Brief Review

Endothelium-Derived Contracting Factors

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The endothelium not only mediates relaxation but is a source of contracting factors. Endothelium-dependent contractions are elicited by physical and chemical stimuli (i.e., hypoxia, pressure, and stretch) and autacoids, local and circulating hormones. The mechanism of endothelium-dependent contractions to hypoxia involves withdrawal of nitric oxide. The endothelial cyclooxygenase pathway can produce thromboxane A$_2$, prostaglandin H$_2$, and superoxide anions. The peptide endothelin is a potent contracting factor; its production is stimulated by vasopressor hormones, platelet-derived factors, coagulation products, and cytokines, whereas endothelium-derived nitric oxide, prostacyclin, and a smooth muscle cell-derived inhibitory factor reduce endothelin production. In hypertension, the release of cyclooxygenase-dependent endothelium-derived contracting factors to stretch, acetylcholine, and platelet-derived products is augmented. Vascular endothelin production in hypertension remains controversial but appears mostly normal; it is augmented in the presence of vascular disease or renal insufficiency. The endothelium-dependent inhibition of endothelin-induced contractions is reduced in hypertension while the reactivity of vascular smooth muscle may be normal, increased, or reduced. The potentiating effects of low concentrations of endothelin on contractions to norepinephrine are augmented with aging and hypertension. In atherosclerosis, the production of the cyclooxygenase-dependent endothelium-derived contracting factors and endothelin is enhanced. Thus, endothelium-derived contracting factors can profoundly affect vascular tone and counteract relaxing factors produced within the endothelium. In hypertension and atherosclerosis, the role of contracting factors appears to become more dominant, leading to an imbalance of endothelium-dependent vascular regulation. (Hypertension 1992;19:117-130)

Soon after endothelium-dependent relaxations were discovered, observations were reported that the presence of endothelial cells augmented rather than inhibited contractile responses of certain blood vessels (for review, see Reference 1). In particular, contractions of canine arteries during anoxia were found to be markedly reduced after removal of the endothelium.2–4 This was the first indication that the endothelium might also produce contracting factors under certain conditions. In the last 10 years, an increasing number of endothelium-derived contracting factors (EDCF) have been characterized, while others remain unidentified (Figure 1).1,5–7 A marked heterogeneity of these responses exists among species as well as among different vascular beds. This article reviews the physiology of EDCFs and their possible role in hypertension and atherosclerosis.1,5–7

Hypoxia-Induced Endothelium-Dependent Contractions

In isolated canine femoral, coronary, and cerebral arteries, hypoxia and anoxia augment contractile responses3,4,8; this response to anoxia is markedly reduced after removal of the endothelium. If isometric tension is recorded in a canine coronary artery without endothelium (bioassay tissue), this hypoxic facilitation of contractile responses can be transferred from a coronary vascular segment with endothelium (donor tissue) to this bioassay tissue.9 Indomethacin augments rather than reduces the hypoxic endothelium-dependent contractions, most likely because the hypoxia-induced release of vasodilating prostanoids (i.e., prostacyclin or prostaglandin E$_2$) partially blunts the response.9 The anoxic endothelium-dependent contractions are not blocked by inhibitors of phospholipase A$_2$ or lipoxigenase, which rules out a product of the metabolism of arachidonic acid as the mediator. Methylene blue (to block the production of cyclic 3':5'-guanosine monophosphate [cGMP]) and nitro-l-arginine (an inhibitor of endo-
FIGURE 1. Schematic diagram shows endothelium-derived contracting factors (EDCF) produced in the blood vessel wall. Cyclooxygenase pathway after stimulation by receptors (open circles) or physical forces can form thromboxane A2 (TXA2), prostaglandin H2 (PGH2), or superoxide radical (O2·−), which inactivate (©) nitric oxide (NO). Hormones such as angiotensin II (All), epinephrine, and arginine vasopressin (AVP) and coagulation factors such as thrombin (Thr) and transforming growth factor β (TGFβ) can stimulate the production of endothelin (ET). An endothelial renin-angiotensin system that provides locally formed angiotensin II also may exist. All, angiotensin II; AA, arachidonic acid; A23187, calcium ionophore; ACE, angiotensin converting enzyme; Ach, acetylcholine; ADP, adenosine diphosphate; ATG, angiotensin; NE, norepinephrine; 5-HT, serotonin (5-hydroxytryptamine). Reproduced from Reference 7, by permission of the American Heart Association.

Pressure-Induced Endothelium-Dependent Contractions

In the middle cerebral artery of the cat, increases in transmural pressure cause vascular smooth muscle cell depolarization and vasoconstriction, respectively.14-16 This pressure-induced vasoconstriction is endothelium-dependent. As the inhibitor of EDRF, hemoglobin does not affect the response, so it cannot be attributed to a reduction in the release of the factor from the endothelium in response to increasing transmural pressure. Thus, it appears likely that transmural pressure releases an EDCF; its nature remains to be determined. In the same preparation, electrical stimulation induces endothelium-dependent, tetrodotoxin-sensitive contractions, which suggests that fast Na+ channels are involved in the release of the EDCF.17 Under physiological conditions, the release of EDCF in response to pressure may contribute to autoregulation of blood flow.

