Anatomy and Pharmacology of Vessels

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Key Concepts

- Arteries are composed of an intima, media and adventitia.
- The endothelium influences artery diameter and prevents adhesion of platelets.
- The media contains concentric layers of smooth muscle and elastic tissue.
- The adventitia contains nerve endings which can modulate vascular diameter.
- Adipose tissue surrounding the vessel releases paracrine mediators.
- Artery structure can be modified in some diseases such as hypertension.

1.1 Artery Structure

Arteries are composed of three major components: endothelial cells, smooth muscle cells and extracellular matrix (ECM). In recent years, the perivascular adipose tissue which surrounds all blood vessels with the exception of cerebral vessels has received increasing attention, and this will be discussed later in the chapter. The ECM is composed of collagen, elastin and glycosaminoglycans (GAGs) and supports the mechanical load of the vessel. The ECM is of great interest in that it can communicate with vascular smooth muscle cells in the vascular media, and changes in the ECM can also contribute to vascular stiffening or arteriosclerosis, a hallmark of ageing and many cardiovascular (CV) diseases (reviewed in Lacolley et al. [1]).

A typical blood vessel is arranged in three distinct layers forming concentric circles. The innermost layer adjacent to the vessel lumen and in contact with the bloodstream consists of a monolayer of endothelial cells on a basement membrane. Collectively this is termed the vessel intima (tunica intima). The basement membrane contains fenestrations through which the endothelial cells can communicate with the underlying layers of the vessel, and there is evidence that the communication and orientation of the intimal cells become altered in some disease such as hypertension [2] and pulmonary hypertension [3]. Myoendothelial gap junctions (MEGJ) are points of very close contact between endothelial cells and vascular smooth muscle cells, and such sites are thought to facilitate the local action of signalling molecules and/or the passage of current between the two cell types. Since these sites have the potential for bidirectional communication between the endothelium and smooth muscle, they are seen as a key pathway for coordinating vascular function and also as a target for therapeutic agents in vascular diseases [4]. Recent work in this area has focussed not only on the integration between various cell types in the artery wall but also on the importance of integration of consecutive segments within the microvasculature. Here, connexins appear to be central, not only acting as gap junctions but also linking with proteins to form signalling complexes (a recent review on the subject was published by Pogoda et al. [5]). A simple representation of vascular structure is shown in Fig. 1.1.

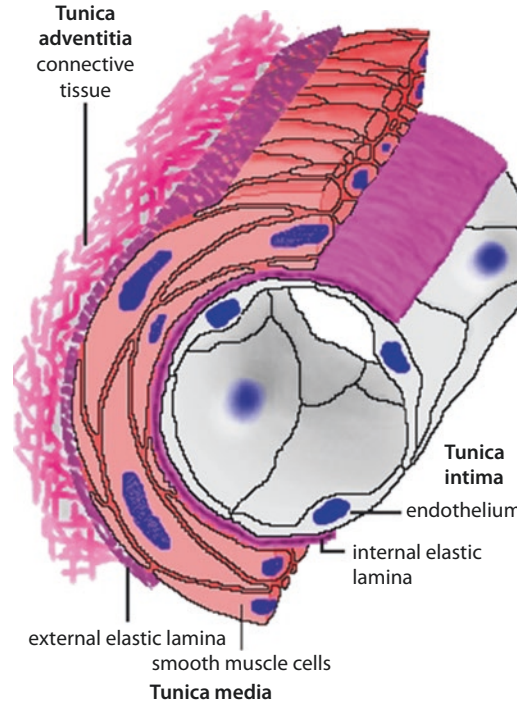

Fig. 1.1 Diagrammatic representation of an artery showing the three main components: endothelium, medial smooth muscle cells separated by the two laminae – the internal and external elastic laminae. (Courtesy of Dr. Craig Daly, University of Glasgow)

