VASCULAR ENDOTHELIUM AS AN ENDOCRINE ORGAN

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Vascular endothelium has a surface area of 400 square meters, weighs about 1·5 kg and contains an estimated 1·2 trillion endothelial cells.[1] It is a selectively permeable barrier for macromolecules, and provides a non-thrombogenic and non-adhesive surface that actively maintains the fluidity of blood. In addition to the barrier and transport functions, vascular endothelium acts as a paracrine/endocrine organ by secreting a wide range of biologically active mediators. These play a key role in regulating immune responses, vascular tone and coagulation, and act on neighbouring smooth muscle cells, monocytes, macrophages, fibroblasts and organ specific cells.

These include:

- vasodilators (prostacycline (PGI_2), nitric oxide (NO), endothelium-derived hyperpolarisation factor (EDRF)) and natriuretic peptides;
- vasoconstrictors (endothelin (ET), thromboxane A2, prostaglandin H2 and components of renin-angiotensin system);
- pro- and anti-thrombotic factors (tissue factor, platelet-activating factor (PAF), von Willebrand factor (vWf));
- fibrinolytic activators and inhibitors (tissue plasminogen activator (t-PA), plasminogen activator inhibitor-1 (PAI-1));
- arachidonate metabolites (prostanoids);
- leukocyte adhesion molecules (intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), E-selectin, P-selectin); and
- multiple cytokines transforming growth factor, pro-inflammatory and anti-inflammatory mediators, tumour necrosis factor, chemokines and steroids.

NITRIC OXIDE (NO)

Furchgott and Zawadzki[2] were the first to show that the endothelium was essential for the vasodilator action of acetylcholine in isolated arterial strips. They postulated that stimulation of muscarinic receptors on the endothelial cells triggered the release of a substance which they named endothelium-derived relaxing factor (EDRF). The chemical nature of EDRF was not known until Palmer, Ferrige and Moncada[3] demonstrated that nitric oxide accounted for most if not all of the biological activity of EDRF. Both EDRF and NO act through the stimulation of soluble guanylate cyclase and subsequent formation of cyclic GMP (cGMP).[4] Cyclic GMP activates cGMP-dependent protein kinases and leads to dephosphorylation of myosin light chains and muscle relaxation.

Nitric oxide is a free radical gas generated by NO synthase (NOS) through the oxygenation of one of the guanidino nitrogen atoms of L-arginine. Nitric oxide is highly unstable with a half-life of seconds. Its action is inactivated by haemoglobin, oxygen and methylene blue, and potentiated by superoxide dismutase. Mechanical stretching, hypoxia, stress, acetylcholine, vasopressin, norepinephrine, endothelin, bradykinin, histamine, adenine nucleotides, thrombin and 5-hydroxy tryptamine cause release of NO.[5] The NOS family is composed of three isoforms: brain NOS (bNOS), endothelial NOS (eNOS) and inducible NOS (iNOS).

Nitric oxide induces vascular smooth muscle relaxation and suppresses platelet aggregation. Nitric oxide also decreases vascular smooth muscle cell collagen types I and III concentration,[6] with a continuous basal release of NO from endothelial cells to keep the vasculature dilated.[7] Administration of NOS inhibitors result in a quick and significant increase in blood pressure.[8] A hypofunctioning NO system could contribute to a number of diseases including hypertension, atherosclerosis, diabetes and vasosplastic disorders.[9] An excess production of NO may cause septic shock, as lipopolysaccharides from anaerobic bacteria induce NOS in vascular smooth muscle cells, macrophages and endothelial cells.

Nitric oxide is a typical paracrine hormone acting only in its immediate environment, as it would be immediately inactivated by haemoglobin when released into the blood stream.

