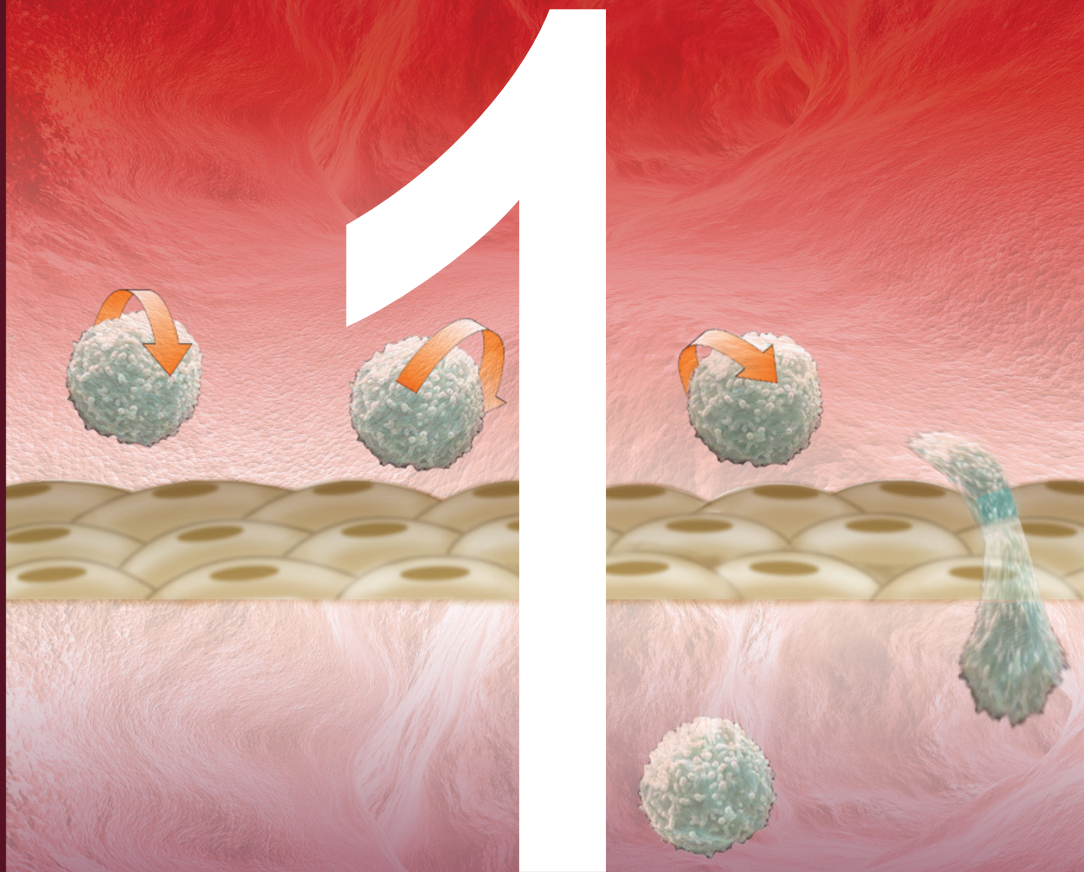
Mechanisms of Vascular Disease:

A Reference Book for Vascular Specialists



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A Reference Book for Vascular Specialists

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Abbreviation List

a1-PI	a1-protease inhibitor
5-HT	5-Hydroxytryptamine/Serotonin
AAA	Abdominal aortic aneurysm
AAS	Acute aortic syndrome
AAV	Adeno-associated viruses
ACE	Angiotensin converting enzyme
ACS	Acute coronary syndrome
ACS	Abdominal compartment syndrome
ACTH	Adrenocorticotropic hormone
ADAMTS	A disintegrin and metalloproteinase with thrombospondin motifs
ADP	Adenosine diphosphate
AIDS	Acquired immune deficiency syndrome
ALI	Acute lung injury
AMP	Adenosine monophosphate
AMPA	α-amino-3 hydroxy-5-methylisoxazole
ANA	Anti-nuclear antibody
ANCA	Anti-neutrophil cytoplasmic antibody
AOD	Aortic occlusive disease
AP1	Activated protein 1
APC	Activated protein C
APC	Antigen presenting cell
APLAS	Antiphospholipid antibody syndrome
ApoAI	Apolipoprotein AI
ApoE	Apolipoprotein E
APS	Antiphospholipid antibody syndrome
APTT	Activated partial thromboplastin time

ARDS	Acute respiratory distress syndrome
AT	Antithrombin
ATP	Adenosine triphosphate
AVP	Ambulatory venous thrombosis
β2-GPI	β2-glycoprotein Ib
bFGF	Basic fibroblast growth factor
BKCa	Large conductance calcium activated potassium channel
BMPs	Bone morphogenetic proteins
BMS	Bare metal stent
CAD	Coronary artery disease
CaM	Calmodulin
CAM	Cell adhesion molecule
cAMP	Cyclic adenosine monophosphate
CCK	Cholecystokinin
cGMP	Cyclic guanine monophosphate
CD	Cluster of differentiation
CD40L	Cluster of differentiation 40 ligand
CEA	Carotid endarterectomy
CETP	Cholesteryl ester transfer protein
CFD	Computational fluid dynamics
CG	Cationized gelatin
CGRP	Calcitonic gene regulated peptide
CHD	Coronary heart disease
CI	Confidence interval
CIMT	Carotid intimal-media thickness
c-JNK	c-Jun N-terminal kinase
CK-MB	Creatinine kinase (Myocardial specific)
CNCP	Chronic noncancer pain
cNOS	Constitutive nitric oxygen synthase enzyme
COX-1	Cyclooxygenase-1
COX-2	Cyclooxygenase-2
CROW	Charcot restraint orthotic walker
CRRT	Continuous renal replacement therapy