Cyclooxygenase-Dependent Endothelium-Derived Contracting Factors

In a variety of blood vessels, endothelium-dependent contractions can be blocked by inhibitors of cyclooxygenase. This suggests that the cyclooxygenase pathway of endothelial cells is the source of the mediator (Figure 1).1,7 Depending on the agonist used, these endothelium-dependent contractions are or are not blocked by more specific synthetase or receptor antagonists of certain prostaglandins. This indicates that the cyclooxygenase pathway can produce several EDCFs.

Agonists Evoking Endothelium-Dependent Contractions

Endothelium-dependent contractions can be elicited by physical forces, precursor substances of cyclooxygenase products, and certain receptor-operated stimuli.

In the isolated canine basilar artery, rat aorta, and porcine ophthalmic artery, quick mechanical stretch induces a rapid increase in active tension in rings...
with endothelium, whereas in those without endothelium a slight decline in tension or only a weak contraction is observed.\textsuperscript{18–20} Stretch-induced endothelium-dependent contractions depend on influx of extracellular calcium since they are blocked by removal of extracellular Ca\textsuperscript{2+} or Ca\textsuperscript{2+} antagonists. The time course of the stretch-induced contractions, with a rapid onset followed by a steady state that lasts for several minutes, resembles that of autoregulatory responses observed in vivo after acute changes in transmural pressure. This suggests that they could potentially play a role in autoregulation of blood flow. Stretch-activated ion channels have indeed been identified in vascular endothelial cells.\textsuperscript{21} Such channels are permeable for Ca\textsuperscript{2+} and adapt their opening frequency to different levels of stretch and therefore may act as mechanotransducers allowing the endothelium to sense changes in mechanical forces exerted by the flowing blood.

Exogenous arachidonic acid induces endothelium-dependent contractions in isolated canine veins, the canine basilar artery, and the rabbit aorta that are blocked by indomethacin.\textsuperscript{3,4,22–26} Although acetylcholine is the classical agonist of endothelium-dependent relaxations in most blood vessels,\textsuperscript{1,27} in the aorta and the renal artery of the spontaneously hypertensive rat (SHR) in particular (see below) and in the basilar artery of the dog and rabbit, the muscarinic agonist induces endothelium-dependent contractions that are prevented by indomethacin.\textsuperscript{23,28–34} In the human saphenous vein, acetylcholine evokes endothelium-dependent contractions in the presence of \textit{N}G\textsuperscript{-}monomethyl L-arginine (LNMMA), suggesting that a cyclooxygenase-dependent EDCF is released concomitantly with nitric oxide thereby limiting the effects of the endogenous nitrovasodilator.\textsuperscript{35} In the basilar artery of the dog, norepinephrine, the Ca\textsuperscript{2+} ionophore A23187, and nicotine cause endothelium-dependent contractions that are prevented by inhibitors of cyclooxygenase.\textsuperscript{23,31,32} The fact that the Ca\textsuperscript{2+} ionophore A23187 evokes endothelium-dependent contractions suggests that the formation or release of EDCF involves a Ca\textsuperscript{2+}-dependent process.

\textbf{Mediators}

Thromboxane A\textsubscript{2} is the major vasoconstrictor product of cyclooxygenase; its formation from endoperoxides is catalyzed by the enzyme thromboxane synthetase.\textsuperscript{36} In the isolated pulmonary artery of the rabbit, thromboxane synthetase inhibitors reduce facilitation of the endothelium-dependent contractions to acetylcholine.\textsuperscript{28} In the human saphenous vein, CGS 13080 (a thromboxane synthetase inhibitor) potentiates endothelium-dependent relaxations to acetylcholine to a similar extent as indomethacin.\textsuperscript{35} In canine cerebral arteries, endothelium-dependent contractions to norepinephrine, nicotine, and the Ca\textsuperscript{2+} ionophore A23187 are blocked by the inhibitor of thromboxane synthetase dazoxiben.\textsuperscript{33,31,37,34,37}

The production of thromboxane A\textsubscript{2} cannot explain endothelium-dependent contractions evoked by arachidonic acid in canine veins or by acetylcholine and endoperoxides in cerebral arteries of the dog since inhibitors of thromboxane synthetase are ineffective.\textsuperscript{22,23,38} In the human saphenous vein, indomethacin as well as the thromboxane/endoperoxide receptor antagonist SQ46619 (but not the inhibitor of thromboxane synthetase CGS 13080) unmask endothelium-dependent relaxations to histamine.\textsuperscript{35} Thus, these differential effects of receptor antagonists versus synthetase inhibitors suggest that under these conditions the release of prostaglandin H\textsubscript{2} (which also activates the thromboxane receptor) rather than that of thromboxane A\textsubscript{2} is involved. The very short biological half-life of this prostanoid does not exclude its role as mediator of this response as nitric oxide (which has a half-life of a few seconds; for review see Reference 1) also is capable to act as potent vasodilator. Prostaglandin H\textsubscript{2} also appears to be the primary mediator of endothelium-dependent contractions to acetylcholine in the aorta of SHR (see below). Prostaglandin H\textsubscript{2} may be directly formed either from arachidonic acid\textsuperscript{39} or from 20-hydroxyeicosatetraenoic acid (20-HETE), a major cytochrome P450-dependent arachidonic acid product\textsuperscript{39,40} via cyclooxygenase.

