Flow-induced arterial remodeling : role of angiotensin II and bradykinin

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Flow-Induced Arterial Remodeling:
Role of Angiotensin II and Bradykinin

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Cell nuclei stained in green, elastin fibers in red. Image kindly provided by:

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Flow-Induced Arterial Remodeling

Role of Angiotensin II and Bradykinin

PROEFSCHRIFT

Ter verkrijging van de graad van doctor
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Table of Contents

CHAPTER 1	Introduction	7
CHAPTER 2	Flow-Dependent Dilatation Mediated by Endogenous Kinins Requires Angiotensin AT ₂ Receptors. <i>Circ Res. In Press</i>	29
CHAPTER 3	Role of Tissue Kallikrein During Arterial Remodeling in Response to Altered Blood. <i>Submitted.</i>	41
CHAPTER 4	Uterine Artery Structural and Functional Changes During Pregnancy in Tissue Kallikrein-Deficient Mice. <i>ATVB. 2003;23:1826-1832.</i>	51
CHAPTER 5	Intra-Arterial Bradykinin and Vasodilatation in relation to Acute and Chronic Changes in Blood Flow. <i>Submitted.</i>	67
CHAPTER 6	Changes in Vascular Distensibility During Angiotensin-Converting Enzyme Inhibition Involve Bradykinin Type 2 Receptors. <i>J Vasc Res. 2004;41:18-27.</i>	77
CHAPTER 7	Tissue Angiotensin-Converting Enzyme in Imposed and Physiological Flow-Related Arterial Remodeling in Mice. <i>ATVB. 2004;892-897.</i>	91
CHAPTER 8	Uterine Artery Dilatation and Remodeling During Postponed Murine Pregnancy. <i>Submitted.</i>	105
CHAPTER 9	Review article: Acute and Chronic Adaptive Responses to Altered Blood Flow in Large and Small Arteries. <i>Submitted.</i>	117
CHAPTER 10	General Discussion.	139
REFERENCES		145
SUMMARY		171
SAMENVATTING		175
DANKWOORD		179
CURRICULUM VITAE		181
GRANTS AND AWARDS		183
LIST OF PUBLISHED ARTICLES		183
LIST OF SUBMITTED ARTICLES		183
LIST OF ABSTRACTS		184

Chapter 1

Introduction

General Introduction

Blood vessels are permanently subjected to mechanical forces. The pulsatile nature of arterial blood flow affects the inner layer of the arterial wall, the endothelium, which senses the frictional dragging force (fluid shear stress) of the blood flow. Transmural pressure causes tangential forces (circumferential wall stress) that affect the cellular and extracellular matrix components of all three layers of the arterial wall (the tunicae intima, media and adventitia)(Figure 1.1).

Figure 1.1. Simplified model of the arterial wall components and the hemodynamic forces which they are exposed to. The endothelial layer is constantly exposed to the frictional, dragging force of the streaming blood flow, the fluid or wall shear stress. Endothelial cells, smooth muscle cells, adventitial cells and their extracellular matrix are permanently exposed to tangential forces resulting from transmural pressure. (Drawing by Rob Hilgers)

Acute changes in pressure and flow result in transient adjustments of the vascular lumen diameter, mediated by myogenic contractile responses or release of paracrine vasoactive compounds that aim to restore shear stress and circumferential wall stress. For instance, an increase in blood pressure causes a narrowing or constriction of the artery (myogenic tone), while a rise in arterial blood flow results in a widening or dilatation of the vessel (flow-induced dilatation).

When these mechanical forces persist for a longer period of time they may cause structural alterations in arterial wall shape, composition and function. This arterial remodeling describes the transformation of the artery (diameter and wall mass changes) in response to chronically altered mechanical forces. It participates in the pathogenesis of many cardiovascular diseases and complications. For example, hypertension is

accompanied by increased wall thickening in small resistance arteries and arterioles resulting in smaller lumen diameters. Atherosclerosis develops at sites that experience reduced or turbulent wall shear stresses, especially near bifurcations of large arteries such as the aorta or carotid artery.

Unfortunately, cardiovascular diseases such as hypertension, atherosclerosis, preeclampsia, myocardial infarction, and heart failure remain the number one cause of death in western societies. Research on vascular remodeling that aims to unravel the mechanisms and mediators involved in the onset of cardiovascular complications is necessary for the development of new therapeutic strategies for the treatment of these diseases.

Acute Arterial Responses to Mechanical Stimuli

Pressure and Myogenic Tone

Vascular smooth muscle cells (VSMCs) are particularly sensitive to stretch and stresses and transmit these mechanical forces into biochemical signals. Intraluminal pressure results in a tangential stress upon the arterial wall or circumferential wall stress (CWS). It is defined by the law of Laplace:

$$CWS = \frac{TP \cdot r}{Wt}$$

where TP is transmural pressure (in N/m), r is the vascular inner or lumen radius (in mm) and Wt is wall thickness (in mm). Small arteries ($\varnothing < 300 \mu\text{m}$) constrict in response to an elevation of pressure, reducing their radius and slightly increasing their wall thickness, hence restoring wall stress toward normal levels. This pressure-induced vasoconstriction is known as the myogenic tone or the myogenic response.¹⁵⁶ It increases with decreasing vessel diameter,¹¹⁵ although the cerebral vascular bed⁴⁰⁴ may be an exception to this rule. Myogenic tone can easily be investigated *in vitro*. When small resistance arteries or arterioles are isolated, cannulated and incubated in a physiological salt solution, raising the intraluminal pressure will result in a pressure-dependent constriction (active, Figure 1.2) which stays stable over a broad pressure range. Withdrawal of Ca^{2+} ions from the incubate and administration of vasodilator agonists abolishes this myogenic tone and fully relaxes the VSMCs so that the arterial diameter continues to increase until a plateau is reached (passive, Figure 1.2).

The myogenic response is mediated by a non-neural¹⁵⁵ and endothelium-independent mechanism. It enables small arteries such as arterioles to (1) autoregulate local blood flow, (2) establish basal vascular tone and peripheral resistance, and (3) regulate capillary hydrostatic pressure.^{116,271} It has long been appreciated that Ca^{2+} ions play a pivotal role in smooth muscle contraction and in the setting of arteriolar tone. This effect of Ca^{2+} is assumed to be mediated through Ca^{2+} -calmodulin activation of myosin light chain kinase. The intracellular signaling mechanisms of this response remain focus of extensive research, but it is agreed upon that a myogenic tone is initiated by VSMC

depolarization, which then regulates Ca^{2+} entry through voltage-gated Ca^{2+} channels³⁵⁷ and stretch-sensitive ion channels.¹¹⁶ For an overview of the mediators involved in myogenic tone the reader is referred to more elaborate review articles.^{117,405}

Figure 1.2. Example of myogenic tone measurement in an isolated small artery. An arterial segment (3 mm) is cannulated between two small glass pipettes and incubated in a physiological salt solution without Ca^{2+} (passive) or with Ca^{2+} (active) at 37°C and intraluminal pressure is raised in small steps (e.g. 10 mmHg) and the diameter is recorded (until a stable diameter is reached). Transmural pressure is plotted against diameter (Pressure-Diameter curve).

Flow (Shear Stress)-Induced Vasodilatation

Endothelial cells (ECs) are directly in contact with the streaming blood and are well adapted to sense the dragging, frictional force (shear stress) of the pulsatile blood flow. Wall shear stress (WSS) is, according to Pousseuille's law, defined by the formula:

$$WSS = \frac{4 \cdot \eta \cdot Q}{\pi \cdot r^3}$$

in which η is the blood viscosity (in $\text{dyne} \cdot \text{s} \cdot \text{cm}^{-2}$), Q is the blood flow velocity (in $\text{cm}^3 \cdot \text{s}^{-1}$), and r is the radius of the arterial lumen (in cm). Normal steady laminar shear stress stimulates cellular responses that are essential for normal endothelial cell function, such as regulating permeability of plasma lipoproteins, adhesion of leukocytes, enabling a balance between anti- and prothrombotic factors, release of endothelium-derived growth factors and vasoactive agents⁴⁶⁰ and maintaining an atheroprotective environment.⁵⁴⁴ Increases in blood flow, and thus shear stress, lead to release of endothelium-derived relaxing factors, such as nitric oxide and prostacyclin, that cause a diameter enlargement, which tends to restore wall shear stress values toward normal.

Interaction Between Myogenic Tone and Flow-Induced Vasodilatation

A distinction and an interaction between pressure-induced and shear stress-induced arterial responses have been clearly demonstrated in an *in vivo* experiment by Pohl and colleagues.⁴²⁶ *In situ* exposed and saline-perfused rabbit small mesenteric arteries were exposed to an elevation of perfusion pressure, which increased both transmural pressure

and flow. After an initial dilatation, the vessels developed a myogenic constriction, followed by a dilatation, which was shear stress-dependent, since nonperfused vessels exposed to the same intraluminal pressure developed a myogenic response without a subsequent dilatation.⁴²⁶ These experiments indicate that myogenic responses can be opposed by flow-induced dilatations, suggesting a mechanism for maintaining adequate tissue perfusion during for instance exercise, when pressure and shear stress increase simultaneously. Also competition between myogenic tone and flow-induced dilatation occurs, since strong levels of myogenic tone in cannulated pressurized coronary arterioles attenuated flow-induced dilatation.³⁰² This competition might be a mechanism to protect tissues from overperfusion and increased permeability leading to edemas.

In some types of isolated arterial segments, flow can cause either a constriction or a dilatation, depending on the level of the pre-existing vasomotor tone and on the membrane potential of the VSMCs.⁴⁴ Resting tone is proposed to be determined by the activity of ATP-sensitive²⁶² and calcium-dependent⁵⁸ potassium channels, where a fraction of these channels is open, such that membrane potential has an intermediate value (approximately -58 mV). The opening of potassium channels would lead to hyperpolarization (-62 mV) and dilatation, whereas closure results in depolarization (-49 mV) and contraction.

Mechanisms of Mechanotransduction

Evidence indicating a role of the endothelium as a shear stress-sensor emerged from *in vitro* studies of cultured endothelial monolayers^{110,112} and isolated cannulated small arteries.^{248,427} In response to sustained steady flows, ECs align and elongate in the direction of the flow,^{110,255} and release vasoactive factors (*e.g.* nitric oxide, prostacyclin, endothelin-1). Conformational changes of proteins are most likely involved. The specialized regions of the plasma membrane known as focal adhesion sites link cytoskeletal proteins to the extracellular matrix, and modulate cellular shape and motility. Shear stress leads to a rapid deformation and reorganization of actin-containing stress fibers, as well as other cytoskeletal intermediate filaments, such as vimentin, at the sites of cell-matrix contacts forming the focal adhesion complexes (Figure 1.3).²¹⁵ Integrins are important linkage proteins and are likely candidates for mechanotransduction in their capacity to bind to the extracellular matrix on the cell surface and to the cytoskeletal proteins in the cytoplasm.⁶⁷ Indeed, shear stress modulates the binding affinity of integrins to the Arg-Gly-Asp (RGD) sequence in integrin receptor proteins such as fibronectin.⁷ The underlying rapid signal transduction pathways involve multiple kinases such as focal adhesion kinase (FAK) that activate downstream mitogen-activated protein kinases (MAPKs, *e.g.* ERK), transcription factors such as nuclear factor- κ B, and receptor-associated G proteins such as Rho.^{417,482,499} Ultimately, the responses to shear stress lead to the rapid phosphorylation and activation of enzymes, such as endothelial nitric oxide synthase (eNOS)(see next section). Other vasoactive compounds can be released in response to fluid shear stress as well. These endothelium-derived factors include prostaglandines,⁴⁶ bradykinin,³⁷ and the still poorly characterized endothelium-derived hyperpolarizing factor (EDHF),²⁴⁴ as well as vasoconstrictors, such as endothelin 1,²⁹⁷ and reactive oxygen species.³²⁴

Figure 1.3. Possible sites of shear stress mechanotransduction in the endothelial cell. **1:** shear stress-sensitive proteins bound on the phospholipid layer on the luminal side of the cell membrane, such as integral membrane proteins are linked to cytoskeletal proteins, potassium channels, and phospholipases. **2:** Focal adhesion sites (integrins and linker proteins) bound to the extracellular matrix and F-actin filament proteins. **3:** Cell to cell contacts via cadherin and adhesion proteins. **4:** Cytoskeletal proteins attached to the nucleus might transduce perturbations in shear stress directly to the nucleus to initiate gene transcription. (*Adapted from*¹¹²).

Endothelial Nitric Oxide Synthase Activation

Numerous studies have been performed to elucidate the mechanism of shear stress-induced activation of eNOS and NO release. In general, eNOS (or NOS3) catalyzes the production of the vasodilator nitric oxide (NO) by the oxidation of L-arginine to L-citrulline.³⁶⁷ The diatomic gas diffuses to the underlying VSMCs where it activates soluble guanylate cyclase leading to cyclic GMP production and subsequent VSMC relaxation (for a review see²²⁸). eNOS is targeted to caveolae (invaginations of the plasma membrane) by cotranslational N-myristoylation and posttranslational palmitoylation,¹⁷⁴ and is held inactive via binding with caveolin-1.²⁸⁰ An increase in intracellular Ca^{2+} , following receptor-dependent and -independent agonist stimulation, enhances NO production and causes vasodilation.¹⁵⁴ Binding of calmodulin and associated proteins is essential for agonist-induced activation of eNOS.^{70,572} However, shear stress activates eNOS via a Ca^{2+} -independent mechanism.¹⁴ Evidence that eNOS can be stimulated by at least two independent signaling pathways came from an observation that disruption of the cytoskeleton attenuated the flow-induced release of NO, but did not affect the agonist-induced vasodilation.²⁵⁰ Studies in cultured ECs^{100,124,298} and in intact vessels^{461,491} demonstrated the production of NO by flow via eNOS activation. Shear stress rapidly activates a signaling pathway involving several tyrosine kinases, FAK, proline-rich tyrosine kinase (PYK2), and vascular endothelial growth factor receptor 2 (Flk-

1/VEGFR2.^{268,321,529} Ultimately, this proximal tyrosine kinase signaling leads to the more downstream signaling via phosphoinositide 3-kinase (PI₃K) and subsequent serine/threonine protein kinase Akt activation that phosphorylates eNOS at amino acid residue serine 1177,^{124,169} leading to activation of eNOS and the release of NO within seconds (Figure 1.4).

Figure 1.4. Schematic model of endothelial nitric oxide synthase (eNOS) activation via shear stress in endothelial cells. Under physiological shear stress levels (resting conditions) low levels of NO are produced because eNOS is linked with caveolin 1 and localized to the caveolae (not shown) which inhibits its activity. Changes in fluid shear stress on the luminal side of the cell disturbs the cytoskeletal proteins and initiates a biochemical signaling cascade via the integrins of the focal adhesion sites. Activation of these integrin proteins activates phosphatidylinositol 3-kinase (PI₃K) to generate phosphatidylinositol (3,4,5)-triphosphate [PI(3,4,5)P₃] from phosphatidylinositol (4,5)-diphosphate [PI(4,5)P₂]. PI(4,5)P₂ directs nonphosphorylated Akt to the plasma membrane, where it is phosphorylated and activated by phosphoinositide-dependent kinase (PDK). Active Akt binds to heat-shock protein 90 (HSP 90), which is bound to eNOS, and then phosphorylates the eNOS protein at amino acid residue Ser 1177, which increases eNOS activity at resting concentrations of intracellular Ca²⁺. (Adapted from⁶⁹).

Shear Stress and Arterial Gene Expression

In contrast to the acute shear stress-induced signaling responses described in the previous section, more sustained (> 1 hour) shear stress-induced responses involve regulation at the level of gene expression. A striking similarity of these shear stress-inducible genes is their atheroprotective role, indicating that normal blood flow is vital for maintaining a healthy balance between a pro- and anticoagulant, -inflammatory, -oxidant, and -proliferative state, and that chronic nonphysiological shear stress values disrupt this balance toward the onset of vascular diseases such as atherosclerosis. Many genes important in thrombosis and haemostasis, inflammation, cell proliferation, and extracellular matrix remodeling are regulated by shear stress, including tissue plasminogen activator (tPA),¹²³ monocyte chemotactic protein 1 (MCP-1),⁵⁰⁰ intercellular adhesion

molecule 1 (ICAM-1),³⁸⁶ vascular cell adhesion molecule 1 (VCAM-1),¹⁰ eNOS,^{395,541} superoxide dismutase 2 (SOD2),⁵⁴¹ and PDGF-A and B.^{287,448} Some of the above named genes have specific shear stress-response elements in their promotor region, including PDGF A- and B, and MCP-1.^{287,448,500} A more indirect pathway of shear-induced changes in gene expression involves the NO/cGMP pathway, which was described above with respect to vasodilatation.⁴²⁵

Chronic Arterial Responses to Mechanical Stimuli

Pressure-Related Arterial Remodeling

Hypertension, a vascular disease with an incidence of nearly 20% in the human population, affects all parts of the cardiovascular system. Studying the vasomotor responses of small vessels exposed to high blood pressure enables better understanding of the underlying signaling events that lead to functional adaptations, which precede structural and more permanent arterial changes.

Since large vessels possess only minor vasomotor tone, their capacity to acutely normalize increases in hemodynamic forces is limited. As a result, large arteries undergo structural remodeling (an increase in arterial wall thickness that normalizes circumferential wall stress) earlier than smaller arteries do. In arterioles, increases in transmural pressure initially induce a constriction, which opposes the passive distension. During this myogenic response, the thickness of the arteriolar wall increases and circumferential wall stress is normalized. Consequently, this hemodynamic sensor will no longer be activated and the proliferative signaling will not occur.⁹ Because of the rearrangement of dynamic focal-adhesion sites, by which the arterial cells are connected to their matrix, the maintained constriction will ultimately become structural, leading to inward eutrophic remodeling.¹⁷ Small arteries ($\varnothing$: 100-300 μm) possess a weak myogenic reactivity and consequently display an intermediate type of remodeling.

