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KININS AND ENDOTHELIAL CONTROL OF VASCULAR SMOOTH MUSCLE

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ABSTRACT

Plasma and vascular kinins stimulate the production of endothelium-derived nitric oxide, prostacyclin and hyperpolarizing factor (which regulates the function of vascular smooth muscle), and endothelial interactions with blood cells. The role of kinins in vasomotion is determined by the rate of production of the peptides by kininogenases and their degradation by kininases, in particular angiotensin-converting enzyme (ACE). Acute increases in plasma kinin levels during excercise or myocardial ischemia indicate that the metabolism of the peptides is fine tuned to the systemic or local metabolic demands. The release of endothelial vasodilators is impaired (or counterbalanced by the release of chemical or functional antagonists) in atherosclerosis, hypertension, diabetes, subarachidonic hemorrhage, and following postischemic injury. ACE-inhibitors potentiate the action of kinins and normalize endothelial function. In septic shock, hypotension triggered by overproduction of kinins leads to cardiovascular impairment and end-organ damage. Thus the balance in the metabolism of kinins modulates the control of blood flow by the endothelium.

INTRODUCTION

By secreting relaxing and contracting factors, the endothelium can induce vasodilatation or vasoconstriction in response to shear stress and a variety of vasoactive substances and, thus, can modulate both humoral and neural control

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of vascular smooth muscle tone (1, 2; for review see 3–5). Furthermore, the endothelium also produces growth-promoting and -inhibiting factors and cytokines, which affect vascular geometry and reactivity (for review see 6–12). Endothelial stimulants are either produced systemically (e.g. catecholamines, growth factors) or generated locally by endothelial cells or vascular smooth muscle (3–12). This review focuses on the regulation of the production of endothelium-derived mediators in an autocrine or paracrine fashion by the vascular kallikrein-kinin system. The rate of production of kinins in blood vessels compared with that of their degradation by kininases, including angiotensin-converting enzyme (ACE), may determine the extent of the contribution of these endogenous peptides to the peripheral regulation of vascular tone and blood flow.

ENDOTHELIUM-DERIVED MEDIATORS

Nitric Oxide

The free radical nitric oxide (NO) (13–16) or a nitrosocompound (17) is the endothelium-derived relaxing factor first described by Furchgott & Zawadzki (1). The vascular wall contains two types of NO synthases: a calcium-dependent one that is expressed constitutively in endothelial cells (18) and one that can be induced by cytokines and endotoxins in both endothelial (19) and vascular smooth muscle cells (20). Constitutive endothelial NO synthase is primarily associated with membranes following the myristilation of its N-terminal glycine (21), whereas inducible NO synthase is a cytosolic enzyme (22–24). Constitutive NO synthase undergoes phosphorylation of a serine residue during stimulation of endothelial cells by shear stress or bradykinin, which leads to a translocation of the enzyme to the cytosol (25). The expression of constitutive NO synthase is enhanced by shear stress, which may be one mechanism whereby chronic elevation in blood flow (26) or exercise-training enhances the endothelial production of NO (27). However, cytokines down-regulate constitutive NO synthase activity, protein, and mRNA (22). Inhibitors of DNA transcription and mRNA translation, as well as corticosteroids (e.g. dexamethasone), interfere with the expression of the inducible NO synthase elicited by cytokines and endotoxins (16, 22–24, 28).

Both types of NO synthases utilize L-arginine, O_2, and reduced nicotinamide adenine dinucleotide phosphate as substrates to form L-citrulline and NO, and require tetrahydrobiopterin, flavin adenine dinucleotide, flavin mononucleotide, and heme as cofactors (for detailed review see 16, 22–24, 28). Calcium dependency distinguishes functionally constitutive NO synthase from the inducible isoform. Thus elevations of cytosolic calcium in endothelial cells allow calmodulin to activate constitutive NO synthase (16, 22, 23, 28). Calmodulin

is tightly bound to inducible NO synthase as a subunit; its stimulatory function on this isoform is evidenced when calcium ions are eliminated by chelation (22–24, 28).

The interaction of NO with its cellular targets, which include heme proteins, ADP-ribosyltransferase, numerous Fe-S enzymes, nonheme Fe proteins, and protein thiols, is determined by the redox status of NO in their microenvironments (28, 29). The neutral free radical NO is in equilibrium with the nitroxyl anion (NO^-), or the cation nitrosonium (NO^+). Thus NO readily reacts with redox metals, oxygen, and superoxide anions; NO^- binds to sulfhydryl groups and metals, whereas NO^+ is involved in nitrosation reactions with aromatic compounds and bases. The activity of NO synthase is regulated by its heme subunit (22–24, 28); thus NO reversibly down-regulates the constitutive isoform (30). NO stimulates soluble guanylate cyclase by forming a nytrosyl complex with its prosthetic heme subunit (31). The resulting increase in guanosine 3′:5′-cyclic monophosphate (cGMP) activates target proteins such as protein kinase G to induce hyperpolarization and relaxation and to inhibit growth of vascular smooth muscle (10, 32, 33). NO may also cause membrane hyperpolarization in the vascular smooth muscle cells by direct activation of potassium channels independently of cGMP (34). Thus when inducible NO synthase is expressed, the overt production of NO may result in the inactivation of functionally important thiol and tyrosyl groups of nucleic adenoribonucleotidase, which is a key enzyme in translation processes (28, 29). Inactivation of thiol groups and loss of iron by NO is cytotoxic by blocking enzymes of the respiratory chain and aconitase in the krebs cycle (28).