ENDOTHELINS (ETs)

Endothelins are a family of peptides with potent vasoconstrictor properties. They originate from a large prepropeptide, from which big ET is generated by a proteolytic cleavage, this is then transformed to the active form by endothelin-converting enzyme in the endothelium.[10] Endothelin-converting enzyme is expressed in endothelial cells and in numerous other tissues including bronchial epithelium, adrenal glands and gonads. Three isopeptides have so far been discovered, ET-1, ET-2 and ET-3.[11] Endothelin-1 and ET-2 resemble each other in their pharmacological actions and in their binding characteristics to receptors; ET-3 has weaker vasoconstrictor action but is a more potent inhibitor of platelet aggregation. It also binds to a different receptor.[12]

Two isoforms of ET receptors ET_A and ET_B have been cloned.[13] $Endothelin_A$ is specific for ET-1 and ET-2, with a low affinity to ET-3 and is distributed in vascular smooth muscle cells, heart, lung and gut. $Endothelin_B$ binds all three ETs equally well and is located in endothelial cells, brain, lung and kidney. ET_A and ET_B in vascular smooth muscle cells mediates paracrine vasoconstrictor effects of ET-1, whereas ET_B on endothelial cells mediates autocrine functions of ET-1 that result in the release of nitric oxide resulting in vasorelaxation.[14]

Although endothelins are produced in various tissues, ET-1 is the only endothelin produced by endothelial cells. Endothelin-1 gene is localised on chromosome 6. Thrombin, angiotensin II, arginine-vasopressin, interleukin-1, TGFß, catecholamines and anoxia cause release of ETs from the vascular endothelium. Nitric oxide, on the other hand, inhibits ET-1 synthesis.

ß-adrenergic receptor agonists and PGI_2 up-regulate ET_A receptor mRNA expression in vascular smooth muscle cells.[15] Indomethacin abolishes both the ET-1-induced inhibition of platelet aggregation and the corresponding rise in platelet cyclic AMP.[16] These effects are most likely due to the release of prostacyclin. Endothelins also affect hormone secretion from a variety of endocrine organs including anterior and posterior pituitary, atria and adrenals.

Endothelin-1 is the most potent vasoconstrictor so far discovered, being ten times more potent than angiotensin II. Its main property is its long-lasting hypertensive action.[17] Increased plasma concentrations of ET-1 are found in severe hypertension and in pre-eclampsia.[18, 19] Big ET, ET-1 and ET-3 are present in circulating blood and probably represent overflow from locally released peptides.[9] Elevated levels of ETs are found in essential hypertension,[20] hypertension following erythropoietin therapy in patients under chronic haemodialysis,[21] cyclosporin-induced hypertension,[22] cardiogenic and septic shock, acute myocardial infarction, diabetes mellitus and renal failure. Endocardial endothelial cells may modulate myocardial contraction through the release of ET-1.

Endothelins have also potent stimulatory effects on vascular smooth cell proliferation. This could be responsible for the development of smooth muscle wall hypertrophy in hypertension.[23] Endothelin-1 is also mitogenic and these mitogenic effects have been shown in cardiovascular smooth muscle cells, in fibroblasts and in mesangial cells.[20] A role of endothelins in the pathogenesis of atherosclerosis has been suggested. Endothelin production and secretion are enhanced in the presence of oxidised low-density lipoprotein (LDL).[24] Patients with elevated total serum cholesterol and lipoprotein (a) concentrations have been shown to have elevated circulating concentrations of endothelin.[25] Endothelin has also been shown to be elevated in association with cigarette smoking.[26] Circulating and tissue endothelin immunoreactivity correlate with the severity of atherosclerosis.[27] In patients presenting with stable and unstable angina, circulating endothelin concentrations have been shown to be elevated[28] and its concentrations have consistently been shown to be increased in the setting of acute myocardial infarction.[29] Plasma endothelin concentrations are also elevated in patients with moderate to severe congestive heart failure and correlate with the severity of the symptoms.[30] Endothelin-1 also functions as an apoptosis survival factor for endothelial cells in an autocrine/paracrine manner via the ET_B receptor.[31]

The vasculature of the kidney is about ten times more sensitive than that of other organs to the vasoconstrictor effects of ET-1.[32] Endothelin affects several aspects of renal function, including vasoconstriction, regulation of tubule function, cellular proliferation and matrix production. Endothelin is involved in the pathogenesis of glomerulosclerosis.[33]