1.2 The Endothelium

Previously viewed as an inert barrier between the blood and the artery wall, the pioneering work of Robert Furchgott and other pharmacologists demonstrated the complexity of the endothelium and its ability to modulate the function of the blood vessel wall and also components of the blood (for a review of the discovery of gasotransmitters and the paracrine function of the endothelium, see Nava and Llorens [6]). Intact endothelium provides an anticoagulant and antithrombotic luminal surface rich in heparin-like glycosaminoglycans. Prostacyclin and nitric oxide (NO) are the most important factors produced and released by endothelial cells that inhibit platelet aggregation and coagulation. The production and release of these molecules from endothelial cells can be activated by agonists involved in platelet aggregation and coagulation, such as thrombin. Thrombomodulin is another important anticoagulant factor expressed by endothelial cells. When binding to thrombin, thrombomodulin reduces thrombin's activity in converting fibrinogen to fibrin and activates the anticoagulant protein C pathway. Endothelial cells are also the source of circulating vWF, which binds and stabilises circulating factor VIII. Endothelial cells also control the fibrinolysis balance by secreting tissue-type plasminogen activator (t-PA) and its inhibitor plasminogen activator inhibitor type 1 (PAI-1). For a recent review of the thrombomodulatory role of endothelial cells, see Urano et al. [7].

1.2.1 Endothelium-Derived Vasoconstrictors

Endothelial cells also regulate vascular tone and blood pressure by synthesising and secreting a spectrum of vasoactive substances (Table 1.1). Endothelin-1 (ET-1) is one of the most potent vasoconstrictors released by endothelial cells, which contributes to the maintenance of endogenous vascular tone. ET-1 also induces tonic NO production in the endothelium to oppose vasoconstriction. In addition to its constrictor effects, ET-1 has mitogenic effects on smooth muscle cells, influences homeostasis of salt and water and stimulates the renin-angiotensin-aldosterone (RAAS) system. ET-1 is synthesised as a precursor peptide which is then enzymatically converted to an inactive intermediate called big ET-1. Big ET-1 is then cleaved by another enzyme known as endothelin converting enzyme (ECE) as well as another route involving a two-step process. ET-1 induces its effects via activation of two G protein-coupled receptors: ETA and ETB. The former mediates vasoconstriction, while activation of ETB on the endothelium relaxes arteries, and activation of ETB on smooth muscle induce constriction. Recent evidence suggests that regional differences in ET receptor expression may determine the response and that ETA and ETB receptors may interact. This is an active area of research, and the system is seen as an attractive target for novel pharmacological agents (reviewed in Houde et al. [8]). However, currently available endothelin receptor antagonists, although effective in reducing systemic blood pressure, are often associated with peripheral oedema and liver toxicity (reviewed in Burnier [9]), and the future of this class of drugs to treat hypertension is uncertain.

Angiotensin II (Ang II) is another important vasoconstrictor released by endothelial cells. Although well known as a circulating hormone regulating the systemic blood pressure through the renin-angiotensin-aldosterone system, the major site for Ang II production is the endothelium [10]. In addition to its vasoconstrictor and proliferative effects on smooth muscle cells, Ang II promotes local production of prostacyclin and ET-1. Interestingly, ACE, the major enzyme for

Table 1.1 Endothelial cell-derived vasoactive substances

Vasoconstrictors	Vasodilators
Endothelin-1	Nitric oxide
Angiotensin II	Prostacyclin
Thromboxane A_2	Endothelium-derived hyperpolarizing factor
Platelet-activating factor	Adenosine
Leukotrienes	C-natriuretic peptide
Superoxide	Hydrogen peroxide

Ang II production in endothelial cells, is the same enzyme responsible for bradykinin degradation to inactive fragments. Bradykinin stimulates NO production and accordingly is a potent vasodilator. Hence activation of ACE leads to vasoconstriction through increased Ang II production as well as through reduced bradykinin-mediated vasodilation. The RAAS has been very successfully targeted to treat hypertension, and agents which either block Ang II receptors (the AT-1 receptor subtype) or prevent its formation by blocking the synthesising enzyme (ACE) are first-line drugs in many patients with hypertension. Recent research has highlighted the importance of aldosterone in blood pressure and that the deleterious effects of aldosterone may not be limited to reabsorption of sodium and water by the kidney (reviewed in Te Riet et al. [11]). The RAAS system is complex, comprising the classical system where ACE cleaves Ang I to produce Ang II and the counter-regulatory system composed of angiotensin converting enzyme 2 (ACE2), angiotensin (1–7), and Mas receptor. In contrast to the classical RAAS, this system generally opposes the actions of Ang II-AT1R. Growing evidence suggests that the counter-regulatory system may be an attractive target in diseases that involve adverse inflammation in the blood vessels and structural changes [12].