The production of NO can be inhibited pharmacologically by use of metabolically stable analogues of L-arginine (e.g. N^{ω}-nitro-L-arginine, N^{ω}-amino-L-arginine), flavoprotein inhibitors (e.g. diphenylene iodonium), depletors of tetrahydrobiopterin (e.g. 2,4-diamino-6-hydroxypyrimidine), or certain inhibitors of cytochrome P450 enzymes (16, 28, 35). Calmodulin antagonists inhibit constitutive NO synthase but not the inducible enzyme (36). Calmodulin antagonists do not affect endothelium-dependent relaxation or elevation of cGMP levels that are mediated by NO in canine and porcine coronary arteries in response to bradykinin (37); thus the calcium-calmodulin complex may be only one of several modes of activation of constitutive NO synthase.

Prostaglandins

Prostacyclin is the major prostaglandin (PG) produced by the endothelium (38, 39). However, in some vascular beds the synthesis of PGD_2 or PGE_2 might prevail over that of prostacyclin (40, 41). Furthermore, in canine veins and cerebral arteries, as in arteries from spontaneously hypertensive rats and diabetic or hypercholesterolemic rabbits or rats, the production of vasoconstrictor prostanoids (2, 3), such as PGH_2, thromboxane A_2, or $PGF_{2\alpha}$, is enhanced (42,

43). This imbalance in favor of proaggregatory prostanoids in vascular diseases curtails the normal vasodilator, homeostatic, and antithrombotic functions of the endothelium. The generation of prostanoids is primarily determined by the mobilization of arachidonic acid from plasma membrane by phospholipase A_2. Arachidonic acid is processed by prostaglandin endoperoxide synthase, which possesses a cyclooxygenase activity yielding PGG_2 and a peroxidase activity that generates PGH_2, the precursor of all prostanoids (38–41). The generation of prostanoids is transient because of the activation kinetics of phospholipase A_2, which is elicited by agonists in endothelial cells (41). Other regulatory mechanisms further determine the output of prostanoids by endothelial agonists; for example, lipid peroxides and protein kinase C modulate cyclooxygenase and prostacyclin synthase, respectively (40). The vasomotor and hemostatic actions of prostacyclin, PGD_2, or PGE_2 are mediated by specific plasma membrane receptors present in some (but not all) vascular smooth muscles, blood elements, and endothelial cells (44). Therefore, prostanoids do not contribute to endothelium-dependent vasodilatation in vascular beds where the smooth muscle cells do not possess receptors for vasodilator prostanoids. The receptors activate adenylate cyclase and induce the production of adenosine 3′:5′-cyclic monophosphate (cAMP) in target cells (3, 44).

Oxygen Reactive Metabolites

Endothelial cells generate oxygen-derived free radicals and hydrogen peroxide (45–47) in response to shear stress and to chemical endothelial stimulants (46, 48). Superoxide anion inactivates NO (49–51). Thus superoxide dismutase (SOD) augments endothelium-dependent relaxations, while inhibition of endogenous SOD reduces the release of NO (52). Superoxide anion is an endothelium-derived contracting factor in canine cerebral arteries (46). Oxygen-reactive metabolites induce the production of platelet-activating factor (PAF) in endothelial cells (53) and affect the secretory functions of the endothelium (54, 55). In rat aorta, oxygen-derived free radicals cause contraction of the vascular smooth muscle after activation of PGH_2 and thromboxane A_2 receptors (56). In contrast, oxygen-derived free radicals mediate the vasodilator response to bradykinin in cat or rabbit cerebral arterioles (57).

Endothelium-Derived Hyperpolarizing Factor

A diffusible endothelium-derived hyperpolarizing factor (EDHF) causes relaxation of the underlying vascular smooth muscle (58–60; for review see 61, 62). The exact nature of EDHF remains unknown. NO and prostacyclin evoke hyperpolarization of vascular smooth muscle cells in a limited number of arteries (63, 64); however, in most blood vessels both the hyperpolarization and the relaxations mediated by EDHF are resistant to inhibitors of NO synthase and cyclooxygenase, respectively (65, 66). Hydrogen peroxide causes

vascular smooth muscle hyperpolarization and relaxation; however, its production by endothelial cells does not account for EDHF (67). Moreover, the transfer of the hyperpolarization in endothelial cells to the vascular smooth muscle cells does not occur by electrotonic coupling (68), in spite of the presence of numerous myoendothelial gap junctions (69, 70). The smooth muscle–dependent hyperpolarization of endothelial cells evoked by isoprenaline through myoendothelial junctions is blocked by the gap junction uncoupler halothane, which does not alter endothelium-dependent hyperpolarization that is mediated by EDHF (71). Conductive gap junctions exist between smooth muscle cells (72, 73) and, thus, may promote the propagation of the hyperpolarization induced by EDHF deep in the vascular wall (61). Hyperpolarization of vascular smooth muscle plasma membrane inactivates voltage-sentive calcium channels, thus inhibiting the extracellular calcium influx mediating the contraction (72). The hyperpolarization induced by EDHF involves potassium channels (61, 62). Endothelial cells synthesize metabolites of arachidonic acid through cytochrome P450 monoxygenases and epoxygenases.

In certain blood vessels, endothelium-dependent relaxations evoked by exogenous arachidonic acid and endothelial agonists are affected by inhibitors of cytochrome P450–dependent metabolism of the fatty acid (74–76). Furthermore, as does EDHF, epoxyeicosanoids cause relaxation in vascular smooth muscle and activate potassium channels (77, 77a). By contrast, 20-hydroxyeicosatetraenoic acid, which is also a cytochrome P450 metabolite, inhibits potassium channels and causes vasoconstriction (78). Thus bioassay experiments in our laboratory show that the amplification of the release of EDHF is accompanied by that of endothelium-derived depolarizing factor(s) (J-V Mombouli & PM Vanhoutte, unpublished observations). Therefore, like the cyclooxygenase pathway that produces prostacyclin and thromboxane A_2, the metabolism of arachidonic acid by cytochrome P450 enzymes generates functional antagonists.

