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Maintenance of vascular integrity: role of nitric oxide and other bradykinin mediators

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In the blood-vessel wall, the endothelium plays a key functional role by generating several substances that modulate vascular smooth muscle tone, as well as growth, and platelet function. This review focuses on the role of the endothelial L-arginine/nitric oxide signal transduction pathway in the maintenance of vascular integrity. Functional alterations of this pathway may be important in cardiovascular disease, because depressed activity of this protective mechanism leads to impaired relaxation and is also associated with reduced antithrombotic properties of the endothelial layer. Many of the beneficial effects of ACE inhibitor therapy may be mediated through their ability to enhance the physiological roles of nitric oxide.

Introduction

Nitric oxide is a vasodilator and potent inhibitor of platelet function that is synthesized from L-arginine by nitric oxide synthase. Nitric oxide enters adjacent smooth muscle cells, leading to increased generation of cyclic guanosine monophosphate and, subsequently, to vascular relaxation. Nitric oxide-mediated vascular dilation is constant, as endothelial cells continuously release small amounts of this relaxing factor. Studies have shown that a basal level of nitric oxide production, as well as agonist-stimulated production, plays a key role in the regulation of vascular tone. Thus, endothelial generation of nitric oxide is involved in the maintenance of normal blood flow and pressure. Given its ability to keep the vascular smooth muscle surface non-adhesive and nonthrombogenic for circulating blood cells, nitric oxide prevents platelet adhesion and aggregation. Depressed activity of the L-arginine/nitric oxide pathway leads to impaired relaxation (vasoconstriction and reduced local blood flow) and is associated with reduced antithrombotic properties of the endothelium. In disease states such as hypertension, endothelium-dependent relaxation may be impaired, despite evidence of increased nitric oxide release. Its haemodynamic roles may be diminished by production of oxidative radicals or other disease-related factors.

In patients with atherosclerosis, the response to endothelium-dependent vasodilators, including nitric oxide, may be impaired, possibly due to increased formation of superoxide radicals or interference by oxidized low-density lipoprotein with the L-arginine/nitric oxide pathway. Angiotensin I converting enzyme (ACE) plays a key role in vascular homeostasis. Pharmacological inhibition of ACE not only prevents the formation of the powerful vasoconstrictor angiotensin II, but augments local concentrations of bradykinin, a potent stimulator of the L-arginine/nitric oxide pathway, thereby enhancing vasodilation. Studies have demonstrated improved endothelial function with these agents, as well as inhibition of platelet aggregation.

Thus the protective effects of ACE inhibitors in various cardiovascular disease states may be attributable to enhancement of the physiological roles of nitric oxide.

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The endothelium and vascular homeostasis

Endothelial cells, which line the intimal surface of blood vessels, play an important role in many physiological processes. They perform a variety of functions, including transportation of water and solute regulation of plasma lipids, participation in inflammatory and immunological reactions, maintenance of the fluidity of blood, and adjustment of the calibre of blood vessels to the ever-changing haemodynamic and hormonal environment. Because of their strategic anatomical location between circulating blood and tissues, endothelial cells have the capacity to sense changes in haemodynamic forces (shear forces and pressure) and in locally produced or circulating mediators, and to respond to these changes by the production of a number of biologically active factors. The endothelium is regarded as one of the most important, and certainly the most extensive, 'organ' in the body that participates in cardiovascular homeostasis.

The seminal observation of Furchgott and Zawadzki[1] 15 years ago, that endothelial cells play an obligatory role in the relaxation evoked by acetylcholine in isolated rabbit aortas, not only stimulated research activity worldwide but truly revolutionized cardiovascular sciences. Now, endothelium-dependent regulation of vascular tone (local regulation of blood flow), platelet function, and mitogenesis (antithrombotic mechanisms) have become a key part of our view of cardiovascular physiology. Perhaps even more important, the achievements in recent years have led to a better understanding of the pathophysiology of hyper-

