, and endothelial cells exhibit cytochrome P-450-dependent monoxygenase activity. The generation of 15-HPETE and 15-HETE by vascular cells was claimed to occur through cooxidation since the biosynthesis of hydroxy-lipids together with that of prostacyclin was inhibited by aspirin and indomethacin. On the other hand, there were claims that in human and calf vascular cells the generation of prostacyclin is only partially inhibited by indomethacin, if at all. In further studies, 12-HETE and other monohydroxylated derivatives of AA were isolated from human, rabbit, and rat blood vessels. The origin of hydroxylated lipids and the preferential routes for their oxygenation within the vascular wall remain an interesting area for investigation.

Some of these lipoxygenase products could be involved in cell adhesion and formation of new capillaries during angiogenesis. Pretreatment of cultured endothelial cells with nordihydroguaiaretic acid (NDGA) or eicosatetraynoic acid (ETYA) decreased adherence of neutrophils. Both of these substances inhibit lipoxygenase, but the selectivity (especially of NDGA) has been questioned. Also, both NDGA and ETYA, but not indomethacin, reduced proliferation and DNA synthesis of endothelial cells in culture. When endothelial cells are stimulated to produce prostacyclin, hydroxyeicosatetraenoic acids (mainly 15-HETE) are formed that intensify the thrombogenic properties of the endothelial surface. On the other hand, all of the products of lipoxygenation of arachidonic and linoleic acids (e.g., 15-HETE, 9-HODE, and 13-HODE) inhibit activation of rabbit platelets.

13-HODE

In 1985, Buchanan and his colleagues announced that monolayers of human cultured endothelial cells, in addition to the membrane-related production of prostacyclin, also synthesized a cytosol-associated lipoxygenase metabolite, which they called LOX. Inhibition of the generation of LOX (by 50 μM sodium salicylate, incubated for 30 minutes) stimulated the production of prostacyclin and, conversely, an inhibition of the biosynthesis of prostacyclin (by 50 μM aspirin, incubated for 5 minutes) increased the generation of LOX. Under either condition, platelet adhesion to cultured endothelial cells was suppressed. However, when endothelial cells were incubated with aspirin (50 μM for 30 minutes, with the assumed formation of salicylate), then the generation of prostacyclin and of LOX was substantially reduced and an enhancement of platelet adhesion to the endothelial cells was observed. Thus, it was suggested that endothelial cells generate a cyclooxygenase metabolite (prostacyclin) and a lipoxygenase metabolite, both of which have the property of increasing the thromboresistance of the endothelium.

In another paper by the same group 13-HODE was identified by high performance liquid chromatography and gas chromatography plus mass spectrometry as 13-HODE, a product of linoleic acid (18:2n-6). Unstimulated human endothelial cells contained more than 3 μg per 10^6 cells, and there was rather less in smooth muscle cells or fibroblasts. Concentrations of 13-HODE were decreased by thrombin or calcium ionophore, and none was detected after trypsin. When 13-HODE was bound to plastic, adhesion of platelets was decreased.

In a later paper, the concentrations of 13-HODE found in endothelial cells were many orders less (17 ng/10^6 cells) and these were suppressed by addition of 10 μM linoleic acid but stimulated by 25 or 50 μM linoleic acid. Exogenous 13-HODE had no effect on platelet binding to endothelial cells, leading to the suggestion that 13-HODE has an antiadhesive effect from inside the cell.

Much of this work remains to be confirmed and, in particular, it is important to show that increased production of 13-HODE stimulated by linoleic acid reduces platelet adhesion and to confirm the assumption that salicylate (but not aspirin) is a selective inhibitor of peroxidase activity, thereby preventing generation of 13-HODE.
The aforementioned in vitro data are in accordance with a reversal of the antithrombotic action of aspirin by salicylate and with the thrombogenic effects of high doses of aspirin in vivo. This concept, however, is difficult to reconcile with the unknown fate of 13-hydroperoxyoctadecadienoic acid (HPODE), which presumably accumulates inside endothelial cells following treatment with salicylate.