Activation of cyclooxygenase not only leads to the production of prostaglandins but also generates superoxide anions.\textsuperscript{41} Superoxide anions evoke contractions of the canine basilar artery; superoxide dismutase, on the other hand, prevents endothelium-dependent contractions to the Ca\textsuperscript{2+} ionophore A23187 in this blood vessel. Superoxide anions therefore have been proposed as the cyclooxygenase-dependent EDCF produced in canine cerebral blood vessels.\textsuperscript{42}

Thus, depending on the agonist and blood vessel studied, activation of the endothelial cyclooxygenase pathway can lead to the formation of several EDCFs such as thromboxane A\textsubscript{2}, prostaglandin H\textsubscript{2} and superoxide anions. The release of EDCF in response to mechanical forces may participate in autoregulatory responses, particularly in the cerebral circulation. The physiological role of endothelium-dependent contractions in response to receptor-operated agonists remains to be defined.

\textbf{Endothelin}

Cultured endothelial cells of various species including humans produce and release the potent vasoconstrictor peptide endothelin.\textsuperscript{43–45}

\textbf{Production}

Three forms of endothelin have been characterized, endothelin-1 (formerly human or porcine endothelin), endothelin-2, and endothelin-3 (formerly rat endothelin); all isoforms contain 21 amino acids but differ in two and five residues, respectively.\textsuperscript{44–47} Endothelial cells appear to produce exclusively endothelin-1, whereas endothelin-3 may be a neural form of the peptide.

Endothelin is generated from precursor molecules (i.e., preproendothelin, a 203-amino acid peptide, \textit{p}roendothelin 1, which is cleaved to the biologically active endothelin-1 and a 34 amino acid fragment that is cleaved to the endothelin-3 precursor).\textsuperscript{48} The synthesis of endothelin is induced in response to hypoxic/ischemic stress and other stimuli that may induce cell injury, leading to intracellular calcium influx.\textsuperscript{49}

\textbf{Mediators}

Endothelin-1 is released in response to hypoxia and shear stress, and its receptor is coupled to the phosphatidylinositol pathway, which leads to an increase in intracellular calcium and activation of protein kinase C.\textsuperscript{50} Endothelin-1 also binds to the endothelin B receptor, which is coupled to the phosphatidylinositol pathway and leads to the release of prostacyclin and nitric oxide.\textsuperscript{51} The release of endothelin-1 is also prevented by inhibitors of cyclooxygenase and superoxide dismutase.\textsuperscript{52} Thus, the release of endothelin-1 is potently inhibited by cyclooxygenase and superoxide dismutase inhibitors, suggesting that it is a cyclooxygenase- and superoxide-dependent process.

\textbf{Activation}

Endothelin-1 binds to the endothelin B receptor, which is coupled to the phosphatidylinositol pathway and leads to the release of prostacyclin and nitric oxide.\textsuperscript{51} The release of endothelin-1 is also prevented by inhibitors of cyclooxygenase and superoxide dismutase.\textsuperscript{52} Thus, the release of endothelin-1 is potently inhibited by cyclooxygenase and superoxide dismutase inhibitors, suggesting that it is a cyclooxygenase- and superoxide-dependent process.

\textbf{Metabolism}

Endothelin-1 is degraded by endothelin-converting enzyme (ECE) and endothelin-degrading enzyme (EDE), which are both present in the endothelium.\textsuperscript{53} The ECE converts endothelin-1 to its biologically inactive form endothelin-3, while the EDE degrades endothelin-3 to its biologically inactive form endothelin-2.\textsuperscript{54} The metabolism of endothelin-1 is also influenced by the presence of other agents that may increase the intracellular calcium, such as calcium ionophores, angiotensin II, and bradykinin.\textsuperscript{55} Thus, the metabolism of endothelin-1 is influenced by the presence of other agents that may increase the intracellular calcium, such as calcium ionophores, angiotensin II, and bradykinin.

\textbf{Pharmacology}

Endothelin-binding proteins are present in the endothelial cell plasma membrane, which may play a role in the modulation of endothelin signaling.\textsuperscript{56} The binding of endothelin to its receptor induces the formation of arachidonic acid and the release of prostaglandins, which may contribute to the inhibition of endothelin signaling.\textsuperscript{57} The binding of endothelin to its receptor induces the formation of arachidonic acid and the release of prostaglandins, which may contribute to the inhibition of endothelin signaling.
and proendothelin or big endothelin, a peptide containing 92 amino acids (see below; Figure 2). Conversion of big endothelin-1 to the 21-residue peptide by the endothelin converting enzyme is essential for the expression of the full vascular activity. The enzyme can be blocked by metalloprotease inhibitors such as phosphoramidon and pepstatin A. The precursor molecule is not stored in intracellular granules. Indeed, protein synthetase inhibitors such as cycloheximide prevent the formation and release of endothelin from endothelial cells in culture and the intact porcine aorta. The preproendothelin messenger RNA (mRNA) is constitutively expressed in cultured endothelial cells and in the intima of intact blood vessels in situ. Human venous umbilical endothelial cells in culture spontaneously release the peptide in sufficient amounts to evoke potent contractions of isolated vascular tissue. In contrast, in intact arteries the amounts of the peptide released abuminally are much lower than those known to evoke vascular effects.