Endothelins are most likely local mediators. The circulating levels of ETs are too low to have a systemic effect. They are rapidly cleared from the blood by the lung, liver and kidney.[34]

NATRIURETIC PEPTIDE (NP)

The NP family consists of three distinct gene products: atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and type C natriuretic peptide (CNP). Atrial natriuretic peptide is mainly synthesised in the cardiac atrium and secreted into the circulation. Brain natriuretic peptide is produced in the cardiac ventricle and CNP is produced by vascular endothelial cells and macrophages.[35] Plasma ANP and BNP levels reflect the haemodynamic overloads to the atrium and ventricle respectively.

Endotoxin, fibroblast growth factor-b (bFGF), transforming growth factor-ß (TGF-ß), cyclic gaunosine monophosphate (cGMP), cyclic adenosine monophosphate (cAMP), vasopressin, thrombin, interlukin-I (IL-1), and tumour necrosis factor-α (TNFα) augment CNP secretion from endothelial cells.[36] Three different NP receptor isoforms have been cloned: natriuretic peptide-A (NP-A), natriuretic peptide-B (NP-B) and natriuretic peptide-C (NP-C). Atrial natriuretic peptide and BNP have a higher affinity for NP-A receptor, whereas CNP has a higher affinity for the NP-B receptor.[37, 38]

Details of the biological actions of CNP are not known. It may act as a neurotransmitter in the central nervous system as the content of CNP in the hypothalamus, thalamus, cerebellum and pituitary gland is tenfold higher than that of ANP and BNP. Intravenous administration of CNP decreases blood pressure, cardiac output, urinary volume and sodium excretion.[39] Apart from its vasorelaxation and hypotensive activity, it strongly stimulates cGMP production and inhibits cell proliferation and DNA synthesis in vascular smooth muscle cells.[40] Type C natriuretic peptide dramatically improves the intimal thickening following vascular injury.[41] Its production is significantly elevated in endotoxic shock, suggesting a pathophysiological role.[42]

The proximity of CNP production in endothelial cells (and macrophages) to its specific NP-B receptor in vascular smooth muscle cells suggests a paracrine mode of action. Atrial natriuretic peptide and BNP suppress thrombin- and angiotensin II-induced ET releases from human endothelial cells.[43] Type C natriuretic peptide strongly inhibits ET-1 secretion in porcine endothelial cells.[44] Thus NP may counteract vaso-constriction by suppressing ET production. Prostaglandin I_2 (PGI_2) also inhibits ET production.[45] Type C natriuretic peptide up-regulates ET_B receptor messenger mRNA expression in cultured vascular smooth muscle cells.[46]

PROSTACYCLIN

Physical or chemical damage to the cell membrane results in the release of prostacyclin. Other stimulators of prostacyclin release are bradykinin, thrombin, serotonin, platelet-derived growth factor (PDGF), interleukin-1 and adenine nucleotides.[5] Prostacyclin acts in a paracrine manner, on the abluminal side causing relaxation of the underlying smooth muscle and in the lumen, preventing platelets clumping onto the endothelium. Vasodilator and anti-platelet actions of prostacyclin are mediated by an increase in the concentrations of cyclic adenosine monophosphate (AMP) in smooth muscle cells and platelets.

Aspirin and similar substances prevent prostacyclin formation, but have little effect on normal blood pressure, suggesting that prostacyclin plays little part in normal control of the blood pressure. The capacity of vascular tissue to generate prostacyclin decreases with age, in diabetes and in atherosclerosis.[5] Prostacyclin also increases the activity of enzymes, which metabolise cholesterol esters in smooth muscle cells, suppresses the accumulation of cholesterol esters by macrophages and prevents release of growth

factors, which cause vascular wall thickening.[47] These data suggest a direct link between prostacyclin biosynthesis in the vascular wall and its susceptibility to thrombotic and atherosclerotic episodes.