Platelet-Activating Factor

Endothelial cells produce PAF [1-alkyl-2(R)-acetyl-glycero-3-phosphorylcholine] following the activation of phospholipase A_2 (79). Although prostacyclin inhibits PAF synthesis, the transient production of the prostanoid (41) may not counteract the sustained generation of PAF by endothelial cells in response to calcium-dependent agonists. PAF causes contraction of vascular smooth muscle and induces desensitization of β-adrenoceptors that mediate vasodilator responses to circulating and neurally released catecholamines in certain vascular beds (80). PAF mediates the platelet-enhanced hypoxia-induced contraction in coronary arteries (81). The adhesion of leucocytes to the endothelium, which promotes the release of deleterious oxygen reactive me-

tabolites, and the activation of platelets are both induced by PAF to participate in postischemic reperfusion induced tissue damage.

Endothelin

Endothelial cells produce big endothelin and contain endothelin-converting enzymes that process this prohormone into endothelin-1 (ET-1), which is the most potent known vasoconstrictor (82; for review see 83). The production of ET-1 is enhanced by thrombin (84) and platelet products (85), but it is inhibited by NO (86). In vivo ET-1 initially evokes a vasodepressor response through the release of nitric oxide, vasodilator prostanoids (87), and EDHF (88). However, in spontaneously hypertensive rats, ET-1 stimulates the production of vasoconstrictor prostanoids from the endothelium (89). In addition to these vasomotor effects, ET-1 promotes vascular smooth muscle growth (9).

Growth Factors and Cytokines

Endothelial cells produce growth inhibitory and promoter factors that contribute to the development of the endothelium, the underlying vascular smooth muscle, and the composition of the extracellular matrix. Several have described these functions in detail (6–9, 11, 12). For instance, heparinoids and transforming growth factor–β (TGF-β) secreted by endothelial cells inhibit vascular smooth muscle growth, whereas platelet-derived growth factor (PDGF) and basic fibroblast growth factor (bFGF) stimulate the proliferation of endothelial and vascular smooth muscle cells. Cytokines, which are key mediators of inflammation, are produced by endothelial cells activated following injury or by endotoxins (12). Cytokines and growth factors induce phenotypic transitions in endothelial cells that alter the antithrombotic and homeostatic properties of the luminal surface of blood vessels (11, 12). Moreover, these factors modulate vasomotion through endothelium-dependent and -independent mechanisms (19, 20). For example, they evoke the release of NO (90) and affect the composition and amounts of prostanoids secreted by endothelial cells in response to agonists (91). The induction of NO synthase by cytokines in vascular smooth muscle cells is inhibited by PDGF and is facilitated by other growth factors, including bFGF, TGF-β, and epidermal growth factor (EGF) (92–94). These and other reports (11, 12) suggest that physiopathological contributions of growth factors and cytokines are modulated by the cross talk between them and are probably also influenced by constitutive endothelial mediators such as NO and prostacyclin.

VASCULAR METABOLISM OF KININS

Circulating high- and low-molecular-weight (HMW and LMW, respectively) kininogens, the precursors of bradykinin and kallidin (lys-bradykinin), are

produced mainly by hepatocytes. However, in the vascular wall they are synthesized and/or stored by endothelial (95, 96) and vascular smooth muscle cells (97). Kininogens undergo processing principally by kallikreins to generate kinins (for review see 98). The latter are rapidly inactivated by kininases and, thus, may only serve autocrine and paracrine functions (98, 99). Kinins are very powerful vasodilators; therefore, uncontrolled elevations in systemic levels of these peptides, as suggested in septic shock, may lead to life-threatening hypotension and cardiovascular impairment.

Kininogens

A single gene codes for HMW and LMW kininogens, which by alternate splicing yields two mRNAs (98). The hormonal regulation of kininogen expression is not fully understood, although cytokines enhance their synthesis in hepatocytes (98). These macromolecules possess multiple bioregulatory functions independent of their kinin moiety (98). Thus they inhibit thiol proteinases such as cathepsins, ficin, papain, and platelet-derived calpains. In the plasma, HMW kininogen is bound to prekallikrein and Hageman factor and is implicated in the intrinsic coagulation pathway (98, 100). Finally, the binding of either HMW or LMW kininogen to platelets inhibits their interaction with thrombin (101). In addition to platelets, endothelial cells and neutrophils express binding sites for HMW kininogen (98); kininogens bound to these different structures are readily available for kinin synthesis (Figure 1).

Kininogenases

Blood vessels contain kinin-generating enzymes (kininogenases), the more important ones being the serine proteases and plasma and tissue kallikreins (98, 99). Plasma kallikrein is found in the blood in its inactive form, prekallikrein; when the latter is activated by Hageman factor, it specifically releases bradykinin from HMW kininogen (98, 100). Plasma kallikrein may also form bradykinin from LMW kininogen, but this requires preprocessing by a neutrophil elastase (98). The circulating serine protease inhibitors—contrapsin-like kallikrein inhibitor, C1-protease inhibitor, α_2-macroglobulin, and anti-thrombin III—control plasma kallikrein activity (98, 100). Tissue kallikrein–like enzymes constitute a family of genetically related serine proteases, although some of them are not kininogenases (98, 99). True tissue kallikreins process LMW kininogen to release kallidin; however, they also process other hormones and enzymes involved in hemodynamics. These enzymes are synthesized in various organs, particularly in the kidney (98, 99) but also in endothelial (97) and vascular smooth muscle cells (102–104). Because the production of tissue kallikreins in blood vessels is time dependent and inhibited by puromycine, synthesis is probably continous and constitutive (105). Proteases in the submaxillary gland and in the kidney rapidly activate tissue kallikreins, which are