CRP	C-reactive protein
CRPS	Complex regional pain syndromes
CT	Computational tomography
CTA	Computed tomographic angiography
CTD	Connective tissue disorders
CTGF	Connective tissue growth factor
CYP	Cytochrome P450
CVD	Cardiovascular disease
CVI	Chronic venous insufficiency
DAG	Diacylglycerol
DES	Drug-eluting stent
DRG	Dorsal root ganglion
DNA	Deoxyribonucleic acid
DSA	Digital subtraction arteriography
DTS	Dense tubular system
DVT	Deep vein thrombosis
EC	Endothelial cell
ECM	Extracellular matrix
EDCF	Endothelium-derived contracting factor
EDH	Endothelium-dependent hyperpolarisation
EDS	Ehlers-Danlos syndrome
EET	Epoxyeicosatrienoic acids
ELAM-1	Endothelial-leukocyte adhesion molecule-1
ELG	Endoluminal grafts
ELISA	Enzyme linked immunosorbent assay
E_K	Equilibrium potential
E_M	Membrane potential
eNOS	Endothelial nitric oxide synthase enzyme
EPC	Endothelial progenitor cells
EPCR	Endothelial protein C receptor
ePTFE	Expanded polytetrafluoroethylene
ERK	Extracellular signal-regulated kinase
ESR	Erythrocyte sedimentation rate

ET	Essential thrombocytosis
ET-1	Endothelin 1
EVAR	Endovascular aortic aneurysm repair
EVLA	Endovenous LASER ablation
FDA	Food and drug administration
FDPs	Fibrin degradation products (soluble)
FGF	Fibroblast growth factor
FGF-2	Fibroblast growth factor 2
FMN	Flavin mononucleotide
FVL	Factor V Leiden
GABA	Gamma-aminobutyric acid
GABA B	Gamma-aminobutyric acid subtype B
G-CSF	Granulocyte colony stimulating factor
GMCSF	Granulocyte-macrophage colony stimulating factor
GP	Glycoprotein
GPCR	G-protein coupled receptor
GSV	Great saphenous vein
HDL	High density lipoprotein
HDL-C	High density lipoprotein cholesterol
HIF	Hypoxia inducible factor
HIT	Heparin induced thrombocytopenia
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HMG Co-A	Hydroxymethylglutaryl coenzyme-A
HMW	High molecular weight
HPETE	Hydroperoxyeicosatetraenoic acid
HETE	Hydroxyeicosatetraenoic acids
HR	Hazard ratio
hsCRP	High-sensitive C-reactive protein
HSP	Heat shock protein
HUV	Human umbilical vein
IAH	Intra-abdominal hypertension

IAP	Intra-abdominal pressure
IAPP	Intra-abdominal perfusion pressure
ICAM-1	Inter-cellular adhesion molecule-1
ICAM-2	Inter-cellular adhesion molecule-2
ICP	Intra-compartmental pressure
ICU	Intensive care unit
IFN	Interferon
IGF-1	Insulin-like growth factor-1
IHD	Ischemic heart disease
IL	Interleukin
IL-1	Interleukin-1
IL-1α	Interleukin-1 alpha
IL1-β	Interleukin-1 beta
IL-6	Interleukin-6
IL-8	Interleukin-8
ILT	Intraluminal thrombus
IKCa	Intermediate conductance calcium-activated potassium channels
IMH	Intramural haematoma
IMP	Inosine monophosphate
iNOS	Inducible nitric oxide synthase enzyme
IP(3)	1,4,5-inositol triphosphate
IRI	Ischemia reperfusion injury
IVIG	Intravenous pooled immunoglobulin
IVUS	Intravascular ultrasound
KGF	Keratinocyte growth factor
KGF-2	Keratinocyte growth factor-2
LAP	Latency associated peptide
LCS	Limb compartment syndrome
LDL	Low density lipoprotein
LDS	Loeys-Dietz syndrome
LLC	Large latent complex
LEC	Lymphatic endothelial cells

LFA-1	Lymphocyte function-associated antigen-1
LO	Lipoxygenase
LOX	Lysyl oxidase
LOPS	Loss of protective sensation
LPA	Lysophosphatidic acid
LPS	Lipopolysaccharide
LTA	Lipoteichoic acid
LTGFBP	Latent TGF binding protein
MAC-1	Macrophage-1 antigen
MAPK	Mitogen activated protein kinase
MCP-1	Monocyte chemoattractant protein-1
M-CSF	Macrophage-colony stimulating factor
MFS	Marfan syndrome
MHC	Major histocompatibility
MI	Myocardial infarction
MIP-1	Macrophage inflammatory protein-1
MLC_{20}	Myosin light $chain_{20}$
MLCK	Myosin light chain kinase
MLCP	Myosin light chain phosphatase
MMP	Matrix metalloproteinase
MODS	Multiple organ dysfunction syndrome
MRA	Magnetic resonance angiography
MRI	Magnetic resonance imaging
mRNA	Messenger RNA
MRSA	Methicillin resistant *Staphylococcus aureus*
MRSE	Methicillin resistant *Staphylococcus epidermidis*
MRTA	Magnetic resonance tomographic angiography
MTHFR	Methylenetetrahydrofolate reductase
MT-MMP	Membrane-type MMP
MVPS	Mitral valve prolapse syndrome
NADPH	Nicotinamide adenine dinucleotide phosphate
NGF	Nerve growth factor

NFκB	Nuclear factor kappa B
NiTi	Nitinol
NJP	Non-junctional perforators
NMDA	N-methyl-D-aspartate
NNH	Number needed to harm
NNT	Number needed to treat
NO	Nitric oxide
NOS	Nitric oxide synthase enzyme
NSAID	Non-steroidal anti-inflammatory drug
NV	Neovascularisation
OCP	Oestrogen/progesterone contraceptive pill
OPN	Osteopontin
OPG	Osteoprotegerin
OR	Odds ratio
OxLDL	Oxidised low density lipoprotein
PAD	Peripheral arterial disease
PAF	Platelet activating factor
PAI	Plasminogen activator inhibitor
PAI-1	Plasminogen activator inhibitor-1
PAR	Protease activated receptor
PAR-1	Protease activated receptor-1
PAR-4	Protease activated receptor-4
PAU	Penetrating aortic ulcer
PC	Protein C
PCA	Poly (carbonate-urea) urethane
PCI	Percutaneous coronary intervention (angioplasty)
PCWP	Pulmonary capillary wedge pressure
PDGF	Platelet-derived growth factor
PDGFβ	Platelet-derived growth factor-β
PDS	Polydioxanone
PECAM-1	Platelet-endothelial cell adhesion molecule-1
PEDF	Pigment epithelium-derived factor
PES	Paclitaxel-eluting stent

