Endothelial dysfunction and inhibition of converting enzyme

PAUL M. VANHOUTTE, M.D., PH.D.
Institut de Recherches Internationales Servier (I.R.I.S.) — Courbevoie — France

ACE INHIBITION AND ENDOTHELIAL DYSFUNCTION

The endothelial cells control the tone of the underlying vascular smooth muscle by secreting vasodilator [prostacyclin, nitric oxide (NO), endothelium-derived hyperpolarizing factor (EDHF)] substances. The vasodilator substances also contribute to the antithrombogenicity of the normal endothelium, and inhibit cellular growth. In coronary vascular disease, the ability of the endothelium to secrete vasodilator substances is reduced, while the propensity to release endothelium-derived contracting factors is increased. In particular, the reduced release of NO in response to aggregating platelets, thrombin and circulating catecholamines favors the occurrence of thrombosis and vasospasm, and plays a key role in the initiation of the atherosclerotic process. From the therapeutic point of view, the best available way to enhance the release endothelium-derived nitric oxide and hyperpolarizing factor is to inhibit converting enzyme. This will protect endogenous bradykinin from breakdown and prolong its action on endothelial receptors.

The discovery by Furchgott and Zawadzki of the obligatory role played by the endothelial cells in relaxations of isolated arteries in response to acetylcholine[1], has initiated a major exploration of the pivotal role of the endothelium in contributing to the normal physiological function of the vascular wall. Endothelium-dependent responses are mediated by the release of several diffusible substances [endothelium-derived relaxing (EDRF) and contracting (EDCF) factors] from the endothelial cells. This brief review focuses on how the secretion by endothelial cells of vasodilator substances which brings about moment-to-moment changes in the tone of the underlying vascular smooth muscle cells, and how dysfunction of the endothelial cells may underlie or accompany several major vascular diseases. It updates similar overviews to which the reader is referred for more exhaustive references[2-13].

ENDOTHELIUM-DERIVED RELAXING FACTORS

Endothelium-derived nitric oxide

The labile diffusible, non-prostanoid substance that mediates the endothelium-dependent relaxation to acetylcholine described by Furchgott and Zawadzki[1] has been identified as nitric oxide (NO) or a closely related compound[3, 14-16]. NO is formed from the guanidine-nitrogen terminal of L-arginine, by an enzyme called NO-synthase, which is constitutive (NO-synthase III)[17] in endothelial cells. The activation of this NO-synthase depends of the intracellular concentration of calcium ions in the endothelial cells, is calmodulin-dependent and requires reduced nicotinamide-adenine-dinucleotide phosphate (NADPH), and 5,6,7,8-tetrahydro-biopterin (BH4) for optimal activity. The enzyme can be inhibited competitively by L-arginine analogs such as N^G-monomethyl-L-arginine or N^G-nitro-L-arginine (Fig. 1). In addition to the constitutive NO-synthase present in endothelial cells, two other isoforms of this enzyme are currently known: a neuronal (type I) form and an inducible (type II) form, which is calcium-independent and can be observed in numerous cell types (including vascular smooth muscle) after exposure to endotoxin, tumour necrosis factor and other cytokines. NO diffuses to the vascular smooth muscle cells and relaxes them by stimulating a cytosolic enzyme, soluble guanylate cylase, that leads to an increase in cyclic 3',5'-guanosine monophosphate (cyclic GMP). The latter increase is associated with inhibition of the contractile apparatus (Fig. 1). The production of NO is a major contributor to endothelium-dependent relaxations in large isolated arteries including coronary, systemic, mesenteric, pulmonary and cerebral arteries. Its significance