1.2.2 Endothelium-Derived Vasodilators

In contrast to vasoconstrictors, endothelial cells also release a number of vasodilators, such as NO and prostacyclin. Nitric oxide is a small gaseous molecule synthesised by nitric oxide synthase (NOS) that induces vascular relaxation by activation of guanylyl cyclase and cGMP accumulation. Nitric oxide was initially discovered as endothelium-derived relaxing factor (EDRF) in 1980 and is released by the endothelium under the challenge of acetylcholine and relaxes isolated vascular beds. EDRF was then proposed to be NO on the basis of pioneering work by Palmer, Ferrige and Moncada using a chemiluminescence assay to detect the EDRF being released in a bioassay set-up. Nitric contains an unpaired electron and thus regarded as a free radical species. Although relatively stable compared to other free radical species such as hydroxyl radicals, nitric oxide is still reactive and readily combines with a number of substances including other radicals, transition metal ions and nucleophiles such as thiols and amines. Nitric oxide is highly hydrophobic and has a very low solubility in water. This characteristic enables nitric oxide to diffuse through cell membranes. Nitric oxide is produced from L-arginine which is oxidised into L-citrulline in the presence of oxygen and nicotinamide adenine dinucleotide phosphate (NADP) by a family of enzymes called NOS. There are three isoforms of NOS: the neuronal NOS (nNOS), the inducible NOS (iNOS) and the endothelial NOS (eNOS). eNOS is constitutively expressed in vascular endothelial cells and maintains tonic release of nitric oxide, which plays a critical role in vascular homeostasis. The activity of eNOS and nNOS requires reversible calcium-dependent calmodulin binding and is influenced by intracellular calcium concentration, while physiological factors such as shear stress and changes in oxygen tension also affect the activity and expression of eNOS. iNOS is not constitutively expressed in most cardiovascular cells. The expression of iNOS is normally induced by pro-inflammatory stimuli. Almost all types of cells including endothelial cells, SMC, fibroblast and leukocytes can express iNOS when activated. Unlike eNOS, iNOS binds calmodulin irreversibly, and the activity of iNOS is not sensitive to the intracellular calcium concentration. iNOS is regarded as a high-output isoform of NOS, and it is of importance in a number of blood vessel pathologies. NO is sometimes referred to as a "gasotransmitter" and also within this category is hydrogen sulphide (H_2S). H_2S is also produced by the vasculature (both endothelium and vascular smooth muscle), and, in contrast to NO which signals through the sGC/cGMP pathway, H_2S produces vasodilation via activation of potassium channels and muscle membrane hyperpolarisation (for a recent review of gasotransmitters, see Gheibi et al. [13]). Therapeutically, many agents in current use to treat conditions such as angina and heart failure do so by liberating NO to promote primarily venous dilatation, and future efforts may be directed towards targeting other gasotransmitters such as H_2S.

Another endothelium-derived vasodilator, prostacyclin, is produced from membrane-bound phospholipid by a series of enzymes including phospholipase A_2, cyclooxygenase and prostacyclin synthase when endothelial cells are activated.

Prostacyclin activates G protein-mediated activation of adenylate cyclase through IP receptors and leads to cAMP formation and then vasorelaxation [14]. In addition, NO and prostacyclin are reported to inhibit vascular SMC proliferation, maintaining the vessel media in a state of quiescence with a very low cell turnover. They are likely to do this by antagonising the cellular effects of growth factors and vasoactive peptides including Ang II and ET-1 (reviewed by Kapakos et al. [15]). The importance of this process in diseases where structural changes occur cannot be overstated. Perturbations in the endothelial layer caused by high blood pressure, atherosclerosis, aneurysms or mechanical injury during percutaneous interventions can result in excessive proliferation and development of a neointima, and this may be a key step in pathologies such as vein graft hyperplasia and in-stent restenosis.