It is surprising that, unlike 15-HETE, 13-HODE is not released into the medium from cultured endothelial cells and its antiadhesive action is said to be exerted from inside the cell. In cultured endothelial cells, the release of prostacyclin is enhanced by thrombin, trypsin, and calcium ionophore A23187. However, all of these stimulators of phospholipases reduce rather than increase the generation of 13-HODE. The phosphatidylinositol in endothelial cells is poor in linoleic acid. Thus, phospholipase C also does not seem to supply the substrate for the biosynthesis of 13-HODE.

According to the hypothesis of Buchanan et al., a continuous turnover of intracellular triglycerides supplies cytosolic 15-lipoxygenase with its proper substrate (linoleic acid), and 13-HPODE and 13-HODE are formed. AA is not as good a substrate for endothelial 15-lipoxygenase as is linoleic acid; nevertheless, endothelial cells convert [3H]AA to 15-HETE. As discussed earlier, 15-HETE intensifies the thrombogenic properties of endothelial cells, but like 9-HODE and 13-HODE, it inhibits activation of rabbit platelets. The balance between lipoxygenase-derived eicosanoids and octadecanoids generated by endothelial cells depends on supplies of substrates and on whether the cell is activated or quiescent.

Exogenous 13-HODE has also been shown to increase prostacyclin production by fetal bovine aortic endothelial cells and to inhibit thrombin-induced thromboxane B2, with a concomitant increase in 12-HETE production in human platelets.

The 13-HODE story is further complicated by the observation that bovine aortic endothelial cells do not make 13-HODE. Rabbit endothelial cells do, but the concentration of extractable 13-HODE increases several hundredfold with time, an accumulation that is not inhibited by aspirin, salicylate, or known lipoxygenase inhibitors such as ETYA or NDGA. It is, however, inhibited by superoxide dismutase. This suggests that 13-HODE in these endothelial cells is a product of autoxidation.

Endothelial cells are not the only ones in the circulation that have the ability to generate 13-HODE. Porcine neutrophils contain a lipoxygenase that, in a partly purified form, converts linoleic acid (18:2n-6) to 13-HODE at a rate twice as high as it converts AA (20:4n-6) to 12-HETE or 5-HETE to 5,12-di-HETE. The biochemical characteristics of this neutrophil lipoxygenase are quite different from those of platelet 12-lipoxygenase. The biological role played by 13-HODE in neutrophils remains unknown.

In epithelial cells, linoleic acid, after its transformation to O-acyl ceramide and to other unusual very-long-chain unsaturated lipids, seems to act as a key factor in the maintenance of the water barrier in the epidermis. There is evidence that linoleic acid is carried by these lipids into the stratum compactum of the epidermis where linoleic acid is converted by lipoxygenases to a series of lipid hydroperoxides. It may be that the latter are responsible for the proper differentiation of the epidermal cells into an effective stratum compactum and a horny layer.

Clearly, endothelial and other cells have a varied capacity to produce prostacyclin, thromboxane A2, PGE2, PGD2, PGF2, 15-HETE, 12-HETE, 5-HETE, 13-HODE, various lipid hydroperoxides, lipid epoxides, leukotrienes, lipoxins, and perhaps 6-keto-PGF1α. This plethora of putative mediators, probably generated in a continuously fluctuating way, makes it more and more difficult to visualize a unified system. Can we identify the main characters within this crowd of biologically active lipids? The antiaggregatory and antiadhesive prostacyclin and perhaps 13-HODE, made by endothelial cells, set off the proaggregatory and adhesive thromboxane A2 and perhaps 12-HETE, made by platelets, form a scheme for a feasible and testable working hypothesis for the control of hemostasis in the circulation. However, lipid derivatives are only part of the story, as is seen in the next section.

Endothelium-Derived Relaxing Factor

Generation of EDRF

EDRF is a labile vasodilator with a half-life counted in seconds. The generation of EDRF by endothelial cells is unaffected by cyclooxygenase inhibitors such as aspirin and indomethacin. This allows for an easy differentiation between EDRF and prostacyclin.