PLASMINOGEN ACTIVATOR INHIBITOR 1 (PAI-1) AND TISSUE PLASMINOGEN ACTIVATOR (t-PA)

Plasminogen activator inhibitor 1 plays an active role in vascular disease, primarily in subjects with android obesity. Elevated plasma PAI-1 activity is elevated in obesity. A strong positive correlation between plasma very low-density lipoprotein (VLDL) triglyceride and PAI-1 activity levels has been demonstrated: VLDL has also been shown to induce PAI-1 secretion from cultured endothelial cells.[48] Insulin stimulates PAI-1 activity in endothelial cells[49] and this may play a role in the thrombogenesis associated with diabetes mellitus. Subjects with glucose intolerance have higher PAI-1 and t-PA levels compared to those with normal glucose tolerance and their levels correlate with insulin levels.[50] Angiotensin increases PAI-1 and transfer factor mRNA expression in cultured rat aortic endothelial cells, thus decreasing the antithrombotic properties of endothelial cells.[51]

The plasminogen activator/plasmin system is involved in various pathological processes considered important for atherogenesis. Tissue plasminogen activator is a key enzyme mediating plasminogen to plasmin conversion. Tissue plasminogen activator plasma concentrations are elevated in patients with advanced atherosclerosis and correlate with an increased risk for myocardial infarction and stroke. Tissue plasminogen activator expression is consistently increased in relation to the severity of the lesion in atherosclerotic coronary arteries.[52] Bradykinin, thrombin and isoproterenol stimulate t-PA release in the human vasculature.[53, 54]

KININS

Kinins are autocoids affecting B2-receptors of many organs. They are involved in autocrine and paracrine mechanisms, which are crucial in organ protection. Vascular kallikrein releases kinins from kininogen, which circulates in the plasma and is also present in the vascular tissue. Kallikrein, its mRNA and kininogen are present in vascular endothelium. Kinins are vasodepressor hormones that participate in local blood flow regulation. Vascular kinins induce potent vasodilatation through the release of prostacyclin, nitric oxide and endothelium-derived hyperpolarisation factor.[55] Angiotensin-converting enzyme (ACE) inhibitors inhibit kinin degradation and so increase their pharmacological effects, resulting in blood pressure lowering, cardioprotection and regression of cardiovascular hypertrophy and increase in insulin sensitivity.

LEUKOCYTE ADHESION MOLECULES

Endothelial activation by inflammatory cytokines induces expression of cellular adhesion molecules, thereby augmenting leukocyte adhesion and recruitment, and subsequent development of atherosclerosis.

Three members of the selectin family of cell adhesion molecules exist: E-, P- and L-selectin. L-selectin is expressed on leukocytes. P-selectin, mainly a product of activated platelets, but also expressed on endothelial cells, mediates the interaction of these cells with leukocytes. It is also involved in cell signalling and inter-cell communication. P-selectin, upon contact with its receptor on monocytes, initiates biosynthesis of tissue factor and other cytokines.[56] Increased concentrations occur in thrombotic disorders, diabetes and ischaemic heart disease.[57-59] E-selectin, an endothelial-specific adhesion molecule, has functional roles in granulocyte rolling and stable adhesion of leukocytes to microvascular endothelium; it may also function in angiogenesis.[60] Cytokines, such as TNFα, interleukin-1ß and lipopolysaccharide, induce E-selectin mRNA expression.[61] Raised plasma concentrations have been reported in variant angina, diabetes mellitus, peripheral atherosclerosis and in ischaemic heart disease.[62-64]

The second major group of adhesion molecules belong to the immunoglobulin supergene family. Vascular cell adhesion molecule-1 (VCAM-1) is a protein expressed on the surface of activated endothelial cells and expressed in early atherosclerosis. Increased levels of circulating adhesion molecules have been identified in diabetic patients. Endothelial expression of VCAM-1 and intimal smooth muscle expression of both VCAM-1 and ICAM-1 was found to be increased in the aortas from diabetic patients.[65] Interleukin-4 up-regulates expression of VCAM-1 on endothelial cells.