PET	Positron emission tomography
PF4	Platelet factor 4
PGI_2	Prostacyclin
PGG_2	Prostaglandin G_2
PGH_2	Prostaglandin H_2
$PGEI_2/PGI_2$	Prostaglandin I_2
PGN	Peptidoglycan
PHN	Postherpetic neuropathy
PHZ	Para-anastomotic hyper-compliant zone
PI3K	Phosphatidylinositol 3-kinase
PIP2	Phosphatidylinositol 4,5-bisphosphate
PLC	Phospholipase C
PLOD	Procollagen lysyl hydroxylase
PMCA	Plasma membrane Ca^{2+} APTases
PMN	Polymorphonuclear leukocyte
POSS	Polyhedral oligomeric silsesquioxanes
PPAR	Peroxisomal proliferation activating receptor
PPI	Proton pump inhibitor
PRV	Polycythaemia rubra vera
PS	Protein S
PSGL-1	P-selectin glycoprotein ligand-1
PT	Prothombin time
PTCA	Percutaneous coronary angioplasty
PTFE	Polytetrafluoroethylene
PTS	Post-thrombotic syndrome
PUFA	Polyunsaturated fatty acid
PVI	Primary valvular incompetence
rAAA	Ruptured AAA
Rac	Ras activated cell adhesion molecule
RANTES	Regulated upon activation, normal T cell expressed and secreted
RAS	Renin angiotensin system
RCT	Randomised controlled trial

RF	Rheumatoid factor
RFA	Radiofrequency ablation
rhAPC	Recombinant human activated protein C
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RR	Relative risk
RSD	Reflex sympathetic dystrophy
S1P	Sphingosine-1-phosphate
SAPK	Stress-activated protein kinase
SCF	Stem cell factor
SCS	Spinal cord stimulation
ScvO2	Superior vena cava venous oxygen saturation
SDF-1	Stromal-cell-derived factor-1
SERCA	Sarco/endoplasmic reticulum CaATPases
SEP	Serum elastin peptides
SES	Sirolimus-eluting stent
SEPS	Subfascial endoscopic perforator surgery
SFA	Superficial femoral artery
SFJ	Sapheno-femoral junction
SIRS	Systemic inflammatory response syndrome
SKCa	Small conductance calcium-activated potassium channels
SLE	Systemic lupus erythematosus
SMA	Smooth muscle alpha actin
SMC	Smooth muscle cell
SMP	Sympathetically maintained pain
SNARE	Soluble N-ethylmaleimide-sensitive factor activating protein receptors
SNP	Single nucleotide polymorphisms
SNRI	Serotonin/Noradrenaline reuptake inhibitors
SPJ	Sapheno-popliteal junction
SPP	Skin perfusion pressure
SR	Sarcoplasmic reticulum
SSRIs	Selective serotonin re-uptake inhibitors
SSV	Small saphenous vein

SVT	Superficial thrombophlebitis
STIM1	Stromal interacting molecule 1
TαCE	TNFα converting enzyme
TAAD	Thoracic aortic aneurysm disease
TAD	Thoracic aortic dissection
TAFI	Thrombin-activatable fibrinolysis inhibitor
Tc-99 MDP	Technetium-99 methylene diphosphonate
TCA	Tricyclic antidepressant
TCC	Total contact cast
TCR	T-cell receptor
TENS	Transcutaneous electrical nerve stimulation
TF	Tissue factor
TFPI	Tissue factor pathway inhibitor
TGF	Transforming growth factor
TGF-α	Transforming growth factor-alpha
TGF-β	Transforming growth factor-beta
TGL	Triglycerides
Th	T helper
TIA	Transient ischemic attack
TIMP	Tissue inhibitors of metalloproteinase
TLR	Toll-like receptors
TNF	Tumour necrosis factor
TNF-α	Tumour necrosis factor-alpha
tPA	Tissue-type plasminogen activator
TRP	Transient receptor potential
TRPC	Transmembrane receptor potential canonical
TRPV1	Transmembrane receptor potential Vanilloid-type
TXA2	Thromboxane A2
uPA	Urokinase
UT	University of Texas
VCAM	Vascular cell adhesion molecule
VCAM-1	Vascular cell adhesion molecule-1
VEGF	Vascular endothelial growth factor

VEGF-R	Vascular endothelial growth factor receptor
VIP	Vasoactive intestinal peptide
VLA-1	Very late activating antigen-1
VOCC	Voltage operated calcium channels
VPT	Vibratory perception threshold
VSMC	Vascular smooth muscle cells
VTE	Venous thromboembolism
VV	Varicose veins
vWF	von Willebrand factor
XO	Xanthine oxidase

1 • Endothelium

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INTRODUCTION

The endothelium, first described over 100 years ago as an inert anatomical barrier between blood and the vessel wall, is now recognized as a dynamic organ with secretory, synthetic, metabolic, and immunologic functions. Forming a continuous lining to every blood vessel in the body, endothelial cells play an obligatory role in modulating vascular tone and permeability, angiogenesis, and in mediating haemostatic, inflammatory and reparative responses to local injury. To fulfil these roles the endothelium is highly dynamic, continuously responding to spatial and temporal changes in mechanical and biochemical stimuli. Such responsiveness is affected through receptors for growth factors, lipoproteins, platelet products and circulating hormones, which regulate changes in protein and mRNA expression, cell proliferation and migration or the release of vasoactive and inflammatory mediators.