EDRF was discovered by Furchgott and Zawadzki, who showed that the presence of the endothelial lining is obligatory for the vasorelaxant action of acetylcholine (ACH) on aortic and arterial strips or rings isolated from rabbits. We now know that when the endothelium is removed from vascular preparations of numerous species, ACh, at concentrations of 0.01 to 1.0 μM, has no vasorelaxant action and often evokes contraction of the naked vascular smooth muscle at high concentrations of 10 to 100 μM. Thus, the activation of muscarinic receptors on endothelial cells triggers the generation of a diffusible and transferable substance that relaxes vascular smooth muscle—hence, the name EDRF.

Other substances that release EDRF from endothelial cells include bradykinin, angiotensin II, histamine (via H1-type receptors), noradrenaline (via α2 adrenergic receptors), 5-hydroxy-
tryptamine, ergometrine, calcium ionophore A23187, thimerosal (a mercurial disinfectant), adenine nucleotides, thrombin, AA, melittin, and various peptides. Pulsatile pressure, visible light, and electrical field stimulation also release EDRF, and more of it is formed in arteries than in veins. Interestingly, most of these stimulants also release prostacyclin, an important point to which we shall be returning. In the arterial wall, prostacyclin is produced by both endothelial and smooth muscle cells whereas EDRF is formed only by endothelial cells.

Entry of extracellular calcium through calcium channels in the endothelial cell membrane does not seem to be part of the release mechanism. On the other hand, a rise of intracellular free calcium is probably a prerequisite for release. This may be linked to a sodium-calium exchange mechanism or a calcium-activated potassium channel. Intracellular events that link receptor stimulation to the release of EDRF include the activation of a guanosine 5'-triphosphate-regulated protein and the increased breakdown of a phosphoinositide. Diacylglycerol activation of protein kinase C is also involved since phorbol esters inhibit EDRF release.

In rabbit aortic strips, basal EDRF release was not affected by inhibitors of mitochondrial ATP generation, whereas stimulated release of EDRF was inhibited. Thus, the mechanism underlying basal production seems different from that for stimulated release.

**Actions of EDRF**

Vascular smooth muscle is the obvious target for the biological action of EDRF. However, EDRF also potently inhibits platelet aggregation and adhesion. These effects are mediated by an increase in intracellular cyclic guanosine 3',5'-monophosphate (cGMP). Adenylate cyclase is stimulated by β-adrenergic receptor agonists and by prostacyclin whereas the soluble guanylate cyclase of smooth muscle cells and platelets is stimulated by nitrovasodilators, including glyceryl trinitrate (GTN, nitroglycerin) and by EDRF.

At least two forms of phosphodiesterase are present in vascular smooth muscle to destroy cyclic nucleotides. One of them prefers cGMP to cAMP, is activated by a calcium/calmodulin complex, and is selectively inhibited by 2-o-propoxyphenyl-8-azopurine-6-one (M & B 22948). This phosphodiesterase inhibitor potentiates selectively GTN-induced and EDRF-induced relaxation of vascular smooth muscle strips and inhibition of platelet aggregation. This reinforces the idea that cGMP is the second messenger for the actions of EDRF and points to a similarity between the mode of action of EDRF and GTN.

That EDRF is a local chemical messenger was demonstrated by a "sandwich" arrangement of two rabbit aortic strips, one with endothelium and the other without. This showed a transfer of EDRF from the endothelium of the first strip to the smooth muscle of the second. Similarly, luminal perfusion of arteries with endothelium demonstrated transfer of EDRF to an endothelium-denuded arterial detector.