The intercellular adhesion molecule (ICAM-1), a product of many cells including endothelium and leukocytes, mediates adhesion and transmigration of leukocytes to the vascular endothelial wall, a step proposed to be critical in the initiation and progression of atherosclerosis. A significant association between increasing concentrations of ICAM-1 and risk of future myocardial infarction has been found.[66] Elevated levels of ICAM-1 have been found in diabetes mellitus.[63] Exposure of endothelial cells to oxidised LDL induces up-regulation of ICAM-1.[67] Hyperglycaemia also induces enhanced monocyte adhesion to vascular endothelial cells via a mechanism involving ICAM-1 and VCAM-1, explaining in part, the accelerated atheroma formation that occurs in diabetics.[68] Angiotensin II also up-regulates ICAM-1 expression by vascular endothelial cells.[69] Tumour necrosis factor-α regulates the expression of ICAM-1 in both human coronary endothelial cells and smooth muscle cells.[70]

OTHERS

The *renin-angiotensin system* (RAS) has traditionally been viewed as an endocrine system though elements of RAS exist in many peripheral tissues. Angiotensin-converting enzyme (ACE) is found mainly in the vascular endothelium. Endothelial cells secrete *Sphingomyelinase* (S-Smase). Interleukin-1ß and interferon γ stimulate secretion of S-Smase.[71] Sphingomyelinase may cause sub-endothelial retention and aggregation of lipoproteins during atherogenesis.

Adrenomedullin, a potent vasorelaxant and natriuretic peptide, is secreted from endothelial cells. It acts as an autocrine/paracrine regulator and exerts an antiproliferative action on endothelial cells.[72]

Thrombomodulin is a proteoglycan component of the endothelial cell membrane with anticoagulant properties. It binds thrombin and converts it into an enzyme that activates the protein C pathway. High levels have been found in diabetic patients with microalbuminuria and in the presence of clinical nephropathy.[73]

Platelet-activating factor (PAF) is an acetylated derivative of phosphatidyl choline, which is produced by endothelial

cells and may act in an autocrine manner. Platelet-activating factor binds to a surface receptor present on platelets as well as many cell types, and it thereby induces platelet aggregation, the release of vasoconstrictive substances and adhesion of platelets to endothelial cells. Platelet-activating factor participates in adhesion or migration of neutrophils and can also stimulate endothelial cells themselves, whereby activating calcium influx and several serine/threonine and tyrosine kinases regulate the endothelial cytoskeleton.[74] Thrombin, elastase and tumour necrosis factor induces the synthesis of PAF by endothelial cells.

Endothelium-derived hyperpolarising factor (EDHF), the identity of which remains to be established, activates K^+ channels and hyperpolarises underlying vascular smooth muscle cells.

Tissue factor is expressed within the vasculature by monocytes and endothelial cells. It is the major cellular initiator of the coagulation cascade and also serves as a cell surface receptor for activation of factor VII.

Vascular endothelial cells also synthesise an inhibitor of tissue factor called *tissue factor pathway inhibitor* (TFPI).[75] Increased TFPI blood levels are found in Type 1 diabetes mellitus and the levels correlate with albuminuria.[76, 77]

Von Willebrand factor is a multimeric glycoprotein mainly synthesised by endothelial cells. It is involved in platelet adhesion and aggregation and acts as the carrier of coagulation factor VIII in plasma. Increased levels of vWf, reflecting activation of or damage to endothelial cells, have been described as atherosclerosis and diabetes.[78] Von Willebrand factor appears to be a predictive marker of diabetic nephropathy.[79]

CONCLUSION

In addition to the barrier and transport functions, vascular endothelium also secretes a range of biologically active mediators, which act in an autocrine, paracrine or endocrine fashion. The vascular system establishes a tight regulation over the production of these endothelium-derived vasoactive factors. Its loss allows local or generalised modifications of the vascular tone. This dysregulation is involved in the pathogenesis of hypertension, atherosclerosis, diabetes mellitus and other vasospastic disorders.

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