**Regulation of Production**

The expression of preproendothelin mRNA is stimulated by vasoressor hormones such as angiotensin II, and arginine vasopressin, substances derived from aggregating platelets such as transforming growth factor β (TGFβ), coagulation products such as thrombin, and cytokines such as interleukin-1 (Figures 1 and 2). The fact that the calcium ionophore A23187 and phorbol ester stimulate its production indicates that it involves activation of phospholipase C and increases intracellular Ca2+ within endothelial cells. In the guinea pig lung, thrombin not only induces the release of the peptide but also elicits a long-lasting pulmonary vasocostriction, suggesting that stimulation of endogenous endothelin production can indeed exert profound vascular effects.

In addition, the production of endothelin is regulated by inhibitory stimuli (Figure 2). In particular, endothelin-derived relaxing factors such as nitric oxide and prostacyclin reduce endothelin production (see below); circulating hormones such as atrial natriuretic peptide also reduce the formation of the peptide via a cGMP-dependent mechanism. In addition, vascular smooth muscle cells produce an inhibitory factor. Indeed, endothelial cells cultured with vascular smooth muscle cells produce much less endothelin than endothelial cells cultured alone. Since smooth muscle cell-conditioned media also exert this inhibitory effect, this strongly suggests that a vascular smooth muscle cell-derived inhibitory factor reduces endothelin production by endothelial cells. The chemical nature of the factor has not been identified yet.

In vivo, the circulating levels of endothelin are very low. The fact that only minimal amounts of circulating endothelin can be detected suggests that under normal conditions there is little activation of the production of the peptide (due to the absence of potent stimuli or due to effective inhibition of its production; or that endothelin is released preferentially toward the vascular smooth muscle rather than toward the lumen). Indeed, in endothelial cells in culture twice as much endothelin is released toward the abluminal than toward the luminal side. This strongly suggests that the circulating levels of endothelin do not reflect the local concentration of the peptide within the blood vessel wall and that the peptide has to be regarded as a local regulatory mechanism rather than a circulating hormone.

**Metabolism and Disposition**

Endothelin-1 injected intravenously remains stable for up to 1 hour in blood, suggesting that little or no enzymatic or spontaneous degradation of the peptide occurs. The decrease of circulating levels of endothelin from the plasma after intravenous injection therefore must be due mainly to removal of the peptide by parenchymal tissue. Indeed, after injection of radio-labeled endothelin into the left ventricle or the femoral vein of anesthetized rats, both endothelin-1 and endothelin-3 are rapidly removed from the circulation. The total radioactivity in the right atrial blood decays with a half-life of 7 minutes. After injection of the
peptide into the left ventricle of anesthetized rats, two thirds is removed within the first minute.\textsuperscript{50} The removal of endothelin-1 occurs somewhat faster than that of endothelin-3. The highest uptake of the peptide occurs in the lung, kidney, and liver, suggesting that these organs possess a high density of binding sites for endothelin and thus play an important role in clearing the peptide from the circulation.\textsuperscript{59–71}

**Effects on Vascular Smooth Muscle**

Endothelin-1 is a potent vasoconstrictor both in vitro and in vivo.\textsuperscript{43,44,72–83} When infused in vivo in experimental animals and in humans, it evokes long-lasting decreases in blood flow and increases in blood pressure, respectively. Similarly, in isolated blood vessels the contractions induced by endothelin are long lasting and difficult to wash out presumably because the peptide binds very tightly to its receptor. In perfused arteries, the vasoconstrictor effects of endothelin-1 are more pronounced with extraluminal than with intraluminal application of the peptide (see below).\textsuperscript{84,85} Of the three endothelins, endothelin-2 is the most potent vasoconstrictor, followed by endothelin-1 and endothelin-3.\textsuperscript{4,45}

Low and threshold concentrations of endothelin-1, which by themselves exert no significant contraction, can potentiate the effects of other vasoconstrictor hormones. In perfused mesenteric resistance arteries of the rat, endothelin-1 amplifies contractions evoked by exogenous norepinephrine or that released from adrenergic nerve endings.\textsuperscript{86} Similar observations have been made with clonidine in the aorta of the rat,\textsuperscript{87} norepinephrine in the human internal mammary artery, and with serotonin in the internal mammary and coronary artery.\textsuperscript{88}

The possibility that endogenous endothelin produced in situ within the blood vessel wall does indeed contribute to the regulation of vascular tone in vivo is supported by the fact that phosphoramidon (which inhibits the conversion of proendothelin into endothelin-1) blocks the pressor activity of proendothelin\textsuperscript{89} and lowers blood pressure when infused intravenously in intact rat.\textsuperscript{90}