The endothelial lining of the preconstricted coronary arteries of isolated rabbit hearts generated EDRF in response to stimulation with ACh or with activated platelets. In dogs, EDRF appears to mediate the ACh-induced vasodilation of the femoral artery and of cerebral arterioles. In pentobarbital-anesthetized dogs, increased blood flow in the femoral artery caused flow-dependent dilation. This response was abolished by removal of the endothelial cells or perfusion with methylene blue, but not by indomethacin. Release of EDRF has also been demonstrated in the iliac and epigastric arteries of conscious dogs and in arteries on the brain surface of anesthetized mice. In rats, an increase in gastric mucosal blood flow induced by ACh or by vagal stimulation was said to be EDRF-mediated. These data obtained in vivo are in accord with the concept that endothelial production of EDRF provides the vascular wall with a shield against the vasospasm brought about by vasoconstrictors released from activated platelets.

Does EDRF (or the lack of it) play a role in cardiovascular disease? The aforementioned findings confirm the view that endothelial injury leading to a lack of EDRF may reverse the indirect vasodilator action to a direct vasoconstrictor action of a number of endogenous vasoactive mediators, even in arteries as small as 150 μm in diameter. Segments of human blood vessels, including coronary and pulmonary arteries as well as isolated perfused umbilical arteries and veins, release EDRF. Damage to the endothelium of pulmonary arteries could therefore lead to the loss of EDRF and the development of pulmonary hypertension.

Arterial strips from rats with genetic, renal, mineralocorticoid or coarctation hypertension, as well as from salt-sensitive Dahl rats, relaxed less effectively to ACh, A23187 and histamine. Rabbits, in which a ligature had been placed round the abdominal aorta, lost sensitivity to the relaxant effects of ACh and A23187 in their hypertensive thoracic but not their normotensive abdominal vessels. Coronary arteries of anesthetized dogs subjected to brief periods of hypertension contracted more to 5-hydroxytryptamine than those that had not been so treated. In all instances, relaxation responses to direct vasodilators were not affected even though hypertension caused some thickening of the intimal layer of the blood vessels.

Endothelium-dependent relaxation is impaired in arteries from hypercholesterolemic rabbits and monkeys and from atherosclerotic humans. In cholesterol-fed rabbits, the attenuation of EDRF-mediated responses occurred without any evidence of...
damage to the endothelial lining. Perfused hind limbs of cholesterol-fed rabbits in vivo showed reduced responses to ACh but not to the directly acting vasodilator sodium nitroprusside.\textsuperscript{245} EDRF-mediated responses of monkeys increased to normal when their atherogenic diet was replaced by standard feed.\textsuperscript{246} This change of diet also resulted in regression of the atherosclerosis. The vascular intimal layer remained abnormally thickened, so the reduced responses could not have been due to an increased diffusion distance between the endothelium and smooth muscle.\textsuperscript{246} It is interesting that endothelium-dependent relaxations of porcine coronary arteries could be facilitated by adding cod-liver oil to their diet.\textsuperscript{247} In humans, an intracoronal infusion of ACh resulted in vasodilation of healthy arteries and vasoconstriction of their stenotic atherosclerotic branches.\textsuperscript{226} Also, coronary arteries from patients who had undergone heart transplantation, which are prone to atherosclerosis, either failed to dilate or paradoxically constricted to ACh.\textsuperscript{248}

Reduced endothelium-dependent relaxations were again seen in isolated aortas from old rats\textsuperscript{249} and in pig coronary arteries made spastic by denudation of the endothelium 3 months previously.\textsuperscript{250} Even slight damage to the endothelium can affect the formation of EDRF, for pial arterioles of anesthetized cats injured by a combination of mercury light and fluorescein dye lost their relaxant responses to ACh and bradykinin.\textsuperscript{251} This type of damage reverses after an hour and the endothelium becomes responsive again.\textsuperscript{252} Reduced EDRF activity during subarachnoid hemorrhage may contribute to cerebral vasospasm, since cerebrospinal fluid from such patients\textsuperscript{253} inhibited relaxations of isolated dog arteries to A23187. This was due to contamination of the cerebrospinal fluid with hemoglobin, which blocks the activity of EDRF.