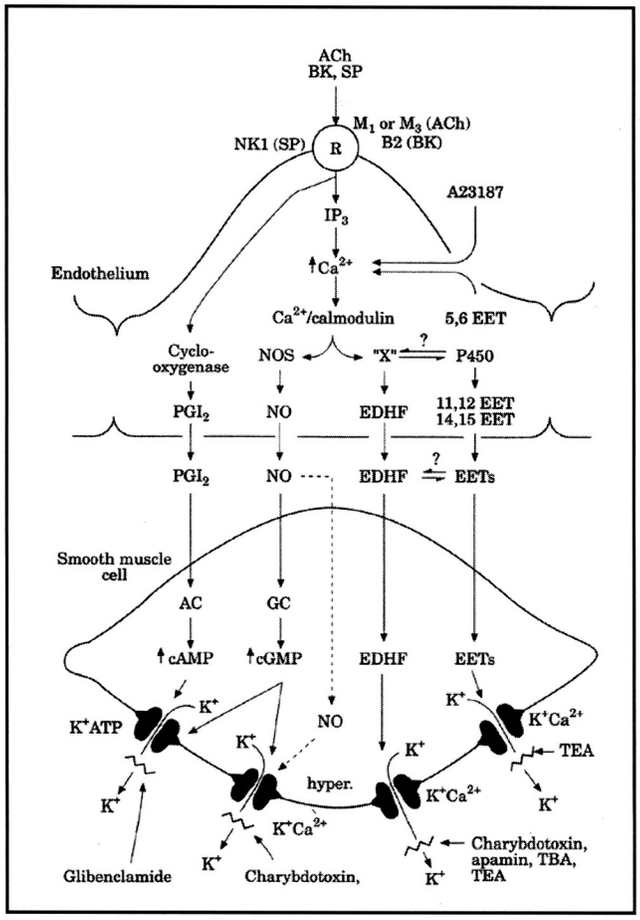

Figure 1. Role of the increase in cytosolic calcium concentration in the release of EDRF(s). Endothelial receptor activation induces an influx of calcium into the cytoplasm of the endothelial cell. Following interaction with calmodulin, this activates NO-synthase (NOS), cyclooxygenase and leads to the release of endothelium-derived hyperpolarizing factor (EDHF). NO causes relaxation by activating the formation of cyclic GMP (cGMP) from GTP by soluble guanylate cyclase (GC). EDHF causes hyperpolarization and relaxation by opening K^+ channels. Prostacyclin (PGI_2) causes relaxation by activating adenylate cyclase (AC) which leads to the formation of cyclic AMP (cAMP). Any increase in cytosolic calcium (including that induced by the calcium ionophore A23187) causes the release of relaxing factors. When agonists activate the endothelial cells, an increase in inositol phosphate (IP3) may contribute to the increase in cytoplasmic Ca^{2+} by releasing it from the sarcoplasmic reticulum (SR). ACh = acetylcholine; BK = bradykinin; EET = epoxyeicosatrienoic acid; SP = substance P; TEA = tetraethylammonium; NK = neurokinin receptor; TBA = tetrabutylammonium.

"in vivo" is suggested by the observations that inhibitors of NO synthase cause vasoconstriction in most vascular beds and an increase in systemic arterial pressure both in animals and in humans[12, 14].

The endothelial cell secretes NO not only towards the underlying vascular smooth muscle but also in the lumen of the blood vessel. Thus, NO inhibits the adhesion of platelets and leukocytes to the endothelium. It acts (synergistically with prostacyclin) to inhibit platelet aggregation[3, 4, 10, 14, 18]. It also inhibits the growth of the vascular smooth muscle cells[19, 20].

The release of NO is modulated by physical[21-25] and humoral stimuli. Among the physical stimuli, the shear stress exerted by the blood on the arterial wall is one of the main factors regulating the local release of NO. Indeed, flow-induced vasodilatation is endothelium-dependent "in vivo"[21, 22].

Several neurohumoral mediators cause the release of NO through activation of specific endothelial receptors (Fig. 2). The endogenous substances stimulating this release are either circulating hormones (e.g. catecholamines, vasopressin), autacoids generated within the vascular wall (e.g. bradykinin, histamine), or mediators released by platelets [serotonin, adenosine diphosphate (ADP)] or formed during coagulation (thrombin)[2-13]. The receptors for these substances are connected to the production of NO by different coupling proteins (Fig. 3). For example, in porcine endothelial cells, alpha$_2$-adrenergic receptors, serotonin receptors and thrombin receptors are coupled to pertussis toxin-sensitive G_i proteins, whereas ADP or bradykinin receptors mediate the production of NO by activation of pertussis toxin-insensitive G_q proteins[26, 27].