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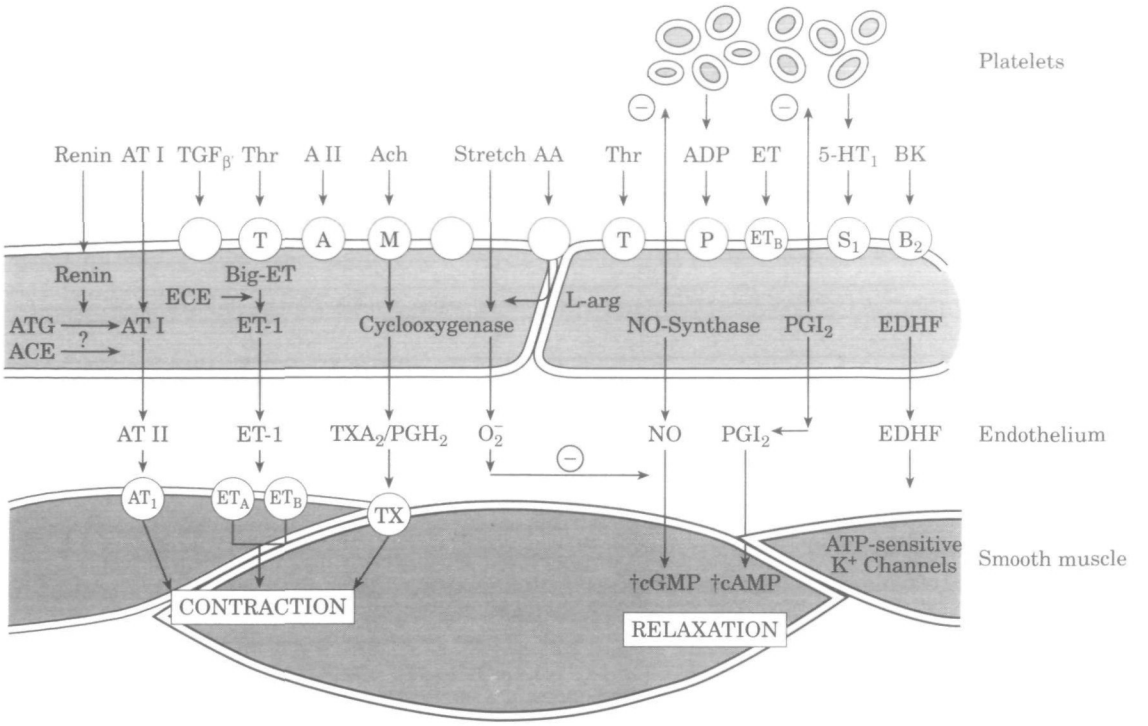

Figure 1 Schematic diagram showing endothelium-derived vasoactive factors produced in blood vessels.

tension, vasospasm, and atherosclerosis and to new therapeutic strategies to fight these pathological conditions.

Indeed, a number of endothelium-derived factors can profoundly modify platelet function as well as the contractile and proliferative state of vascular smooth muscle cells[2]. These factors include nitric oxide and prostacyclin, which are both vasodilators and potent inhibitors of platelet function, and a putative endothelium-derived hyperpolarizing factor. By contrast, endothelial cells can also produce vasoconstrictors and growth promoters, such as thromboxane A_2, prostaglandin H_2, endothelin, and angiotensin II (Fig. 1).

This review will focus on the current knowledge concerning the role of the nitric oxide signal transduction pathway in the maintenance of vascular integrity.

L-Arginine/nitric oxide pathway

The demonstration in 1980 of the phenomenon of endothelium-dependent relaxations and of the release of endothelium-derived relaxing factor (EDRF)[1] led to a search for the chemical identity of this factor. In the next few years, EDRF was shown to be an extremely labile molecule[3] and some of its properties were described. Eventually, it was shown that vascular endothelial cells release nitric oxide and that this compound accounted for the vasodilatory and platelet inhibitory effects of EDRF[4]. Nitric oxide is synthesized from L-arginine by nitric oxide synthase (NOS) through a five-electron oxidation of the guanidine-nitrogen terminal of L-arginine[5]. Many advances in the understanding of the L-arginine/nitric oxide pathway have come from molecular studies of NOS. Three distinct genes encoding different NOS isoforms have been cloned. Neuronal NOS was the first form of the enzyme to be purified and cloned[6]. More recently, both endothelial[7–9] and macrophage forms have also been cloned[10–12]. Macrophages have negligible NOS activity under basal conditions, but after stimulation with lipopolysaccharide and/or cytokines, massive increases in NOS activity occur within 2 to 4 h[13–16]. The macrophage enzyme, which is calcium independent, has thus been referred to as 'inducible NOS' (iNOS) in contrast to the enzyme in neuronal tissues or endothelium, which appears to be constitutively expressed.