The \textit{nitrovasodilators} share nitric oxide (NO) as a final common pathway, for NO acts directly to activate the soluble guanylate cyclase of smooth muscle cells.\textsuperscript{254} NO can be liberated directly from organic nitrates in the presence of thiol such as cysteine.\textsuperscript{254} Formation of NO from sodium nitroprusside and from SIN-1 (a metabolite of molsidomine) occurs spontaneously by a thiol-independent mechanism.\textsuperscript{254} Methylene blue inhibits the activation of guanylate cyclase by NO and SIN-1, partially by GTN, but not by sodium nitroprusside.\textsuperscript{255}

In contrast, hemoglobin\textsuperscript{206} inhibits the vasorelaxant action of NO, SIN-1, GTN, and sodium nitroprusside. Hemoglobin is a direct inhibitor of the active ferroheme center of guanylate cyclase\textsuperscript{256} and also effectively removes NO from solution.\textsuperscript{257} The vasorelaxant actions of EDRF are antagonized by both methylene blue and hemoglobin.\textsuperscript{212} Since activation of guanylate cyclase is common to both GTN and EDRF, this justifies the use of the nick-name \textit{endogenous nitroglycerin} for EDRF. The similarity is further emphasized since tissues made tolerant to the relaxant effects of GTN fail to respond to EDRF.\textsuperscript{258} EDRF is one of the few endogenous mediators that exerts its physiological actions by activation of the soluble guanylate cyclase of smooth muscle cells.\textsuperscript{259} Removal of the endothelium results in a 70% reduction of smooth muscle cyclase activity\textsuperscript{260} together with some loss of reactivity to nitrovasodilators.\textsuperscript{261}

\textbf{Structure of EDRF}

The chemical structure of EDRF has been investigated intensely. The search was complicated by its short half-life and by the use of whole-artery preparations, which did not allow distinction between inhibition of the generation of EDRF, of its activity, or changes in its inactivation.

Many attempts have been made to implicate a metabolite of AA, either arising from cyclooxygenation\textsuperscript{186} or lipoxgenation,\textsuperscript{180} as well as the collection, separation, and analysis of the EDRF-containing effluent. Unfortunately, cells cultured on beads were generally insensitive to most EDRF releasers except bradykinin and ionophore A23187.\textsuperscript{21, 269}

\textbf{Superoxide dismutase} and \textbf{ferriytochrome c}\textsuperscript{274} reveal the spontaneous release of EDRF from endothelial cells, potentiate the stimulated release, and antagonize the inactivation of EDRF by ferrous ions. Since ferrous ions generate superoxide anions in oxygenated buffers,\textsuperscript{49} the simplest interpretation of the aforementioned results is that EDRF is destroyed by $O_2^-$ whereas scavengers of $O_2^-$ (superoxide dismutase and ferricytochrome c) protect EDRF from destruction. Many of the EDRF "inhibitors" that we listed may partially act by generating $O_2^-$, since their activity is considerably attenuated by superoxide dismutase or ferricytochrome c.\textsuperscript{274}

Some of these results and an analogy between the mechanisms of stimulation of guanylate cyclase by
EDRF\(^{21, 22}\) and GTN\(^{212}\) led Khan and Furchgott\(^{225}\) and Ignarro et al.\(^{224}\) to speculate that EDRF was NO.

Indeed, recent studies show that EDRF released from cultured porcine endothelial cells\(^ {277}\) is NO or a chemically related radical. EDRF released from perfused cultured endothelial cells by bradykinin\(^ {21}\) or NO gas dissolved in deoxygenated water both relaxed the bioassay cascade of vascular smooth muscle in a dose-dependent manner. The relaxation induced by EDRF or NO declined at the same rate (1½ ~ 4 seconds) during passage down the isolated tissue cascade.\(^ {272}\) The actions of both EDRF and NO were inhibited by hemoglobin (which binds NO)\(^ {257}\) and potentiated by superoxide dismutase.\(^ {273}\) Superoxide anions must, therefore, contribute to the inactivation of both substances.

Finally, NO was assayed chemically as the chemiluminescent product of its reaction with ozone. NO was released from porcine endothelial cells by bradykinin in amounts that accounted for the actions of EDRF, showing that this EDRF was identical to NO.\(^ {277}\) EDRF released from endothelial cells of bovine pulmonary artery and vein by ACh or bradykinin also behaved chemically like NO.\(^ {278}\) This EDRF produced identical shifts in absorption spectrum peaks to genuine NO during its reaction with hemoglobin.