All vascular endothelial cells have a common embryonic origin but show clear bed-specific heterogeneity in morphology, function, gene and protein expression, determined by both environmental stimuli and epigenetic features acquired during development. Thus, the endothelium should not be regarded as a homogenous tissue but rather a conglomerate of distinct populations of cells sharing many common functions but also adapted to meet regional demands.[1]

The continuous endothelial cell layer provides an uninterrupted barrier between the blood and tissues in the majority of blood vessels and ensures tight control of permeability of the blood-brain barrier. In regions of increased trans-endothelial transport such as capillaries of endocrine glands and the kidney, the presence of fenestrae, transcellular pores approximately 70 nm in diameter with a thin fenestral diaphragm across their opening, facilitate the selective permeability required for efficient absorption, secretion, and filtering. In hepatic sinuses, the presence of a discontinuous endothelium with large fenestrations (0.1–1 mm in diameter) lacking a fenestral diaphragm, provides a highly permeable and poorly selective sieve essential for transfer of lipoproteins from blood to hepatocytes.

Beyond these structural variations, endothelial heterogeneity is also manifest in regional differences in the release of vasoactive and inflammatory mediators, in response to changes in shear stress and hypoxia, and in expression of pro- and anti-coagulant molecules. For example, endothelial expression of the pro-thrombotic mediator von

Willebrand factor (vWF) is a function of endothelial cells found in vessels of discrete size and/or anatomic location. Similarly, the contribution of nitric oxide (NO) to endothelium-dependent vasodilation is far greater in large conduit arteries compared to small resistance vessels. These regional biochemical and phenotypic differences between endothelial cells extend to their susceptibility to injury in the face of cardiovascular risk factors such as hypercholesterolemia, diabetes and smoking and thus impact the function of the vasculature both in health and disease.

This chapter provides an overview of how the endothelium regulates four key aspects of cardiovascular homeostasis-vascular tone, angiogenesis, haemostasis and inflammation.

ENDOTHELIUM-DEPENDENT REGULATION OF VASCULAR TONE

Since the first report by Furchgott and Zawadski[2] of endothelium-dependent modulation of the contractile state of smooth muscle cells in the artery wall, it has become apparent that endothelial cells release a plethora of vasoactive factors in response to a wide range of mechanical and chemical stimuli. That many of these factors also modulate processes such as inflammation, cell adhesion and coagulation, highlights the crucial physiological role of the endothelium and why endothelial dysfunction is pivotal in the development of cardiovascular diseases such as atherosclerosis and hypertension. This section will focus on the four major pathways underlying endothelium-dependent modulation of vascular tone; NO, arachidonic acid metabolites, endothelium-dependent hyperpolarisation (EDH) and endothelin.

Nitric oxide

The first endothelium-derived relaxing factor described by Furchgott and Zawadski was subsequently identified as NO, a short-lived free radical synthesized from L-arginine by endothelial NO synthase (eNOS) and destroyed by reactive oxygen species (ROS). NO activates the haem-dependent enzyme, soluble guanylyl cyclase in surrounding smooth muscle cells, leading to formation of cyclic guanosine monophosphate (cGMP). Subsequent protein kinase G-mediated phosphorylation of a diverse range of target proteins such as large conductance calcium-activated potassium (BK_{Ca}) channels, RhoA, Rho kinase, transient receptor potential (TRP) channels, myosin light chain phosphatase and phospholamban, leads to smooth muscle cell relaxation and hence vasodilation.[3]

eNOS is a bidomain enzyme; an N-terminal oxygenase domain with binding sites for haem, tetrahydrobiopterin, oxygen and the substrate L-arginine supports the catalytic activity, and a C-terminal reductase domain binds nicotinamide adenine dinucleotide phosphate (NADPH), flavin mononucleotide (FMN) and flavin adenine dinucleotide co-factors. Transfer of electrons from NADPH to flavins in the reductase domain and then to the haem in the oxygenase domain is required so that the haem iron can bind oxygen and catalyze the synthesis of NO from L-arginine. Binding of the ubiquitous calcium regulatory protein calmodulin (CAM) facilitates transfer of electrons from the reductase to the oxygenase domain and is critical for activation of the enzyme.

eNOS is constitutively expressed in all endothelial cells but regulation of the enzyme by physiological and pathophysiological stimuli occurs via a complex pattern of transcriptional and post-translational modifications. For example, both eNOS

mRNA and protein levels are increased by fluid shear stress via activation of a pathway involving both c-Src-tyrosine kinase and transcription factor NFκB. At the post-translational level, eNOS activity is highly regulated by substrate and cofactor availability as well as by endogenous inhibitors, lipid modification, direct protein-protein interactions, phosphorylation, O-linked glycosylation, and S-nitrosylation. Agonists at endothelial G-protein coupled receptors (GPCRs) such as bradykinin, and acetylcholine, elicit calcium-CAM-dependent NO production by via phospholipase C-mediated generation of inositol 1,4,5-trisphosphate (IP_3) and subsequent release of calcium from intracellular stores. However, activation of tyrosine kinase linked receptors such as the vascular endothelial growth factor (VEGF) receptor, and mechanical stimulation of the endothelium by shear stress, lead to phosphorylation of eNOS at Ser^{1177} to increase the calcium sensitivity of the enzyme so that it can be activated at resting calcium levels. Distinct kinase pathways can mediate eNOS phosphorylation; shear stress elicits phosphorylation of Ser^{1177} via protein kinase A whereas insulin and VEGF cause phosphorylation of the same residue via the serine/threonine protein kinase Akt. Conversely, phosphorylation of the enzyme at Tyr^{657} within the FMN domain or Thr^{495} within the CaM-binding domain, inhibit enzyme activity.[4]

Within endothelial cells, eNOS is targeted to invaginations of the cell membrane called caveolae, membrane microdomains enriched in cholesterol and sphingolipids, and defined by the presence of the scaffolding protein caveolin. Caveolae sequester diverse receptors and signaling proteins including GPCRs, growth factor receptors and calcium regulatory proteins such as CAM. Thus, targeting of eNOS to this region facilitates communication with upstream and downstream pathways. Within caveolae, caveolin-1 tonically inhibits eNOS activity, thereby limiting the production of NO; binding of calcium-CAM leads to disruption of the caveolin-1/eNOS interaction and increases eNOS activity. Other associated proteins such as platelet endothelial cell adhesion molecule-1 (PECAM-1), modulate eNOS activity by virtue of their function as scaffolds for the binding of signaling molecules such as tyrosine kinases and phosphatases.