Many cell types throughout the body, including hepatocytes, neurons, neutrophils, endothelial cells, vascular smooth muscle cells, and cardiac myocytes, appear capable of iNOS expression[17–19]. Once expressed, the inducible isoform generates large amounts of nitric oxide over an extended period of time (48 to 72 h)[20]. In humans, iNOS has been found to be expressed in a variety of cytokine-induced pathologic states, including tumors[21], cirrhosis[22], ulcerative colitis[23], and endotoxemic shock[24].

To understand the role of NOS in vascular physiology and pathology, discrimination between the involvement of the various isoforms of NOS may be important.

Constitutive NOS synthesizes nitric oxide within seconds in response to ligand-receptor-coupling events at the cell surface and displays a strict dependence on Ca^{2+} and calmodulin[25–27]. In vascular endothelium, Ca^{++} may be made available through stimulation by agonists such as acetylcholine and bradykinin, which generate inositol 1,4,5-triphosphate (IP_3) production via activation of the so called 'phosphoinositide second messenger system'. IP_3 elicits Ca^{2+} release from intracellular stores by binding to IP_3 receptors on the endoplasmic reticulum (Fig. 2). Furthermore, a portion of mobilized Ca^{2+} is thought to arise

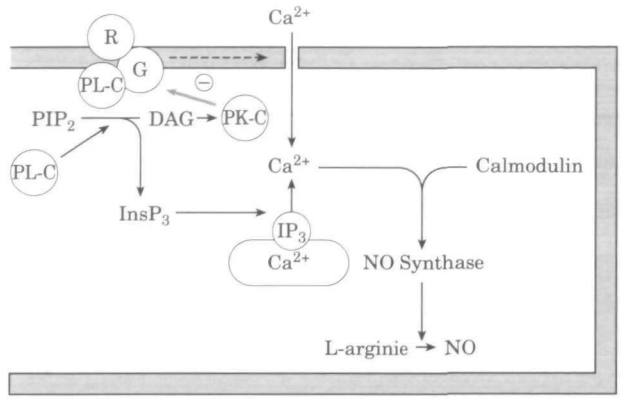

Figure 2 Pathways that can increase intracellular calcium in endothelial cells, which in turn leads to activation of nitric oxide synthase: phospholipase C activation by receptor and G protein coupling; influx of extracellular calcium. (Modified from[122].)

extracellularly. Alternatively, agonist-independent nitric oxide release also contributes to vascular tone. Both shear stress[28] and deformation of endothelium[29], due to pulsatile flow in blood vessels, stimulate nitric oxide release through poorly characterized mechanisms. Once released, nitric oxide enters adjacent smooth muscle cells, where it activates soluble guanylate cyclase to generate cyclic guanosine monophosphate (cGMP). The increased concentration of cGMP in these cells causes vascular relaxation[30].

Physiological roles of nitric oxide

REGULATION OF VASCULAR TONE

The vasculature is in a constant state of active dilation mediated by nitric oxide. Endothelial cells continuously release small amounts of nitric oxide, producing a basal level of vascular smooth muscle relaxation. However, the expression of constitutive enzyme and the release of nitric oxide can be enhanced above basal levels after receptor stimulation by different agonists. Both in vitro and in vivo studies have demonstrated that basal and stimulated production of nitric oxide in endothelial cells plays a key role in the regulation of vascular tone. The strongest evidence comes from results obtained in studies with N^G-substituted analogs of L-arginine, which are potent and selective inhibitors of nitric oxide synthesis (Fig. 3)[5,31]. L-Arginine analogs, such as N^G-monomethyl-L-arginine (L-NMMA), N^G-nitro-L-arginine-methyl ester (L-NAME) and N^G-nitro-L-arginine (L-NA), cause endothelium-dependent contractions in a number of isolated arteries. These contractions are mediated by inactivation of basal production of nitric oxide[5,32,33]. The inhibitory effect of these compounds is prevented by L-arginine (but not D-arginine). This stereospecific inhibition indicates that L-arginine analogs compete with L-arginine for the NOS active site to prevent production of nitric oxide. Intravenous injections of L-NMMA into anaesthetized rabbits resulted in an immediate and substantial rise in blood pressure, which could be reversed by L-arginine[34]. The blood pressure-elevating and vasoconstrictive effects of L-NMMA have now been demonstrated in a number of species, including man[35–37]. Similar results have been obtained with the other known NOS inhibitors[38]. These inhibitors have no intrinsic constrictor activity on vascular smooth muscle; their activity is entirely endothelium dependent and results from the inhibition of endogenous vasodilatation.