A general characteristic of hypertension is that peripheral vascular resistance is increased especially in arterioles and capillaries.^{53,377} Besides rarefaction ($\downarrow$ vessel number) and inward eutrophic remodeling, this is due to an augmented myogenic response,²⁴³ via enhanced release of constrictor factors such as endothelin-1,¹⁴³ prostaglandin H_2 , and thromboxane A_2 ,²⁴² which increase the Ca^{2+} sensitivity of the contractile apparatus.

The cellular signaling cascades involved in VSMC proliferation are intricate and are mostly derived from cell culture experiments. However, recent information came from intact vessels, which were cannulated and pressurized *in vitro*.^{352,585} Increased transmural pressure leads to the release of angiotensin II,^{21,218} which via the specific angiotensin II receptor subtype 1 (AT_1), activates phospholipase C and protein kinase C (PKC) in rat VSMC.²²⁷ PKC activates the Ras-Raf pathway, and MAPKs such as extracellular signal-regulated kinase (ERK). ERK1/2 is a key enzyme in mediating the VSMC proliferative actions in response to angiotensin II³⁵² and pressure.^{150,550,585} ERK1/2 stimulates the expression of several proto-oncogenes such as *c-fos* and *c-myc* causing proliferation and protein expression necessary for important remodeling events.³⁸⁵ One of these processes is extracellular matrix degradation in which matrix metalloproteinases (MMPs) especially the elastinolytic MMPs, such as MMP-2 (type IV collagenase), MMP-9 and MMP-12 play

a pivotal role. Proto-oncogenes of the AP-1 family such as *c-fos* regulate MMP-9 promoter activity.¹⁰² Platelet-derived growth factor (PDGF)-A and angiotensin II are also expressed in response to an increase in pressure and appear to be critical for VSMC hypertrophy and hyperplasia through autocrine mechanisms.^{40,385,389}

Flow (Shear Stress)-Related Arterial Remodeling

Flow-related arterial remodeling is the process involving arterial adaptations to chronic alterations in flow, that aim to normalize the imposed wall stresses on the endothelium and the arterial wall, via structural changes of the lumen diameter (WSS) and wall thickness (CWS). The arterial remodeling of an arterial bed in response to changes in blood flow can occur via two mechanisms: capillary network expansion via the sprouting of new capillaries from existing capillaries (angiogenesis) and enlargement of existing collateral arterioles (arteriogenesis).^{13,259} Changes in architecture of the arterial wall in response to alterations in hemodynamic stimuli occur under physiological as well as under pathological conditions, but can also be experimentally induced via surgical manipulations outlined in the following sections.

Several remodeling responses can be distinguished (Figure 1.5). The classification that was proposed in 1996 for arterial structural responses in hypertension³⁷⁹ is based on the changes of the structural arterial lumen diameter (inward or outward) and on changes in wall mass (increased: hypertrophic, decreased: hypotrophic, and unchanged: eutrophic). For example, an increase in lumen diameter without a change in medial cross-sectional area, would be denoted as an outward eutrophic remodeling. This remodeling classification can also be applied to flow-related arterial diameter and wall mass changes. Sustained elevated blood flow will eventually result in a structural luminal expansion, most likely from rearrangement of existing wall mass leading to a thinner wall. This thinning of the arterial wall will increase circumferential wall stress, which triggers growth and proliferation of arterial wall cells. The resulting wall hypertrophy normalizes this wall stress.^{71,434}

Chronic reductions in arterial blood flow lead to a reduction in lumen diameter that normalizes wall shear stress. This narrowing of the lumen diameter is structural, since the diameter can not be restored to control values after application of several vasodilator compounds 3 days postligation in mesenteric arteries⁴³⁴ and 7 days postligation in carotid arteries.^{62,462} Reductions in arterial diameter decrease circumferential wall stress which not only induces hypotrophic signaling but also stimulates atrophy and apoptosis of the VSMCs.⁷¹

Figure 1.5. Arterial remodeling events (adapted from³⁷⁹) resulting from exposure to chronic alterations in transmural pressure and/or blood flow. On the vertical axis are lumen diameter changes denoted as either inward (decrease in diameter), outward (increase in diameter) or unchanged. Similarly, on the horizontal axis are wall mass changes termed hypotrophic, eutrophic, and hypertrophic as, respectively, a decreased, unchanged, and increased wall mass.

Physiological Flow-Related Arterial Remodeling

Pre- and Postnatal development

The development of the embryonic vasculature depends on the *de novo* formation of new blood vessels (vasculogenesis) and sprouting of new vessels from preexisting vessels (angiogenesis). These processes are driven by locally released growth factors such as fibroblast growth factor and vascular endothelial growth factor.⁷⁹ Further vessel development, via expanding of capillary beds and arterialization of feed arteries by enlargement and recruitment of pericytes and smooth muscle cells, becomes thereafter largely sensitive to local haemodynamic forces such as pressure and shear stress. This concept was first appreciated by Thoma,⁵³⁵ who observed that arteries in the chicken embryo grew rapidly when blood flow was high and regressed at low blood flows.

Marked birth-related blood flow changes occur in the perinatal period. In the fetus, placental blood flows accounts for nearly 40% of maternal total cardiac output.²²² Immediately after birth pulmonary vascular resistance dramatically falls in the newborn, resulting in a 10-fold increase in pulmonary blood flow, allowing pulmonary gas exchange.⁴⁶⁴ Closure of the umbilical arteries at birth has dramatic consequences for the abdominal aorta experiencing a 95% blood flow reduction.³¹¹ As a result the diameter of the abdominal aorta decreases roughly 30% with a marked reduction in wall mass 2 to 3 weeks postpartum in lambs.³¹¹ This was accompanied by a reduction of smooth muscle cell proliferation (with no net DNA synthesis) and the occurrence of apoptosis.⁹⁰ Perfusion of the kidneys and gastrointestinal tract increases 3-fold in the first 10 days

postpartum.⁴⁵⁴ The thoracic aorta supplying these organs increases 34% in diameter and undergoes a doubling in wall mass in a period of 2-3 weeks after birth.³¹¹ Changes in vessel diameter and the cellular arrangements of the arterial wall constituents affect arterial viscoelasticity. Collagen-to-elastin ratio of the thoracic aorta decreased by 45% at 3 weeks postpartum in lambs.³² Indeed the aortic wall became substantially less elastic at this period after birth in neonatal lambs.⁵⁸³ These findings indicate that cell turnover plays an important role in postnatal arterial growth and development.

These observations confirm the direct sensitivity of arterial growth to altered blood flow, a phenomenon which was shown to be endothelium-dependent.³¹² They furthermore indicate that shear stress is the trigger for arterial diameter adjustments and that blood flow regulates the remodeling of blood vessels in such manner that wall shear stress remains comparable in many arteries and arterioles in newborn and adult animals.^{459,577}

Chronic Exercise

During physical exercise an increase in cardiac output and distribution of blood flow to skeletal muscle and the coronary circulation develops. Regular exercise reduces the incidence of cardiovascular diseases¹⁶² and lowers risk factors for atherosclerosis.⁵¹⁶ Furthermore, exercise has been demonstrated to improve endothelial function by increasing endothelium-dependent release of nitric oxide in the rat,^{86,120} rabbit,⁸⁷ dog,⁵⁷⁹ and human,^{208,569} and by increasing eNOS expression in dogs.⁴⁹² In patients with coronary artery disease this improvement of endothelial function during chronic exercise was attributed to increased phosphorylation of eNOS.²⁰⁸ Larger lumen diameters of the common femoral artery were observed in young human adults performing regular exercise compared to untrained healthy controls.^{127,249,292,589} During treadmill exercise in rats, a significant increase in blood flow (reaching up to 50-fold) to working skeletal muscle develops,¹² resulting in significant morphological changes in the vascular wall and in the structure of arteriolar networks.^{314,488}

Pregnancy

During mammalian pregnancy uterine blood flow increases dramatically. This ranges from 3.5-fold in humans,⁵³³ 30-fold in sheep⁴⁵⁶ to even 70-fold in rabbits.⁶⁴ Adaptation of the uterine vascular bed to enable sufficient perfusion to the growing fetus(es) occurs through augmented dilatation^{99,337,393,581} outward remodeling of pre-existing vessels and the development of new vessels (angiogenesis).⁶⁰⁷ Sex hormones such as estrogens increase blood flow to the uterine tissue⁵⁶ via multiple actions such as upregulation of endothelial and neuronal NOS^{175,337,391} and vascular endothelial growth factor (VEGF).³⁹² VEGF can act as an angiogenic factor⁵³⁸ and as a vasodilating factor through stimulation of endothelial flt-1 and flk-1/KDR receptors²¹² leading to NOS activation⁵⁸² and subsequent production of NO and prostacyclin.²¹²

Also pregnancy-induced remodeling of the afferent main uterine artery (Figure 1.6) contributes substantially to the decrease in uterine resistance. It has been described in a number of species, including mouse,^{561,571} rat,^{394,406-408,517,518} guinea pig,^{145,363} rabbit,⁵⁰⁸ pig,¹⁹⁹ ewe,^{11,168,195,279} and humans.^{59,533}

Immediately after birth, uterine blood flow decreases rapidly and the uterine artery regresses toward pre-pregnant vessel structure representing a physiological model of reduced blood flow.

Figure 1.6. Schematic diagram of the left uterine arterial system in rodents such as rat and mouse. In this thesis the main uterine artery (see arrow) is represented as a segment of the uterine arcade, and not as a segment of the segmental arteries (also known as radial arteries), which are bifurcating from the main uterine artery.

(Figure adapted from³⁹⁴).

Flow-Related Arterial Remodeling in Pathologies

Most vascular disorders are associated with reduced or inadequate tissue perfusion that ultimately affect arterial remodeling and the severity of the disease. In humans, regions of low shear stress develop inward remodeling and progressive atherosclerosis,^{186,338,519,544} whereas areas of physiological shear stress remain quiescent, and areas of elevated shear stress show outward remodeling.⁵¹⁹ The pathobiology of restenosis after percutaneous coronary artery interventions involves arterial remodeling and neointimal hyperplasia.^{403,418,419,431,432}

Insulin-dependent diabetes mellitus is associated with endothelial dysfunction (reduced vasodilator responses to agonists such as acetylcholine⁴³³ and reduced flow-induced NO-mediated vasodilatation).^{165,545} Absence of a normal flow response could contribute to decreased microvascular circulation leading to long-term vascular complications. Decreased MMP activity has been demonstrated in internal mammary arteries of diabetic patients which may contribute to increased collagen deposition and pathological remodeling in diabetes.⁴³⁰

Aging is an important risk factor for cardiovascular diseases such as ischemic heart disease. Although the mechanisms associated with vascular aging are multifactorial, one underlying pathological cause may be an age-associated endothelial dysfunction^{135,603} leading to impaired vasodilator production²⁵⁸ and flow-induced dilatation,¹⁸¹ which predisposes to an increased vascular resistance.²⁰⁴ Furthermore, the ability of arteries, and in particular the shear stress-sensing ECs, to remodel in response to altered blood flow decreases with advancing age. Reduced flow-related arterial remodeling is observed in both large arteries^{62,361} and resistance arteries.^{258,511,554}

Arterial remodeling is often associated with cardiovascular diseases. Striking examples in large elastic conduit arteries include (1) the formation of a neointima that may evolve into a vulnerable plaque⁴³¹ and (2) aneurysm's which might ultimately burst.⁵¹⁵ On the other hand, arterial remodeling can favourably influence the outcome of cardiovascular disease. For instance, influences of a growing neointima or atherosclerotic

plaque on the patency of the arterial lumen can be limited by timely expansion (outward remodeling) of the underlying tunica media and adventitia.^{260,418,419} Furthermore, evolution of newly formed or pre-existing collateral arteries into feed arteries can help maintain or restore perfusion of an organ endangered by a flow-limiting stenosis.⁷⁹

Collateral Arteries

While mechanical forces such as circumferential wall stress and wall shear stress can explain the structure and build up of individual arterial segments^{79,439,440} and branching arterial trees,²⁴⁷ there are notable exceptions. Collateral arteries interconnect arterial vessels of the same branching order. They can be formed by angiogenesis and arteriogenesis during pathological or experimentally imposed obstruction of a major feed artery.^{213,387,556,557} Herein, shear stress, hypoxia, (endothelium-derived) growth factors and monocyte/macrophages play important roles. Pre-existing collateral arteries are, however, also an integral part of certain vascular beds. In the cerebral circulation of most mammalian species (the gerbil being an exception) all major feed arteries are interconnected at the base of the brain into a structure called the circle of Willis. The uterine arteries that provide the blood supply to the uterus before and during pregnancy, interconnect the ovarian arteries (branches of the abdominal aorta or of the renal arteries) to the vaginal arteries (branches of the iliac arteries). The circulation to the small intestine is intertwined by numerous arterial trees branching off the superior or inferior mesenteric arteries. These mesenteric arterial trees are all interconnected at either a proximal level (*e.g.* pig) or a distal level close to the gut wall (*e.g.* rat). In the coronary circulation, the presence of pre-existing collaterals differs extensively between species (*e.g.* dog, many; pig and human, few) which might explain interspecies differences in cardiac consequences of coronary artery occlusion.³⁷⁵

In theory, blood pressure is equal at both entry points of a pre-existing collateral artery and there is thus no pressure gradient or net average blood flow over the centre of the collateral vessel. If wall shear stress would be the only determinant of vessel lumen diameter, collateral arteries would be readily lost during development. Their presence in the adult and their occurrence in organs that are characterized by marked fluctuations in blood flow such as the gastro-intestinal tract and the female uro-genital system, continues to be a major challenge for current vascular biology. The characteristics of these vessels are of great importance because of their remarkable plasticity in health and disease and because they allow the cardiovascular system to cope both functionally and structurally with major physiological challenges (digestion of large quantities of food; early growth and development of the offspring) and subclinical pathological situations (obstruction of certain feed arteries).

Experimental Models of Flow-Related Arterial Remodeling

Most experimental animal models to study arterial remodeling in response to chronic elevations in blood flow involve creation of an arteriovenous fistula, which can be performed in a number of large arteries such as the femoral artery,^{119,329} iliac artery,^{201,601} carotid artery,^{119,282,348,350,498,546,547} and aorta^{33,202,595} in animals such as the baboon, rabbit, rat and mouse. A major advantage of this method is that supraphysiological blood flow levels are obtained in large arteries. For example, an approximate 30-fold increase in iliac

artery blood flow was obtained in monkeys⁶⁰¹ and an 11-fold increase in carotid artery blood flow in the rat.⁵³⁹ Another method is the unilateral ligation of the carotid artery, first introduced by Guyton and Hartley,²⁰⁶ which leads to a reduction of blood flow in the ipsilateral (ligated) artery and a elevation in blood flow in the contralateral right carotid artery. Collaterals interconnecting the facial, cranial and cerebral areas, perfused by both carotid arteries form the anatomical basis for this shunting.²⁵ A comparable method was applied in mice by Kumar and Lindner,³⁰⁰ resulting in a complete cessation of blood flow in the ligated common carotid artery and a nearly doubling in blood flow in the contralateral carotid artery. A variant of this method is ligation of the external carotid artery resulting in an approximately 90% reduction in carotid artery blood flow.⁴⁶³ Analogous to the unilateral carotid artery ligation, this surgical approach can be applied to side branches of the superior mesenteric artery, resulting in decreased blood flow in ligated mesenteric arteries and shunting of blood flow through collaterals resulting in flow-loaded mesenteric arteries.^{71,326,327,434,435,554,555}

The Renin-Angiotensin System

Physiological Function and Mediators

The renin-angiotensin system (RAS) has traditionally been viewed as an endocrine and circulating system that is important in the short-term homeostasis of cardiovascular and renal function. It is nowadays well accepted that in addition a local RAS operates in many tissues and blood vessels that regulate local vascular resistance and tissue perfusion.¹³¹ A decrease in blood pressure leads to sympathetic activation and in renal vasoconstriction and hypoperfusion. This stimulates the release of renin from the renal zonae glomerulosae into the circulation, where it catalyses the transformation of angiotensinogen into the decapeptide angiotensin I. The key enzyme of the RAS is angiotensin I-converting enzyme (ACE), a zinc-containing carboxy-di-peptidase that cleaves the two carboxyterminal aminoacids from angiotensin I to form the potent vasoconstrictor octapeptide angiotensin II (Ang II),¹³⁹ the main biological mediator of the RAS. (Figure 1.7). ACE is found mainly in the vascular endothelium, where it is bound to the membrane by a hydrophobic membrane-spanning region near the carboxy terminal²³⁵ and is therefore also referred to as tissue ACE. Protease activity releases ACE into the blood, generating an enzymatically active but truncated plasmatic isoform. Recently, another ACE-related carboxypeptidase and ACE homologue has been reported (ACE2), which like ACE is membrane-bound and predominantly expressed on the endothelium and restricted in humans to heart, kidney and testis.¹²⁹ It cleaves Ang I into Ang 1-9, which can be further broken down to Ang 1-7, which has opposing effects compared to Ang II, such as diuresis and vasodilatation.¹⁵⁰ On the other hand, Ang II has numerous, acute and chronic actions, including modulation of vasomotor tone,⁸ cell growth,⁴¹ apoptosis,⁴²⁸ cell migration,³³⁰ extracellular matrix deposition,²⁴¹ inflammation,¹⁰⁸ and stimulation of the production of other vasoconstrictor- and growth factors.^{82,159}

Figure 1.7. Scheme of the renin-angiotensin system. Renin cleaves angiotensinogen to form the decapeptide angiotensin I (Ang I). Ang I is converted by angiotensin I-converting enzyme (ACE) in the lung, tissue and blood vessels to active angiotensin II (Ang II), which can further be metabolized into smaller peptides by ACE, ACE2, and other aminopeptidases, such as chymase, cathepsin G, or carboxypeptidase. Ang II can bind to its specific receptors to induce vasoconstriction and growth (via AT₁), or to cause vasodilatation (via AT₂).