A vast range of stimuli such as shear stress generated by the viscous drag of blood flowing over the endothelial cell surface, circulating hormones (e.g. catecholamines, vasopressin), plasma constituents (e.g. thrombin), platelet products (e.g. 5-HT) and locally-produced chemical mediaors (e.g. bradykinin) each evoke NO-mediated vasodilation. Release of endothelium-derived NO by such stimuli plays a critical role in mediating acute changes in local blood flow and tissue perfusion. Shear stress-stimulated NO production is central to exercise-induced increases in blood flow in skeletal muscle. Production of NO in response to 5-HT released from aggregating platelets, dilates coronary arteries thus preventing the clot from occluding the vessel. Mice lacking eNOS are hypertensive and infusion of L-arginine analogues, competitive inhibitors of eNOS, cause alterations in local blood flow and in systemic blood pressure, demonstrating the importance of endothelium-derived NO in long-term control of blood pressure and blood flow in vivo. In humans, elevated levels of an endogenous inhibitor of eNOS, asymmetric dimethylarginine, are associated with hypertension and increased cardiovascular risk.

In addition to its vasodilator actions, NO is now recognized as playing myriad other protective roles in the vasculature as a regulator of clot formation, inflammation and vessel repair. Loss of NO-mediated vasodilation, due to reduced expression or activity of eNOS and/or oxidative stress-mediated

reductions in NO bioavailability, is a hallmark of endothelial dysfunction associated with cardiovascular risk factors such as hypercholesterolemia, smoking, diabetes and obesity. Loss of NO tip the homeostatic balance in favour of vasoconstriction, proliferation, activation of platelets and blood clot formation, and inflammation. These pathological processes contribute to clinical manifestations such as hypertension, atherosclerosis and arterial thrombosis, which are associated with significant morbidity and mortality.

Metabolites of arachidonic acid: Arachidonic acid, released from cell membrane phospholipids by phospholipases, is metabolized by cyclooxygenase (COX), lipoxygenase (LO), and cytochrome P450 monooxygenase (CYP) enzymes to yield an array of endothelium-derived vasoactive factors.

Cyclooxygenases: COX enzymes metabolize arachidonic acid to endoperoxide intermediates which are then converted to a range of eicosanoids (e.g. prostacyclin; PGI_2, thromboxane A_2) through the actions of various synthases. Two isoforms of cyclooxygenase are found in the endothelium. The constitutively expressed COX-1 has long been regarded as vasculoprotective, the predominant product being PGI_2 which acts on prostanoid (IP) receptors to cause vasodilation and inhibition of platelet aggregation via activation of adenylyl cyclase and subsequent elevation of intracellular cyclic-adenosine monophosphate (cAMP). PGI_2 also inhibits platelet and lymphocyte adhesion to endothelium, limits vascular smooth muscle cell proliferation and migration, and counteracts the production of growth factors.

However, evidence is now emerging that GPCR-mediated activation of endothelial COX-1 can generate other products such as TXA_2 and PGH_2 which activate thromboxane (TP) receptors on smooth muscle cells and so function as endothelium-derived contracting factors (EDCFs). Stimulation of TP receptors elicit not only vasoconstriction but also proliferation of vascular smooth muscle cells, platelet adhesion and aggregation and expression of adhesion molecules on endothelial cells. COX-1 shows basal activity and is activated by endothelial GPCRs. A shift from production of endothelium-derived relaxing factors to COX-dependent EDCFs is implicated in endothelial dysfunction associated with ageing, diabetes and hypertension.[6]

COX-2 was first identified as an inducible form of the enzyme, regulated at the level of gene expression and associated with inflammation. However, it is expressed in some blood vessels in the absence of overt signs of inflammation and may be a major source of vasculoprotective PGI_2; hence the deleterious cardiovascular consequences seen in some patients treated with selective COX-2 inhibitors.[7]

Lipoxygenases: LO enzymes deoxygenate polyunsaturated fatty acids to hydroperoxyl metabolites. The three LO isoforms expressed in endothelial cells are 5-LO, 12-LO, and 15-LO, which correspond to the carbon position of arachidonic acid oxygenation. Each LO oxygenates arachidonic acid to form a stereospecific hydroperoxyeicosatetraenoic acid (HPETE). HPETEs are unstable and are reduced to the corresponding hydroxyeicosatetraenoic acids (HETEs). 5-LO is the initial enzyme in the synthesis of leukotrienes but 5-LO products do not seem to be involved in regulation of vascular tone. In contrast, products from the 12-LO and 15-LO pathways are vasoactive but show species and vessel variation in the responses they elicit. 12-HETE elicits relaxation of a number of peripheral arteries including human coronary vessels, but causes vasoconstriction in dog renal arteries. 15-HPETE and 15-HETE cause slight vasorelaxation at lower concentrations but contractions at higher concentrations mediated by activation

of TP receptors. Although vasoactive LO metabolites are produced by endothelial cells, elucidation of their physiological role has been hindered by the lack of selectivity of pharmacological inhibitors.

Cytochrome P450 monooxygenases: CYP enzymes add oxygen across the double bonds of arachidonic acid to produce four cisepoxides, 14,15-, 11,12-, 8,9-, and 5,6-epoxyeicosatrienoic acids (EETs). Two CYP enzymes have been cloned from human endothelium CYP2C8/9 and CYP2J2 both of which produce mainly 14,15-EET with lesser amounts of 11,12-EET. The latter are also the major EETs released from endothelial cells stimulated by GPCR agonists (e.g. acetylcholine, bradykinin) and physical stimuli such as cyclic stretch and shear stress. EETs are rapidly metabolized by esterification into phospholipids or hydration to dihydroxyeicosatrienoic acids by soluble epoxide hydrolase.

EETs are vasoactive causing vasoconstrictions in the lung but eliciting vasodilatation of systemic arteries via activation of iberiotoxin-sensitive, BK_{Ca} channels on the vascular smooth muscle cells. EETs are proposed mediators of EDH in systemic arteries, acting either as transferable factors that hyperpolarize and relax smooth muscle cells, or acting in an autocrine manner to cause hyperpolarisation of the endothelial cell membrane potential which is then spread to the underlying smooth muscle through gap junctions (see below).