**Mechanism of Action**

The structural similarity with neurotoxins (apamin, \(\alpha\)-scorpion toxin) originally suggested that endothelin directly activates voltage-operated \(\text{Ca}^{2+}\) channels.\textsuperscript{44,91,92} However, the binding of endothelin to the vascular smooth muscle membrane is not affected by \(\text{Ca}^{2+}\) antagonists\textsuperscript{89}; thus, the peptide binds to specific receptors on the cell membrane of vascular smooth muscle\textsuperscript{93–104} and cannot be considered as a natural agonist at voltage-dependent \(\text{Ca}^{2+}\) channels. Indeed, two distinct G protein–coupled receptors for endothelin have been cloned recently, one that shows high specificity for endothelin-1 and most likely represents the vascular smooth muscle receptor (\(\text{ET}_1\) receptor\textsuperscript{105}) and one that equally binds all isoforms of the peptide, is not expressed in vascular smooth muscle cells, and most likely represents the receptor present on endothelial cells (\(\text{ET}_\text{B}\) receptor) responsible for the release of prostacyclin and EDRF (see below) (Figure 2).\textsuperscript{106,107}

In certain vascular smooth muscle, such as the porcine coronary artery, the peptide indirectly, via a G protein, activates voltage-operated \(\text{Ca}^{2+}\) channels and in turn evokes the influx of extracellular \(\text{Ca}^{2+}\).\textsuperscript{108} Similar mechanisms might be operative in the human forearm circulation where the contraction induced by endothelin-1 is prevented by calcium antagonists.\textsuperscript{81} The potentiating effects of low concentrations of endothelin-1 are also sensitive to \(\text{Ca}^{2+}\) antagonists and may be related to an increased influx of extracellular \(\text{Ca}^{2+}\) through voltage-operated \(\text{Ca}^{2+}\) channels or an increased sensitivity to extracellular \(\text{Ca}^{2+}\).\textsuperscript{88}

In cultured vascular smooth muscle and glomerular mesangial cells after binding to its receptors, endothelin-1 activates phospholipase C and in turn leads to the formation of inositol trisphosphate (\(\text{IP}_3\)) and diacylglycerol.\textsuperscript{109–113} The increase in \(\text{IP}_3\) is more pronounced and lasts longer than with other vasoconstrictor hormones. Endothelin also evokes a rapid and in part transient increase in cytosolic \(\text{Ca}^{2+}\) that is associated with a rapid efflux of \(\text{Ca}^{2+}\), whereas the influx of the cation remains unaffected.\textsuperscript{113,114} Thus, the rise in cytosolic \(\text{Ca}^{2+}\) induced by the peptide is mainly derived from intracellular stores due to the formation of \(\text{IP}_3\). In line with this interpretation, in the human internal mammary artery, removal of extracellular \(\text{Ca}^{2+}\) only slightly reduces endothelin-induced contractions, whereas removal of intracellular \(\text{Ca}^{2+}\) markedly depresses the response.\textsuperscript{115} This also may explain why \(\text{Ca}^{2+}\) antagonists of different classes do not prevent endothelin-induced contractions and only partially reverse the response if added after the contraction has fully developed.\textsuperscript{115}

Endothelin affects the membrane potential of vascular smooth muscle cells, particularly in veins.\textsuperscript{80} Activation of \(\text{Ca}^{2+}\)-sensitive \(K^+\) channels by the peptide provokes a transient hyperpolarization followed by a sustained depolarization due to the opening of nonspecific cation channels permeable to \(\text{Ca}^{2+}\) and \(\text{Mg}^{2+}\).\textsuperscript{113} The depolarization leads to an activation of L-type \(\text{Ca}^{2+}\) channels, which may explain why \(\text{Ca}^{2+}\) antagonists are more effective in reversing than in preventing endothelin-induced contractions.\textsuperscript{80,113–115}

**Endothelin Derived From Vascular Smooth Muscle Cells**

Although in the intact porcine aorta immunoreactive endothelin can no longer be detected after removal of the endothelium,\textsuperscript{55,56} vascular smooth muscle cells in culture obtained from blood vessels of experimental animals as well as of humans produce significant amounts of the peptide after stimulation with various agonists.\textsuperscript{116} The biological significance of this phenomenon remains to be determined; it is possible, however, that vascular smooth muscle cells in atherosclerotic plaques, which also undergo a change in phenotype, produce and release endothelin. The increased local levels of the peptide may contribute to proliferative
responses as well as to the enhanced contractility typical of the atherosclerotic blood vessel wall.

**Interactions With Endothelium-Derived Relaxing Factors**

Endothelin can interact with the release and action of EDRF and prostacyclin, and the latter can interfere with the production and effects of the former.