Ang II plays important roles in the functional and structural integrity of the arterial wall and in the pathogenesis of cardiovascular diseases.^{104,478} In the vasculature, Ang II mediates its effects by binding to specific angiotensin type 1 (AT₁) and type 2 (AT₂) cell membrane receptors. AT₁ receptors have been shown to mediate most of the Ang II-induced vascular functions.⁴⁶⁵ In the vasculature, AT₁ receptors are present at high levels in the media and adventitia, but not on the endothelium.^{9,604} Most adult arteries express predominantly AT₁ receptors, and the AT₂ subtype is expressed relatively weakly in large arteries such as the aorta, while a high level of expression is often observed in resistance arteries.^{509,573} The AT₂ receptor opposes some of the actions mediated by the AT₁ receptor. AT₂-deficient mice are hypertensive and have an enhanced pressor action of Ang II,^{214,252} suggesting that the AT₂ receptor plays a role in blood pressure regulation. Ang II has been demonstrated to release NO via an endothelium-dependent AT₂ receptor activation,⁴⁷⁶ that is coupled to the kinin-NO-cGMP pathway.^{189,263,493} The AT₂ receptor is also involved in flow-induced dilatation of mesenteric resistance arteries.³⁵³ This AT₂ receptor-dependent vasodilatation is mediated by local production of bradykinin in these arteries.²⁸³ These studies indicate that the AT₂ receptor mediates vasodilatation in resistance arteries and hereby decrease peripheral resistance and lowering mean arterial pressure.

Renin-Angiotensin System and Arterial Remodeling

Chronic hypertension is associated with VSMC hypertrophy and increased media deposition of collagen leading to encroachment of the lumen diameter and an increase in vascular resistance (reviewed in Mulvany).³⁷⁹ Pulse pressure, but not the increase in mean arterial pressure is the trigger for the increase in wall thickness, since coarctation of the thoracic aorta in monkeys³³³ and dogs²³¹ reduced pulse pressure without affecting mean blood pressure, yet still decreased vascular hypertrophy. The importance of the RAS in vascular remodeling processes came from pharmacological intervention studies using ACE inhibitors (captopril) to prevent the development of hypertension in spontaneously hypertensive rats.¹⁸³ ACE inhibitors reverse cardiovascular remodeling through reduction of blood pressure, which is a non-specific effect, and through a direct, specific arterial effect (inhibition of VSMC hyperplasia/hypertrophy) that is unrelated to its hypotensive action. Other antihypertensive drugs, like propranolol, have been shown to lower blood pressure without affecting medial hypertrophy, suggesting that smooth muscle hypertrophy is not simply a response to increased blood pressure, but that other factors, such as angiotensin II, may be important in modulating medial hypertrophy.^{410,481} Experimental and clinical studies with ACE inhibitors and AT₁ receptor antagonists consistently documented beneficial effects of these agents in treating and preventing cardiovascular diseases such as hypertension, atherosclerosis and restenosis.^{36,423,443,444,481}

Although vasoconstriction is the primary function of VSMCs, during development and vascular remodeling their phenotype changes from a contractile to a synthetic phenotype.^{66,91,284,411} *In vitro* studies have documented hypertrophic effects of Ang II on VSMCs.^{41,180,480} Ang II, acting via AT₁ and AT₂ receptors,⁵⁹⁹ plays an important role in the hypertrophic and/or hyperplastic growth of VSMCs during increased workload of the arterial wall occurring during hypertension and during the inflammatory responses involved in atherosclerosis.^{20-22,543} Infusion of Ang II in the rat resulted in a significant DNA synthesis in VSMCs of the aorta and carotid artery, which was suppressed by co-infusion with the α_1 -adrenergic receptor blocker prazosin.⁵⁶⁶ Additionally, infusion of Ang II increased media thickness in densely innervated mesenteric arteries, whereas no change was observed in the sparsely innervated superior epigastric artery, indicating that perivascular nerves are involved in the hypertrophic growth stimulus.⁵¹³ The hypertrophic effects of Ang II were independent of its blood lowering effect⁵²¹ and its increased vasoconstrictor activity.⁵⁵ Infusion of non-hypertensive doses of Ang II in apolipoprotein E-deficient mice lead to serious abdominal aortic aneurysms and adventitial remodeling,¹⁰⁸ that was inhibited by AT₁ blockade and augmented by AT₂ antagonism.¹⁰⁹

The progression of neointimal thickening induced by Ang II was mediated via AT₁ receptors, in balloon-injured rat carotid arteries.⁵⁶⁵ AT₂ receptors may antagonize the AT₁-mediated effects by inhibiting cell growth and by inducing apoptosis.^{125,171} However, conflicting findings are observed in AT₂ receptor knockout mice, showing that AT₂ receptors mediated the Ang II-induced cardiovascular growth.^{253,489}

Balloon injury of rat aortas resulted in an increased vascular ACE expression in the proliferating neointima.^{152,443,444} The carotid artery revealed an early induction of ACE at 2 days following balloon injury.¹⁴⁹ Glucocorticoids²⁶ and fibroblast growth factor (FGF)¹⁵² are known to stimulate ACE expression as well. Transfection of noninjured porcine iliac arteries with recombinant FGF-1 increased ACE expression in neointimal VSMC to the

same extent as injured, nontransfected arteries.¹⁵² Interestingly, greater ACE expression was observed close to the lumen which gradually decreased to the media,¹⁵² suggesting that shear stress and FGF could be involved in the regulation of ACE expression in the neointima. Laminar shear stress increased ACE activity and mRNA levels of both ACE and bFGF in cultured ECs, VSMCs and fibroblasts.¹⁹² Taken together, these data imply that FGF induces ACE, generating Ang II to stimulate VSMC growth in response to shear stress and arterial injury.

The Kallikrein-Kinin System

Physiological Function and Mediators

Kallikreins release kinins from high and low molecular weight kininogens (Figure 1.8).⁸⁰ Similarly to ACE, plasma and tissue isoforms exist for kallikreins. Human tissue kallikrein cleaves low molecular weight kininogen to release Lys-bradykinin (kallidin), whereas plasma kallikrein releases bradykinin (BK).³⁴⁶ Both peptides are equipotent. Kinins such as BK, kallidin and Met-Lys-BK act mainly as local hormonal oligopeptides by autocrine-paracrine mechanisms. Kinins play a role in several (patho)biological processes including renal blood flow regulation,⁵¹⁴ inflammation,³⁴⁴ and endotoxic shock.⁵⁹⁰ In cell culture experiments it was shown that kinins can induce the release of endothelial NO and prostacyclin, both of which have antiproliferative and -antimigratory effects on VSMCs.⁵³⁴ Furthermore, kinins can induce the release of interleukins and tumor necrosis factor from macrophages, followed by inducible NOS expression in VSMCs.^{30,273} Kininases degrade the kinins into (in)active fragments. They are found in the blood, endothelial cells and other tissues.⁷⁶ ACE (kininase II) removes the C-terminal Phe-Arg from bradykinin and kallidin, linking the renin-angiotensin system with the kallikrein-kinin system.⁵⁸⁷

Based on their pharmacological responses to various BK analogues, two kinin receptors, B₁ and B₂, have been characterized.⁴⁴⁵ The B₂ receptors mediate most of the physiological effects of BK and kallidin. B₁ receptors are induced by certain pathological conditions.³⁴⁵ Another coupling of the renin-angiotensin system with the kallikrein kinin system is via AT₂-mediated vasodilator responses to Ang II. Several studies suggest that the AT₂ receptor is coupled to the kinin-NO-cyclic GMP pathway.^{189,263,493,551} Also shear stress can stimulate AT₂ receptors and vasodilatation through local production of BK in rat mesenteric resistance arteries²⁸³ and mouse carotid arteries.^{37,359} This mechanism probably counterbalances the AT₁-induced vasoconstriction *in vivo*. Tissue kallikrein localized on ECs likely releases BK in response to shear stress, but the mechanism by which the activity of tissue kallikrein is regulated remains poorly defined. Tsutsumi and colleagues demonstrated that AT₂ receptor activation causes cellular acidification through inhibition of amiloride-sensitive Na⁺/H⁺ exchanger activity that is supposed to activate tissue kallikrein.⁵⁵¹

Figure 1.8. Scheme of the kallikrein-kinin system. Kinins are released from big precursor molecules, the kininogens. Low molecular weight (LMW) kininogen is cleaved by tissue kallikrein to release Lys-bradykinin (kallidin), which can be cleaved into bradykinin (BK). High molecular weight (HMW) kininogen is cleaved by plasma kallikrein to release BK, that binds to specific receptors (B₂R) to cause vasodilatation. Kallidin and BK can be further metabolized by specific kininases (see also text).

Kallikrein-Kinin System and Arterial Remodeling

Potentiation of the endogenous kinins via ACE inhibition has antihypertensive actions. This was demonstrated in a number of different rat models of hypertension after administration of ACE inhibitors such as enalaprilat, ramipril and captopril. Blockade of the B₂ receptors has been shown to attenuate the blood-lowering effect of ACE inhibitors in these studies.^{18,35,72,105} In rabbits on a long-term atherogenic diet, ACE inhibitor treatment protected against the progressive endothelial dysfunction observed in atherosclerosis.^{31,452}

The protective effects do not firmly discriminate between reduced Ang II production, increased levels of BK or increased NO and prostacyclin production by ACE inhibition. In apolipoprotein E-deficient mice the antiatherogenic effects of ACE inhibition were independent of its blood lowering and BK relating effects,²⁸⁵ suggesting that the disadvantageous effects are mediated by Ang II. Kinins have been documented to mediate the antiproliferative actions of ACE inhibition after rat carotid artery endothelial denudation,¹⁴⁸ an effect which was NO-mediated and B₂ receptor-dependent.¹⁴⁹ Other studies have shown absence of antiproliferative actions of ACE inhibition on VSMCs, whereas AT₁-blockade did. Prescott and colleagues demonstrated that in the carotid artery

balloon injury model, ACE inhibition reduced neointima formation by inhibiting SMC migration alone without affecting SMC proliferation.⁴³⁸ They found a more pronounced reduction in lesion size after AT₁ antagonism, however, when both SMC migration and proliferation were inhibited. Thus, combined ACE inhibition and AT₁-blockade reduced neointima formation, which further demonstrates the importance of Ang II in VSMC migration and proliferation after arterial injury.⁴³⁸

In rabbits, the AT₁ receptor, and not the AT₂ receptor, was implicated in neointimal hyperplasia after carotid artery balloon injury.²⁶⁵ In another model of neointima formation, ACE inhibition markedly reduced the neointimal formation after blood flow cessation in mouse carotid arteries.¹³⁸ Furthermore, gene transfer of human tissue kallikrein, the kinin-forming enzyme in the endothelium, inhibited neointima formation in the same model, which was mediated via B₂ receptor signaling.¹³⁷ These results demonstrate a role for kinins in suppressing VSMC growth and migration, but again do not discriminate evidently between the beneficial actions of kinins and the proliferating actions of Ang II in neointima formation. Perhaps receptor heterodimerization of AT₁ and B₂ receptors occurs, just as has been shown in preeclampsia.⁵

Relations Between Acute and Chronic Arterial Responses to Mechanical Stimuli

Although mechanical stimuli such as CWS and WSS can modulate vasomotor tone and arterial structure, the relation between the acute functional and chronic structural responses remains unclear. Several possibilities have been considered: (1) the functional response (*e.g.* more or less constriction) might give rise to the functional response (*e.g.* more or less growth and reorganization of the extracellular matrix),^{16,17} (2) the same mediators might participate such as the vasodilator and growth-inhibitory substances NO, prostacyclin and bradykinin or the vasoconstrictor agonist and growth factor angiotensin II and (3) distinct processes might be involved. In the case of the second hypothesis, conditions that characterize cardiovascular disease such as endothelial dysfunction^{135,433,602} and activation of the renin-angiotensin system¹⁷⁹ would entail alterations of both the acute and the chronic compensatory responses to mechanical stimuli. In the case of the third hypothesis, the chronic structural responses would come into play as a secondary back-up protective mechanism when the acute functional responses would fail to normalize the mechanical forces.

Below, components of these working hypotheses are combined with respect to flow-induced vasodilatation and flow-induced outward arterial remodeling (Figure 1.9). It is proposed that an acute moderate rise in blood flow, for instance during physical activity, will lead to an increase in WSS, causing release of endothelium-derived vasodilatory compounds that dilate the vessel. This flow-induced vasodilatation restores the WSS value toward normal (upper part Figure 1.9). However, when the rise in blood flow is large and persistent, flow-induced dilatation can not fully restore WSS. Structural rearrangement of pre-existing wall material leads to a wider diameter and hence a thinner wall. The former will tend normalize WSS, while as a result of the latter CWS will be increased and hypertrophy will be stimulated (lower part, Figure 1.9).

Small BF increases:

Large BF increases:

Figure 1.9. Possible relation between acute flow-induced dilation (FID) and flow-induced outward hypertrophic remodeling. See text for details. WSS: wall shear stress; CWS: circumferential wall stress; Wt: wall thickness; Ø: structural diameter; →: consequence; thick arrow: structural response; ↑: increase; ↓: decrease; ⊥: negative feedback regulation; dotted line: insufficient action.

Novel Experimental Animal Models

Traditionally surgical and pharmacological intervention studies have been applied as a model for (cardio)vascular remodeling. The use of drugs as a tool for chronic inhibition of an enzyme or a receptor system is limited to (1) the antagonists specificity and affinity for the enzyme, (2) the metabolic degradation rate, (3) transport to the site of action. Nowadays, a growing number of transgenic mouse models are being used for disease research and drug development. Transgenic animals are animals in which foreign genes have been experimentally introduced or endogenous DNA deleted from. Transgenic mice can be defined as a gain of function or loss of function. Gain of function is associated with an overexpression of randomly integrated transgene fragments (foreign DNA) into fertilized eggs of mice. Targeting the deletion or mutation of a specific gene using embryonic stem cell technology creates loss of function or knock-out models. Both of these two groups of transgenic mice offer a myriad of models to study individual genes and gene products in the process of, for instance, development and disease pathogenesis. Many transgenic mouse models mimic the phenotypes of the human diseases. Transgenic models have the main advantage that the effect of one specific gene or gene product can be studied, although many genes have pleiotropic roles. This problem can be overcome by cell type-selective and conditional transgenic technology. The potential disadvantages of transgenic animals are outweighed by the unique opportunity for developing and testing

different therapeutic strategies before they are introduced in the clinic. Many transgenic mice have been used in the field of vascular biology to study the mediators involved in neointima formation, smooth muscle cell migration, matrix degradation, atherosclerotic plaque, etcetera. However, few transgenic mice models were used to study flow-related arterial remodeling. Mice lacking one cytoskeletal protein (such as desmin or vimentin) show blunted acute flow-induced dilatation of mesenteric resistance arteries.^{220,327} Despite this acute arterial dysfunction, longterm arterial structural adaptations to blood flow were unaltered in these knock-out mice compared to wild-type mice.^{327,479} In this thesis two strains of genetically-altered mice were used: tissue kallikrein-deficient (TK^{-/-}) mice and tissue angiotensin I-converting enzyme-deficient (t-ACE^{-/-}) mice. The lack of tissue kallikrein has been shown to abolish the kinin-forming capacity in most tissues and to induce cardiac and vascular abnormalities, without changing blood pressure.³⁵⁹ Flow-induced vasodilatation in response to shear stress of carotid arteries was blunted in these TK^{-/-} mice.^{37,359} Some phenotypical characteristics of t-ACE^{-/-} mice are reduced (15-20 mmHg less than normal) mean arterial blood pressure, reduced male fertility, and renal vascular thickening.¹⁴¹ In the future, many more genetically engineered animal models will be valuable for gaining important insights into the molecular mechanisms underlying development, disease pathogenesis, prevention and treatment.

Aim and Outline of the Thesis

The present thesis aims to determine the roles of vasoactive peptides/growth factors in flow-induced arterial dilatation and flow-related arterial remodeling. Two interdigitating systems, the renin-angiotensin system and the kallikrein kinin system, which are regarded as opposing systems in blood pressure control, were evaluated to elucidate the contribution of these systems and their main players in acute and chronic responses of both small and large arteries to altered blood flow (Figure 1.10).

Two models of flow-related arterial remodeling were studied. The first model is murine pregnancy, which like all mammalian species, represents a physiological model of increased uterine blood flow to the growing fetus(es), resulting in considerable arteriogenesis of the afferent uterine vascular bed. The second model is unilateral carotid artery ligation of the mouse, which represents an experimentally-imposed model of a chronic cessation of blood flow in the ligated carotid artery and a doubling of blood flow in the contralateral carotid artery. In order to investigate arterial function (vasomotor responses to mechanical stimuli, vasoconstrictors and vasodilators) and remodeling (diameter and wall mass changes), reactivity studies and histological techniques were performed in isolated, pressurized uterine and carotid arteries.

Tissue kallikrein-deficient mice were studied in Chapters 2 to 4 to analyze the effect of an absence of membrane-bound kallikrein (and reduced bradykinin synthesis) on acute vasomotor responses and chronic structural responses of carotid arteries (Chapter 2 and 3) and uterine arteries (Chapter 4) to flow. The renin-angiotensin system, and especially tissue angiotensin-I-converting enzyme, was the focus of Chapters 6 and 7. Arterial structure and elasticity of large conduit arteries and peripheral arteries were analyzed in tissue angiotensin I-converting enzyme deficient mice (chapter 6). Agonist- and flow-induced dilatation were investigated in isolated uterine arteries of nonpregnant, late

pregnant and 7-days postpartum mice (Chapter 5). The effects of chronic exposure to altered blood flow, applying the two models of flow-related arterial remodeling, were determined in these mice (Chapter 7). The consequences of aging on acute and chronic uterine artery responses to flow were investigated in one-year old wild-type nonpregnant and late pregnant mice in comparison to young (3-months) mice (Chapter 8). A review of flow-related arterial remodeling and its relationship to flow-induced vasodilatation is presented in Chapter 9. Finally, a general discussion of this thesis is outlined in Chapter 10.