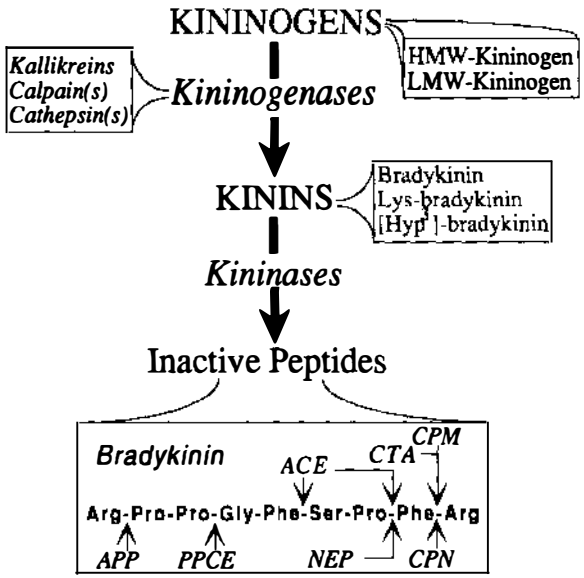

Figure 1 Kinin metabolism. Kininogens, the precursors of kinins, can be processed into kinins by kininogenases, including tissular (from endothelium, vascular smooth muscle) and plasma kallikreins, cathepsin(s) (from neutrophils and vascular smooth muscle), and calpain(s) (from platelets). Bradykinin is the major kinin, although kallidin (lys-bradykinin) and hydroxylated kinins (e.g. [Hyp^3]-bradykinin) can also be found in significant amounts. The minimal structure for activation of endothelial B_2 kinin receptors is that of bradykinin, which is cleaved (at sites indicated by arrows) by kininases, including aminopeptidase P (APP, plasma membranes in vascular cells), postproline cleaving enzyme (PPCE, intracellular), angiotensin-converting enzyme (ACE, plasma membranes in vascular cells, plasma), neutral endopeptidase (NEP, plasma membranes in vascular cells), cathepsin A (CTA, platelets), and carboxypeptidase N (CPN, plasma) and M (CPM, plasma membranes in vascular cells).

secreted as proenzymes (98, 99). α_1-Antitrypsin and kallikrein-binding protein inhibit tissue kallikreins (98).

Kininases

Kinins are substrates for a variety of peptidyl peptidases (Figure 1), including ACE, aminopeptidases, carboxypeptidases, cathepsin A (deamidase), and neutral endopeptidase, or intracellular enzymes, such as postproline cleaving enzyme (for review see 106). Carboxypeptidases N (CPN) and M (CPM) are kininase I–type enzymes: They delete the C-terminal arginine (Figure 1). CPN is the major kininase in human plasma (107), whereas ACE (the major kininase at the surface of endothelial cells) and neutral endopeptidase are type II kininases, which release the C terminus dipeptide (Figure 1). Endothelial ACE (EC 3.4.15.1) is a single-chain transmembrane glycoprotein with two active

sites (108); a soluble form of the enzyme is secreted into the plasma, possibly following proteolytic cleavage of the small transmembrane anchor (108). Ninety-five percent of the exogenous bradykinin administered to humans is degraded by endothelial ACE after a single passage through the pulmonary circulation (109). Large, genetically determined interindividual variations in plasma levels of ACE (108) may have predictive value in the prognosis of myocardial infarctions (110). Endothelial ACE activity may be further regulated by hormones or local vasoactive substances (for review see 111, 112).

VASOMOTOR ACTIONS OF KININS

Kinin Receptors

Two classes of kinin receptors, termed B_1 and B_2, have been found in vascular tissues (for review see 113–116). B_2 receptors are predominant in endothelial cells, and both bradykinin and kallidin are potent agonists at these receptors, whereas metabolites produced by kininases I and II are inactive, or much less potent than their precursors (115–117). This class of receptors comprises subtypes that are currently associated with new generations of bradykinin agonist and antagonist analogues (113, 114, 116, 118). In endothelial cells, B_1 kinin receptors are expressed constitutively in only a few blood vessels (113–117); however, they can be induced by endotoxins (119) or spontaneously in vitro (120). B_1 receptors are activated by the kininase I metabolites of kinins desArg^9-bradykinin or desArg^{10}-kallidin (113–117). In certain blood vessels, B_1 and/or B_2 receptors mediate endothelium-independent vasomotor responses through the generation of vasoconstrictor or vasodilator prostanoids by vascular smooth muscle cells (115). In addition, these prostanoids help kinins modulate sympathetic neural activity in the blood vessels (121).

Transduction Mechanisms in Endothelial Cells

B_2 kinin receptors belong to the superfamily of G protein–coupled receptors (113, 114). The majority of G proteins activated by these constitutive receptors in endothelial cells are ADP-rybosylated by botulinium toxin (122). These G proteins are coupled to phospholipase C, which generates inositol phosphates and diacylglycerides (123, 124). Thereafter, there is a stimulation of protein kinase C and the mobilization of calcium ions from both intracellular and extracellular pools. The influx of calcium ions involves nonselective cationic channels (activated by strech or inositol polyphosphates) and possibly voltage-gated channels (for review see 54, 125). In endothelial cells, hyperpolarization enhances the electrochemical gradient of the plasma membrane that drives current through the cationic channels (54, 125). Whether EDHF mediates hyperpolarization in endothelial cells is unknown because of the lack of