From the physiological point of view, the substances produced during platelet aggregation are important releasers of NO. This conclusion is based on the findings that in various species, including humans, aggregating platelets induce endothelium-dependent relaxations and that the presence of endothelial cells substantially inhibits the vasoconstriction induced by thromboxane A_2 and platelet-derived serotonin. There are two major mediators of the endothelial response to platelets: serotonin and ADP, which act on 5-HT_{1D}-serotonin and P_{2y}-purinergic receptors, respectively (Fig. 3). The endothelial action of thrombin and platelet products is crucial for the protective role played by the normal endothelium against unwanted coagulation (Fig. 4). Thus, local platelet aggre-

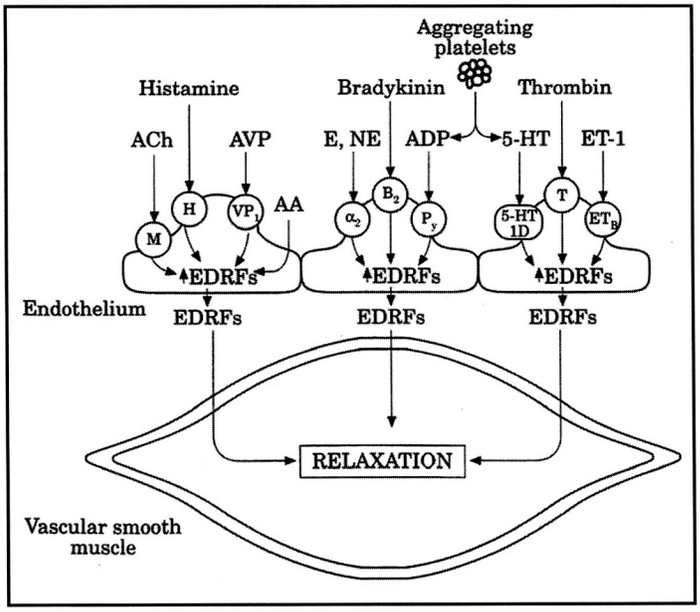

Figure 2. Some of the neurohumoral mediators which cause the release of endothelium-derived relaxing factors (EDRF) through activation of specific endothelial receptors (circles). In addition, EDRF can be released independently of receptor-operated mechanisms by the calcium ionophore A23187 (not shown). E = epinephrine (adrenaline); AA = arachidonic acid; ACh = acetylcholine; ADP = adenosine diphosphate; a **= alpha adrenergic receptor; AVP = arginine vasopressin; B = kinin receptor; ET = endothelin, endothelin-receptor; H = histaminergic receptor; 5-HT = serotonin (5-hydroxytryptamine), serotoninergic receptor; M = muscarinic receptor; NE = norepinephrine (noradrenaline); P = purinergic receptor; T = thrombin receptor; VP = vasopressinergic receptor.**

gation, with the release of serotonin and ADP, as well as the production of thrombin (because of the local activation of the coagulation cascade) leads to massive local release of NO, which diffuses toward the underlying vascular smooth muscle, induces its relaxation, and thus dilatation of the artery. This reaction helps to eliminate the microaggregate. The release of NO towards the blood vessel lumen also inhibits platelet adhesion at the endothelium-blood interface and (in synergy with prostacyclin) exerts a major feedback on platelet aggregation, thereby eliminating the imminent danger of vascular occlusion. Conversely, if the endothelial barrier has been removed, there is a breakdown in the feedback control of platelet aggregation by NO (and prostacyclin). Aggregation proceeds with the continuous release of serotonin and thromboxane A_2; both of these have unrestricted access to smooth muscle which contracts and the blood vessel closes down, constituting the vascular phase of haemostasis (Fig. 4)[3, 8, 10, 28].

Prostacyclin

Prostacyclin, a product of cyclooxygenase, is formed primarily in endothelial cells in response to shear stress, hypoxia and several mediators that also release NO. Prostacyclin causes relaxation of certain vascular smooth muscle by activating adenylate cyclase and increasing the production of cyclic 3',5'-adenosine monophosphate (cyclic AMP). In most blood vessels the contribution of prostacyclin to endothelium-dependent relaxation is negligible, and its effect is essentially additive to that of NO. However, the two substances act synergistically to inhibit platelet aggregation (Fig. 4)[18, 29].

Endothelium-dependent hyperpolarizing factor

Electrophysiological studies in various arteries, including the human coronary artery, demonstrate that acetylcholine, and other endothelium-dependent dilators cause endothelium-dependent hyperpolarizations and relaxations which are due to a diffusible endothelium-derived hyperpolarizing factor (EDHF) different from NO and prostacyclin[30-34]. The chemical nature of EDHF remains speculative. In some blood vessels, epoxyeicosatrienoic acids (EETs), formed from arachidonic acid by the action of cytochrome P450, may correspond to EDHFs[31, 34, 35] (Fig. 1).