Figure 1.10. Interplay between the kallikrein-kinin system (left) and the renin-angiotensin system (right). (Tissue) angiotensin-converting enzyme (ACE) links both systems in that it inactivates bradykinin and activates angiotensin II.

Chapter 2

Flow-Dependent Dilatation Mediated by Endogenous Kinins Requires Angiotensin AT₂ Receptors

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Abstract

Objective. The vascular kallikrein-kinin system contributes to flow-induced dilatation by activating bradykinin B₂ receptors coupled to endothelial nitric oxide (NO) release. Because the bradykinin/NO pathway may mediate some of the effects of angiotensin II AT₂ receptors, we examined the possible contribution of AT₂ receptors to the kinin-dependent response to flow.

Methods and Results. Changes in outer diameter following increases in flow rate were evaluated in perfused arteries from wild-type (TK^{+/+}) and tissue kallikrein deficient (TK^{-/-}) mice in which the presence of AT₂ receptor expression was verified. Saralasin, a non-selective angiotensin II receptor antagonist, significantly impaired flow-induced dilatation in TK^{+/+}, whereas it had no effect in TK^{-/-} mice. In both groups, blockade of AT₁ receptors with losartan or candesartan did not affect the response to flow. Inhibition of AT₂ receptors with PD123,319 significantly reduced flow-induced dilatation in TK^{+/+} mice. Combining PD123,319 with the bradykinin B₂ receptor antagonist HOE-140 had no additional effect to AT₂ receptor blockade alone in TK^{+/+} arteries. Flow-dependent dilatation was also impaired in AT₂ receptor deficient mice (AT₂^{-/-}) when compared to AT₂^{+/+} littermates. Furthermore, HOE-140 significantly reduced the response to flow in the AT₂^{+/+}, but not in AT₂^{-/-} mice.

Conclusion. This study demonstrates that functional AT₂ receptors play a key role in flow-induced dilatation mediated by the vascular kallikrein-kinin system.

Introduction

Kallikreins are a distinct group of serine proteases, which are capable of generating vasoactive kinins from kininogen *in vitro*. Vascular tissue contain kininogen and kallikrein mRNA suggesting the existence of an endogenous kallikrein-kinin system (KKS). Tissue KKS is present in both endothelial and smooth muscle cells of various vascular territories and is different from the circulating plasma KKS.^{397,413,483,596} Indeed, tissue kallikrein (TK) is encoded by the kallikrein *k1k1* gene, and experimental evidence suggests that endogenously formed kinins could participate in the regulation of vascular tone.^{176,365,366,469,587} Recently we reported that tissue kallikrein plays an essential role in kinin synthesis within the vascular wall of murine carotid arteries.³⁷ In addition, this tissue KKS contributes to the flow-induced dilatation through activation of bradykinin B₂ receptors leading to NO release in these arteries.³⁷ Flow-induced dilatation enables arteries to increase their diameter in response to elevations in blood flow following the release of endothelial-derived factors such as NO or prostacyclin to ensure appropriate organ perfusion.^{110,269,427,461} Flow-induced dilatation is a complex response involving not only local mediators (such as kinins), but also mechanical activation of endothelial cells. Other peptide systems such as angiotensin II (Ang II) can also contribute to flow-induced dilatation by activating AT₂ receptors, as demonstrated in resistance arteries.³⁵³

Interestingly, several studies suggested a possible link between Ang II receptors and bradykinin B₂ receptors regarding NO production.^{189,219,323,493,504,530,588} In vascular endothelial cells, the production of NO by Ang II is caused by activation of the angiotensin type 2 (AT₂) receptor.⁵⁸⁸ In addition, AT₂ receptor stimulation by exogenous Ang II leads to an increase in cyclic GMP, through a mechanism involving bradykinin B₂ receptors and NO release.^{189,219,283,493,504,530,563} Furthermore, overexpression of AT₂ receptors increases bradykinin production presumably by activating kininogenase(s).⁵⁵¹ However, there is no information regarding a possible relation between AT₂ and bradykinin B₂ receptor pathways during flow-induced dilatation. Therefore, the purpose of the present study was to determine whether AT₂ receptors contribute to flow-induced dilatation mediated by endogenously formed kinins using isolated carotid arteries from TK^{+/+} and TK^{-/-} mice.

Methods

Animals

Twelve-week old male littermate wild-type (TK^{+/+}) and tissue kallikrein knockout (TK^{-/-}) mice were used as previously described.^{37,359} Twelve-week old male AT₂ receptor deficient mice (AT₂^{-/-}) and their age-matched AT₂^{+/+} littermates were generated as described.^{252,489} They were backcrossed ten times in the C57Bl/6J genetic background.

RNA Extraction and RT-PCR Analysis

For each group of mice, carotid arteries from ten mice were pooled and total RNA was extracted according to the Trizol reagent protocol (Life Technologies, Cergy Pontoise, France). The quality of the RNA preparation was confirmed by ethidium bromide staining.

Single-strand cDNA synthesis was carried out in 20 μ l of reaction buffer, consisting of first-strand buffer 5X (GibcoBRL, Cergy Pontoise, France), RNase inhibitor (40 UI/ μ L), di-nucleotidetriphosphate (25 mmol/L), DTT (100 mmol/L, Amersham, Orsay, France), and reverse MMLV (200 UI/ μ L). The reverse transcriptase reaction was performed by incubating the reaction mixture for 90 min at 37°C followed by 10 min at 65°C, using a 3'-oligo-(5'-GGTTTTCCAAGGAAGGGGTATGAG). The polymerase chain reaction (PCR) included three steps: denaturation (94°C, 45 s), annealing (65°C, 45 s), and extension (72°C, 105 s), for 35 cycles using the above mentioned 3'-oligo and the 5'-oligo (5'-CATGCTTTTGTCTGGGCTTCGTC). These primers were taken from the unique exon from position 2,176 to 2,749 of the angiotensin AT₂ receptor gene, localized in chromosome X, thus producing a 0.573 kb fragment of the AT₂ receptor cDNA.³⁸⁸ The cDNA was amplified using 5 UI/ μ L of *Taq* DNA polymerase (Life Technologies, Cergy Pontoise, France) and 20 μ mol/L of each set of primers in 50 μ l buffer 10X (22 mmol/L Tris-HCl; pH 8.4, 55 mmol/L KCl, 1.65 mmol/L MgCl₂, 25 mmol/L di-nucleotidetriphosphate, and 10 μ L of loading dye (0.02 % red cresol and 60% sucrose). The PCR products were sequenced and corresponded to the mouse AT₂ receptor sequence.

In addition, expression of GAPDH was evaluated in parallel to that of the AT₂ receptor. Briefly, total RNA was reverse transcribed as mentioned above using the 3'-oligo-(5'-CATGTAGGCCATGAGGTCCACCAC). Then, the RT product was amplified by 35 cycles as described above, using the 3'-primer and the 5'-oligo (5'-TGAAGGTCGGTGTGAACGGATTGGC). The migration of both PCR products was then followed by electrophoresis on a 2% agarose gel containing ethidium bromide.

In Vitro Measurement of Arterial Diameter

Carotid arteries were carefully exposed and quickly excised. The procedure was in accordance with the European Community guidelines on the care and use of laboratory animals (Ministère de l'Agriculture, France, authorization 07430). Carotid arteries were cannulated at both ends and then perfused continuously *in vitro* in a video-monitored arteriograph set-up (Living Systems Instrumentation, Burlington, VT, USA), where flow and pressure can be modified independently, as previously described.³⁷ Briefly, arteries were bathed in a physiological salt solution warmed (37°C) and gassed (95% O₂/5% CO₂) throughout the experiments. Intraluminal pressure was monitored by a servoperfusion system. The outer diameter as well as proximal and distal intraluminal pressures were continuously recorded. The integrity of the endothelium was assessed by extraluminal administration of acetylcholine (1 μ mol/L) during phenylephrine-induced (1 μ mol/L) constriction. Arteries were excluded when vasodilatation by acetylcholine was <60% of that induced by Na-nitroprusside (0.1 mmol/L). At the end of each experiment, passive diameter was obtained following incubation of the artery (40 min) with a Ca²⁺-free physiological salt solution and Na-nitroprusside (0.1 mmol/L), in the absence of smooth muscle tone. Phenylephrine and all antagonists used were administered both intra- and extraluminally.

In Vitro Protocols

All experiments evaluating the response to increases in intraluminal flow were performed in the presence of phenylephrine (1 $\mu\text{mol/L}$). When a stable constriction was reached, intraluminal flow was applied in a stepwise manner (from 10 to 800 $\mu\text{L/min}$). Each flow was applied for 3-9 min until a stable diameter was reached, and then augmented to the next level. Experiments were performed in the presence of either, saralasin (a nonspecific AT₁ and AT₂ blocker; 1 $\mu\text{mol/L}$), losartan (an AT₁ blocker; 0.1 $\mu\text{mol/L}$), candesartan (an AT₁ blocker; 10 nmol/L), PD123,319 (an AT₂ blocker; 1 $\mu\text{mol/L}$), or HOE-140 (a bradykinin B₂ receptor blocker; 1 $\mu\text{mol/L}$). Tissues were preincubated for 40 min with each blocker.

Some experiments were performed on isolated mesenteric arteries, which were mounted as described above. Mesenteric artery basal diameters in AT₂^{+/+} and AT₂^{-/-} mice were $177 \pm 12 \mu\text{m}$ ($n=7$) and $181 \pm 20 \mu\text{m}$ ($n=6$), respectively. The arteries were perfused (40 $\mu\text{L/min}$) at an intraluminal pressure of 80 mmHg, and dilatation to cumulative concentrations of extraluminally administered bradykinin (0.1 nmol/L - 10 $\mu\text{mol/L}$) was recorded during preconstriction with the thromboxane analogue U46619 (1-10 nmol/L).

Drugs

The compounds used for *in vitro* studies included acetylcholine chloride, bradykinin diacetate salt, L-phenylephrine hydrochloride, U46619 (Calbiochem, France), PD123,319, saralasin, and losartan (from Sigma, St-Quentin-Fallavier, France). HOE-140 was kindly provided by Drs. H.J. Lang and B.A. Schölkens (Hoechst-Marion-Roussel, Frankfurt, Germany). Candesartan was kindly provided by ASTRA-ZENECA.

Data Analysis and Statistics

Data are given as changes in diameter (microns) from the arterial diameter obtained during constriction with phenylephrine. Results are expressed as means $\pm$ SEM of n experiments; n represents the number of animals used for each experimental protocol. pD₂ values represent the negative logarithm of the concentration of agonist causing 50% of its maximal response. Statistical evaluation was performed by using ANOVA for factorial or repeated measurements, followed by Scheffe t -test.⁵⁷⁵ Values of $P < 0.05$ were considered to be statistically significant.

Results

RT-PCR revealed the expression of AT₂ receptors in carotid arteries of TK^{+/+} and TK^{-/-} mice (Figure 2.1). In perfused carotid arteries of TK^{+/+} and TK^{-/-} mice, administration of phenylephrine, acetylcholine or Na-nitroprusside caused similar changes in outer diameter for each set of experiments (Table 2.1).

Figure 2.1. Expression of the angiotensin II AT₂ receptor gene in carotid arteries of TK^{+/+} and TK^{-/-} mice. This typical RT-PCR experiment is a representative of a total of two different experiments obtained on different pools of 10 carotid arteries. Expression of GAPDH was observed in the same samples in parallel experiments.

Step increases in intraluminal flow augmented the carotid artery diameter in both TK^{+/+} and TK^{-/-} mice (Figure 2.2). At high flow rates flow-induced dilatation (FID) was significantly lower in arteries from TK^{-/-} mice compared to TK^{+/+} as previously observed.³⁷

Table 2.1. Changes in outer diameter of carotid arteries from TK^{+/+} and TK^{-/-} mice, in response to phenylephrine, acetylcholine, and Na-nitroprusside in the absence and presence of antagonists.

Genotype	Agonist	Control	Losartan	Losartan + PD123,319	Saralasin	PD123,319	PD123,319 + HOE 140
TK ^{+/+}	Phe	-58±5	-56±11	-74±8	-65±14	-67±14	-70±14
	ACh	94±5	91±4	93±6	100±2	99±3	99±3
	SNP	83±12	80±16	93±11	91±2	77±15	83±15
TK ^{-/-}	Phe	-58±8	-71±7	-72±9	-63±12	-68±9	ND
	ACh	94±4	85±5	84±4	101±1	95±4	ND
	SNP	72±10	79±8	78±9	74±14	92±9	ND

Abbreviations used: Phe (phenylephrine); ACh (acetylcholine); SNP (Na-nitroprusside); ND (not determined). The constrictions to Phe and the dilatations to SNP are given as changes in μm from the basal diameter (positive and negative numbers indicate a gain and loss in diameter, respectively). Dilatations to ACh are expressed as percent inhibition of the constriction evoked by Phe. Statistical comparison between treatments was performed using ANOVA.

In TK^{+/+} mice, FID (between 200 and 800 $\mu\text{L}/\text{min}$) was significantly impaired by the nonspecific angiotensin receptor antagonist saralasin (Figure 2.2, *left*). Conversely, saralasin did not significantly modify FID of TK^{-/-} carotid arteries (Figure 2.2, *right*). We next investigated the effect of preferential AT₁ and AT₂ receptor antagonists on FID. The AT₁ receptor blocker losartan did not affect FID in both TK^{+/+} and TK^{-/-} arteries (Figure 2.3). Similarly, candesartan had no effect on FID (data not shown).

Figure 2.2. Changes in diameter (μm) following step-increases in intraluminal flow in carotid arteries from TK^{+/+} (*left*, closed symbols; $n=7$) and TK^{-/-} (*right*, open symbols; $n=6$) mice. Experiments were performed under control condition (circles) or in the presence of saralasin (squares). * $P < 0.05$ versus control condition.

Interestingly, the AT₂ receptor blocker PD123,319 significantly blunted the response to flow at high flow rates in TK^{+/+} arteries exposed to losartan (Figure 2.3, *left*). However, PD123,319 had no significant effect on FID in TK^{-/-} arteries exposed to losartan (Figure 2.3, *right*). In TK^{+/+} arteries, blockade of AT₂ receptors with PD123,319 alone significantly reduced FID (from 200 to 800 $\mu\text{L}/\text{min}$, Figure 2.4). Interestingly, the magnitude of dilatation of TK^{+/+} arteries exposed to PD123,319 was comparable to that of TK^{-/-} arteries in the absence of any antagonists (Figure 2.4). Furthermore, PD123,319 had no significant effect on FID of TK^{-/-} arteries (Figure 2.4).

The bradykinin B₂ receptor antagonist HOE-140 also decreased FID in TK^{+/+} arteries confirming previous observations.³⁷ A combination of both PD123,319 and HOE-140 significantly altered FID compared to control conditions (Figure 2.4). However, the impaired FID in the presence of both antagonists was not different from the FID observed in the presence of PD123,319 alone.

FID was also significantly reduced in carotid arteries of AT₂^{-/-} mice when compared to their wild-type littermates (Figure 2.5). In addition, HOE-140 significantly blunted FID in AT₂^{+/+}, but had no significant effect in AT₂^{-/-} mice (Figure 2.5). Dilatation to exogenously added bradykinin was investigated in perfused (40 $\mu\text{L}/\text{min}$) mesenteric resistance arteries ($n=6$) of AT₂^{+/+} and AT₂^{-/-} mice. There was no significant difference in pD₂ values (7.89 ± 0.31 versus 8.18 ± 0.33) and the maximal responses ($52 \pm 12\%$ and $47 \pm 7\%$) to bradykinin between AT₂^{+/+} and AT₂^{-/-} mesenteric arteries, respectively.

Figure 2.3. Changes in diameter (μm) following step-increases in intraluminal flow in carotid arteries from $\text{TK}^{+/+}$ (left; closed symbols; $n=6$) and $\text{TK}^{-/-}$ (right; open symbols; $n=6$) mice. Experiments were performed under control condition (circles), in the presence of losartan (squares), or in the presence of both losartan and PD123,319 (triangles). Control experiments and experiments with losartan were performed on carotid arteries from the same mice, while experiments with both losartan and PD123,319 were performed on carotid arteries of a different set of animals. * $P < 0.05$, pD2 values (7.89 ± 0.31 versus 8.18 ± 0.33) and the maximal responses ($52 \pm 12\%$ and $47 \pm 7\%$) to bradykinin between $\text{AT}_2^{+/+}$ and $\text{AT}_2^{-/-}$ mesenteric arteries, respectively.

Figure 2.4. Changes in diameter (μm) following step-increases in intraluminal flow in carotid arteries from $\text{TK}^{+/+}$ (left; closed symbols; $n=6$) and $\text{TK}^{-/-}$ (right; open symbols; $n=6$) mice. Experiments were performed under control condition (circles), in the presence of PD123,319 (squares), or in the presence of both PD123,319 and HOE-140 (triangles). Control experiments and experiments with PD123,319 were performed on carotid arteries from the same mice, while experiments with both PD123,319 and HOE-140 were performed on carotid arteries of a different set of animals. * $P < 0.05$.

Figure 2.5. Changes in diameter (μm) following step-increases in intraluminal flow in carotid arteries from AT₂^{+/+} (closed symbols; $n=7$) and AT₂^{-/-} (open symbols; $n=6$) mice. Experiments were performed under control condition (circles) or in the presence of HOE-140 (squares) in carotid arteries from the same mouse. * $P < 0.05$.

Discussion

Tissue kallikrein activation contributes to flow-induced dilatation by activating bradykinin B₂ receptors coupled to endothelial NO release.^{37,176,197,366,469} In this study we demonstrate that angiotensin AT₂ receptors mediate the tissue kallikrein-dependent dilatation induced by shear stress in murine carotid arteries.