Thus, it appears that the endothelial generation of nitric oxide by NOS is involved in the maintenance of normal blood flow and pressure. The observations that in porcine coronary arteries and in small canine cerebral arteries endothelium-dependent relaxations to bradykinin are resistant to inhibitors of nitric oxide formation or cyclooxygenase[39–41] strongly suggest that an endothelium-derived relaxing substance distinct from nitric oxide and prostacyclin is formed as well. Recent studies suggest that bradykinin and acetylcholine hyperpolarize vascular smooth muscle cells in an endothelium-dependent manner[42–45]. These data would be compatible with the concept that endothelial cells release a biochemically unidentified substance that has the capacity to hyperpolarize vascular smooth muscle cells via adenosine triphosphate-dependent potassium channels[46,47]. Hyperpolarization of vascular smooth muscle cells is associated with a decreased sensitivity to vasoconstrictor substances and may also contribute to vasodilator responses induced by prostacyclin and nitric oxide.

INHIBITION OF PLATELET FUNCTION

Endothelium-derived relaxing factors, such as nitric oxide and prostacyclin, not only serve to relax the underlying smooth muscle but also keep the surface nonadhesive and nonthrombogenic for circulating blood cells. Both mediators increase cGMP and cyclic adenosine monophosphate (cAMP) in platelets and thereby prevent platelet adhesion and aggregation[48–52]. In addition, platelets themselves possess an L-arginine/nitric oxide pathway that modulates the reactivity of the cells to aggregatory stimuli[53].

In isolated human coronary and internal mammary arteries, aggregating platelets cause endothelium-dependent

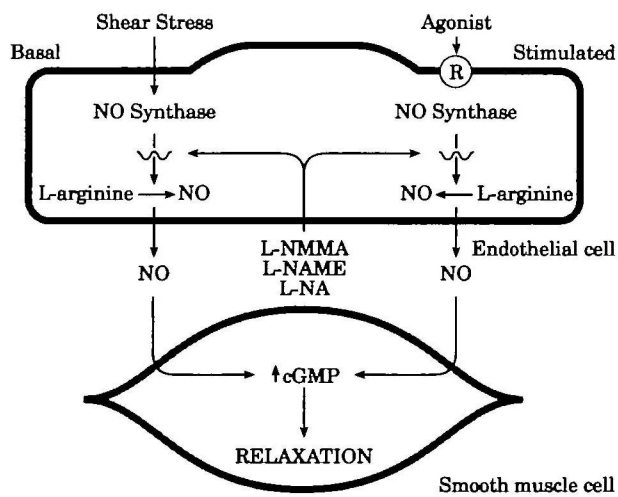

Figure 3 Schematic representation of inhibitory effect of L-arginine analogs on basal and stimulated production of nitric oxide. (Modified from[33].)

relaxations that are mediated by nitric oxide[54,55]. The platelet-derived mediator primarily responsible for the stimulation of nitric oxide formation is adenosine diphosphate, although serotonin may contribute under certain conditions[56,57]. In contrast to normal arteries, arteries devoid of endothelial cells or with dysfunctional endothelium markedly contract in response to aggregating platelets[55]. Platelet-induced vasoconstriction is mediated primarily by serotonin and thromboxane A_2, which activate specific receptors on vascular smooth muscle cells[55,58]. At sites where platelets are stimulated, the coagulation cascade also is activated, leading to the formation of thrombin. Thrombin is an enzyme that is responsible for the formation of fibrin from fibrinogen. In addition to its activity in the coagulation cascade, thrombin is a potent activator of platelets[48], but it also exerts effects in endothelial cells. Indeed, thrombin causes endothelium-dependent relaxations in human coronary arteries and internal mammary arteries[59,60]. These relaxations are partially inhibited by indomethacin and L-NAME[59]. This indicates that both nitric oxide and prostacyclin contribute to the response. Hence, in the presence of an intact endothelium, thrombin not only causes vasodilatation, but it also inhibits platelet function via an endothelium-dependent mechanism. These effects counteract the direct activating effects of thrombin in platelets and thereby represent a protective negative feedback mechanism, preventing further activation of platelet-vessel wall interaction.