An EET receptor on smooth muscle cells has not been identified but development of 14,15-EET analogues such as 14,15-epoxyeicosa-5Z-enoic acid has revealed strict structural and stereoisomeric requirements for relaxations suggesting a specific binding site or receptor and BK_{Ca} channel activation by EETs requires a G protein indicating that a GPCR for EETs exists.

Some EETs activate vascular TRP channels, non-selective cation channels that can mediate calcium influx. Endothelium-dependent flow-induced dilation is linked to 5,6-EET-mediated activation of vanilloid type 4, TRPV4, channels. Formation of a complex of TRPV4 with BK_{Ca} channels in smooth muscle cells may couple local increases in calcium due to activation of TRPV4 by EETs to membrane hyperpolarisation and vasorelaxation.[8] In contrast, endothelial stimulation by bradykinin or hypoxia is associated with activation of TRPC3 and TRPC6 channels. In addition to stimulating channel activity, EETs elicit the rapid intracellular translocation of TRP channels into caveolae, a process dependent on activation of protein kinase A by cAMP, and consistent with the activation of a GPCR.

In some models of endothelial dysfunction, reduced bioavailability of NO is counteracted by increased production of EETs which can maintain endothelium-dependent vasodilator responses. Thus, strategies aimed at enhancing production of endothelium-derived EETs or inhibiting their degradation, may represent a new therapeutic approach to endothelial dysfunction.

Endothelium-dependent hyperpolarisation (EDH): Observations of agonist-induced endothelium-dependent vasorelaxation which persisted in the presence of inhibitors of prostaglandin and NO synthesis and was accompanied by hyperpolarisation of the vascular smooth muscle cell membrane potential, led to identification of a third endothelium-derived relaxing factor, EDHF. Hyperpolarisation of the smooth muscle cells reduces the open probability of voltage-dependent calcium channels thus reducing calcium influx to cause relaxation. A range of agents have been proposed to account for the actions of EDHF including K^+ ions, EETs and C-type natriuretic peptide. However, in many arteries, endothelium-dependent hyperpolarisation of vascular smooth muscle (EDH)

actually reflects direct electrical coupling between endothelial and smooth muscle cells via myoendothelial gap junctions rather than the actions of a diffusible factor.[9]

Irrespective of the mediator, the initiating step in EDH-mediated vasorelaxation is activation of small- (SK_{Ca}) and intermediate–conductance (IK_{Ca}) calcium-activated potassium channels on endothelial cells. Inhibition of endothelium-dependent relaxation by a combination of SK_{Ca} and IK_{Ca} channel blockers is now regarded as the hallmark of EDH and has been documented in response to many agonists, in a wide range of blood vessels from a number of species.[10] SK_{Ca} and IK_{Ca} channels, activated by increases in intracellular calcium via CAM which is constitutively associated with the channels, are voltage-independent and thus can operate at negative membrane potentials close to the K^+ equilibrium potential.

The lack of selective inhibitors of EDH, aside from the SK_{Ca} and IK_{Ca} channel blockers, has hampered investigations of the physiological role of this pathway but it is now clear that EDH becomes progressively more important as a mediator of endothelium-dependent vasodilation with decreased vessel size. The importance of EDH as a regulator of blood flow and blood pressure in vivo is demonstrated by enhanced resistance artery tone and elevated systemic blood pressure seen in mice lacking endothelial SK_{Ca} or IK_{Ca} channels. Loss of EDH, due to changes in expression or activity of SK_{Ca} and/or IK_{Ca} channels, contributes to experimental hypertension and diabetes-related erectile dysfunction. In contrast, resistance of the EDH pathway to the deleterious actions of ROS may allow EDH-mediated vasodilation to be maintained in the face of reduced bioavailability of NO in atherosclerosis and heart failure. Thus, selective activation of endothelial SK_{Ca} and IK_{Ca} channels is a potential therapeutic avenue for the future.

Endothelin: Endothelins are a family of 21 amino acid peptides, of which there are three members (ET-1, ET-2, ET-3). Endothelial cells produce only ET-1; endothelin ET-2 is produced in the kidney and intestine, while ET-3 has been detected in the brain, gastrointestinal tract, lung and kidney. ET-1 is a potent vasoconstrictor inducing long-lasting vasoconstriction at a half maximum effective concentration in the nano molar range, at least one order of magnitude lower than values reported for other vasoconstrictor peptides such as angiotensin II.

ET-1 is produced constitutively by the endothelium but production is regulated at the level of gene expression; inflammatory factors such as transforming growth factor-β (TGFβ) and tumour necrosis factor-α (TNFα, insulin, and angiotensin II up-regulate ET-1 mRNA whereas NO, PGI_2 and shear stress cause down-regulation.) ET-1 is synthesized as a large protein, the pre-proET-1 (203 amino acids) that is cleaved to pro-ET-1 (39 amino acids) and then to ET-1 by ET-converting enzymes. The half life of ET-1 protein and mRNA is 4–7 minutes and 15–20 minutes, respectively, and the majority of plasma ET-1 (90%) is cleared by the lung during first passage.

The biological effects of ET-1 are mediated by two GPCR subtypes, ET_A and ET_B which have opposing effects on vascular tone. ET_A receptors present on vascular smooth muscle are responsible for the majority of ET-1 induced vasoconstriction; activation of phospholipase C increases formation of IP_3 and diacylglycerol, and the resultant increase in intracellular calcium and activation of protein kinase C cause vasoconstriction. ET_B receptors are mainly present on endothelial cells and play an important role in clearing ET-1 from the plasma in the lung. Activation of endothelial ET_B receptors induces vasodilatation by stimulating the release of

PGI_2 and NO. Inhibition of ET_B increases circulating ET-1 levels and blood pressure in healthy subjects demonstrating that although ET-1 is regarded as primarily a vasoconstrictor, ET_B-mediated vasodilation is physiologically important.[11]

ET-1 is not only a vasoactive factor. Acting via ET_B receptors, ET-1 modulates the expression and degradation of extracellular matrix (ECM) and thus plays a role in vascular remodelling. Acting via ET_A, ET-1 promotes smooth muscle proliferation contributing to neointima formation following vascular injury and to thickening of the arterial wall in pathological conditions such as pulmonary arterial hypertension, atherosclerosis and vein graft occlusion. As NO strongly inhibits the release of ET-1 from the endothelium and ET-1 attenuates NO-mediated dilation, ET-1 and NO are functionally closely interdependent and many of the cardiovascular complications associated with endothelial dysfunction are due to an imbalance in this relationship.