Before investigating the putative contribution of AT₂ receptors in flow-induced dilatation mediated by the endogenous kallikrein-kinin system, we first verified the presence of AT₂ receptors in carotid arteries, because these receptors are either absent or expressed at a low level in blood vessels from adult animals.²⁵¹ RT-PCR experiments demonstrated the presence of AT₂ receptor mRNA in carotid arteries from wild-type and mice lacking tissue kallikrein (TK^{-/-}). In perfused carotid arteries we observed that flow-induced dilatation was significantly smaller in TK^{-/-} compared to TK^{+/+} mice, confirming our previous study.³⁷ The first hint regarding the involvement of angiotensin receptors in flow-induced dilatation mediated by endogenous kinins came from experiments with saralasin, a nonspecific AT₁ and AT₂ receptor antagonist. Saralasin impaired the flow-induced dilatation in TK^{+/+} arteries, but not in TK^{-/-} arteries, which are unable to generate endogenous kinins.^{37,359}

To further determine which of the angiotensin receptor is involved in flow-induced dilatation coupled to the vascular kallikrein-kinin system, we studied the effect of flow-induced dilatation of selective antagonists to either AT₁ or AT₂ receptors. Neither losartan nor candesartan modified flow-induced dilatation in both TK^{+/+} and TK^{-/-} mice, demonstrating that AT₁ receptors do not contribute to the kinin-mediated dilatation induced by shear stress. This interpretation is in agreement with other studies showing absence of AT₁ receptor antagonism in flow-induced dilatation,³⁵³ but it may contrast with previous studies showing that angiotensin II increases cyclic GMP levels by

activating an endothelial AT₁ receptor.^{57,77} The apparent discrepancy might result from the absence of shear stress and the use of high concentrations of exogenous angiotensin II in these studies.

Unlike the blockade of the AT₁ receptor alone, exposing arteries to antagonists of both AT receptor subtypes decreased the flow-induced dilatation in TK^{+/+} arteries, thus implying that AT₂, but not AT₁ receptors, contribute to flow-induced dilatation in wild-type arteries. The inhibitory effect of PD123,319 on this response in wild-type arteries reinforce this interpretation. Interestingly, AT₂ receptor antagonism reduced flow-induced dilatation in wild-type mice to reach comparable levels to those observed in TK^{-/-} mice in the absence of any antagonists. The effect of AT₁ and AT₂ receptor antagonists was also examined in TK^{-/-} arteries. Unlike TK^{+/+} arteries, blockade of AT₁ and/or AT₂ receptors did not modify flow-induced dilatation in TK^{-/-} arteries. As TK^{-/-} arteries express both AT₂ and B₂ receptors,^{37, this study} we conclude that the participation of AT₂ receptors to flow-induced dilatation requires the presence of a functional kallikrein-kinin system.

We further confirmed the results obtained with saralasin and PD123,319 in wild-type mice by investigating flow-induced dilatation of carotid arteries obtained from AT₂ receptor deficient mice (AT₂^{-/-}).²⁵² Flow-induced dilatation was blunted in carotid arteries of these mice compared to arteries of wild-type mice. In addition, B₂ receptor antagonism reduced flow-induced dilatation in AT₂^{+/+}, but not in AT₂^{-/-} mice, although deletion of the AT₂ receptor gene did not affect the functional response to B₂ receptor activation with exogenous bradykinin. Taken together, these findings indicate that the participation of bradykinin B₂ receptors in flow-induced dilatation requires the presence of functional AT₂ receptors.

We next studied the possible contribution of bradykinin B₂ receptors to the AT₂-dependent flow-induced dilatation in wild-type mice. Antagonism of the B₂ receptor did not further decrease flow-induced dilatation in wild-type arteries already exposed to AT₂ receptor blockade. Previous results demonstrated that B₂ receptor blockade in these arteries significantly blunted flow-induced dilatation.³⁷ Taken together, these observations suggest that there is no additional inhibitory effect on flow-induced dilatation by AT₂ receptor antagonism when B₂ receptors are blocked or the kallikrein-kinin system is inactivated. Similarly, if AT₂ receptors are blocked or not expressed, B₂ receptor blockade no longer inhibits flow-induced dilatation. Thus, the present data demonstrate that the involvement of AT₂ receptors in flow-induced dilatation requires the presence of both functional bradykinin B₂ receptors and an active kallikrein-kinin system. Furthermore, this study supports the conclusion that the flow-induced dilatation mediated by the local kallikrein-kinin system requires the presence of functional AT₂ receptors. This interpretation is in agreement with previous studies indicating that AT₂-dependent dilatation, following exposure to exogenous angiotensin II, involves bradykinin B₂ receptor activation.^{189,283,493}

Although the exact mechanism linking AT₂ and B₂ receptors in flow-induced dilatation remains to be elucidated, several hypotheses can be stated. The increase in shear stress may favor and augment the boundary layer mass transport of kinins and angiotensin II to their receptors therefore decreasing their degradation rate.^{130,364} However, we cannot exclude the possibility that shear stress might also modulate endogenous peptide synthesis. Indeed, as a short-term increase in shear stress enhanced ACE activity, flow

stimulation may augment the local production of angiotensin II.⁴⁵¹ However, an increase in ACE activity would also contribute to an enhanced degradation of locally formed kinins,²⁹⁴ thus counterbalancing the effect of an augmented angiotensin II synthesis. Interestingly, the lack of an additional effect of AT₂ and B₂ receptor blockade supports the conclusion that these two receptors do not act synergistically. This observation also favors the interpretation that these two receptor pathways lie upstream or downstream from each other. Activation of the AT₂ pathway might precede stimulation of endogenous kinin synthesis and B₂ receptor activation, as indicated in a recent study.²⁸³ This interpretation is also supported by data from this study and previous work³⁷ as the dilatation in response to bradykinin was unchanged following inactivation of either kallikrein or AT₂ receptors. Furthermore, overexpression of AT₂ receptors in vascular smooth muscle cells increases intracellular acidosis, resulting in an enhanced kininogenase activity and subsequent kinin generation.⁵⁵¹ However, activation of kininogenase(s) by lowering intracellular pH, following AT₂ receptor activation, is not fully compatible with the known *in vitro* characteristics of tissue kallikrein activation.⁴⁴¹ Alternatively, the present results may suggest a direct molecular interaction between B₂ and AT₂ receptors, comparable to the one recently proposed for AT₁ and AT₂,⁵ but these different hypotheses would require further investigations.

In conclusion, the present study demonstrates that flow-induced dilatation mediated by the vascular kallikrein-kinin system requires both AT₂ and B₂ receptors. This pathway would contribute to the regulation of vascular tone by offsetting vasoconstrictor and pro-atherogenic effects of angiotensin II under physiological shear stress values in the vessel wall.

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Chapter 3

Role of Tissue Kallikrein During Arterial Remodeling in Response to Altered Blood Flow

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Abstract

Objective. The tissue kallikrein-kinin system (KKS) participates in acute arterial responses to changes in flow. Thus, the aim of this study was to elucidate the potential role of the vascular KKS during arterial remodeling in response to chronic changes in blood flow.

Methods and Results. We ligated the left common carotid artery of mice lacking the tissue kallikrein gene ($TK^{-/-}$) and their wild-type littermates ($TK^{+/+}$). Sham-operated mice were used as controls (normal flow). Pressure-diameter curves were obtained in isolated carotid arteries 4 weeks after surgery. In the ligated group, carotid artery blood flow was significantly higher in the right carotid artery (high flow) than in the left carotid artery (no flow) and in normal flow carotid artery. Normal flow carotid arteries were smaller in $TK^{-/-}$ than in $TK^{+/+}$ mice ($740 \pm 19 \mu m$ versus $791 \pm 21 \mu m$ at 100 mmHg, respectively). In both strains, diameter and structure were similar in normal and high flow carotid artery. Diameters of no flow carotid artery were significantly reduced compared to normal flow carotid artery, being more pronounced in $TK^{-/-}$ mice ($\Delta 260 \mu m$) than in $TK^{+/+}$ ($\Delta 179 \mu m$). In both strains, unilateral carotid artery ligation resulted in similar increases in media cross-sectional area without changes in collagen and elastin contents.

Conclusion. The vascular KKS seems to protect against excessive inward remodeling in response to flow cessation. Moreover, mechanisms involved in acute vasomotor responses to shear stress likely differ from those operating in structural responses to chronic changes in blood flow.

Introduction

Tissue kallikrein is a serine protease expressed in the vascular wall that cleaves kininogen to generate kinins.^{359,397} In endothelial cells, kinins stimulate the release of nitric oxide (NO) and prostacyclin (PGI₂) through the activation of bradykinin B₂ receptors. NO and PGI₂ exert relaxing, antiproliferative, and antimigratory effects on vascular smooth muscle cells.^{170,177,463,470} Furthermore, the vascular kallikrein-kinin system (KKS) participates in flow-induced dilatation (FID) through the activation of bradykinin B₂ receptors coupled to endothelial NO release.³⁷

Wall shear stress is a major determinant of the structural arterial lumen diameter and, indirectly, of arterial wall mass.^{184,185,308,434,553} In response to changes in shear stress, endothelial cells modulate their release of vasoactive mediators and alter their pattern of gene expression.¹¹⁰ Arterial structural responses to changes in blood flow have been suggested to be endothelium-dependent³¹² and to display similarities to acute flow-induced dilatation.⁴³⁵ In this regard, endothelial NO synthase (eNOS) appears to be a major determinant of flow-related arterial remodeling.^{463,547} Indeed, lack of eNOS leads to smooth muscle cell hypertrophy in response to a drastic chronic decrease in blood flow. Kinins, which can stimulate NO release, also appear to contribute to arterial remodeling, since bradykinin B₁ and B₂ receptors participate in the inhibition of neointima formation caused by either ACE inhibition or overexpression of the human tissue kallikrein (TK) gene in experimental models of altered blood flow.^{137,138,380}

Because TK is involved in acute NO-mediated flow-induced dilatation and because of the putative role of kinins in arterial remodeling, we investigated whether deficiency of TK affects arterial remodeling during chronic alterations in blood flow. We therefore performed unilateral carotid artery ligations,³⁰⁰ resulting in a cessation of blood flow in the ligated and a doubling of blood flow in the contralateral (right) carotid arteries, in tissue kallikrein deficient (TK^{-/-}) and wild-type (TK^{+/+}) mice. We focused our attention on the central part of the arteries where no signs of neointimal formation developed.²¹⁰

Materials and Methods

Animals

Tissue kallikrein deficient (TK^{-/-}) mice were obtained by targeted disruption of the TK (*kkl1*) gene by replacing 100 bp of exon 4 with the neomycin-resistance gene in embryonic stem cells as described earlier.³³⁴ Breeding of TK^{+/-} mice derived from these embryonic stem cells led to TK^{+/+} and TK^{-/-} littermate mice. Experiments were performed on 12-week old male mice with a mixed genetic background (129/SV-C57Bl/6J). To identify the genotype, DNA was extracted from the tail, and the presence of targeted TK alleles was detected by RT-PCR.

Unilateral Carotid Artery Ligation

Male TK^{+/+} and TK^{-/-} mice (3 to 4 months old) were used in all experiments. The animals were anesthetized with a gas mixture (O₂/isoflurane). Carotid arteries were carefully exposed by a midline incision (1 cm) in the neck of the mouse. The left common carotid

artery was dissected *in situ* and ligated near the carotid bifurcation with a 5.0 surgical silk suture as previously described.³⁰⁰ Since mouse carotid arteries lack side branches, left carotid arteries ligated near the bifurcation will have no net forward flow and will be referred to as “no flow”, while the ipsi- or contralateral (right) carotid artery will be referred to as “high flow”. In parallel, carotid arteries of sham-operated mice will be referred to as “normal flow”. All surgical procedures were in accordance with the European Community guidelines concerning the care and use of laboratory animals (Ministère de l'Agriculture, France, authorization 07430).

Carotid Artery Blood Flow Measurements

Four weeks after surgery, both carotid arteries were carefully dissected before measuring *in situ* blood flow. We used a transit-time ultrasonic flow probe (0.5 mm V series, Transonic Systems) that was mounted on a micromanipulator and positioned halfway between the aortic arch and the carotid artery bifurcation. Mean BF was obtained by averaging during a 5-minute period for the left and right carotid artery of each animal.

Pressure-Diameter Curves

Carotid artery segments (5 mm long, halfway between the aortic arch and the carotid bifurcation) were isolated and placed in ice-cold calcium-free modified *Krebs-Ringer* buffer (composition in mmol/L: 144 NaCl, 4.7 KCl, 1.2 MgSO₄, 1.2 KH₂PO₄, 14.9 HEPES, 2.5 CaCl₂, and 5.5 glucose; pH 7.4). The proximal end of each vessel was cannulated on a glass cannula in an organ chamber (arteriograph system, Living System Instrumentation, Burlington, VT, USA) and the distal part of the segment was closed with a nylon knot. The organ chamber was filled with calcium-free *Krebs-Ringer* buffer containing 10 µmol/L sodium nitroprusside (SNP) to assure maximal vasodilatation. The cannulated arterial segment was pressurized using a feedback-controlled pressure source (Living Systems Instrumentation) and placed under a binocular lens connected to a video camera for recording of arterial diameter. Due to the intransparency of the carotid artery, outer diameters were recorded.

A pressure-diameter (P-D) relationship was established by recording the diameter during stepwise (10 mmHg) increases in intraluminal pressures from 20 to 150 mmHg. After each step the vessel was allowed to equilibrate until a stable diameter was reached. After the experiment, the arteries were fixed at a transmural pressure of 100 mmHg in 4% phosphate-buffered formaldehyde and stored in 70% ethanol.

Morphometry

Fixed vessels were embedded in paraffin and cross-sections (4 µm) were stained with Lawson's solution (Boom, Meppel, the Netherlands) to visualize the internal and external elastic laminae. In the case of carotid arteries the morphometric analysis focussed on the central part of the vessel, halfway between the aortic arch and the carotid bifurcation. Unlike the distal part³⁰⁰ near the ligation, the central part showed no signs of neointima formation or inflammation. Video images were made from cross-sections using a Zeiss axioscope and a standard charge-coupled device camera (Sony). Using JAVA software (Sigma Scan, Jandel Scientific, Corte Madera, CA, USA) the circumference of the internal and external elastic laminae were determined. From these values the media cross-sectional

area (mCSA) was calculated. The average number of medial nuclear profiles per cross-section was measured by counting the nuclear profiles in the smooth muscle layers of hematoxylin-eosine stained cross-sections ($n=3$ for each artery and individual mouse). Digital images of Lawson stained cross-sections were made at 400X magnification. Using Leica QWin[®] software the density of elastin was determined in two areas of the media and averaged. Elastin content was calculated by multiplying elastin density with the medial cross-sectional area. To stain collagen, cross sections were deparaffinised and incubated in phosphomolybdic acid (0.2%) for 5 minutes, followed by incubation with Sirius Red (0.1%) in saturated picric acid solution for 90 minutes. After washing with 0.1 M HCl for 2 minutes, sections were dehydrated and protected with coverslips. The density and content of collagen in media were quantified as described for elastin.

Data Analysis and Statistics

Results are expressed as mean $\pm$ SEM of n experiments, n represents the number of animals used for each experimental protocol. Statistical evaluation was performed by ANOVA for factorial or repeated measurements, followed by Scheffe t test.⁵⁷⁵ Values of $P<0.05$ were considered statistically significant.

Results

General Observations

Body weights did not differ between the four groups of mice. In the sham groups, carotid arterial blood flow (mean of left and right artery) did not differ significantly between TK^{+/+} and TK^{-/-} mice (0.69 ± 0.20 mL/min versus 0.59 ± 0.08 mL/min). In both groups of mice, left carotid artery ligation resulted in a cessation of blood flow in the occluded artery and a comparable substantial increase of blood flow in the right carotid artery (1.25 ± 0.20 mL/min in TK^{+/+} mice and 1.28 ± 0.23 mL/min in TK^{-/-} mice).

Arterial Structure of TK^{+/+} and TK^{-/-} Mice

Outer diameters of pooled left and right carotid arteries were significantly smaller in TK^{-/-} compared to TK^{+/+} sham-operated mice (Figure 3.1). However, media thickness (not shown) and media cross-sectional area were comparable in TK^{+/+} and TK^{-/-} mice (Figure 3.1, *insert*). There was no difference in collagen and elastin content and density in carotid arteries of both strains (Table 3.1).

Carotid Artery Structural Changes in Response to Altered Blood Flow

In both TK^{+/+} and TK^{-/-} mice, outer diameters of high flow arteries were not different from normal flow arteries (Figure 3.2). Medial cross-sectional area, collagen and elastin were not significantly altered in carotid arteries of both strains exposed to elevated blood flow (Figure 3.3, Table 3.1).

Figure 3.1. Relations between distending intraluminal pressure and outer diameter in isolated carotid arteries (pooled left and right) of TK^{+/+} (open symbols) and TK^{-/-} (filled symbols) mice. *Insert:* media CSA of isolated carotid arteries (pooled left and right) of TK^{+/+} (open bars) and TK^{-/-} (filled bars) mice. * $P < 0.05$ between the two groups of mice.

Table 3.1. Effect of unilateral artery ligation on collagen and elastin in carotid arteries from TK^{+/+} and TK^{-/-} mice.

	Strain	Unit	Normal Flow	High Flow	No Flow
Collagen	TK ^{+/+}	%	35.7 ± 6.7	37.2 ± 8.9	15.1 ± 2.0 *,#
		x 10 ³ μm ²	9.8 ± 1.3	10.5 ± 3.0	6.5 ± 1.0
	TK ^{-/-}	%	36.4 ± 4.0	34.7 ± 9.5	10.1 ± 3.0 *,#
		x 10 ³ μm ²	9.8 ± 0.8	10.1 ± 2.5	4.3 ± 1.9
Elastin	TK ^{+/+}	%	38.1 ± 1.9	41.9 ± 5.6	21.2 ± 4.0 *,#
		x 10 ³ μm ²	10.4 ± 0.8	10.8 ± 1.1	8.4 ± 1.3
	TK ^{-/-}	%	36.0 ± 2.5	38.6 ± 1.4	28.0 ± 6.7
		x 10 ³ μm ²	9.9 ± 0.7	10.3 ± 0.6	11.6 ± 2.1

The data are expressed as percentage (%) and surface (10³ μm²) of media area and are given as mean ± SEM. * $P < 0.05$ versus normal flow (sham-operated carotid artery), # $P < 0.05$ versus high flow (right carotid artery) and no-flow (left carotid artery).