In intact animals and in the human forearm circulation studied in vivo, endothelin causes a transient vasodilatation that precedes its pressor effect.44,81,117,118 This vasodilator effect of endothelin in the human forearm circulation can be observed at each concentration tested (i.e., 0.5–50 ng/ml/100 ml forearm tissue) of the peptide, but it is transient and followed by sustained contractions at higher concentrations.81 Indeed, in the presence of a Ca2+ antagonist, endothelin-1 only causes vasodilatation at any concentration.81 During intravenous infusion of endothelin in the intact organism and after exposure of cultured endothelial cells to the peptide, the formation of prostaglandins is stimulated.119 Indomethacin, on the other hand, augments the pressor effects of the peptide in the rabbit.120 In cultured human vascular smooth muscle cells, endothelin-1 activates phospholipase A2 and in turn the metabolism of arachidonic acid.121 In perfused mesenteric resistance arteries from rats, the vasodilator effects of endothelin-1 are prevented by indomethacin but not LNMMMA, indicating that endothelin-derived prostacyclin mediates the response.85

Endothelin-1 also can release EDRF and cause endothelin-dependent relaxations.122,123 Conversely, endothelin-derived nitric oxide can affect the release of endothelin from the intima of the intact porcine aorta (Figure 2).55 Indeed, LNMMMA (to inhibit nitric oxide formation) or methylene blue (to inhibit soluble guanylate cyclase) both augment the thrombin-induced production of endothelin.55,60 Conversely, the stable analogue of cGMP 8-bromo cGMP as well as superoxide dismutase (to inhibit the breakdown of endothelin-derived nitric oxide), various nitrovasodilators, and atrial natriuretic peptide prevent the thrombin-induced rise in endothelin release.55,60,61,124 This strongly suggests that endothelium-derived nitric oxide released concomitantly after stimulation with thrombin exerts a negative feedback on endothelin production via a cGMP-dependent mechanism. Although some authors have also demonstrated cGMP-dependent regulation of endothelin production in endothelial cells in culture,60,61 others were unable to observe this effect under culture conditions (Reference 125 and R. Busse, personal communication, 1990). Most likely, during the culture process endothelial cells can lose this regulatory mechanism of the production of endothelin. Since the activity of soluble guanylate cyclase is maintained,122 the defect must involve a decreased expression of cGMP-dependent protein kinase as demonstrated in vascular smooth muscle cells in culture.126 Prostacyclin, via the formation of cyclic 3',5'-adenosine monophosphate (cAMP) within the endothelium, also can inhibit the vascular production of endothelin.53 Since the peptide stimulates the production of both prostacyclin and EDRF, this appears to represent a negative feedback mechanism of endothelin production (Figure 2). The impaired formation of EDRF and prostacyclin in hypertensive and atherosclerotic vascular disease may in part explain the enhanced endothelin production occurring under these conditions (see below).

In conduit arteries of various species, including those obtained from humans, acetylcholine as well as nitrovasodilators such as nitric oxide, glycyl dintrate, sodium nitroprusside, and 3-morpholino sydnonimine (SIN-1) (the active metabolite of molsidomine) fully reverse contractions induced by endothelin at the level of vascular smooth muscle.44,45,77,80 Similarly, in isolated mesenteric resistance arteries of the rat, EDRF and SIN-1 both inhibit endothelin-induced contractions.85,127 In contrast, in the human forearm circulation, acetylcholine and sodium nitroprusside do not prevent endothelin-induced contractions, whereas Ca2+ antagonists of various classes are most effective.81

**Effects on Humoral Systems**

In vivo, the release of atrial natriuretic peptide induced by endothelin also may contribute to the decrease in blood pressure observed when the peptide is infused intravenously.128,129 By contrast, endothelin-1 inhibits the basal and stimulated release of renin from isolated glomeruli of the rat.130,131 In the intact dog, however, this direct inhibitory effect of the peptide on renin production is overrun by a pronounced indirect stimulatory effect due to the marked decrease in renal blood flow, at least with high circulating levels of endothelin.72 Under the latter conditions, endothelin also increases the circulating levels of aldosterone and vasopressin.72 In normal humans, intravenous infusion of smaller amounts of endothelin-1, however, increase blood pressure and serum potassium and lower serum sodium in the absence of significant changes in the renin, aldosterone, and atrial natriuretic peptide.132

**Renal Effects**

In the renal circulation, exogenous endothelin-1 causes profound decreases in renal blood flow and glomerular filtration rate (for review see Reference 133)72,134,135 and also may be formed within the kidney, in particular within the inner medulla.136,137 In addition, as judged from rat mesangial cells in culture, the peptide also may act as a mitogen.111 When infused in vivo, endothelin appears to exert on renal arterioles profound contractile effects that precede its hypertensive action.44 Thus, endothelin may take part in the regulation of blood pressure through its renal effect.

Thus, endothelin exerts multiple effects in the circulation that may profoundly affect blood pressure and tissue perfusion. Physiologically, it appears that relatively little endothelin is produced; the precise physiological role of the peptide pro-
duced within the blood vessel wall awaits experiments with specific inhibitors of the production and/or action of endothelin.

**Putative Endothelium-Derived Contracting Factors**

Endothelial cells are the major site of conversion of angiotensin I to angiotensin II in the circulation (e.g., References 138 and 139). Moreover, cultured bovine endothelial cells contain a prorenin-activating enzyme, reninlike activity, and immunoreactive angiotensinogen, angiotensin I, II, and III.140-142 If endothelial cells were to produce enough angiotensin II locally and to release it toward the underlying smooth muscle, the peptide could function as an EDCF. Of particular interest in this context is the fact that angiotensin II stimulates the expression of endothelin RNA in both endothelial cells obtained from large conduit arteries and resistance vessels.45,143

With electron microscope immunocytochemical studies, serotonin can be detected in some endothelial cells in femoral and mesenteric arteries of the rat.144,145 Bovine aortic endothelial cells contain a decarboxylase that catalyzes the formation of histamine from histidine and thus are capable of producing histamine.146,147 At least in certain blood vessels, the vasoconstrictor effect of histamine and serotonin on vascular smooth muscle is partially or fully inhibited by the concomitant release of EDRF (for review see Reference 1). Lipoxygenase and cytochrome P450 products can evoke contractions of vascular smooth muscle, and endothelial cells are capable of forming such arachidonic acid products.39,40,148 The putative role of all these substances as EDCFs remains to be elucidated.