ANGIOGENESIS

Angiogenesis is the growth of new blood vessels as a result of endothelial cells sprouting from existing vessels. In adults, it is a protective mechanism initiated in response to tissue hypoxia and ischemia or injury. It is also a key process in pathological conditions such as the proliferative diabetic retinopathy and neovascularization of tumours and as such, inhibitors of angiogenesis have received considerable interest as potential therapeutic strategy. The angiogenic process depends on a complex transcriptional network coordinating production and release of numerous cytokines and growth factors. Recruitment and proliferation of bone marrow–derived endothelial progenitor cells to form new vessels (vasculogenesis) is a distinct but complimentary process which occurs simultaneously in ischemic and wounded tissue to augment perfusion.[12]

Angiogenesis requires a sequence of individual processes: degradation of ECM by metalloproteinase enzymes, proliferation and directional migration of endothelial cells to form endothelial tubes, maturation of new vessels by recruitment of pericytes (connective tissue cells) to stabilize endothelial sprouts and secrete ECM molecules to form the vascular basement membrane and apoptosis to prune back immature vessels into a vascular network.[13] The endothelial cells that sprout from the parent vessel, tip cells, possess long and motile filopodia that extend towards the source of pro-angiogenic growth factors and respond to other guidance cues to enable directional vessel growth. Endothelial cell migration requires the dynamic regulation of interactions between integrins and the surrounding ECM. Integrins are cell surface receptors which provide adhesive and signaling functions and link the actin cytoskeleton of the cell to the ECM at areas called focal adhesions. Phosphorylation of focal adhesion kinase, a cytoplasmic non-receptor tyrosine kinase, in response to pro-angiogenic signal molecules stimulates cell contraction, thus allowing cell movement on adhesive contacts. Subsequent integrin inactivation destroys the adhesive complex and allows detachment of the cell in its new location.

Cell-cell contacts between endothelial cells, essential for development of patent vessels, are mediated by cell surface receptors such as PECAM-1, a 130 kDa member of the immunoglobulin superfamily, which acts like a docking molecule to allow other proteins to provide further strength to vascular structures. Cadherins such as vascular endothelial cadherin are transmembrane proteins which provide weak adhesive cell-cell forces, further stabilized by catenins, intracellular proteins linking the cadherin cell surface molecule to the actin cytoskeleton.

Angiogenesis in response to hypoxia and ischemia is largely controlled by the transcription factor hypoxia-inducible factor-1 (HIF-1).[14] HIF-1 has multiple subunits; HIF-1α which is produced continuously but is rapidly degraded in the presence of oxygen and HIF-1β which is constitutively expressed. Under hypoxic conditions, HIF-1α degradation is inhibited and the stabilized protein translocates to the nucleus, where it dimerizes with HIF-1β and binds to hypoxia response elements on more than 60 HIF–responsive genes that function to enhance oxygen delivery and increase metabolism. Central angiogenic signals driven by increased HIF-1 activity include VEGF, fibroblast growth factor (FGF), platelet-derived growth factor (PDGF) and angiopoietin. After injury, local platelets release TGFβ and PDGF, which stimulate vessel growth.

FGF and VEGF stimulate endothelial cell proliferation and migration. Their high affinity for heparan sulfate glycosaminoglycans on the endothelial cell surface facilitates binding to receptors and provides a reservoir of both factors in the ECM, which can be released during wounding or inflammation. VEGF stimulates endothelial replication and migration and increases vessel permeability facilitating extravasation of plasma proteins to form a provisional matrix for cell migration. PDGF is required for the recruitment and survival of pericytes for vessel stabilization and maturation. Angiopoietins have multiple effects the angiogenic process, particularly the interactions between endothelial cells, pericytes and the basement membrane. For example, angiopoietin-1 stimulates secretion of growth factors from endothelial cells, which in turn stimulate differentiation of surrounding pericytes into smooth muscle cells. Conversely, angiopoietin-2 is an antagonist of the actions of angiopoietin-1 and so acts as a naturally occurring inhibitor of angiogenesis. Overall regulation of angiogenesis is a balance between angiogenic versus angiostatic factors.

There is a fuller description of the angiogenic process in Chapter 6, which also deals with therapeutic angiogenesis.

HAEMOSTASIS

The endothelium plays a pivotal role in regulating blood flow by exerting effects on the coagulation system, platelets and fibrinolysis.[15] Under normal physiological conditions, the endothelium provides one of the few surfaces which can maintain blood in a liquid state during prolonged contact.

A key factor in blood clot formation is activation of the serine protease thrombin which cleaves fibrinogen, producing fragments that polymerise to form strands of fibrin. It also activates factor XIII, a fibrinoligase, which strengthens fibrin-to-fibrin links, thereby stabilising the clot and stimulates platelet aggregation. Heparan sulfate proteoglycan molecules provide an anti-thrombotic endothelial cell surface by serving as co-factors for antithrombin III, causing a conformational change that allows this inhibitor to bind to and inactivate thrombin and other serine proteases involved in the clotting cascade. The endothelium also prevents thrombin formation by expressing tissue factor pathway inhibitor which binds to clotting factor Xa. Tissue factor pathway inhibitor and antithrombin III both contribute to physiological haemostasis, and both show impairment in acquired thrombotic states. A third endothelial anti-coagulation mechanism is expression of thrombomodulin; binding of thrombin to cell surface thrombomodulin removes its pro-coagulant activity, and the thrombin-thrombomodulin complex activates protein C a vitamin K-dependent anticoagulant. Activated protein C, helped by its cofactor protein S, inactivates clotting factors Va and VIIa.