Ligation of the left carotid artery resulted in a significant reduction of the outer diameter and distensibility (Figure 3.2). The observed inward remodeling was more pronounced in ligated arteries of TK^{-/-} mice (811 ± 11 to 553 ± 17 μm; -32%) than in TK^{+/+} mice (from 899 ± 23 to 734 ± 31 μm; -18%). In both strains of mice, ligation of the left carotid artery resulted in a significant medial hypertrophy, which was not significantly different between TK^{+/+} and TK^{-/-} mice (Figure 3.3). In TK^{+/+} mice, unilateral carotid artery ligation decreased both elastin and collagen density in the no flow artery without altering the contents of these extracellular matrix proteins (Table 3.1). However in TK^{-/-} mice, ligation decreased collagen density in the no flow artery, but did not significantly affect elastin density (Table 3.1).

Figure 3.2. Effects of unilateral carotid artery ligation on relations between distending intraluminal pressure and outer carotid artery diameter in TK^{+/+} (left) and TK^{-/-} (right) mice. SHAM: sham-operated; NF: no-flow (ligated) vessels; HF: high flow (hyperperfused) vessels. * $P < 0.05$ versus NF arteries.

Figure 3.3. Effects of unilateral carotid artery ligation on medial CSA of hyperperfused (left) and ligated (right) carotid arteries in TK^{+/+} and TK^{-/-} mice. SHAM: sham-operated; NF: no-flow (ligated) vessels; HF: high flow (hyperperfused) vessels. * $P < 0.05$ versus sham, * $P < 0.05$ versus SHAM.

Discussion

The arterial wall expresses tissue kallikrein (TK), which contributes to flow-induced NO-mediated dilatation.³⁷ The present study shows that the vascular kallikrein-kinin system participates in the morphogenesis of large elastic arteries and limits inward remodeling in flow-limited arteries. These results also demonstrate that the contribution of the kallikrein-kinin system to flow-induced remodeling unlikely involves similar pathways to those activated during flow-induced dilatation.

Local mechanical forces that govern arterial structure include circumferential wall stress (CWS) and wall shear stress (WSS).¹¹⁰ Chronic changes of these forces result in arterial remodeling.^{137,138,184,185,300,312,434,435,463,547,553} These arterial adaptive structural responses involve partly interrelated alterations in (a) arterial wall mass, (b) mechanical properties of the arterial wall material and (c) arterial lumen diameter. Because WSS can stimulate intra-arterial production of bradykinin,³⁷ we compared flow-related arterial remodeling in large conduit arteries of TK^{-/-} and TK^{+/+} mice using the unilateral carotid artery ligation method introduced by Kumar and Lindner.³⁰⁰

The outer diameter of normal flow carotid arteries was significantly smaller in TK^{-/-} than in littermate wild-type mice over a broad pressure range. A thinner arterial wall in TK^{-/-} arteries can not explain the difference in outer diameter, as media thickness was comparable between TK^{+/+} and TK^{-/-} mice. Since blood pressure and *in vivo* carotid artery blood flow were similar in both strains,³⁷ this study the present results indicate that TK is involved in the development of the arterial lumen diameter during pre- and postnatal growth. They also suggest that the arterial wall of TK^{-/-} mice may be chronically exposed to slightly higher shear stress values compared to carotid arteries of wild-type mice. This interpretation, together with the previously observed blunted flow-induced dilatation in TK^{-/-} arteries,³⁷ would suggest that the arterial wall was unable to normalize wall shear stress.

Ligation of the left common carotid artery was successful, since a complete cessation of blood flow was measured in the occluded arteries and a doubling of blood flow developed in the contralateral artery, where, as a consequence, shear stress was augmented. Connections between the left and right carotid circulation, such as the circle of Willis, form the anatomical basis for this shunting. We did not observe a significant outward remodeling of carotid arteries of both strains exposed to a 4-week period of a doubling in blood flow. Flow-related remodeling has been demonstrated to be age- and strain-dependent. Aging blunts outward carotid artery remodeling in rats³⁶¹ and rabbits.³¹⁰ Harmon and colleagues applied the unilateral ligation model in a variety of inbred strains of mice. They observed that carotid arteries of the 129/SV strain developed the least outward remodeling in response to a doubling of blood flow 4-weeks post ligation.²¹⁰ Since the genetic background of the mice used in this study is 129/SV-C57Bl/6J and the average age is 4 to 5 months, absence of significant outward arterial remodeling might depend on these factors.

In carotid arteries of both strains, a chronic cessation of blood flow resulted in a compensatory inward arterial remodeling accompanied by a drastic reduction in arterial distensibility. These changes in arterial diameter were more pronounced in TK^{-/-} than in TK^{+/+} mice, while the increase in media cross-sectional area was comparable between the

two strains. The absence of an additional effect on medial hypertrophy in TK^{-/-} mice in response to blood flow cessation is in agreement with studies investigating the effect of bradykinin B₂ receptor deficiency and adenovirus-mediated human tissue kallikrein gene delivery in the same model of unilateral carotid artery ligation.¹³⁷ Considering the role of tissue kallikrein in shear stress-induced NO release³⁷ and the role of NO as a negative regulator of vascular smooth muscle cell proliferation in response to a remodeling stimulus,⁴⁶³ absence of an additional effect on medial hypertrophy in TK^{-/-} mice is paradoxical. An explanation for this discrepancy might be the difference in the experimental model between the latter study (ligation of the external carotid artery) and the present study (ligation of the common carotid artery). One possible explanation is that the interruption in blood flow observed in our study may decrease basal NO level to such an extent that an additional decrease in NO release, caused by a deficiency in endogenous kinins, would only have minor effects. Alternatively, other endothelium-derived factors could compensate for the deficiency in NO release in TK^{-/-} carotid arteries, as suggested from a previous study investigating flow-related arterial remodeling in rats chronically treated with the NO synthase blocker L-NAME.⁸⁴ The present study also contrasts with results obtained in vimentin-deficient mice using the same surgical model.⁴⁷⁹ Flow-induced dilatation is blunted in mesenteric arteries of these mice,²²⁰ but inward remodeling was smaller and medial hypertrophy was larger compared to wild-type mice.⁴⁷⁹ Taken together, these data suggest that the mechanisms implicated in acute and chronic responses are different. In this respect, the flow-related arterial adaptations in TK^{-/-} mice likely involve different pathways than those mediating TK-dependent endothelial NO release.³⁷

It is not clear which arterial component(s) is (are) crucial in re-shaping the arterial architecture in response to chronic changes in blood flow. Since the medial cross-sectional area increase was comparable in carotid arteries of both strains after blood flow cessation despite a more substantial inward remodeling in TK^{-/-} mice, indicates that the medial mass is not the main determinant. Since extracellular matrix proteins such as collagens and elastin determine arterial elasticity, we measured the contents and density of these proteins. Surprisingly, the marked medial hypertrophy and reduction in arterial distensibility resulting from blood flow cessation were not accompanied by increases in either collagen or elastin content. Rather, the density of these extracellular matrix proteins was reduced. This seems largely due to the medial hypertrophy but might also involve increased activity of matrix metalloproteinases (MMP-2 and -9) associated with smooth muscle cell migration, macrophage accumulation and neointima formation in the proximal and distal (but not central) parts of the murine carotid artery exposed to blood flow cessation.^{172,173,187,260,293,303} If anything, collagen density dropped more and elastin density dropped less in the occluded TK^{-/-} carotid arteries, which encroached more on their lumen than occluded TK^{+/+} arteries. The greater narrowing of TK^{-/-} arteries in response to blood flow cessation is in line with the reduced expansion during development described above. The vascular kallikrein-kinin system may thus be proposed to reduce inward arterial remodeling independent of collagen and elastin changes. Other extracellular matrix components such as fibronectin⁵³² and cytoskeletal proteins such as desmin³⁰⁴ merit attention in this respect.

These results indicate that the vascular kallikrein-kinin system (a) is involved in the development of the arterial lumen diameter, and (b) reduces inward arterial remodeling in response to chronic reductions in blood flow. They also dissociate between short- and long-term arterial responses to shear stress.

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Chapter 4

Pregnancy-Related Arterial Structural and Functional Alterations Despite Blunted Flow-Induced Dilatation in Tissue Kallikrein-Deficient Mice.

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Abstract

Objective: Tissue kallikrein (TK) participates in acute flow-induced dilatation (FID) of large arteries. We investigated whether TK-deficiency blunts FID and alters chronic flow-related arterial changes in resistance-sized muscular arteries.

Methods and Results: Vasomotor responses and structural parameters were determined in uterine arteries isolated from nonpregnant, 18 to 19 days pregnant and 7 days postpartum TK^{-/-} and TK^{+/+} littermate mice. In TK^{-/-}, diameter, media cross sectional area (CSA), myogenic tone and dilatation in response to acetylcholine were comparable to those in TK^{+/+}, but FID (0-100 μ l/min) was significantly reduced ($55 \pm 4\%$ versus $85 \pm 4\%$ in TK^{+/+}). For both TK^{-/-} and TK^{+/+}, FID was not significantly reduced in the presence of 100 μ M L-NAME, but reduced to 50% in the presence of both L-NAME and 10 μ M indomethacin. In TK^{+/+}, pregnancy resulted in significant increases of diameter and CSA, and in the L-NAME-sensitive component of FID. In uterine arteries of pregnant TK^{-/-}, outward hypertrophic remodeling and the increase of FID were similar to those in TK^{+/+}. By 7 days postpartum, uterine artery diameter and CSA did no longer differ from nonpregnant values and FID was markedly reduced in both TK^{-/-} and TK^{+/+}.

Conclusions: These observations (1) confirm at the level of resistance arteries the key role of TK in FID and (2) indicate that TK deficiency and blunted FID do not compromise the arterial remodeling and the changes in the contribution of nitric oxide to FID during and after pregnancy.

Introduction

Tissue-kallikrein (TK) is a serine protease that generates kinins, such as bradykinin, by enzymatic cleavage of high and low molecular weight kininogens.^{396,398} Bradykinin modulates vascular tone by stimulating vascular endothelial B₂ receptors and the subsequent release of NO and prostacyclin (PGI₂).^{69,89} Recently, Bergaya and colleagues demonstrated a reduced flow-induced dilatation (FID) in isolated carotid arteries of tissue-kallikrein-deficient (TK^{-/-}) mice, indicating a key role of the local kallikrein-kinin system in vasomotor responses of large arteries to increased shear stress.³⁷

Pressure and flow are hemodynamic determinants of circumferential wall stress (CWS) and wall shear stress (WSS) in the arterial system. On an acute basis they modulate arterial smooth muscle tone: an increase in transmural pressure triggers a myogenic contractile response,^{43,156,157,404} while an increase in flow induces an endothelium-dependent FID.^{42,43,110,506} Long-term changes in blood flow lead to arterial structural adaptations in order to normalize WSS.^{33,308,434,479,547,556} In general, chronic blood flow elevations lead to a widening of lumen diameter, whereas sustained reductions in blood flow lead to a narrowing of lumen diameter. These structural arterial adaptations have been shown to be endothelium-dependent.³¹²

Pregnancy may be considered a model of physiological flow-related remodeling of uterine arteries. It has previously been studied in rats,⁴⁰⁶ guinea pigs,⁴²⁰ and ewes.¹⁶⁸ During pregnancy, blood flow through the uterine circulation increases substantially.^{160,335} In order to accommodate this increase in uterine blood flow, the uterine vasculature undergoes luminal expansion and an increase in wall mass,⁴⁰⁶ a process termed outward hypertrophic remodeling or arteriogenesis. The increase in wall mass involves both hypertrophy and hyperplasia of vascular smooth muscle cells as observed in the uterine artery of late pregnant rats.⁴⁰⁸ The driving force for this outward hypertrophic remodeling of uterine arteries during pregnancy is increased WSS. Previous studies have demonstrated that chronic elevations in blood flow lead to an upregulation of FID, mainly due to enhanced production of endothelial factors, such as NO and prostaglandins. For instance, chronic exercise in rats resulted in increased FID and expression of endothelial nitric oxide synthase (eNOS),²⁹⁰ and arterial wall mass increases in the arteriolar network in skeletal muscle.⁴⁸⁸ These observations suggest that long-term elevations in blood flow increase acute FID, but do not explain whether FID is required for successful structural remodeling of arteries.

The purpose of this study was to investigate whether TK-deficiency alters FID and chronic flow-related arterial structural and functional alterations in resistance-sized muscular arteries. Vasomotor responses and structural parameters were determined in uterine arteries isolated from nonpregnant, 18 to 19 days late pregnant and 7-days postpartum TK^{-/-} and TK^{+/+} mice.

Methods

Mice lacking tissue kallikrein.

Tissue kallikrein (TK)-null mice were obtained by targeted disruption of the TK *k/l* gene that was accomplished by replacing 100 bp of exon 4 with the neomycin-resistance gene in embryonic stem cells.³⁵⁹ Breeding of heterozygous mice derived from these embryonic stem cells led to wild-type (TK^{+/+}), heterozygous (TK^{+/-}) and homozygous (TK^{-/-}) littermate mice. Mice had a mixed genetic 129/SV-C57BL/6 background.

At an age of 15 weeks, four to five TK^{+/+} female virgin mice were placed together in a cage with one male TK^{-/-} mouse. Female TK^{-/-} mice were put together with one male TK^{+/+} mouse. This allowed to compare pregnant TK^{-/-} and TK^{+/+} mice, each carrying heterozygous fetuses. Males were separated from the females after 24 hours. When mating was successful, the day of placing a male mouse with the females was considered as day 0. For the late pregnant group, pregnant mice were sacrificed 18 to 19 days after day 0. For the postpartum group, mothers were sacrificed 7 days after delivery of their pups. For the nonpregnant group, age-matched (18 week-old) virgin mice were used.

Preparation of blood vessels and experimental set up.

Mice were sacrificed with an overdose of pentobarbital (i.p.). The mesentery and uterus were dissected and pinned out on a petridish (coated with a layer of silicon). Adipose- and connective tissue were carefully removed from the arteries. From every mouse one segment (3 mm) of a first-order mesenteric artery and one segment (3 mm) of the uterine artery at midpoint of the uterine arcade were isolated. Both preparations were mounted in an arteriograph system (Living System Instrumentation, Burlington, VT, USA), in which wall thickness and lumen diameter could be continuously monitored while controlling intraluminal pressure. Both ends of the vessels were cannulated on 120-150 μ m wide glass micropipettes (Living System Instrumentation, Burlington, VT, USA), and tied with two 17- μ m thin nylon threads. The two micropipettes were selected such that both had the same resistance to flow. Arterial segments were bathed in a 10-ml organ chamber filled with physiological salt solution of the following composition (mmol/L): 144 NaCl, 4.7 KCl, 2.5 CaCl₂, 1.2 MgSO₄, 1.2 KH₂PO₄, 14.9 HEPES and 5.5 glucose, pH 7.4. The artery was superfused at a rate of 4 ml/min with physiological salt solution warmed at a temperature of 37°C and continuously gassed with 95 % O₂ and 5 % CO₂. The pressure at both ends of the artery was monitored using two pressure transducers. Intraluminal flow (0-100 μ l/min) could be applied without changes in average pressure.

At the beginning of each experiment, the artery was incubated for 45 minutes at 80 mmHg. The integrity of the endothelium was evaluated by assessing the response to acetylcholine (1 μ mol/L) during phenylephrine (1 μ mol/L)-induced constriction.

Pressure-induced (myogenic) tone was determined by submitting the arteries to 10 mmHg pressure steps ranging from 10 to 140 mmHg in the absence of intraluminal flow. Pressure was only increased (not decreased or randomly changed) from 10 to 140 mmHg, and augmented to the next level until a stable diameter was reached.

Flow-induced dilatation was studied by increasing the flow rate (0, 4, 10, 25, 50, 75 and 100 μ l/min) at an intraluminal pressure of 80 mmHg (Figure 1). Arteries were

Morphological Properties.

After reactivity measurements, arteries were pressurized at 80 mmHg for one hour in phosphate-buffered 10% formaldehyde solution. Fixed vessels were embedded in paraffin, cross sections (4 μ m) were stained with Lawson's solution (Boom, Meppel, the Netherlands) to visualize the internal and external elastic laminae. Video images were made from cross sections using a Zeiss axioscope and a standard charge-coupled device camera (Sony). Using JAVA software (Sigma Scan, Jandel Scientific, Corte Madera, CA, USA) the circumference of the internal and external elastic laminae were determined, and media cross-sectional area (CSA) was defined as the area enclosed between both laminae. The average number of medial nuclear profiles per cross-section was measured by counting the nuclear profiles in the smooth muscle layers of hematoxylin-eosine stained cross-sections at a magnification of 400X (three cross-sections were counted for each artery and were averaged).

Drugs.

N^ω-nitro-L-arginine methyl ester (L-NAME), indomethacin, HOE-140 and sodium nitroprusside (SNP) were purchased from Sigma Chemical Co. (St Louis, MO). Other reagents were purchased from Prolabo (Paris, France).

Statistical analysis.

Results are shown as means $\pm$ SEM. Significance of the differences between the different groups was determined by ANOVA. Means were compared by paired *t* test or student-Newman Keuls test. *P* < 0.05 was considered to denote statistical significance.

Results

General Findings.