Nitric oxide has also been shown to inhibit leukocyte activation both in vitro and in vivo[61,62]. Thus, nitric oxide even appears to be involved in regulating the interactions between leucocytes and the vascular endothelium.

REGULATION OF VASCULAR GROWTH

Removal of the endothelium by balloon catheter invariably leads to intimal proliferation. This strongly suggests that the presence of endothelial cells exerts an antiproliferative effect[63], which may be related to the fact that endothelial cells inhibit the adhesion and aggregation of circulating platelets[48–51] and of monocytes[64], both important sources of platelet-derived growth factor and transforming growth factor beta-1[65]. Furthermore, endothelial cells produce inhibitors of migration and proliferation, such as nitric oxide[66,67] and heparin-like substances[68], as well as transforming growth factor beta-1, which under certain conditions is an inhibitor of vascular proliferation[69,70]. On the other hand, endothelial cells can also produce growth promoters, such as platelet-derived growth factor, basic fibroblast growth factor, and endothelin-1[69,71,72]. Thus, the secretion of growth inhibitors or promoters by endothelial cells, as well as their capacity to inhibit circulating blood cells, modulates vascular structures[63].

Nitric oxide in cardiovascular disease

Functional alterations of the endothelial L-arginine/nitric oxide pathway may be important in cardiovascular disease, because a depressed activity of this protective mechanism would lead to impaired relaxation (vasoconstriction and reduced local blood flow) and be associated with reduced antithrombotic properties of the endothelial layer. Hypertension and atherosclerosis are well-recognized pathophysiological contributors to the progression of cardiovascular disease.

HYPERTENSION

The role of endothelium in hypertension is still controversial. The endothelium-dependent relaxations are heterogeneously affected in this condition. In some vascular beds of hypertensive rats, such as the aorta and mesenteric, carotid, and cerebral vessels, endothelium-dependent relaxations are impaired[73–75]. In contrast, in coronary and renal arteries of spontaneously hypertensive rats (SHR), endothelial function does not seem to be affected by high blood pressure[75,76]. Although the endothelium-dependent relaxations are either diminished or normal in spontaneous hypertension, the production of nitric oxide seems to be increased. The release of breakdown products of nitric oxide (NO_2–NO_3) from isolated coronary vessels is augmented in SHR[77]. The activity of constitutive NOS is also enhanced in the SHR heart[78]. In addition, it has been demonstrated that pharmacologically induced elevations in blood pressure increase the release of nitric oxide in normotensive rats[79]. These data suggest that blood pressure per se is a stimulus for nitric oxide release. This interpretation is reinforced by the fact that constitutive NOS activity is normal in prehypertensive 4-week-old SHR[80]. Despite its increased release, nitric oxide is functionally unable to perform its haemodynamic role in the vasculature of genetically hypertensive rats[80], probably because higher production of oxidative radicals, such as superoxide anion, or diminished activity of superoxide dismutase (SOD) accounts for increased degradation of nitric oxide. This effect may contribute to the impaired endothelium-dependent relaxations in this model of hypertension[73]. In addition, increased production of endothelium-dependent contracting factors can explain the abnormal endothelial function of some vascular beds.

Nitric oxide production and inactivation might be heterogeneously affected in different forms of hypertension[81]. Indeed, in Dahl salt-sensitive rats, endothelium-dependent relaxations are impaired[81,82], but no release of vasoconstrictor prostanoids can be demonstrated. This suggests that decreased nitric oxide production could contribute to the pathogenesis of this form of hypertension.

Studies in humans have demonstrated diminished basal and stimulated nitric oxide production[37,83]. The decrease in forearm blood flow induced by L-NMMA is smaller in hypertensive than in normotensive patients[37]. Most studies have shown reduced endothelium-dependent vasodilatation in patients with primary or secondary hypertension[84–86]. The impaired endothelial response in hypertensive patients can be improved by indomethacin, suggesting that vasoconstrictor prostanoids also contribute to impaired endothelium-dependent relaxation[85].