Inflammation in the vascular tree is characteristic of many cardiovascular diseases, and mediators produced by the endothelium may also play a role. Endogenous NO inhibits the expression of adhesion molecules and pro-inflammatory cytokines. Inhibition of basal nitric oxide production results in increased leukocyte adhesion in the post-capillary venules, while addition of exogenous nitric oxide reduces leukocyte infiltration in acute inflammation. Induction of iNOS in inflammation might contribute to limit the extent of the inflammatory response. However, a high concentration of NO is also well known to react with ROS to produce cytotoxic peroxynitrite and cause tissue damage (for a recent review on the biochemistry of peroxynitrite and how it damages biological membranes, see Bartesaghi and Radi [16]). Paradoxically, acute exposure of isolated segments of artery to peroxynitrite actually induces vasodilation which may have a physiological role. Interestingly, in atherosclerotic vessels from experimental animals, the response to some vasodilators is attenuated although the response to peroxynitrite is maintained [17].

1.3 Vascular Smooth Muscle Cells

Immediately underlying the basement membrane is a fenestrated layer of ECM called the IEL. This represents the innermost layer of the vessel media (tunica media). All blood vessels, with the exception of the smallest capillaries, contain a muscular media. Arteries have a degree of muscularisation which is related to function. Resistance arterioles are particularly well muscularised relative to their size in order to dissipate arterial blood pressure prior to blood entering the capillaries, while conduit vessels such as the aorta and carotid arteries have a comparatively less muscular media with more connective tissue, making for a more elastic vessel. Indeed, a term sometimes coined to describe conduit vessels is "windkessel" which literally means "air-chamber". When blood pressure rises during ejection of blood from the heart (systole), windkessel vessels distend and then recoil as blood moves out and blood pressure falls during diastole. There is a net storage of blood during systole, and, by their distensibility, these vessels act like capacitors. Vascular stiffness increases with age, likely due to increased calcification and loss of elastin, and so the ability of the elastic vessels to dampen the fluctuations in blood pressure over the cardiac cycle diminishes. This can give rise to isolated systolic hypertension and an increased pulse pressure.

The medial smooth muscle cells are arranged concentrically, and their coordinated contraction and relaxation are responsible for changes in blood vessel diameter. Vascular smooth muscle (VSM) is continuously active with the concentration of cytosolic calcium (Ca^{2+}) being the major determinant of vasoconstriction or vasodilation (for a recent review of calcium and contractile processes in VSM, see Liu and Khalil [18], Touyz et al. [19]). The movement of Ca^{2+} into the cytosol results from a change in membrane potential (and the opening of voltage-operated calcium channels) or the activation of receptors by contractile agents. This results in the production of inositol 1, 4,5-trisphosphate (IP_3) and diacylglycerol from the hydrolysis of phosphatidylinositol-bisphosphate by phospholipase C. Following activation, IP_3 causes Ca^{2+} release from its internal store, the sarcoplasmic reticulum (SR) via the IP_3 receptor. The calcium-binding protein calmodulin forms a complex with Ca^{2+} which in turn activates myosin light-chain kinase. The activated enzyme phosphorylates smooth muscle myosin light chain (SM MLC) with this process driven by adenosine triphosphate (ATP). This results in the interaction of SM MLC with alpha smooth muscle actin (αSMA) filaments, initiating crossbridge cycling and the generation of force which in turn produces contraction. For relaxation to occur, the level of free

cytosolic Ca^{2+} must decrease which occurs by the movement of Ca^{2+} back into the lumen of the SR via the sarco-/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) pump with the hydrolysis of ATP. Other pumps may extrude calcium across the plasma membrane to reduce intracellular calcium such as the plasma membrane calcium ATPase (PMCA) or sodium-calcium exchanger (NCX). Many of these processes are regulated by reactive oxygen species in vascular cells. Expression of these calcium extrusion pumps is altered in atherosclerotic mice [20], and there may be compensatory changes that may maintain vasodilator function of the vessel wall as atherosclerotic plaques develop. Thus, VSM regulates the luminal diameter of the vessel and therefore is fundamental in vascular tone as well as arterial blood flow and pressure. In addition, vascular dysfunction which is characteristic of many CV diseases appears to be associated with abnormalities in PKC and ROCK activity, and active efforts are underway to develop modulators of these signalling proteins which could be future therapeutic agents.