In TK^{-/-} and TK^{+/+} mice, body weight (29 $\pm$ 1 versus 26 $\pm$ 1 g, respectively) as well as heart weight (140 $\pm$ 6 versus 134 $\pm$ 7 mg, respectively) and uterine weight (0.33 $\pm$ 0.06 versus 0.33 $\pm$ 0.04 g, respectively) did not differ significantly. During pregnancy, in TK^{-/-} and TK^{+/+} mice, body weight (46 $\pm$ 2 versus 43 $\pm$ 2 g, respectively) and uterine weight (12.13 $\pm$ 0.77 versus 11.21 $\pm$ 0.98 g, respectively) increased to the same extent. Heart weight was significantly increased by day 18 to 19 of pregnancy in TK^{-/-} mice but not in TK^{+/+} mice (155 $\pm$ 3 versus 147 $\pm$ 6 mg, respectively). When TK^{-/-} mice were compared with TK^{+/+} mice, the number of fetuses (10 $\pm$ 0 versus 8 $\pm$ 1, respectively) and their body weights (0.84 $\pm$ 0.04 versus 0.97 $\pm$ 0.14 g, respectively) did not differ. At day 7 after pregnancy, in TK^{-/-} and TK^{+/+} mice, body weight (40 $\pm$ 1 versus 35 $\pm$ 2 g, respectively) and heart weight (200 $\pm$ 5 versus 164 $\pm$ 9 mg, respectively) remained elevated, but uterine weight (0.19 $\pm$ 0.02 versus 0.20 $\pm$ 0.03 g, respectively) was decreased to below the original value.

Arterial structure.

The structure of uterine arteries and mesenteric resistance arteries did not differ between TK^{-/-} and TK^{+/+} mice (Figure 4.2). Neither the arterial lumen diameter, as determined by pressure-diameter curves under passive conditions (Figure 4.3A) nor the media cross

sectional area and the number of medial cells, determined on cross sections (Table 4.1), differed between TK^{-/-} and TK^{+/+} mice.

Pregnancy was accompanied by a regionally selective and statistically significant outward hypertrophic remodeling of the uterine artery (Figure 4.4A). The increases in arterial structural diameter and in media cross sectional area (Table 4.1) were comparable in TK^{-/-} and TK^{+/+} mice.

Table 4.1. Morphometric analysis of uterine and mesenteric arteries of wild-type and tissue kallikrein-deficient mice.

Measurement	NP		LP		PP	
	TK ^{+/+}	TK ^{-/-}	TK ^{+/+}	TK ^{-/-}	TK ^{+/+}	TK ^{-/-}
Uterine Arteries						
Diameter (μm)	335 $\pm$ 9	357 $\pm$ 16	457 $\pm$ 8*	466 $\pm$ 14*	366 $\pm$ 11	399 $\pm$ 16
mCSA ($\times 10^3 \mu\text{m}^2$)	4.4 $\pm$ 0.6	4.2 $\pm$ 0.4	6.3 $\pm$ 0.7*	6.2 $\pm$ 0.4*	3.8 $\pm$ 0.2	5.1 $\pm$ 0.5*
No. medial nuclear profiles/ cross-section	23 $\pm$ 3	25 $\pm$ 3	19 $\pm$ 2	19 $\pm$ 1	22 $\pm$ 3	22 $\pm$ 1
Mesenteric Resistance Arteries						
Diameter (μm)	239 $\pm$ 18	258 $\pm$ 14	271 $\pm$ 13	289 $\pm$ 12	308 $\pm$ 19*	310 $\pm$ 15*
mCSA ($\times 10^3 \mu\text{m}^2$)	1.9 $\pm$ 0.4	2.2 $\pm$ 0.4	1.9 $\pm$ 0.2	2.6 $\pm$ 0.3	2.6 $\pm$ 0.3	2.7 $\pm$ 0.4

Diameters in the absence of smooth muscle tone obtained at an intraluminal pressure of 140 mmHg, media cross-sectional areas (mCSA), and the number of medial nuclear profiles per cross-section of paraffin-embedded uterine arteries and mesenteric resistance arteries of nonpregnant (NP), late pregnant (LP), and postpartum (PP) wildtype (TK^{+/+}) and tissue kallikrein-deficient (TK^{-/-}) mice. * $p < 0.05$ compared with NP. Values are mean $\pm$ SEM.

Figure 4.2. Cross-sections of uterine arteries. Hematoxyline-cosine-stained cross-sections (4 μm) of uterine arteries of nonpregnant (a+d), late pregnant (b+e), and postpartum (c+f) TK^{+/+} (a-c) and TK^{-/-} (d-f) mice. Due to the small size and manipulation during the process of paraffin embedment, cross sections appear not circular. *Insert:* portions of uterine arteries stained with Lawson's solution visualizing the internal and external elastic laminae (4X higher magnification).

By 7 days after pregnancy, most of the structural changes in the uterine artery were reversed (Figure 4.5A). In TK^{+/+} mice, the structural lumen diameter and the media cross sectional area did no longer differ from findings before pregnancy (Table 4.1). In TK^{-/-} mice, the structural lumen diameter had regressed to normal (Figure 4.5A) but a significant media hypertrophy persisted (Table 4.1). The changes in media cross sectional area of the uterine artery during and after pregnancy were not accompanied by significant changes in the number of arterial smooth muscle cells/cross-section in both TK^{-/-} and TK^{+/+} mice (Table 4.1). It might be noteworthy that mesenteric resistance arteries, which were not modified during pregnancy, were enlarged in the postpartum period. This was statistically significant for the lumen diameter in both TK^{-/-} and TK^{+/+} mice, but the 20-30 % media hypertrophy did not reach statistical significance in either strain (Table 4.1).

Figure 4.3. Effects of TK-deficiency on structure and reactivity of uterine arteries. (A) Pressure-diameter relationships in the absence of calcium for nonpregnant TK^{-/-} (open squares) and TK^{+/+} (filled squares) mice. No significant differences between the two groups were observed. (B) Pressure-diameter relationships in the presence of calcium. No differences in myogenic tone were observed between the two strains. (C) Flow-induced dilatation response to all flow rates was observed in uterine arteries of nonpregnant TK^{-/-} mice (open squares). * $p < 0.05$ compared to TK^{+/+}. (D) Effect of 0.1 μmol/L HOE-140 on the FID response of uterine arteries of TK^{+/+} mice. * $p < 0.05$ compared to control arteries. Data are shown as means ± SEM.

Vasoconstrictor Reactivity.

Myogenic vasoconstriction (Figure 4.3B) and constriction induced by 1 $\mu\text{mol/L}$ phenylephrine (Table 4.2) did not differ between uterine arteries of $\text{TK}^{-/-}$ and $\text{TK}^{+/+}$ mice. In the case of myogenic tone, neither the pressure at which the response was induced nor the maximal amplitude (Table 4.2) of the response differed significantly (Figure 4.3B). In uterine arteries of term pregnant mice, when compared to nonpregnant animals, the myogenic constriction at 100 mmHg and the constriction induced by 1 $\mu\text{mol/L}$ phenylephrine were larger when expressed in absolute values (not shown) but comparable when expressed as percentage reduction of the passive diameter (Figure 4.4 and Table 4.2). This was the case for both $\text{TK}^{-/-}$ and $\text{TK}^{+/+}$ mice. In uterine arteries of late pregnant $\text{TK}^{+/+}$, unlike $\text{TK}^{-/-}$, myogenic constriction was not well maintained at high levels of transmural pressure (> 100 mmHg; Figure 4.4B).

Figure 4.4. Effects of pregnancy on structure and reactivity of uterine arteries. (A) Pressure-diameter relationships in the absence of calcium for uterine arteries isolated from nonpregnant (squares) and late pregnant (circles) $\text{TK}^{-/-}$ (open symbols) and $\text{TK}^{+/+}$ (filled symbols) mice. Significantly larger diameters were measured at all pressures for late pregnant mice, indicating an outward remodeling. No differences were observed between the two strains. (B) Pressure-diameter relationships in the presence of calcium. Myogenic tone measured at 100 mmHg (see Table 2) tended to be higher in uterine arteries of late pregnant mice. (C) Flow-induced dilatation in uterine arteries of nonpregnant (squares) and late pregnant (circles) $\text{TK}^{-/-}$ (open symbols) and $\text{TK}^{+/+}$ (filled symbols) mice. FID is expressed as percentage dilatation of U46619- and/or myogenic-induced tone. Data are shown as means $\pm$ SEM.

Figure 4.5. Properties of uterine arteries of $TK^{+/+}$ and $TK^{-/-}$ mice 7 days after pregnancy. (A) Pressure-diameter relationships in the absence of calcium for uterine arteries isolated from nonpregnant (squares) and postpartum (diamonds) $TK^{-/-}$ (open symbols) and $TK^{+/+}$ (filled symbols) mice. No significant differences were observed, indicating an almost complete remodeling to the original diameters of nonpregnant mice. (B) Pressure-diameter relationships in the presence of calcium. Myogenic tone was significantly stronger in uterine arteries of postpartum $TK^{+/+}$ mice compared to nonpregnant $TK^{+/+}$ mice (see also Table 2), but were not statistically different between uterine arteries of postpartum $TK^{+/+}$ and $TK^{-/-}$ mice. (C) FID in uterine arteries isolated from nonpregnant (squares) and postpartum (diamond) $TK^{-/-}$ (open symbols) and $TK^{+/+}$ (filled symbols) mice. FID is expressed as percentage dilatation of U46619- and/or myogenic-induced tone. A significantly decreased response to all flow rates was observed in arteries of postpartum mice compared with nonpregnant mice. * $p < 0.05$ versus nonpregnant mice. Data are shown as means $\pm$ SEM.

At 7 days after pregnancy, the relative constriction induced by phenylephrine did not differ from observations before and during pregnancy in $TK^{-/-}$ and $TK^{+/+}$ mice (Table 4.2). Sensitivity to myogenic vasoconstriction was increased in the postpartum period when compared to before and during pregnancy (Figure 4.5B). Yet, the amplitude of the myogenic constriction at physiological pressures (80-100 mmHg) was for both $TK^{-/-}$ and $TK^{+/+}$ comparable to that before pregnancy (Table 4.2).

Agonist-Induced Vasodilatation.

Dilator responses to 1 $\mu\text{mol/L}$ acetylcholine and intraluminal flow (see below) were evaluated during 40–45% constriction induced by a transmural pressure of 80 mmHg and 1 to 100 nmol/L of the thromboxane A_2 analogue U46619 (Figure 4.1). This level of preconstriction was comparable for all groups of uterine arteries investigated (Table 4.2). Dilator responses were expressed as a percentage of the response to 100 $\mu\text{mol/L}$ sodium nitroprusside, which induced near maximal dilatation in all cases.

Acetylcholine (1 $\mu\text{mol/L}$) caused vasodilatation in all uterine arteries studied. The amplitude of this response did not differ between $\text{TK}^{-/-}$ and $\text{TK}^{+/+}$ mice and was not modified in pregnant and postpartum mice (Table 4.2).

Flow-Induced Vasodilatation.

Increases in flow rate from 0 to 100 $\mu\text{l/min}$ caused flow-dependent increases in diameter in constricted uterine arteries (Figure 4.1). Because the response to 25 $\mu\text{l/min}$ did not differ significantly from responses to higher flow rates, only responses from 0 to 25 $\mu\text{l/min}$ are depicted in the figures 4.3 to 4.5.

Flow-induced dilations were significantly smaller in uterine arteries of $\text{TK}^{-/-}$ than $\text{TK}^{+/+}$ mice at all flow rates tested (Figure 4.3C). Maximal responses averaged $55 \pm 4\%$ ($n=8$) and $85 \pm 4\%$ ($n=7$) in $\text{TK}^{-/-}$ and $\text{TK}^{+/+}$, respectively. In the presence of the B_2 -receptor antagonist HOE-140 (0.1 $\mu\text{mol/L}$; icatibant) flow-induced dilatation of $\text{TK}^{+/+}$ arteries was reduced to the level observed in $\text{TK}^{-/-}$ arteries (Figure 4.3D). In the presence of the NO-synthase inhibitor L-NAME (100 $\mu\text{mol/L}$), flow-induced dilatation was moderately but consistently reduced in uterine arteries of both $\text{TK}^{-/-}$ and $\text{TK}^{+/+}$ mice, and the significant difference between both strain persisted (Table 4.3). In the presence of

Table 4.2. Vasomotor responses of uterine arteries to phenylephrine, acetylcholine, pressure (100 mmHg) and U46619 for wild-type and tissue-kallikrein deficient mice.

mouse	state	% constriction to 1 $\mu\text{mol/L}$ Phe	% dilatation to 1 $\mu\text{mol/L}$ ACh	% MT	% induced tone before FID
$\text{TK}^{+/+}$	NP	51 ± 4	85 ± 7	32 ± 5	45 ± 2
	LP	44 ± 8	94 ± 2	43 ± 7	38 ± 4
	PP	52 ± 6	87 ± 3	$47 \pm 5^*$	45 ± 4
$\text{TK}^{-/-}$	NP	50 ± 8	93 ± 2	35 ± 5	45 ± 2
	LP	47 ± 10	87 ± 5	45 ± 4	45 ± 4
	PP	51 ± 4	79 ± 4	39 ± 7	40 ± 4

Abbreviations used: NP: nonpregnant; LP: late pregnant; PP: postpartum; MT: myogenic tone; Phe: phenylephrine; ACh: acetylcholine. Constrictions of uterine arteries to 1 $\mu\text{mol/L}$ Phe are shown as a percentage of dilatation induced by 100 $\mu\text{mol/L}$ sodium nitroprusside. Dilatations of uterine arteries to 1 $\mu\text{mol/L}$ ACh during 1 $\mu\text{mol/L}$ Phe constriction are shown as percentage of the diameter obtained at 80 mmHg in the absence of smooth muscle tone and in the presence of 100 μM sodium nitroprusside. Percentage MT of uterine arteries was measured at an intraluminal pressure of 100 mmHg. Constriction before flow-induced dilatation was induced by myogenic tone, U46619 or a combination of both. $^*p < 0.05$ compared with uterine arteries of nonpregnant mice. (mean $\pm$ SEM).

both L-NAME and the cyclooxygenase inhibitor indomethacin (10 $\mu\text{mol/L}$), flow-induced dilatation was further reduced in arteries of both strains and, again, the significant difference between both strains persisted (Table 4.3). In the presence of both inhibitors, 50% of the maximal flow-induced dilatation persisted in uterine arteries of both TK $^{-/-}$ and TK $^{+/+}$ mice. The level of constriction was not altered by L-NAME or by the combination of L-NAME and indomethacin (not shown).

Pregnancy was accompanied by increased flow-induced uterine artery dilatation in both TK $^{-/-}$ and TK $^{+/+}$ mice (Figure 4.4C). Both the sensitivity and the maximal effect were increased. The latter increased from $85\pm4\%$ to $94\pm2\%$ in TK $^{+/+}$ and from $55\pm4\%$ to $76\pm8\%$ in TK $^{-/-}$ mice. During pregnancy, flow-induced dilatation remained significantly smaller in TK $^{-/-}$ than TK $^{+/+}$. As judged from findings with L-NAME, the pregnancy related increase in flow-induced vasodilatation was primarily due to a larger contribution of NO to the process. In uterine arteries of pregnant TK $^{+/+}$ mice, L-NAME inhibited 50% of the flow-induced response while this was only 18 % before pregnancy (Table 4.3). Also in TK $^{-/-}$ mice the effect of L-NAME was larger during pregnancy (43% inhibition) than before pregnancy (15% inhibition). The L-NAME and indomethacin-resistant part of flow-induced dilatation was not significantly modified by pregnancy. However, in uterine arteries of pregnant mice flow-induced dilatation that can not be attributed to NO and prostaglandins, did no longer differ significantly between TK $^{-/-}$ and TK $^{+/+}$ mice (Table 4.3).

While pregnancy was accompanied by increased flow-induced dilatation, this response was reduced to below pre-pregnancy values in the postpartum period (Figure 4.5). Maximal flow-induced dilatation averaged $31\pm5\%$ and $38\pm5\%$ in TK $^{-/-}$ and TK $^{+/+}$ uterine arteries, respectively (Table 4.3). Although all components seemed to be affected, primarily the L-NAME sensitive part of the flow-induced dilatation was blunted or even abolished. In uterine arteries obtained at 7 days after pregnancy, differences between TK $^{-/-}$ and TK $^{+/+}$ mice were no longer statistically significant but this might be due to the small and variable nature of the responses at this time point.

Table 4.3. Flow-induced dilatation at a flow rate of 100 $\mu\text{L/min}$ under control conditions, in the presence of L-NAME or in the presence of L-NAME + indomethacin for uterine arteries of nonpregnant, late pregnant and postpartum wildtype and tissue kallikrein-deficient mice.

Condition	NP		LP		PP	
	TK $^{+/+}$	TK $^{-/-}$	TK $^{+/+}$	TK $^{-/-}$	TK $^{+/+}$	TK $^{-/-}$
Control	85 ± 5	55 ± 4 ‡	94 ± 2 †	76 ± 8 †‡	38 ± 5 †	31 ± 5 †
L-NAME	70 ± 4 *	47 ± 7 ‡	48 ± 6 *†	44 ± 6 *	25 ± 7 †	30 ± 6 †
L-NAME + indomethacin	43 ± 5 *	27 ± 7 *‡	34 ± 8 *	36 ± 6 *	16 ± 4 *†	16 ± 4 *†

Flow-induced dilatation (FID, at 100 $\mu\text{L/min}$) of uterine arteries of nonpregnant (NP), late pregnant (LP), and 7-days postpartum (PP) wildtype (TK $^{+/+}$) and tissue kallikrein-deficient mice (TK $^{-/-}$) under control conditions, in the presence of 100 $\mu\text{mol/L}$ L-NAME, or 100 $\mu\text{mol/L}$ L-NAME in combination with 10 $\mu\text{mol/L}$ indomethacin. * $p<0.05$ versus control; † $p<0.05$ versus NP; ‡ $p<0.05$ versus TK $^{+/+}$. Mean $\pm$ SEM.