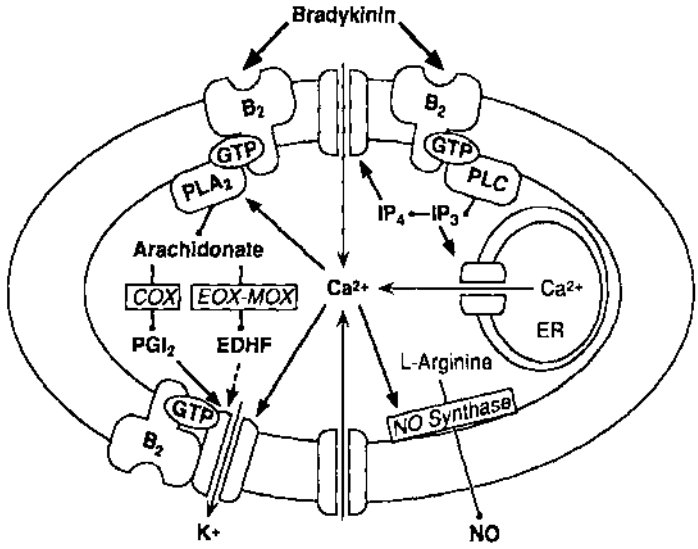

Figure 2 Endothelial metabolism of calcium and relaxing factors. Calcium ions (Ca^{2+}) are central to the activation of the synthesis of nitric oxide (NO) by the constitutive NO synthase, and contribute to the release of arachidonic acid by activating phospholipase A_2, which can alternatively be activated by G protein–coupled B_2 receptors. The increase in cytosolic calcium results from the depletion by inositol-1,4,5-trisphosphate (IP_3) of intracellular stores of Ca^{2+} [i.e. endoplasmic reticulum (ER)]. IP_3, which is generated following G protein–dependent activation of phopholipase C, may also be processed into inositol-1,4,5,6-tetrakisphosphate (IP_4) to open Ca^{2+} permeable channels. The opening of the latter is facilitated by the hyperpolarization of the plasma membrane. Arachidonic acid is metabolized into prostacyclin (PGI_2) or EDHF, which may be a product of cytochrome P450–dependent epoxygenase (EOX) or monoxygenase (MOX). The hyperpolarization induced in endothelial cells by kinins results from the activation of potassium channels, either by G protein–coupled B_2 receptors, Ca^{2+}, or cyclic AMP–elevating substances such as PGI_2. Whether EDHF also modulates potassium channels in endothelial cells is unknown (*dashed arrow*).

identification of EDHF. In some endothelial cells, the release of prostanoids requires high concentrations of cytosolic calcium (126); this reflects a calcium-dependent activation of phospholipase A_2, which is the major source of unesterified arachidonic acid. In other endothelial cells, the activation of phospholipase A_2 by bradykinin is mediated through pertussis toxin–sensitive G proteins (127) (Figure 2).

The influx of extracellular calcium and the intracellular alkalinization induced by bradykinin are sufficient to sustain the release of NO (126, 128). Thus concentration-response curves to bradykinin for the calcium mobilization differ from the release of NO (125). Second messengers other than calcium participate in the modulation of other endothelial secretory functions as well (129). Intracellular calcium and protein kinase C mediate the exocytosis of

von Willebrand factor (126), and protein kinase C inhibits the activation of constitutive NO synthase (130). The expression of growth factors, cytokines, and substances implicated in coagulation and fibrinolysis is partly regulated by protein kinase C (126). cAMP elevations cause membrane hyperpolarization that enhances bradykinin-induced calcium influx into endothelial cells (131); this explains facilitation of NO release by prostacyclin (132). cAMP enhances ACE activity (112) and stimulates exocytosis (126). cGMP inhibits the secretion of endothelin (133). Even though bradykinin is a powerful activator of endothelial cells, the concentration-dependent release of the different substances produced by the endothelium is influenced by the cross talk between the different second messengers and the metabolic pathways that are involved.

Hemodynamic Functions of Kinins

BLOOD PRESSURE REGULATION In humans, as in animals, plasma levels of kinins (10–50 pg ml^{-1}, as detected by radioimmunoassay techniques) are much lower than the threshold concentration (approximately 100 pg ml^{-1} in humans) for the systemic hypotensive effect of bradykinin (109). Thus circulating kallikreins and kininogens may not participate in the regulation of systemic peripheral arterial resistance. Patients deficient in the kininase I CPN have slightly elevated plasma kinins and are hypotensive (134); therefore, modulation of kininase activities may lead to vasodilatation. Administration of bradykinin or its analogues elicits complex responses. Agonists evoke vasodilatation followed by pressor effects, whereas antagonists induce the opposite sequence (135). This observation partially reflects the multiple actions of these compounds in endothelial cells, vascular smooth muscle, and nerve endings; furthermore, kinins influence vasopressor mechanisms in the brain and in the kidney (135). The synthesis of kinins in the kidney or in the brain may affect systemic blood pressure, via renal and central neural mechanisms, respectively. Indeed, defects in the expression of the renal kallikrein-kinin system are involved in the etiology of hypertension (99), possibly through sodium retention (135). Moreover, intracerebroventricular injection of either bradykinin or an ACE inhibitor elicits a rise in blood pressure in rats; both effects are inhibited by B_2 kinin receptor antagonists (136). Because of their agonistic actions, first-generation bradykinin antagonists (115) have not yielded conclusive evidence as to the role of kinins in blood pressure regulation (135). Studying metabolically stable antagonists that are devoid of agonistic actions (137, 138) may lead to a better definition of the role of kinins in the peripheral regulation of systemic vascular resistance.