EDRF from porcine aortic endothelial cells and NO gas have also been tested on platelet aggregation. The antiaggregatory activity of both\(^ {211}\) was potentiated by superoxide dismutase and the selective inhibitor of cGMP phosphodiesterase, M & B 22948, and inhibited by hemoglobin and Fe\(^ {2+}\). Moreover, there was synergism\(^ {279}\) between the antiaggregatory effect of prostacyclin and subthreshold concentrations of EDRF or NO. In addition to inhibiting human platelet aggregation, EDRF (NO) released from cultured bovine aortic endothelial cells by bradykinin reduced the adhesion of platelets to endothelial cells.\(^ {274, 275}\)

We have also used bovine aortic endothelial cells grown on microcarrier beads and perfused with Krebs-Ringer solution to investigate the release of EDRF.\(^ {270}\) EDRF was bioassayed on a cascade of four strips of rabbit aorta and prostacyclin by radioimmunoassay of 6-keto-PGF\(_1\alpha\). Our cultures of endothelial cells released EDRF and prostacyclin when stimulated with bradykinin and its congeners, adenosine 5'-diphosphate, ATP, AA, or phospholipase C. The release of both EDRF and prostacyclin was inhibited by phorbol myristate acetate, a diacylglycerol kinase inhibitor (R59022), or gentamycin. Our results lead to the important concept that the receptor-mediated release of EDRF and prostacyclin are coupled and the initial common step is activation of a phospholipase C.\(^ {270}\)

Our conclusion that activation of the same receptors ultimately leads to release of both EDRF and prostacyclin suggests that these substances act in concord as a common mechanism of defense for the endothelial cells. The synergism already shown between EDRF (NO) and prostacyclin\(^ {279}\) in preventing platelet activation might take on a new dimension if a similar synergism is demonstrated for other cells in the circulation. Interestingly, rat neutrophils generate a neutrophil-derived relaxing factor that relaxes smooth muscle,\(^ {280}\) and human neutrophils and mononuclear cells inhibit platelet aggregation by elaborating a factor indistinguishable from EDRF.\(^ {281}\) This factor even shows synergism with prostacyclin on platelet aggregation,\(^ {282}\) whereas there may not be synergism between the vasodilator effects of prostacyclin and EDRF on smooth muscle.\(^ {283}\)

The concept that EDRF and NO are identical has been challenged. One group\(^ {284}\) claims that NO, but not EDRF, relaxes guinea pig teniae coli, and another\(^ {285}\) that tracheal smooth muscle relaxes to NO but not to EDRF. Nevertheless, the results are not consistent and the first group was unable to demonstrate relaxations of guinea pig trachea to either NO or EDRF.\(^ {284}\) Clearly, EDRF is a potent vasorelaxant of arterial smooth muscle and may not reliably relax other types of muscle.\(^ {286, 287}\) In addition, it is said that EDRF can be absorbed on to an anion exchange resin and NO cannot.\(^ {286}\) Finally, the elaboration of more than one EDRF has been postulated.\(^ {289}\)

**Conclusions**

The relative importance of prostacyclin, EDRF, and 13-HODE for homeostasis at the blood-endothelium interface in vivo remains to be elucidated. At present, we can offer only a working hypothesis.

Normally, the vascular endothelium remains thromboreistant because of a continuous basal release of minute amounts of prostacyclin and EDRF, possibly in response to pulsatile pressure. The role of 13-HODE requires further investigation. This basal release of prostacyclin and EDRF can be supplemented by an increased coupled release through receptor activation by 5-hydroxytryptamine or adenine nucleotides discharged from activated platelets.\(^ {290}\) In addition, a direct transfer of prostaglandin endoperoxides from platelets to the endothelium amplifies the release of prostacyclin. Prostacyclin and EDRF encourage platelets and leukocytes from sticking to the endothelial lining of blood vessels. Besides preventing platelet aggregation, EDRF and prostacyclin cause vasodilation and oppose the effects of platelet-derived vasoconstrictors (Figure 3).