The hyperpolarization of smooth muscle cells induced by EDHF is mediated by an increased movement of potassium ions. The type of potassium channels involved is not definitively established, but the channels seem more likely to be calcium-dependent than ATP-dependent[34, 36] (Fig. 1).

The contribution of hyperpolarization in endothelium-dependent vascular relaxation varies in function of the size of the arteries and is prominent in resistance vessels[33, 35]. In large arteries, both mediators can contribute to endothelium-dependent relaxations, but the role of NO predominates under normal circumstances. However, in these arteries, EDHF can mediate near normal endothelium-dependent relaxations when the synthesis of NO is inhibited[34, 37].

ENDOTHELIAL DYSFUNCTION

In several types of vascular disease and

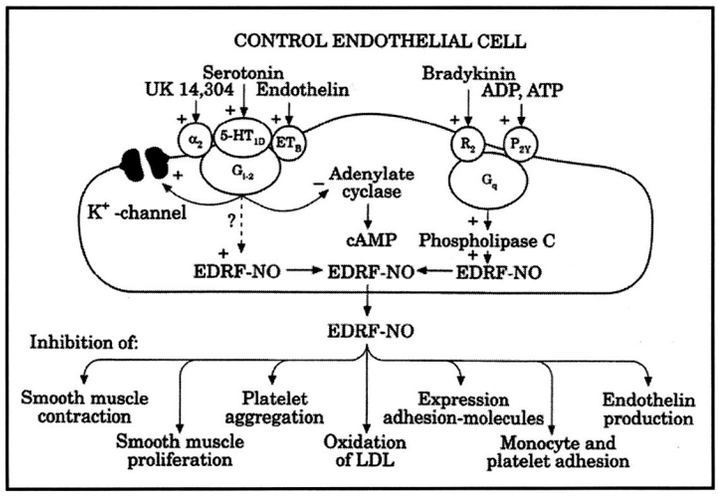

Figure 3. Postulated signal transduction processes in a normal endothelial cell. Activation of the cell causes the release of EDRF-NO which has important protective effects in the vascular wall. a **= alpha-adrenergic; 5-HT = serotonin-receptor; ET = endothelin receptors; B = bradykinin receptor; P = purinoceptor; G = coupling proteins; cAMP = cyclic adenosine monophosphate; NO = nitric oxide; LDL = low density lipoproteins; EDRF = endothelium-derived relaxing factor.**

hypertension, the endothelial cells become dysfunctional[3, 4, 8, 10-13]. This dysfunction usually expresses itself as an impairment in endothelium-dependent relaxations, due mainly to a reduced release (or action) of EDRFs although production of endothelium-derived vasoconstrictor substances may contribute[38-47].

Regenerated endothelium

Even the normal ageing process induces a turnover and regeneration of endothelial cells, resulting in an abnormal function. Regenerated endothelial cells have lost some of their ability to release EDRF, particularly in response to platelet aggregation and thrombin[48, 49]. Indeed, regenerated endothelium responds poorly to serotonin, and other substances using the pertussis toxin-sensitive pathway controlling the release of EDRF (Fig. 3). In cultured regenerated endothelial cells, pertussis toxin sensitive G_i proteins are expressed normally, but have a reduced activity[50]. The loss of pertussis toxin-sensitive response is selective, and does not apply to endothelium-dependent responses induced by ADP or bradykinin. The area of regenerated endothelium becomes a site of predilection for triggering exaggerated vasoconstriction in response to serotonin or ergonovine[51].

Hypercholesterolemia and atherosclerosis

Hypercholesterolemia induced by high-fat and/or high cholesterol diets in experimental animals impairs endothelium-dependent relaxations[3, 7, 10, 52, 53]. By contrast, endothelium-independent relaxations to nitroglycerin, sodium nitroprusside or adenosine are normal or only slightly impaired. A progressive deterioration of endothelium-dependent relaxations is also observed in genetically hyperlipidaemic rabbits[54]. Endothelium-dependent relaxations are reduced also in the coronary microcirculation in hypercholesterolaemic animals[55, 56]. They are impaired in humans with atherosclerosis and/or hypercholesterolemia[57-62].