**Endothelium-Derived Contracting Factors in Hypertension**

**Cyclooxygenase-Dependent Endothelium-Derived Contracting Factors**

The endothelium-dependent component of stretch-induced contractions in the aorta of the rat is augmented in aortas obtained from deoxycorticosterone acetate (DOCA) hypertensive rats,19 suggesting that hypertension promotes stretch-induced endothelium-dependent contractions.

Acetylcholine causes endothelium-dependent contractions in the aorta of the adult SHR but not in that of normotensive Wistar-Kyoto (WKY) rats of the same age.30 In old rats (12 months of age), when the contractions become most pronounced in the hypertensive animals, acetylcholine does induce endothelium-dependent contractions in normotensive rats, suggesting that the response reflects the premature aging of the hypertensive blood vessel wall.149 The endothelium-dependent contractions to acetylcholine are noted with higher concentrations of the muscarinic agonist than those required to release endothelium-derived nitric oxide.20 The response can be prevented by inhibitors of phospholipase A2 and cyclooxygenase, whereas inhibitors of prostacyclin and thromboxane synthetase are ineffective. In contrast, thromboxane A2/endoperoxide receptor antagonists prevent the endothelium-dependent contractions to acetylcholine in the aorta of the SHR, indicating that prostaglandin H2 (PGH2), while the changes in the production and action of endothelin-1 (ET-1) are controversial. The concomitant reduced formation and responsiveness of vascular smooth muscle to endothelin-derived nitric oxide (NO) leads to an imbalance between NO and EDCF, which may contribute to the increased peripheral vascular resistance and complications of hypertension. 5-HT, serotonin (5-hydroxytryptamine); Ach, acetylcholine; L-arg, L-arginine; AA, arachidonic acid; cGMP, cyclic GMP.

**FIGURE 3.** Schematic diagram shows endothelium-derived contracting factors (EDCF) in hypertension. Hypertension is associated with an increased formation of prostaglandin H2 (PGH2), while the changes in the production and action of endothelin-1 (ET-1) are controversial. The concomitant reduced formation and responsiveness of vascular smooth muscle to endothelin-derived nitric oxide (NO) leads to an imbalance between NO and EDCF, which may contribute to the increased peripheral vascular resistance and complications of hypertension. 5-HT, serotonin (5-hydroxytryptamine); Ach, acetylcholine; L-arg, L-arginine; AA, arachidonic acid; cGMP, cyclic GMP.

In the aorta of the SHR, the contractions evoked by aggregating platelets and serotonin also are enhanced in rings with but not in those without endothelium.153 The endothelium-dependent component of the contractions to serotonin can be prevented by indomethacin.154 Similarly, serotonin increases coronary flow and dilates cerebral blood vessels of normotensive rats but decreases coronary flow and constricts cerebral blood vessels of the SHR, an effect that also is inhibitable by indomethacin.155-157 In the cerebral circulation of hypertensive rats, the attenuated vasodilator effects of adenosine diphosphate also are normalized by indomethacin.156,157 These results are consistent with the concept that in hyper-
tension, an EDCF, most likely prostaglandin H$_2$, is released in response to platelet-derived products, which offsets the effects of EDRF. These altered responses to platelet-derived products may contribute to the increased peripheral vascular resistance and/or to the vascular complications of hypertension such as ischemic stroke, myocardial infarction, and peripheral vascular disease.

**Endothelin**

The circulating levels of endothelin appear decreased in rats with spontaneous hypertension and unaltered in rats with DOCA-salt hypertension. In patients, some studies also found normal circulating levels of the peptide, but others reported increased levels of the peptide in systemic or pulmonary hypertension (Table 1). The plasma levels of endothelin appear to be positively correlated with blood pressure in normotensive subjects but negatively correlated in hypertensive subjects. Since most forms of vascular disease as well as congestive heart failure and renal insufficiency are associated with increased circulating levels of endothelin, these discrepancies may, at least in part, be related to the presence or absence of these conditions in hypertensive patients. Alternatively, a subgroup of endothelin-dependent hypertension may exist. In both normotensive and hypertensive subjects, the circulating endothelin levels increase with age. As the peptide appears to be released preferentially toward the abluminal side of the endothelium, measurements of circulating endothelin may not necessarily reflect its contribution to the increased peripheral vascular resistance occurring in hypertension. Furthermore, local endothelin production may differ considerably within the body; indeed, in the SHR tissue endothelin levels appear to be decreased in the renal medulla but normal in the lung.