A further stimulation of platelets,\(^ {291}\) neutrophils\(^ {56, 292}\) and monocytes\(^ {293}\) leads to the generation of a burst of free radicals. Lipid peroxides, hydroxyl radicals, or superoxide anions\(^ {294}\) released by white cells onto the endothelial lining could overcome a prostacyclin-EDRF defense mechanism. This breakdown of the endothelial defense would be amplified by a lowering of the blood antioxidant barrier, elevation of oxidized low density lipoprotein plasma...
levels,285 or a hypertensive insult to the vascular wall.219 White cells would begin to adhere to the endothelium in the form of mural white thrombi.

Local vasoconstriction or stenosis will follow the pathological interaction of activated white cells with arterial segments deprived of the prostacyclin-EDRF protective mechanism. Thrombin formation will be initiated by platelet factors and by an interaction of plasma factors with subendothelial layers. Thus, a local deficiency in prostacyclin and EDRF may underlie atherogenesis and thrombogenesis, with a subsequent stenosis of coronary, cerebral, and peripheral arteries manifested by angina pectoris, myocardial infarction, stroke, and ischemic ulcers. These diseases may be ameliorated by a compensatory overproduction of prostacyclin, EDRF, or t-PA in healthy arterial segments adjacent to a vasospastic or stenotic region or by pharmacological agents that release the vasodilators prostacyclin and EDRF,300 and when given intravenously to the vasodilators of platelets (P) by raising platelet cAMP and cGMP levels simultaneously. They both also diffuse to the underlying smooth muscle (SM) and produce vasorelaxation by increasing cAMP and cGMP concentrations there. A basal release of EDRF would maintain high levels of cGMP in the SM and produce a constant state of relaxation.

We are aware of how hypothetical our description of these interactions among only three out of the many endothelial mediators is, especially since EDRF and 13-HODE are at such an early stage of exploration. However, the recent flood of data on the endothelium-dependent homeostatic mechanisms in the circulation has prompted us to try to bring some order into the growing amount of information.

Other Mediators

It is beyond the scope of this review to discuss factors such as platelet-derived growth factor, said to cause vasoconstriction,297 t-PA, and others. However, at the time of writing, another important mediator released by the endothelial cells, endothelin, has been discovered and characterized.14 Endothelin is a peptide of 21 amino acids, generated by endothelial cells through an unusual proteolytic processing. Levels of the messenger RNA that makes preproendothelin were increased by thrombin, calcium ionophore, and epinephrine—stimuli that also release the vasodilators prostacyclin and EDRF. The most striking property of endothelin, which is ten times more potent than angiotensin II as a vasoconstrictor, is the long duration of hypertensive action as demonstrated on rat blood pressure. After a single intravenous injection, the blood pressure was elevated for more than one hour. Moreover, the sensitivity of renal artery segments to endothelin was greater in spontaneously hypertensive rats than in Wistar-Kyoto rats.298 In a survey of the actions of endothelin on vascular smooth muscle, we299 found venous tissue to be more sensitive than arterial smooth muscle to endothelin.

Interestingly, endothelin also releases prostacyclin and EDRF,300 and when given intravenously to anesthetized rats, the pressor activity is strongly limited by the action of these vasodilators. Indeed, when the basal blood pressure is high, intravenous endothelin may only cause a fall in blood pressure. Furthermore, endothelin is removed in the pulmonary circulation in vivo and in vitro; about 60% disappeared in one passage through isolated guinea pig lungs.

Our working hypothesis is that endothelin is a local hormone, released by the endothelial cells to constrict the underlying smooth muscle. The pressor activity of any endothelin released luminally into the circulation will be strongly ameliorated by removal in the lungs and by release of the vasodilators prostacyclin and EDRF.

The discovery of endothelin clearly adds a new dimension to the role of endothelial cells in disease states such as atherosclerosis and hypertension.