1.4 The Adventitia

The adventitial layer (tunica adventitia) surrounds the media and is comprised of connective tissue within which nerve fibres and a network of small blood vessels called the vasa vasorum are located. The vasa vasorum supplies oxygen and other nutrients to the adventitia and outer portion of the media, while the inner portion of the media and intima receive nutrients from the luminal blood by the process of diffusion. The innervation of the adventitia consists of varicosities of the sympathetic branch of the autonomic nervous system. Varicosities, which are small enlargements along the nerve fibres, are the point at which noradrenaline is released. By activating postsynaptic alpha adrenoceptors, these nerves induce contraction of the medial smooth muscle cells and a reduction in vessel diameter. It has been known for some time that varicosities release more than one neurotransmitter, a process known as cotransmission. There can be interaction between the transmitters released by the nerves (e.g. noradrenaline and adenosine 5′-triphosphate (ATP)) as well as interactions between nerve-derived transmitters and locally derived mediators released by the blood vessel (e.g. prostanoids). For an authoritative review, the reader is directed towards Burnstock [21].

Parasympathetic fibres are found to be associated with blood vessels in certain organs such as salivary glands and gastrointestinal glands and in genital erectile tissue. These specialised nerves release acetylcholine (ACh) which binds to muscarinic ACh receptors to induce vasodilation through NO formation and guanylyl cyclase activation. The tone of vessels is thus an amalgamation of the influence of the autonomic nervous system coupled with factors released by the endothelium (and also other parts of the vessel which will be discussed below). Many currently used pharmacological agents reduce blood pressure by altering this balance – for example, by blocking the alpha adrenoceptors or blocking the production, metabolism or action of endothelium-derived vasoconstrictors. The outer regions of blood vessels may also contain sensory neurones which release vasoactive substances such as calcitonin gene-related peptide (CGRP) which can dilate blood vessels and may have a role in diseases such as migraine [22].

1.5 Perivascular Adipose Tissue

Surrounding the adventitia of the vast majority of blood vessels (capillaries and cerebral blood vessels being the exception) is a cushion of perivascular adipose (PVAT) tissue (Fig. 1.2), and it is becoming increasingly apparent that it too can influence blood vessel function [23]. PVAT has a mixed composition of both white adipose (WAT) and brown adipose tissue (BAT), with varied WAT/BAT ratio according to its anatomical location. The PVAT around abdominal and mesenteric vessels is mainly WAT, while the thoracic aorta appears to have more BAT. Besides adipocytes, PVAT contains other cells grouped together as the stromal vascular fraction (SVF), including fibroblasts, mesenchymal stem cells, lymphocytes, macrophages and endothelial cells derived from structures such as the vasa vasorum (reviewed in Almabrouk et al. [24]). In addition to the release of free fatty acids/non-esterified fatty acids (NEFAs) by lipolysis, adipose tissue secretes bioactive proteins. These are collectively termed as adipokines and have endocrine, autocrine and paracrine actions. PVAT-