In the early stage of the atherosclerotic process, the endothelial dysfunction appears to be limited to the pertussis toxin-sensitive, G_i protein-dependent pathway which leads to NO formation (Fig. 3). Thus, the ability of regenerated endothelial cells, chronically exposed to high cholesterol levels, to ADP-ribosylate pertussis toxin is reduced[63]. Consequently, in coronary arteries from hypercholesterolemic pigs, endothelium-dependent relaxations evoked by agents that activate the pertussis toxin-sensitive G_i protein (e.g. serotonin, alpha$_2$-adrenergic agonists, aggregating platelets, thrombin) are depressed while those induced by ADP, bradykinin, or the Ca^{2+}-ionophore A23187 are preserved[26, 27, 48, 49, 53, 54, 63]. Oxidized low-density lipoproteins (LDL), which are considered to be more atherogenic than native LDL, induce, "in vitro", a similar selective endothelial dysfunction for stimuli activating the pertussis-sensitive G_i protein pathway, while at higher concentrations they inhibit also endothelium-dependent responses evoked by receptor-independent stimuli[3, 64, 65].

The most important mechanism in the reduction in endothelium-dependent responses is a lessen release of NO. Nevertheless, as the disease progresses and the artery thickens and stiffens, it becomes increasingly difficult for NO to reach smooth muscle that is still able to relax. Endothelial dysfunction is probably a fundamental initial step in the progression of atherosclerosis. This hypothesis argues that ageing and prolonged exposure to shear stress, coupled with risk factors such as hypertension, smoking and stress, accelerate endothelial ageing and hence the process of endothelial regeneration. As a result, larger and larger sections of the endothelium become unable to resist platelet adhesion and aggregation and respond less well to thrombin formation. The feedback effect of NO (together

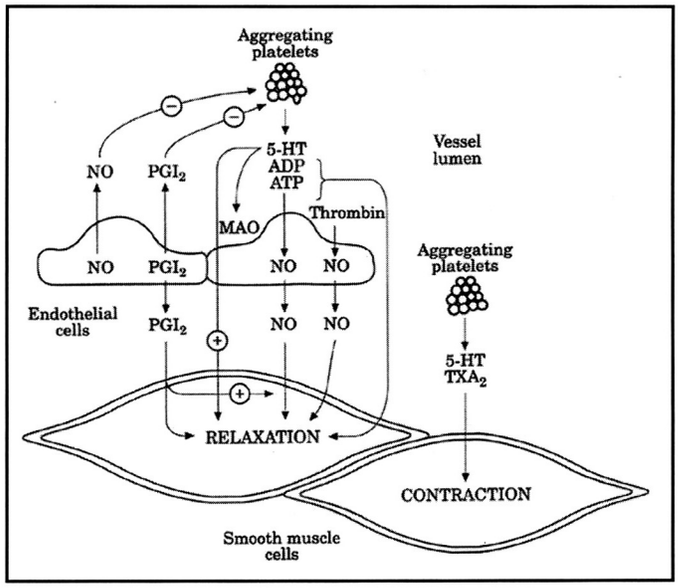

Figure 4. Interaction between platelet products, thrombin and endothelium. If the endothelium is intact, several of the substances released from the platelets [in particular, the adenine nucleotides (ADP and ATP) and serotonin (5-HT)] cause the release of EDRF and prostacyclin (PGI_2). The same is true for any thrombin formed. The released EDRF will relax the underlying vascular smooth muscle, opening up the blood vessel, and thus flushing the microaggregate away; it will also be released towards the lumen of the blood vessel to brake platelet adhesion to the endothelium and, synergistically with prostacyclin, inhibit platelet aggregation. In addition, monoamine oxidase (MAO) and other enzymes will break down the vasoconstrictor serotonin, limiting the amount of the monoamine that can diffuse toward the smooth muscle. Finally, the endothelium acts as a physical barrier that prevents the access to the smooth muscle of the vasoconstrictor platelet products serotonin and thromboxane A_2 (TXA_2). These different functions of the endothelium play a key role in preventing unwanted coagulation and vasospastic episodes in blood vessels with a normal intima. If the endothelial cells are removed (e.g. by trauma), the protective role of the endothelium is lost locally, platelets can adhere and aggregate, and vasoconstriction follows; this contributes to the vascular phase of haemostasis. + = activation; - = inhibition.

with prostacyclin) on platelet aggregation decreases steadily, while vasoconstrictor factors (serotonin and thromboxane A_2) are released in increasingly greater amounts, together with growth factors, such as platelet-derived growth factor (PDGF), which probably are responsible for initiating the characteristic morphological changes in atherosclerosis[3, 7, 8, 10, 26-28].