References

2. Moncada S, Gryglewski R, Bunting S, Vane JR. An enzyme isolated from arteries transforms prostaglandin endoper-

2. Moncada S, Gryglewski R, Bunting S, Vane JR. An enzyme isolated from arteries transforms prostaglandin endoper-

Downloaded from https://hyper.ahajournals.org/ by guest on April 19, 2017
oxides to an unstable substance that inhibits platelet aggregation. Nature 1976;263:663–665
8. Van Mourik JA, Lawrence DA, Loskutoff DJ. Purification of an inhibitor of plasminogen activator (anti-activator) synthesized by endothelial cells. J Biol Chem 1984;259:14914–14921
42. Gryglewski RJ, Bunting S, Moncada S, Flower JR, Vane JR. Arterial walls are protected against deposition of platelet thrombi by a substance (prostaglandin X) which is unique from prostaglandin endoperoxides. Prostaglandins 1976;12:685–713
43. Siegel M, McConnel RT, Cuatrecasas P. Aspirin-like drugs interfere with arachidonate metabolism by inhibition of 12-hydroxyperoxy-5,8,20,14 eicosatetraenoic acid peroxidase activity of the lipoxigenase pathway. Proc Natl Acad Sci USA 1979;76:3774–3780
46. Sagone AL, Wells RM, De Mocko C. Evidence that OH production by human PMNs is related to prostaglandin metabolism. Inflammation 1980;4:66-71


55. Kappas A. Overview of enzyme systems involved in bio-reduction of drugs and in redox cycling. Biochimica et Biophysica Acta 1986;835:3-16


80. Marnett LJ. Peroxyl free radicals potential mediators of tumor initiation and promotion. Carcinogenesis (Lond) 1987;8:1365-1374


MEDIATORS FROM THE ENDOTHELIAL CELL/Gryglewski et al.

90. Chavy WC, Murata S, Nakano J, Orimo H. Age-related decrease in prostacyclin biosynthetic activity in rat aortic smooth muscle cells. Biochim Biophys Acta 1980;620:159-166
95. Sundar S. Prostacyclin in (extracted) plasma of essential hypertensives. Acta Cardiol 1987;42:135-139
130. Simpson PJ, Lucchesi BR. Myocardial ischaemia: The potential role of prostacyclin and its analogues. In: Gry-


175. Galton SA, Sneddon JM, Vane JR. The formation of 13-hydroxyoctadecadienoic acid (13-HODE) by endothelial cells (EC) from different species [Abstract]. Br J Pharmacol 1988;93:223P
186. Singer HA, Peach MJ. Endothelium-dependent relaxation of rabbit aorta: II. Inhibition of relaxation stimulated by methacholine and A 23187 with antagonists of arachidonic acid metabolism. J Pharmacol Exp Ther 1983;226:796–801
198. Piomelli D, Pinto A, Mullane K. The mechanism of release of endothelium-derived relaxing factor by acetylcholine [Abstract 79]. Hypertension 1986;8:934
205. Sneddon JM, Vane JR. Endothelium-derived relaxing factor reduces platelet adhesion to bovine endothelial cells. Proc Natl Acad Sci USA 1988;85:2800–2804


250. Yamamoto Y, Tomoihe K, Egashira K, Nakamura M. Attenuation of endothelium-related relaxation and enhanced responsiveness of vascular smooth muscle to histamine in...


261. Cox RH, Haas KS, Moisey DM. cGMP-mediated relaxations are chronically depressed in canine coronary arteries following endothelial denudation [Abstract]. Circulation 1987;75(suppl IV):IV-382


301. Antunes E, de Nucci G, Vane JR. Endothelin releases eicosanoids from and is removed by perfused lungs of the guinea pig [Abstract]. J Physiol (Lond) 1988 (in press)
Mediators produced by the endothelial cell.
R J Gryglewski, R M Botting and J R Vane

Hypertension. 1988;12:530-548
doi: 10.1161/01.HYP.12.6.530

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1988 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/12/6/530

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/