REGIONAL BLOOD FLOW The presence of kininogenases and kininogens in the vascular wall has been demonstrated indirectly in vitro. In endothelial cells in

culture, kinins accumulate during ACE inhibition and elicit the generation of NO and PGI_2 (139, 140); this accumulation is blocked by a serine protease inhibitor, 3,4-dichloroisocoumarin (140). In canine isolated coronary arteries, exogenous tissue kallikrein mobilizes kinins in the vascular wall to cause endothelium-dependent relaxations (141). Together with shear stress, vascular kinins stimulate the basal release of vasodilator mediators in perfused isolated canine arteries (142) and rat hearts (140). During exercise, kinin levels increase in vascular beds perfusing skeletal muscles (143). However, the regulatory mechanisms governing acute or chronic changes in the vascular kinin output are unclear. Kininogens are abundant in both the vascular wall and the plasma; therefore, they are not the limiting step. Thus, the regulation of kininogenase activity through protein synthesis, posttranslational processing, and protease inhibitor production (98, 144) warrants further investigation to establish the molecular basis of the autocrine and paracrine functions of kinins in blood vessels. Differences in vascular reactivity may affect the impact of the vascular kallikrein-kinin system on smooth muscle function. For instance, endothelium-dependent relaxations to bradykinin are augmented in coronary arteries from exercise-trained dogs (JV Mombouli & PM Vanhoutte, unpublished observations) or pigs (145), when compared with confined sedentary ones. Therefore, the cardiovascular inadaptation of sedentary individuals to intense physical efforts may result from kinins less potent in the release of endothelial vasodilator substances.

SEPTIC SHOCK Septic shock is characterized by a blood stream microbial invasion that is accompanied by profound hypotension, which leads to multiorgan failure and death (146). The multiplicity of inflammatory mediators that aggravate this syndrome render its treatment very difficult (147). Toxic microbial products, including endotoxins, dramatically activate plasma kallikrein-kinin system and deplete circulating serine protease inhibitors (for review see 100). The massive generation of bradykinin (148) that ensues may initiate the life-threatening hypotension, which is mediated by NO and other endothelium-derived vasodilator mediators (Figure 3). Thus B_2 kinin receptor antagonists (149) and the blockade of plasma kallikrein (148, 150) prolong the survival rate in animal models of septic shock. Endotoxins induce B_1 receptors, thus allowing $desArg^9$-bradykinin to become a very potent vasodilator (115, 119, 120, 148). Under these circumstances, CPN, which is normally a kininase in the absence of constitutive B_1 receptors, becomes a converting enzyme. Together with bradykinin and $desArg^9$-bradykinin, endotoxins stimulate macrophages and vascular tissues to produce cytokines, which in turn induce calcium-calmodulin–independent NO synthesis by endothelial and vascular smooth muscle cells (16, 19, 20). These processes, which positively feed into each other (Figure 3), result in an overwhelming and sustained production of

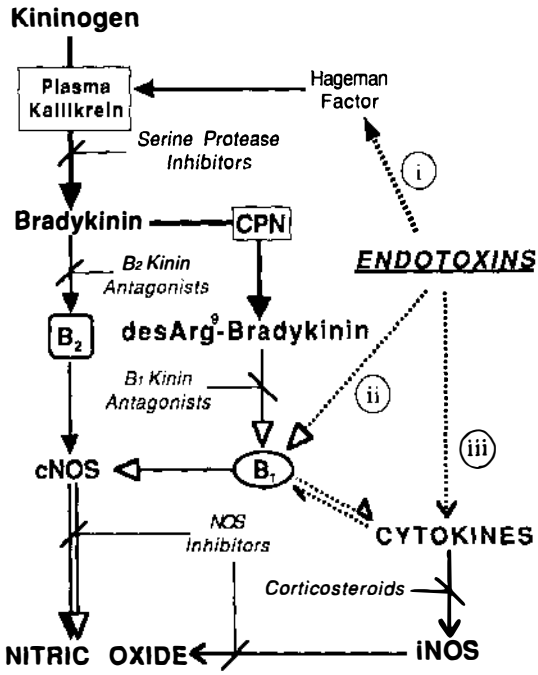

Figure 3 Kinins, nitric oxide, and septic shock. Microbial products, such as endotoxins, activate (i) the plasma kallikrein-kinin system. The bradykinin thus generated evokes the production of NO through activation of the constitutive NO synthase (cNOS) pathway. Moreover, endotoxins induce (iii) the calcium-independent NO synthase (iNOS) and (ii) B_1 kinin receptors (activated by desArg^9-bradykinin) in vascular endothelial and smooth muscle cells. Endotoxins also stimulate the production of cytokines in the vascular wall. A positive feedback may occur between the secretion of cytokines by macrophages and the expression of B_1 receptors in different components of the vascular wall. Therefore, using B_1 and B_2 receptor antagonists, together with specific inhibitors of constitutive or inducible NO synthase, may interrupt the vicious cycle in the production of NO at different stages of the development of septic shock. The timetable of expression of the different processes and their importance relative to other pathologic hypotensive mechanisms may vary from one species to another or among individuals.

NO that ultimately affects host-defense mechanisms and impairs cardiovascular function (148, 151).

KININS IN CARDIOVASCULAR DISEASES AND THERAPY

ACE inhibitors were first designed to impair the renin-angiotensin system and prevent the deleterious effects of an excessive production of angiotensin II

(152). However, the available experimental evidence supports the hypothesis that endothelium-derived mediators contribute to the therapeutic actions of these agents independently of the renin-angiotensin system (111, 112). Thus endothelial mediators modulate the renin-angiotensin in the kidney and in the vascular wall (112). Following the inhibition of ACE, the increased levels of angiotensin (1–7) may result in further release of endothelial vasodilators (153). ACE inhibitors are used for the treatment of hypertension and heart failure; kinins may contribute significantly to the beneficial effects of these substances, possibly because they are among the endothelial stimulants that are least affected by endothelial dysfunctions, which occur during cardiovascular pathologies.