INHIBITION OF CONVERTING ENZYME

Angiotensin-converting enzyme is located mainly at the cell membrane of the endothelial cells. It converts the less active peptide angiotensin I into the powerful vasoconstrictor angiotensin II, which acts both as direct activator of vascular smooth muscle, and as an amplifier of the sympathetic nervous system. Hence, it is not surprising that inhibitors of the enzyme can cause peripheral vasodilatation by reducing the local and circulating levels of angiotensin II, particularly in patients with high renin. Converting enzyme also is the main pathway for the breakdown of bradykinin into inactive peptides; thus, the vasodilator effects of converting enzyme inhibitors is due in part to their protective effect against the breakdown of locally produced bradykinin. Indeed, chronic treatment with an converting enzyme inhibitor not only decreases the ratio of plasma angiotensin II to angiotensin I but also increases the plasma level of bradykinin[66-71].

Exogenous bradykinin

All converting enzyme inhibitors tested so far cause a marked shift to the left of the concentration-relaxation curve to bradykinin in isolated blood vessels with endothelium, without affecting the lack of response in the absence of endothelial cells. If rings of isolated arteries with endothelium suspended are exposed under control conditions to increasing concentrations of a converting enzyme inhibitor, no changes in tension are observed. However, if the preparations are studied in the presence of a sub-threshold concentration of bradykinin[66], or even hours after a previous exposure to the peptide[72], the converting enzyme inhibitors causes a marked relaxation. The potentiating effect of converting enzyme inhibitors on the endothelium-dependent relaxation to bradykinin is accompanied by an increased production of cyclic GMP, illustrating the greater release of NO[73]. Likewise, converting enzyme inhibitors augment the endothelium-dependent hyperpolarizing effect of the kinin[74]. Both components are inhibited equally by B_2-kinin receptor antagonists either in the absence or during converting enzyme inhibition. These experiments demonstrate that both the

greater release of NO and the larger endothelium-dependent hyperpolarization contribute to the augmented endothelium-dependent relaxations to bradykinin caused by converting enzyme inhibition (Fig. 5)[66, 67].

Endogenous bradykinin

Plasma and tissue kallikreins are the main enzymes involved in the formation of kinins from kininogens. Kininogens can cause endothelium dependent relaxations[75]. Rings of isolated arteries with, but not those without, endothelium relax to kallikrein, a response that is potentiated by the converting enzyme inhibitor perindoprilat. Both the response to kallikrein and its augmentation by perindoprilat are prevented by an inhibitor of kallikrein[75]. These studies indicate that the arterial wall contains a precursor of kinins, and that the local activation of the kallikrein-bradykinin system can yield enough kinins to activate the endothelial cells to release relaxing factors, particularly when converting enzyme is inhibited by perindoprilat.

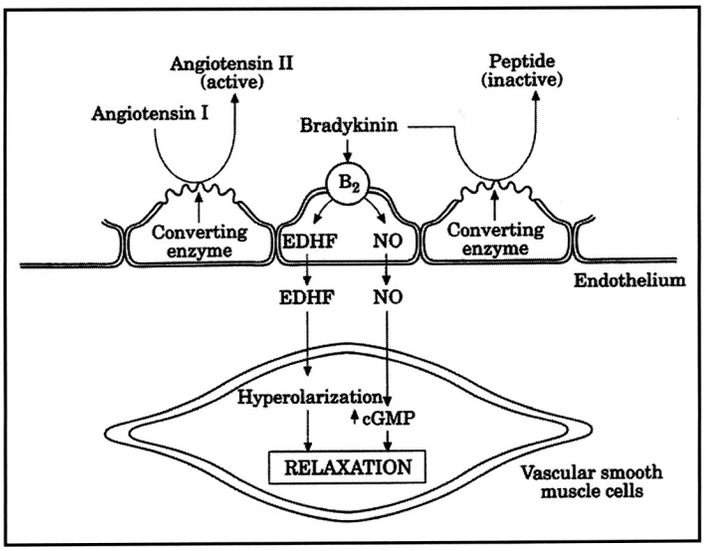

Figure 5. Converting enzyme and endothelium-derived vasodilator mediators. Converting enzyme, which is abundantly expressed at the surface of endothelial cells, catalyzes the generation of the vasoconstrictor angiotensin II and the degradation of the endothelium-dependent vasodilator bradykinin. Bradykinin, through activation of endothelial B_2-kinin receptors, induces the release of endothelium-derived hyperpolarizing factor (EDHF) and/or NO. Inhibitors of converting enzyme potentiate both actions (modified with permission[12]).