The anti-platelet properties of the endothelium are largely mediated by release of PGI_2 and NO. As in smooth muscle, PGI_2 inhibits platelet aggregation through the activation of IP receptors and activation of adenylyl cyclase whereas NO inhibits platelet adhesion, activation, secretion, and aggregation through a cGMP-dependent mechanism. NO inhibits agonist-dependent increases in intra-platelet calcium to suppress the calcium-sensitive conformational change in the heterodimeric integrin glycoprotein IIb– IIIa required for fibrinogen binding. NO also promotes platelet disaggregation by impairing the activity of phosphoinositide 3-kinase, which normally supports conformational changes in glycoprotein IIb–IIIa, rendering its association with fibrinogen irreversible. Should a blood clot form, fibrinolysis depends primarily on the action of plasmin, an active protease formed from its precursor, plasminogen, upon stimulation by tissue-type plasminogen activator.

Under physiological conditions, there is a haemostatic balance and in addition to these anti-thrombotic mechanisms, the endothelium also synthesises several key haemostatic components; vWF and plasminogen activator inhibitor-1 (PAI-1) being particularly important. PAI-1 is secreted in response to angiotensin IV, providing a link between the renin-angiotensin system and thrombosis. In addition to anti-coagulant activity, binding of thrombin to thrombomodulin accelerates its capacity to activate thrombin-activatable fibrinolysis inhibitor (TAFI) which cleaves fibrin and other proteins, resulting in the loss of plasminogen/plasmin and tPA binding sites and thus retarding fibrinolysis. Perturbations, such as those that may occur at sites of injury, inflammation or high hydrodynamic shear stress, tip this haemostatic balance in favour of a pro-thrombotic and anti-fibrinolytic microenvironment. Critical steps include loss of cell surface heparin proteoglycan molecules and increased expression of the transmembrane glycoprotein tissue factor (TF) which initiates coagulation by stimulating the activation of clotting factors IX and X, and pro-thrombinase, with subsequent fibrin formation. TF accumulates in experimentally injured vessels and accumulation in some atherosclerotic plaques likely accounts for their high thrombogenicity.

INFLAMMATION

Development of inflammatory reactions by the endothelium in response to injury or infection is critical for the maintenance and/or repair of normal structure and function of the vessel wall. However, excessive inflammatory reactions can lead to severe tissue damage and contribute to the development of atherosclerosis.

The interaction between endothelial cells and inflammatory cells such as leukocytes depends on the production of inflammatory cytokines (e.g. interleukin 8; IL-8) to attract leukocytes and expression of adhesion molecules (e.g. selectins) to facilitate their migration towards the site of infection. Loosely tethered leukocytes first roll over the endothelial surface, then arrest, spread, and finally migrate between endothelial cells to attach on to underlying ECM components.[16]

Leukocyte rolling involves endothelial adhesion molecules of the selectin family which transiently bind to carbohydrate ligands on leukocytes to slow passage through the blood vessel. E- and P-selectin are expressed only on the surface of activated endothelial cells whereas L-selectin is constitutively expressed on leukocytes and binds to ligands induced on the endothelium at sites of inflammation or on other leukocytes. The role of individual types of selectins in leukocyte rolling shows stimulus- and time-dependent variation. Immediate stimulation

of leukocyte rolling induced by histamine or thrombin depends on rapid expression of P-selectin, surface levels of this adhesion molecule declining after only 30 minutes. In contrast, TNFα stimulates delayed leukocyte rolling and adhesion to endothelial cells through the induction of E-selectin, surface levels of which peak after 12 hours and decline after 24 hours. Both E- and P-selectin are expressed on the surface of endothelial cells overlying atherosclerotic plaques, affirming the importance of these molecules in the development of atherosclerosis.

Firm adhesion of leukocytes is promoted by binding of chemokines such as IL-8 to leukocyte GPCRs resulting in rapid activation of β1 and β2 integrins to increase their affinity for adhesion molecules of the immunoglobulin superfamily, intercellular adhesion molecule (ICAM-1) and vascular cell adhesion molecule (VCAM-1). ICAM-1 is constitutively expressed on endothelial cells but levels are increased by stimuli such as TNFα peaking at 6 hours and remaining elevated for 72 hours. ICAM-1 mediates firm adhesion of blood cells by acting as a ligand for leucocyte beta2 integrins. VCAM, a ligand for integrins α4β1 and α4β7, principally mediates the adhesion of monocytes, lymphocyte, eosinophils, and basophils to the endothelial surface. Expression of VCAM-1 is induced by cytokines, oxidized low-density lipoproteins and ROS acting, as with induction of ICAM-1, primarily via NF-κB.

Migration of leukocytes through the endothelium requires the transient disassembly of endothelial cell junctions. Firm adhesion of leukocytes to the endothelium induces clustering of adhesion molecules like ICAM-1 and VCAM-1 triggering activation of intracellular signaling pathways which induce endothelial cell actin cytoskeleton and cell junction remodelling. The remodelling process involves numerous pathways including Rho GTPase signaling, protein phosphorylation and ROS generation but a key event is alteration of the dimerization of PECAM-1. PECAM-1 localizes to intercellular junctions of endothelial cells, forming homodimers linking two cells. Leukocytes also express PECAM-1 and the dissociation of PECAM-1 dimers between endothelial cells to form dimers between emigrating leukocytes and endothelial cells is critical for leukocyte migration.

CONCLUSIONS

The endothelium, once viewed as an inert physical barrier, is a dynamic secretory organ fulfilling numerous roles in the maintenance of cardiovascular homeostasis. Endothelial cells from different parts of the vasculature show highly differentiated functions as a consequence of both environmental stimuli and epigenetic modifications. Advances in defining many endothelial functions at the molecular level may lead to targeted therapies to alleviate chronic endothelial dysfunction associated with the progression of cardiovascular disease.

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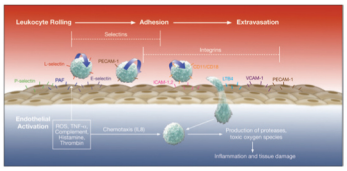

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Mechanisms of Vascular Disease

Edited by Robert Fitridge and Matthew Thompson

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