Potentiation of Kinins and Other Endothelial Agonists

The activation of endothelial B_2 kinin receptors is primarily regulated by membrane-bound ACE. The intensity of ACE activity relative to the density and/or coupling efficiency of the B_2 receptors and the role of other kinin-disposition mechanisms may determine the ability of ACE inhibitors to actually potentiate the actions of kinins (111). For example, despite the presence of ACE activity in large porcine coronary and femoral arteries, ACE inhibitors do not significantly affect the concentration-relaxation curves to bradykinin (111, 154). By comparison, human, canine, or bovine coronary arteries show a 10-fold potentiation (155–157). Resistance arteries of the pig exhibit a significant potentiation (154); hence such differences are organ but not species related. ACE inhibitors in canine coronary arteries cause a striking amplification of the release of EDHF by bradykinin (65). Hecker et al (158) have suggested a direct interaction of ACE inhibitors with the B_2 kinin receptors to explain a potentiation of kinins independently of ACE activity. Campbell et al have shown that ACE inhibitors significantly augment plasma kinin levels at concentrations that do not affect the renin-angiotensin system (159). Therefore, ACE inhibitors may induce kinin levels to increase independently of ACE. ACE inhibitors potentiate endothelium-dependent relaxations evoked by platelets and by ADP (155). These compounds also augment endothelium-dependent relaxations to acetylcholine through a kinin-dependent mechanism (160).

Preservation of Endothelial Function

HYPERTENSION The endothelial control of vascular function is consistently impaired in hypertension. However, the mechanisms underlying the impairment may vary among forms of hypertension in humans (161) as well as in experimental models (3). These mechanisms include a decrease in the production of NO, as found in Dahl salt–sensitive hypertensive rats (162). In this

model, supplementing the rats' diets with L-arginine (163) or antihypertensive treatment alleviates the impairment (164). By contrast, spontaneously hypertensive rats experience a simultaneous production of several vasoconstrictor products of cyclooxygenase (42). In humans the blunting of the endothelium-dependent vasodilatation that occurs in essential, but not in primary aldosteronism or renovascular, hypertensives is mediated by cyclooxygenase-dependent vasoconstrictor mechanisms (161). Impairment of the vasodilator and antiplatelet functions of the endothelium may contribute to the cardiovascular risks associated with hypertension. The kallikrein-kinin system opposes the development of hypertension in DOCA-salt hypertension (165). Benetos et al (166) have suggested that sodium-induced suppression of endogenous bradykinin may participate in the initiation of salt-dependent hypertension. Infusion of aprotinin to spontaneously hypertensive rats accelerates the rise in blood pressure, which suggests that alterations in the kallikrein-kinin system(s) may be involved in the establishment of hypertension (167). Levels of kininogenase activity are low in arteries from spontaneously hypertensive rats (168) and one-kidney, one-clip hypertensive rats, which indicates a reduced vascular production of kinins (99). ACE inhibitors, therefore, might help overcome this deficit by slowing down kinin catabolism in the vascular wall. In various rat models, B_2 receptor antagonists reduce the antihypertensive action of ACE inhibitors both acutely (99, 169, 170) and chronically (171). Furthermore, this treatment restores endothelial vasodilator function, inhibits smooth muscle hyperplasia, and prevents the margination of monocytes in the subendothelial space (172); however, whether the latter action is caused by vascular kinins or the prevention of angiotensin II generation remains to be established.

DIABETES Morbidity and mortality in diabetes mellitius are associated with vascular complications. In humans, vasodilatation to endothelium-dependent relaxing agents is reduced in diabetic compared with nondiabetic patients (173). Bradykinin-induced vasodilatation is also impaired in the renal vasculature of diabetic rats (174). These alterations result from an overproduction of vasoconstrictor prostanoids and oxygen radicals promoted by high glucose (43, 51, 175). Therefore, inhibitors of cyclooxygenase or SOD can restore endothelium-dependent relaxations (43, 51, 175, 176). Normal vessels exposed to elevated glucose levels display impaired endothelium-dependent relaxations, which can be restored by aldose reductase inhibitors, as can those in diabetic vessels (176). In addition, end glycation products, which accumulate in vessels during the development of the diabetic vascular disease, quench NO; this action further reduces the relaxations (177, 178). Kinins mediate improvements of tissue sensitivity to insulin and of the glucose and lipid metabolism induced by ACE inhibitors (143, 179). ACE inhibitors reduce urinary albumin and protein excretion in both normotensive and hypertensive diabetic patients, in

part through reductions in blood pressure and glomerular filtration rate (for review see 180, 181). Early alterations in the renal kallikrein-kinin system contribute to the development of hypertension in diabetes (182). Along with other intrarenal mechanisms, the kidney kallikrein-kinin system contributes to the beneficial effects of ACE inhibitors on diabetic nephropathy (180, 181).