The vascular reactivity to endothelin-1 also has yielded conflicting results. When infused in vivo into

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**Table 1. Studies Reporting Circulating Endothelin Levels in Systemic and Pulmonary Hypertension**

<table>
<thead>
<tr>
<th>Type of hypertension</th>
<th>Control</th>
<th>Hypertension</th>
<th>$p$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systemic hypertension</td>
<td>1.4±0.1*</td>
<td>1.4±0.1*</td>
<td>NS</td>
<td>159</td>
</tr>
<tr>
<td></td>
<td>1.6±0.1†</td>
<td>1.7±0.1†</td>
<td>NS</td>
<td>159</td>
</tr>
<tr>
<td></td>
<td>5.1±0.5‡</td>
<td>6.7±0.5‡</td>
<td>NS</td>
<td>160</td>
</tr>
<tr>
<td></td>
<td>44.7±3.5</td>
<td>42.3±4.9</td>
<td>NS</td>
<td>161</td>
</tr>
<tr>
<td></td>
<td>0.24±0.02§</td>
<td>0.23±0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.4±0.5</td>
<td>2.3±1.1</td>
<td>0.025</td>
<td>163</td>
</tr>
<tr>
<td></td>
<td>0.5±0.2</td>
<td>1.1±0.7</td>
<td>0.05</td>
<td>164</td>
</tr>
<tr>
<td></td>
<td>18.5±0.9</td>
<td>30.2±1.4</td>
<td>0.01</td>
<td>165</td>
</tr>
<tr>
<td></td>
<td>18.5±0.9</td>
<td>30.1±1.4</td>
<td>0.01</td>
<td>166</td>
</tr>
<tr>
<td>Pulmonary hypertension</td>
<td>0.3±0.2</td>
<td>1.5±0.5</td>
<td>0.001</td>
<td>167</td>
</tr>
</tbody>
</table>

*Data are expressed as mean±SEM in picograms per milliliter.
*At the age of 20–34 years, male.
†At the age of 35–39 years, male.
‡Data were expressed as picomoles per liter in this reference.
§Data were expressed as femtomoles per milliliter in this reference.
*Severe uncontrolled hypertension.
amidon causes a marked decrease in arterial blood pressure in the SHR.89

Endothelium-Derived Contracting Factors in Atherosclerosis

Cyclooxygenase-Dependent Endothelium-Derived Contracting Factors

In endothelial cells in the regenerated state (after balloon angioplasty), which may represent a very early stage of endothelial dysfunction, the contractions induced by higher concentrations of serotonin are enhanced in porcine coronary arteries with endothelium but not in denuded rings of the same blood vessel, suggesting the involvement of an EDCF.183 Similarly, in atherosclerotic porcine coronary arteries, the contractions to serotonin are facilitated by the endothelium.184 As this endothelin-dependent facilitation of serotonin-induced contractions can be prevented by inhibitors of cyclooxygenase, a product of this enzyme is the most likely mediator.

Endothelin

In the atherosclerotic blood vessel wall, lipoproteins and in particular modified forms (i.e., oxidized low density lipoproteins) accumulate.185,186 In endothelial cells in culture obtained from the human and porcine aorta, oxidized low density lipoproteins, but not the native form of the lipoprotein, induce the expression of preproendothelin mRNA and induce an increase in endothelin release.186 These findings are of great pathophysiological interest since the lipoproteins found in human atherosclerotic plaques resemble much more modified forms than native low density lipoprotein.185 Locally increased levels of endothelin may contribute to the enhanced vasoconstrictor responses of atherosclerotic blood vessels as well as, through their proliferative effects on vascular smooth muscle (see References 45 and 111), to the development of the atherosclerotic plaque.

In patients with hyperlipidemia188 and atherosclerosis,189,188,189 the circulating levels of endothelin are also increased. In atherosclerosis, endothelin levels exhibit a positive correlation with the extension of the disease.189 It remains uncertain, however, whether the enhanced production of the peptide occurring in the presence of modified lipoproteins and atherosclerosis is sufficient to alter vascular function. In this context, it is of interest that even threshold concentrations of endothelin may potentiate contractile responses of vascular smooth muscle cells to other agonists such as norepinephrine and serotonin.80

In conclusion, endothelium-derived EDCFs are formed and released in response to physical and chemical stimuli as well as to autacoids and hormones. The endothelial cyclooxygenase pathway can form thromboxane A2, prostaglandin H2, and superoxide anions. The peptide endothelin is a potent and long-lasting contracting factor. EDRFs, such as prostacyclin and nitric oxide, can interact with EDCFs both at the level of the endothelium and at that of vascular smooth muscle. In the aging, hypertensive, and atherosclerotic blood vessel wall, the formation of the cyclooxygenase-dependent EDCF, most likely prostaglandin H2, is enhanced. Although endothelin can profoundly affect blood pressure and its circulating levels are increased in patients with vascular disease and renal insufficiency, its role in hypertension remains undefined. Indeed, normal and augmented circulating levels of the peptide have been reported; in addition, the sensitivity of hypertensive blood vessels to endothelin have been found to be normal, augmented, or even reduced.

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KEY WORDS • endothelins • thromboxanes • prostaglandin endoperoxides • superoxide • hypoxia • angiotensin II
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