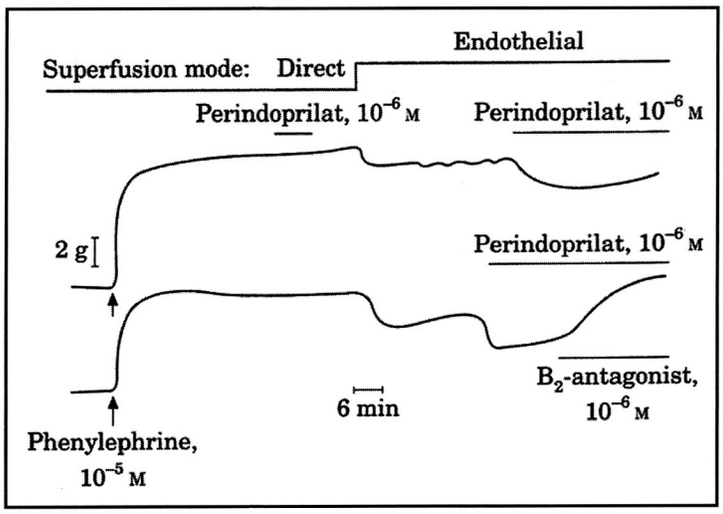

Figure 6. Kinins and flow-induced release of endothelium-derived relaxing factors. Experiments were performed in isolated canine carotid arteries using a perfusion-superfusion bioassay which consisted of a perfused carotid artery (donor) and a downstream ring of carotid artery without endothelium used as a bioassay ring. When given directly to vascular smooth muscle (direct superfusion) the ACE-inhibitor perindoprilat has no effect (left). When infused through an artery with endothelium (endothelial superfusion), perindoprilat causes the release of EDRF (as measured by the relaxation of bioassay rings), which is antagonized by D-Arg[Hyp³, D-Phe⁷]-bradykinin (10^{-6}M; a selective B_2-kinin antagonist) (modified with permission[73]).

Increases in shear stress augment the release of endothelium-derived relaxing factors (see above). When the converting enzyme inhibitor perindoprilat is given into perfused isolated arteries with (but not in those without) endothelium, it causes a marked relaxation which can be attributed to the release of endothelial factors (Fig. 6)[76]. The relaxations are reversed by a selective B_2-kinin antagonist, but are not affected by losartan, a selective antagonist of angiotensin AT_1-receptors. Even the basal release of endothelium-derived relaxing factors is inhibited in part by the B_2-kinin receptor antagonist. These experiments strongly suggest that shear stress activates the local kallikrein-kinin system in the arterial wall, and that this system contributes to the increased release of endothelium-derived relaxing factors which underlies flow-induced vasodilatation[71, 76]. A similar conclusion has been reached in the human coronary circulation[77].

Significance

Although a major pharmacological target for converting enzyme inhibitors remains the reduced production of angiotensin II, in the face of an increased production of renin, augmented endothelium-dependent relaxations to locally produced bradykinin may help to explain the acute vasodilator properties of these compounds, particularly in hypertensive patients with low levels of renin. Although converting enzyme inhibitors are not considered as anti-anginal drugs, they may be useful in the treatment of ischaemic heart disease and acute myocardial infarction. The beneficial effect of converting enzyme inhibitors may be due , in part, to the augmentation of coronary diameter by preventing the degradation of bradykinin generated by shear stress, and therefore increasing the formation of endothelium-derived NO and EDHF.

Since NO is involved not only in the regulation of vascular tone, but also inhibits the adhesion and aggregation of platelets as well as the growth of vascular smooth muscle (Fig. 3), the potentiated release of endothelium-derived NO may contribute to the vascular protective effect of converting enzyme inhibitors such as perindoprilat.

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