HYPERLIPIDAEMIA AND ATHEROSCLEROSIS

Although the morphology of the vascular endothelium is not altered in the early stage of atherogenesis[65], its function as a regulator of vascular homeostasis is profoundly

modified. By contrast, the presence of overt atherosclerosis is associated with morphological changes in the intima of large arteries (intimal thickening, accumulation and proliferation of smooth muscle cells and lipid containing macrophages)[65].

Functional studies report that the response of atherosclerotic arteries to endothelium-dependent vasodilators is impaired at a very early stage in rabbit[87], porcine[88] and human coronary arteries[89], whereas relaxations in response to the nitric oxide-donor molsidomine SIN-1 are well maintained excluding reduced responsiveness of vascular smooth muscle to nitric oxide. In vivo, acetylcholine and serotonin have even produced paradoxical vasoconstriction[58,90]. Furthermore, bioassay experiments with atherosclerotic arteries have shown that the release of nitric oxide is reduced in porcine coronary arteries with hypercholesterolaemia and atherosclerosis[91,92]. However, recent research in hypercholesterolaemic rabbit aortas has revealed that the production of nitric oxide is markedly enhanced rather than impaired[93,94]. The latter observation suggests increased formation of superoxide radicals in the endothelium, inactivating nitric oxide, and/or decreased activity of SOD[95]. Indeed, superoxide anion production in these preparations is increased[96] and treatment with exogenous SOD partially improves endothelium-dependent relaxations of this artery[97].

In the porcine coronary artery, oxidized low-density lipoprotein (ox-LDL) inhibits endothelium-dependent relaxations to different agents, such as platelets, serotonin, and thrombin[98,99]. This inhibition of endothelium-derived relaxation is specific for ox-LDL, and it is not induced by comparable concentrations of native LDL[99]. A receptor distinct from that for LDL, the scavenger receptor, appears to be activated by ox-LDL, since the endothelial effect of modified LDL can be prevented by dextran sulphate, a competitive antagonist of ox-LDL for this receptor[99].

Oxidized LDL may interfere with the L-arginine pathway, since the inhibition of the endothelium-dependent relaxation that it produces is similar to that of L-NMMA. However, the effect of ox-LDL can be reversed by L-arginine, suggesting that NOS is not directly affected. Furthermore, the pretreatment of isolated vessels with L-arginine improves endothelial function in response to serotonin that was blunted by ox-LDL. These results suggest that ox-LDL decreases the intracellular availability of L-arginine. Accordingly, in humans with hypercholesterolaemia, L-arginine enhances the blunted increase in local blood flow in response to acetylcholine[100].

Vascular effects of ACE inhibition

As indicated above, endothelium-derived mediators regulate vascular integrity. There is evidence that in cardiovascular disease states, the protective role of the endothelium appears to diminish, while the production of vasoconstrictive, proaggregatory, and promitogenic mediators is maintained or enhanced[101,102]. One of the enzymes with a key role in vascular homeostasis is angiotensin I converting enzyme (ACE). ACE is located on the endothelial cell membrane and is responsible for the conversion of angiotensin I into angiotensin II as well as for the breakdown of bradykinin.

Bradykinin is a potent stimulator of the L-arginine/nitric oxide pathway[4]. Hence, ACE inhibitors not only prevent the formation of a powerful vasoconstrictor with proliferative properties, but also augment the local vascular concentrations of bradykinin and, in turn, the activation of the L-arginine/nitric oxide pathway (Fig. 4)[102–106].

Endothelial function has been shown to be improved by ACE inhibitors in several animal models of cardiovascular disease including SHR[107] and hypercholesterolaemic rabbits[108], as well as models of experimental heart failure[109].

Several animal models have shown that ACE inhibitors can reduce neointima formation[110,111] following vascular injury. Further analysis of the mechanisms involved in the inhibition of miointimal formation[112] points to a role for