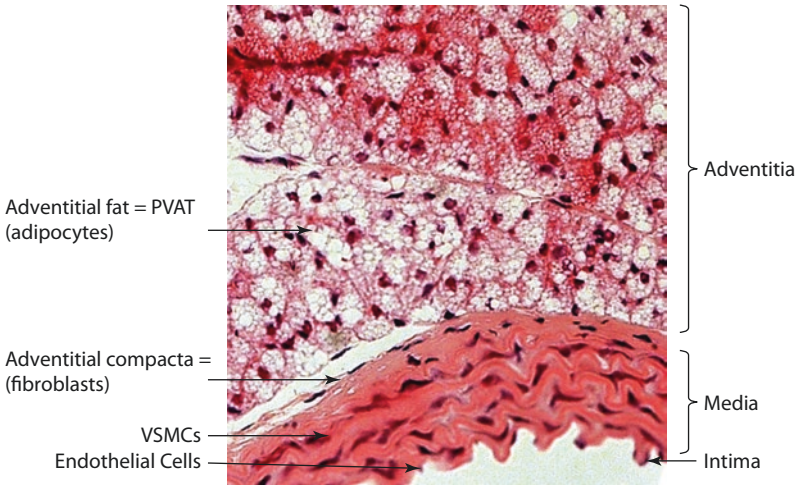

Fig. 1.2 Haematoxylin- and eosin-stained mouse aorta showing the structure of the blood vessel and the surrounding perivascular adipose tissue (PVAT). The blood vessel wall is composed of three layers: tunica intima (endothelium), tunica media (VSMCs layer) and adventitia which includes the PVAT. PVAT contains primarily adipocytes but also vasa vasorum and other cells (macrophages, adipocyte stem/progenitor cells, lymphocytes, fibroblasts, etc.). (Photograph courtesy of Dr. T.A.M. Almabrouk, University of Zawia. Libya)

derived adipokines such as adiponectin, leptin, omentin and resistin participate in the regulation of vascular function (reviewed in Mattu and Randeva [25]). Many of the substances released by PVAT from healthy blood vessels are beneficial, and in vitro studies have shown that adiponectin can augment the ability of vessels to relax [26]. PVAT can also release a whole host of other substances with direct or indirect effects on the underlying blood vessel such as angiotensin, reactive oxygen species (ROS) including superoxide, hydrogen peroxide (H_2O_2) and gaseous molecules such as H_2S and NO. Recent work has demonstrated that an enzyme involved in controlling cellular metabolism known as AMPK and which is expressed in PVAT may be pivotal in release of some adipokines [26].

PVAT also produces classical chemokines (or cytokines) including IL-6, IL-8, monocyte chemoattractant protein-1 (MCP-1) and PAI-1 (plasminogen activator inhibitor-1). Interestingly, inflammatory cells such as macrophages and T-lymphocytes, fibroblasts and capillary endothelial cells have also been demonstrated to contribute to the chemokine and cytokine profile of adipose tissue, and in disease states or in animal models of disease, inflammation within the PVAT increases [27]. In addition, dysfunctional fat can affect the activity of other tissues within the vessel wall such as the endothelium, and adiponectin has been proposed to mediate crosstalk between PVAT and vascular endothelium [28]. Cytokines generated from within the PVAT may also induce changes in vascular reactivity indirectly through upregulation of iNOS. Currently, there is a large research effort underway to understand the function of PVAT better and in particular to appreciate whether changes in PVAT underlie the vascular dysfunction often seen in obese, insulin-resistant patients.

Conclusions and Clinical Perspectives

The basic structure of a blood vessel consists of three concentric layers: endothelium, vascular smooth muscle cells, adventitia and, in most vessels, perivascular adipose tissue associated with the adventitia. The thickness of the medial smooth muscle and the amount of elastic fibres within the medial layer depend on the position of the vessel within the vascular tree and the pressure of the blood within the vessel. Veins are adapted to their function of moving low pressure blood back to the heart and storing the majority of blood by virtue of having valves and distensible, thin walls. The function of blood vessels is to conduct blood ejected by the heart around the body. The diameter of a blood vessel affects the flow of blood within that vessel, and diameter can be varied by a huge number of factors including release of mediators by the blood vessel itself, circulating vasoactive factors, neural

control within the adventitia, release of adipocytokines by the perivascular adipose tissue and metabolic activity of the tissue served by the blood vessel. With increased understanding of the physiological regulation of the structure and function of blood vessels, novel therapeutic targets for vascular disorders may become apparent.

Gaps in Knowledge

- How cells within the artery wall communicate with each other is not fully understood.
- The processes of remodelling in response to diseases such as high blood pressure are incompletely understood.
- It is unclear whether vascular remodelling is a cause or effect of increased blood pressure.
- The perivascular adipose tissue, which produces adipokines and other vasoactive factors, modulates vascular function, although exact mechanisms and the clinical relevance still remain unclear.

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