ATHEROSCLEROSIS Atherosclerosis is characterized by progressive dysregulation of lipid metabolism, increased subintimal proliferation, and migration of smooth muscle. The inability of the endothelium to restrict the infiltration of low-density lipoproteins in the vascular wall results in the accumulation of lipids by smooth muscle cells and macrophages (6, 8, 11). These events are a consequence of the impaired antithrombotic and homeostatic functions of the endothelium that occur following traumatic injury to the endothelium or activation of endothelial cells by atherogenic stimuli. In atherosclerotic porcine coronary arteries, a loss of pertussis toxin–sensitive G protein function occurs at early stages of the disease (183, 184). Consequently, the release of endothelium-derived vasodilators induced by platelets or by 5-hydroxytryptamine is severely reduced (183, 184). The ability of the endothelium to oppose platelet aggregation, adhesion to the endothelium, and secretion of mitogens is therefore dramatically impaired. In this disease monocytes also increasingly infiltrate the subendothelial space. These cells, together with platelets recruited to the vascular wall, provide growth promoters and cytokines, in addition to those produced by activated endothelial and/or vascular smooth muscle cells (6, 8, 11). These changes in phenotype are exaggerated by hypercholesterolemia (184), which also causes the endothelium to produce an excess of oxygen radicals (185). Oxidized low-density lipoproteins acutely impair endothelium-dependent relaxations to thrombin and aggregating platelets in vitro but do not affect those evoked by bradykinin (186), in which EDHF provides a major contribution (66). The impairment can be corrected by exogenous L-arginine (186); interestingly, vasodilatation to endothelium-dependent relaxing agents in hypercholesterolemic patients can similarly be normalized by administration of L-arginine (187, 188). Oxidized low-density lipoproteins carry lipid peroxides that inhibit the formation of prostacyclin and thus curtail its protective role (40). Bradykinin-induced endothelium-dependent relaxations are more resistant to hypercholesterolemia and atherosclerosis in porcine coronary arteries (183, 184). However, oxidized low-density lipoproteins attenuate selectively the G_i-dependent component of the responses to bradykinin and other endothelial agonists (183, 189).

In humans, however, both receptor-dependent and -independent stimulation of endothelial cells is impaired in the latter stages of the formation of atherosclerotic plaques (190, 191). The myointimal smooth muscle proliferation and migration induced in rat carotid arteries after endothelial injury by a balloon-

catheter is blocked by ACE inhibitors (192–194). The action of ACE inhibitors in blocking smooth muscle proliferation and migration is mimicked in part by angiotensin receptor antagonists; however, it is also sensitive to B_2 kinin receptor antagonists (194). ACE inhibitors prevent the atherosclerosis in hyperlipidemic rabbits and cholesterol-fed monkeys (195); in rabbits, this antiatherogenic effect is inhibited by B_2 kinin receptor antagonists (196).

CARDIOPROTECTION Abnormal endothelial responses, such as those following atherosclerosis, contribute to the pathogenesis of myocardial ischemia (197). Thus substances that evoke the release of nitric oxide in healthy coronary arteries, such as catecholamines, serotonin, and thrombin, induce a paradoxical vasoconstriction when the endothelium is dysfunctional, which leads to ischemic episodes. Following prolonged ischemia, reperfusion itself impairs the endothelium-dependent relaxations in the isolated coronary arteries induced by bradykinin (55) and other endothelium-dependent vasodilators (55, 198, 199). However, postischemic reperfusion does not alter the endothelium-independent relaxation elicited by direct vascular smooth muscle relaxants, including exogenous NO (55, 199). The tolerance to endothelial agonists, which is induced by endothelium-derived oxygen-reactive species, precedes the subendothelial margination and accumulation of neutrophils, and myocardial necrosis (55), and can be prevented by SOD (55).

Activation of the plasma kallikrein during myocardial infarction in humans induces an elevation of cardiac kinin levels (200, 201), such as that observed in myocardial ischemic models in dogs (201, 202). Under these conditions, either ACE inhibitors or exogenous bradykinin prevents reperfusion-induced cardiac dysfunction (203). In contrast, B_2 kinin receptor antagonists aggravate postischemic injury and counteract the cardioprotective action of bradykinin and ACE inhibitors (203); the cardioprotective effects of ACE inhibitors may not involve the impairment of the cardiac renin-angiotensin system (204). ACE inhibitors and bradykinin also prevent the deleterious effects of PAF-activated neutrophils, which amplify the damage to both vascular and myocardial tissues during postischemic reperfusion (205).

In addition to these kinin-dependent effects, the thiol group in certain ACE inhibitors provides additional protection (206), possibly through potentiation of the actions of NO (207) and the activation of potassium channels in the vascular smooth muscle (208). Non-antihypertensive doses of ACE inhibitors have antihypertrophic effects independent of afterload and blockade of angiotensin II formation and, thus, improve left ventricular performance and metabolism (209–211).

SUBARACHNOID HEMORRHAGE In a canine experimental model, endothelium-dependent relaxations to agonists, including bradykinin and the calcium iono-

phore A23187, are reduced in cerebral arteries (212). The impairment in the relaxations does not result from a reduced production of NO (212); it reflects the inability of guanylate cyclase to generate cGMP (213). However, in the cerebral arteries, bradykinin elicits vasodilatation through NO-independent mechanisms also, including EDHF (62). Thus protection of the peptide with ACE inhibitors may alleviate the vasospasm.

CONCLUSION

The endothelium controls the vascular smooth muscle via systemic and local mechanisms. Systemic regulation of blood vessel tone involves shear-stress and humoral factors, such as catecholamines and vasopressin; these mechanisms modulate blood pressure and perfusion of vital organs in harmony with the general metabolic requirements of the organism. Local mechanisms are implicated in the regulation of the blood perfusion of organs, as a function of their own specific demands, and also contribute to hemostasis by preventing vascular occlusion and ischemia, which lead to tissue damage. Kinins are among the most powerful endogenous stimulants of endothelial cells, as determined experimentally both in vivo and in vitro. Because they are produced in the immediate vicinity of their receptors in endothelial cells, they probably participate in the regulation of vasomotion by the endothelium. Their great efficiency commands tight regulation of kinin levels through proteolytic inactivation, principally by ACE, to avoid an excessive vasodepressor tone. However, acute changes in the local ouput of kinins may be critical to the proper perfusion of certain organs during exercise or restoration of blood flow during ischemic episodes. As illustrated for some of the actions of ACE inhibitors obtained in experimental models, proper management of kinin metabolism may yield therapeutic benefits in the treatment of hypertension, diabetes, atherosclerosis, and various cardiomyopathies.

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