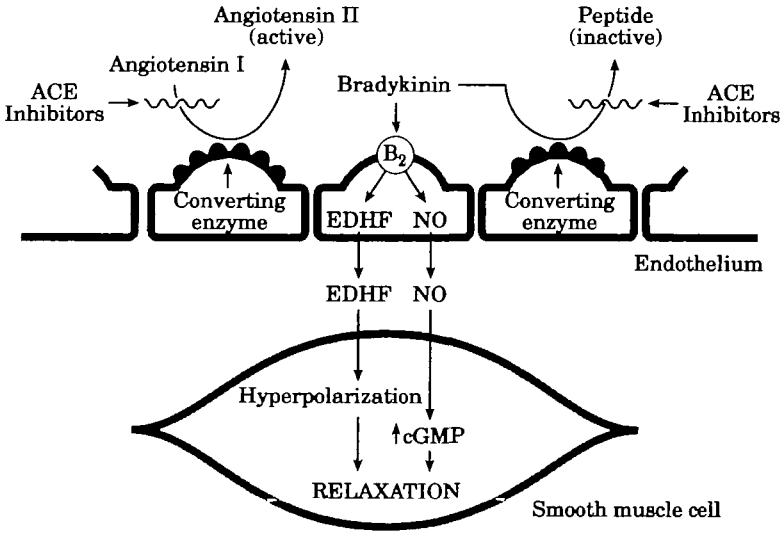

Figure 4 Local vascular effects of ACE inhibitors in the blood vessel wall.[121]

bradykinin. These findings establish a link between the ability of ACE inhibitors to increase kinin concentrations and their antiproliferative effects following vascular injury. It is likely that the inhibitory effect of kinins on neointima formation is mediated through their stimulation of nitric oxide synthesis, which is known to inhibit smooth muscle cell proliferation and migration[66,67]. Another connection between ACE inhibition and the actions of bradykinin is indicated by the finding that the endothelium-dependent relaxation evoked by ACE inhibition is attenuated by a B2-kinin receptor antagonist[113].

Evidence points to the positive activity of ACE inhibitors on arterial thrombosis through their effects on platelet function and the endogenous fibrinolytic system. Several studies have demonstrated inhibition of platelet aggregation by ACE inhibitors[114,115]. These inhibitory actions may be related to the inhibition of angiotensin II formation, which is associated with an increase in thrombotic activity. In addition, the increase in bradykinin levels resulting from the inhibition of ACE kininase activity induces the generation of elevated levels of prostacyclin and EDRF, both of which are associated with modulation of platelet aggregation[49]. The ability of ACE inhibitors to attenuate platelet aggregation and adhesion may have positive effects in the prevention of atherosclerosis.

ACE inhibitors also appear to have a protective effect against endothelial damage caused by oxygen-derived free radicals. In rabbit aorta, ACE inhibition attenuated the reduction of the vasodilatory response to acetylcholine[116], normally seen when oxygen-derived free radicals are generated. L-arginine also significantly reduced oxygen-derived free radical damage, while L-NA attenuated the protective effects of both ACE inhibitors and L-arginine. The results of this study suggest that the protective effects of ACE inhibitors may be mediated by facilitation of nitric oxide release, with a subsequent reduction in lipid peroxidation.

In recent clinical trials, ACE inhibitors have been shown to reduce mortality and morbidity in patients with heart failure. In the SOLVD study[117], patients with asymptomatic left ventricular dysfunction who received ACE inhibitors were found to have significantly reduced mortality rates, progression to heart failure, and heart failure-related hospitalizations. In addition, a statistically significant risk reduction for death, MI, and unstable angina of 23% was observed in the SOLVD population[118]. In the SAVE study[119], ACE inhibitors administered to patients following myocardial infarction resulted in a 25% reduction in recurrent myocardial infarction, as well as a 19% reduction in all-cause mortality. In the more recent AIRE study[120] of more than 2000 patients following myocardial infarction, the all-cause mortality rate was reduced by 27% over the 15-month study period. It is interesting to note that the risk reduction was similar in patients with different levels of systolic and diastolic blood pressure at baseline. These observations suggest that the reduction in major ischaemic events seen with ACE inhibition is due, at least in part, to mechanisms separate from their hypotensive effects. Some probable mechanisms include the attenuating effects of ACE inhibitors on the progression of atherosclerosis, stabilization of atherosclerotic lesions, inhibition of cardiac hypertrophy and remodelling, and antithrombotic effects.

Conclusions

In summary, the L-arginine/nitric oxide pathway has undoubtedly reached the clinical arena and is currently under intensive investigation in various forms of cardiovascular disease, including essential hypertension, atherosclerosis, and vasospasm. Many of the beneficial effects of ACE inhibitor therapy may be explained by the inhibition of bradykinin degradation. Thus, ACE inhibition may restore cardiovascular homeostasis, at least in part, by enhancing the physiological roles of nitric oxide.

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