Discussion

In this study we observed in uterine arteries of the mouse: (1) that flow-induced vasodilatation (FID) is markedly reduced in the absence of tissue kallikrein, (2) that pregnancy is accompanied by rapidly reversible outward remodeling and upregulation of FID, and (3) that these pregnancy-related arterial structural and functional changes are comparable in the absence and presence of TK.

In large conduit arteries a reduced FID was previously observed in mice lacking TK, pointing out the importance of locally produced kinins in regulating vasomotor responses to shear stress in these vessels.^{37,359} The objectives of the present study were 1) to determine whether a blunted FID was also present in resistance-sized arteries of TK^{-/-} mice and 2) whether blunted acute responses to shear stress hamper structural arterial adaptations in response to chronically altered blood flow. We have chosen pregnancy as a physiological model representing an increased blood flow during gestation and reduced blood flow after birth (7 days postpartum) in the uterine vasculature of mice. In many species normal pregnancy is associated with a marked increase in uterine blood flow.^{63,455} Uterine blood flow has not yet been measured in mice, but cardiac output has been demonstrated to be 64% higher in late pregnant mice compared to nonpregnant mice.⁵⁹³

Uterine arteries of TK^{+/+} and TK^{-/-} mice had similar responses to acetylcholine and sodium nitroprusside, indicating that the release of NO, prostaglandins, and endothelium-derived hyperpolarizing factor (EDHF) by acetylcholine is unaffected by the lack of TK. In this study we have not addressed the direct vasodilatation induced by bradykinin on pre-constricted arteries. However, bradykinin has been shown to elicit similar vasoactive responses in TK^{+/+} and TK^{-/-} carotid arteries.³⁷

Uterine arteries of nonpregnant mice responded to intraluminal pressure by developing myogenic tone and to flow by vasodilatation. Myogenic tone developed at sub-physiological pressures (50–60 mmHg) where no further increases in diameter were recorded and subsequent decreases in diameter were followed at higher pressures until a stable tone was maintained over a wide pressure range. Myogenic tone was similar in arteries of TK^{+/+} and TK^{-/-} mice, suggesting no contribution for the kallikrein-kinin system, which is in line with the endothelium-independent nature of the process.⁴³ The exact role of myogenic tone in the uterine vasculature is uncertain. In radial arteries bifurcating from the uterine arcuate artery of the rat almost no myogenic tone has been observed, but was present during pregnancy.⁴⁰⁶ Myogenic responses in the uterine circulation might be important in maintaining constant blood flow and in controlling capillary hydrostatic pressure at the site of placentation during pregnancy.

FID was markedly reduced in uterine arteries of TK^{-/-} mice, demonstrating the participation of the kallikrein-kinin system in FID of not only conduit³⁷ but also resistance arteries. Also in systemic resistance arteries such as the mesenteric resistance artery of male mice, FID was found to be reduced (personal observations, S.B.). If a similar reduction in FID occurs *in vivo*, one would expect an increase in total peripheral resistance. However, no difference in mean arterial pressure was measured between the two mice strains,³⁷ possibly as a result of reduced cardiac function in TK^{-/-} mice.³⁵⁹ The contribution of the kallikrein-kinin system was around one-third of the FID observed in uterine arteries of TK^{+/+} mice. This is in agreement with observations in carotid arteries.³⁷

The difference in response to flow was not due to a difference in myogenic and/or U46619-induced tone before the application of intraluminal flow. Kinins, such as bradykinin, act on bradykinin B₂ receptors that can stimulate the release of endothelium-derived relaxing factors (EDRFs).^{69,89,396,398} To demonstrate the involvement of the local kallikrein-kinin system in mediating the FID in uterine arteries, the B₂ receptor antagonist HOE-140 was used. Since HOE-140 was shown not to affect FID in TK^{-/-} carotid arteries,³⁷ experiments on uterine arteries of TK^{-/-} mice were omitted. Indeed, FID was significantly reduced in uterine arteries incubated in the presence of the B₂ receptor antagonist, indicating the participation of bradykinin via B₂ receptors in the FID response. FID depended to a large extent on the production of prostaglandins, since the NO synthase blocker L-NAME only marginally suppressed the response to flow, whereas incubation with both L-NAME and indomethacin induced a fifty percent reduction in the response to flow in both strains. There was no significant difference in the relative contribution of NO, PGI₂, and the non-NO-non-PGI₂ compound(s) to the FID response between TK^{+/+} and TK^{-/-} arteries. This rules out any compensation by other mediators for the lack of the kallikrein-kinin system as judged by these *in vitro* responses, but argues for a role of bradykinin as an autocrine mediator during FID. Compensation by other vasoactive compounds has been observed in eNOS deficient mice.^{244,524} The remaining response to flow might be mediated by EDHF, as was demonstrated in carotid arteries of the mouse.³⁷

At late pregnancy, heart weight increased in both TK^{+/+} and TK^{-/-} mice, which reached statistical significance in the latter mice. Pregnancy results in a marked increase in cardiac output, due to an increase in stroke volume and heart rate, which might have caused the cardiac hypertrophy.⁵⁹³

At late pregnancy myogenic tone tended to be increased. FID of uterine arteries of both strains of mice was significantly elevated. Predominantly NO was responsible for this upregulation in FID, since the contribution of the L-NAME-sensitive compound was significantly increased in both TK^{+/+} and TK^{-/-} mice. Because NO has been reported to inhibit the production of other endothelium-derived factors,⁵³⁶ the observed upregulation of the role of NO in FID during pregnancy might be accompanied with a drop in the relative importance of prostaglandin and non-prostaglandin/non-NO mediators. Pregnancy increases uterine artery NO-mediated vasodilatation, via increased basal NO production (NOS) in rats,⁹⁸ guinea pigs,⁵⁸¹ ewes,³³⁶ and humans.³⁹¹ Expression of eNOS mRNA and eNOS protein is increased in ovine pregnancy,³³⁷ which likely follows estrogen-induced upregulation of eNOS⁵⁵⁹ and vascular endothelial growth factor,⁵²¹ causing an increase in uterine blood flow.⁴⁵⁷ Several studies have demonstrated an increased FID of arteries experiencing a rise in blood flow. For instance, exercise training augmented FID in rat skeletal muscle arterioles.⁴⁸⁸ The augmented dilator response was due to increased release of NO and prostaglandins. More recently, Loufrani and colleagues used a surgical model to apply an increased blood flow in mesenteric resistance arteries and observed a greater FID and eNOS expression in these flow-loaded arteries.³²⁷ In view of our similar findings in TK^{+/+} and TK^{-/-} mice, TK does not seem to be required for this chronic upregulation.

During pregnancy, blood flow through the uterine circulation increases substantially.^{160,335} Due to this increase in wall shear stress the uterine artery undergoes a

structural increase in diameter, termed outward remodeling. A limitation of the present study is the absence of a quantitative uterine blood flow measurement. However, we have reasons to speculate that blood flow increases substantially in late pregnant mice. Firstly, uterine blood flow has been shown to increase dramatically (± 40 -fold) at term in rats.⁶³ Secondly, cardiac output increases 64% during murine pregnancy.⁵⁹³ Because uterus weight and fetal weight and number are not statistically different between TK^{+/+} and TK^{-/-} late pregnant mice, we believe that the uterine blood flow rise is comparable in both strains. However, the presence of a reduced acute FID in TK^{-/-} uterine arteries may indicate a somewhat higher wall shear stress. Since wall shear stress is the primary stimulus to initiate flow-induced arterial remodeling, we questioned whether a blunted FID would result in an altered remodeling response during and after pregnancy.

In previous studies, chronic elevations and reductions in blood flow have been reported to respectively induce outward and inward arterial remodeling.^{33,312,406,434,479,547,556} This adaptive response allows a normalization of wall shear stress and is accompanied by changes in arterial smooth muscle cell number, size and phenotype in order to restore circumferential wall stress.^{71,555} Here we confirm that chronic increases in blood flow, as they occur during pregnancy, lead to an outward hypertrophic remodeling of uterine arteries. The increase in arterial wall mass was due to vascular smooth muscle cell hypertrophy rather than hyperplasia, since no difference in the number of medial smooth muscle cells were observed. The outward remodeling and increase in media cross-sectional area were similar in TK^{+/+} and TK^{-/-} mice, despite a significantly different acute response to flow before and during pregnancy. This indicates that TK deficiency and blunted FID do not compromise structural and functional arterial changes in response to long-term blood flow elevations. A similar conclusion was drawn recently in a study using desmin knock-out mice. Desmin is a constitutive subunit of the intermediate filaments in smooth muscle cells. Although a blunted FID of mesenteric resistance arteries was measured in these mice, the outward hypertrophic remodeling in response to a 4-week period of a doubling of blood flow was even higher compared to wild-type mice.³²⁷ Vimentin, another intermediate filament of the cytoskeleton, is involved in the transduction of mechanical strain, and shear stress. We previously showed an excessive luminal enlargement of carotid arteries when exposed to a chronic increase in blood flow, despite reduced FID in vimentin-deficient mice.^{220,479} Also, during chronic L-NAME treatment, which blunts FID, flow-induced outward hypertrophic remodeling is maintained in the rat.⁸⁴ All together, these observations suggest a dissociation between acute and chronic arterial responses to wall shear stress.

TK and bradykinin B₂ receptor mRNA and protein are expressed in several tissues of the uterus and myometrical blood vessels during human pregnancy.⁵⁶⁰ However, lack of TK does not cause any noticeable complications in murine pregnancy, as far as uterine arterial remodeling, fetus weight and the number of pups delivered are considered. More subtle consequences can, however, not be excluded. To this end, cardiovascular and renal function will have to be critically compared in the heterozygous offspring of TK^{-/-} and TK^{+/+} mothers.

Body weight remained elevated in postpartum mice, possibly because of increased food intake to facilitate lactation. Heart weight also remained increased compared to heart weights of nonpregnant mice. The cardiac hypertrophy most likely resulted from the

increased cardiac output and heart rate at late pregnancy and at 3 days postpartum as measured by Wong and colleagues.⁵⁹³ Myogenic tone was stronger in uterine arteries of 7 days postpartum mice compared to virgin mice. Remarkably, the FID response was markedly reduced in uterine arteries 7 days postpartum, suggesting a reduced shear stress sensing of the endothelium, despite normal acetylcholine-mediated vasorelaxation. We have currently no explanation for this phenomenon. An increased myogenic tone and a decreased FID lead to an increased contractility, possibly reducing the risk of excessive blood loss after delivery.

As observed from the passive pressure-diameter curves of uterine arteries of postpartum mice, diameters no longer differ from their nonpregnant values. Media cross-sectional area of uterine arteries of 7 days postpartum mice were similar to nonpregnant values, reflecting an inward hypertrophic remodeling compared to uterine arteries of mice near term. Again, the blunted acute responses to flow are not sufficient to disturb structural arterial adaptations in response to long-term changes in blood flow.

A surprising finding was the outward remodeling observed in mesenteric resistance arteries of 7 days postpartum mice. Feeding of the pups requires a higher food intake than normal, likely to increase blood flow through the mesentery, leading to this increase in diameter. In rats with streptozotocin-induced diabetes, blood flow to the small intestine is markedly increased due to marked hyperphagia.²²⁵ Our group previously showed that this rise in mesenteric blood flow resulted in a larger diameter of the mesenteric resistance artery of diabetic rats compared to control rats.¹⁰¹

In conclusion, we confirmed the role of the kallikrein-kinin system in acute FID via B₂ receptors at the level of resistance arteries. Absence of this kallikrein-kinin system seems not to be compensated by other mediators, that can mediate FID in TK^{-/-} arteries. Furthermore, we demonstrated the dynamic nature of physiological arterial remodeling and arteriogenesis during and after pregnancy, and show that TK deficiency and blunted FID do not compromise arterial structural and functional changes during and after pregnancy, which are accompanied by marked changes in local blood flow. These observations point to a dissociation between acute and chronic arterial responses to wall

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Chapter 5

Intra-Arterial Bradykinin and Vasodilatation in Relation to Acute and Chronic Changes in Blood Flow

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Abstract

Objective. Investigate the role of endogenous intra-arterial bradykinin in chronic modulation of endothelium-dependent vascular reactivity.

Methods and Results. We investigated the vasodilator response to flow (shear stress) and acetylcholine (ACh) in pre-constricted isolated uterine arteries of nonpregnant, late pregnant and 7-days postpartum tissue angiotensin-converting enzyme-deficient (t-ACE^{-/-}) and wild-type (t-ACE^{+/+}) mice. Vasodilatation was measured in the absence and presence of either the bradykinin B₂-receptor antagonist HOE 140, the nitric oxide synthase inhibitor N^ω-nitro-L-arginine (NLA) or a combination of both. Under control situations, ACh and shear stress induced similar relaxations in arteries of t-ACE^{+/+} and t-ACE^{-/-} mice. HOE 140 reduced the responses to ACh and shear stress to a larger extent in uterine arteries of t-ACE^{-/-} mice compared to t-ACE^{+/+} mice. NLA reduced these responses to the same extent in both strains. No changes in the responses to ACh were observed during and after pregnancy in both strains. The inhibitory action of NLA on shear stress-induced vasodilatation was more substantial in uterine arteries of late-pregnant t-ACE^{+/+} mice compared to t-ACE^{-/-} mice, suggesting enhanced release of nitric oxide in late pregnant t-ACE^{+/+} mice. The role of bradykinin in ACh and shear stress-induced vasodilatations was more pronounced in uterine arteries of late pregnant t-ACE^{-/-} mice. By 7 days after pregnancy, shear stress-induced vasodilatation was markedly blunted in uterine arteries of both mouse strains.

Conclusion. These observations confirm (1) the release of vasodilator concentrations of bradykinin by chemical and mechanical endothelium-dependent stimuli, and (2) the modulatory role of bradykinin during flow-induced dilatation of uterine arteries in situations of chronically altered uterine blood flow.

Introduction

The endothelium is a main determinant of vasomotor tone *in vivo*.^{43,110} Endothelium-dependent vasodilatation can be triggered by both chemical and mechanical stimuli. Endothelium-dependent agonists include bradykinin, which in turn can be released by the endothelium in response to an increase in wall shear stress.^{37,223} The physiological significance of endogenous bradykinin in endothelium-dependent vasodilatation largely remains to be established.

Endothelium-dependent vascular reactivity is susceptible to chronic modulation. Various cardiovascular diseases are associated with endothelial dysfunction.⁵³¹ In several cases this can be reversed by ACE inhibition and angiotensin type 1 (AT₁) receptor antagonists.^{36,444,481} On the other hand, chronic increases in local blood flow and estrogens have been found to enhance endothelium-dependent vasodilatation through an upregulation of endothelial nitric oxide synthase (eNOS).⁵⁵⁹ The latter processes contribute to the dramatic increase in uterine blood flow during pregnancy. The role of bradykinin in chronic modulation of endothelium-dependent vascular reactivity has not been elaborated in great detail.

We evaluated the contribution of endogenous bradykinin to acute and chronic modulation of agonist- and flow-induced arterial vasodilatation. To this end we studied uterine arteries before, during, and after pregnancy. We used both wild-type mice and mice deficient in tissue angiotensin-converting enzyme (t-ACE), which degrades bradykinin. Responses of isolated arteries were recorded in the absence and presence of the bradykinin B₂ receptor antagonist HOE 140 and the NOS inhibitor N^ω-nitro-L-arginine.

Methods

Animals

The generation of mice with C57Bl6/SV genetic background and lacking tissue-bound angiotensin converting enzyme (t-ACE^{-/-}) has been described by Esther and colleagues.¹⁴¹ Mice heterozygous for the mutated ACE allele (t-ACE^{+/-}) were mated to obtain mice homozygous for the mutated allele (t-ACE^{-/-}) and their wild-type littermates (t-ACE^{+/-}). All animals were housed in groups of 2 to 4 and had free access to standard food (SRMA-1210; Hope Farms, Woerden, the Netherlands) and tap water. Adult female mice (age 4-5 months) were used to study their vasculature; comparing t-ACE^{-/-} with t-ACE^{+/-}. In view of the infertility of male t-ACE^{-/-} mice, male t-ACE^{+/-} mice were used for mating virgin (in the remainder of this article referred as nonpregnant) t-ACE^{+/-} and t-ACE^{-/-} mice. All experiments were conducted according to institutional guidelines. Experiments were performed in nonpregnant, late pregnant (day 18 of first pregnancy), and postpartum (7 days after first delivery).

Preparation of Blood Vessels and Experimental Set-Up

After euthanasia (overdose of pentobarbital), the uterus was dissected and pinned out on a petridish (coated with a layer of silicon) filled with modified Krebs-Ringer buffer

(composition in mmol/L: 144 NaCl, 4.7 KCl, 2.5 CaCl₂, 1.2 MgSO₄, 1.2 KH₂PO₄, 14.9 HEPES, and 5.5 glucose, pH 7.4). Adipose- and connective tissue were carefully removed from the arteries. From every mouse a segment (3 mm) of the main uterine artery at midpoint of the uterine arcade was isolated. The artery was mounted in an arteriograph system (Living System Instrumentation, Burlington, VT, USA) as previously described.²²³

At the beginning of each experiment, the artery was incubated for 45 minutes at 80 mmHg. The integrity of the endothelium was evaluated by assessing the response to acetylcholine (1 μ mol/L) during phenylephrine (1 μ mol/L)-induced constriction. Arteries that dilated <60% of passive diameter (see below) were excluded.

Passive lumen diameter at 80 mmHg and medial cross-sectional area of fixed uterine arteries were determined as described previously.²²³ Flow-induced dilatation (FID) was studied by applying an intraluminal flow resulting in a wall shear stress of 5 dyne/cm² at an intraluminal pressure of 80 mmHg. Arteries were constricted either spontaneously via myogenic tone or in combination with the thromboxane analogue U46619 to a level of approximately 50-60% of the passive diameter. Flow-induced dilatation was calculated as follows:

$$FID = \frac{(D_{\text{dilatation}} - D_{\text{constriction}})}{(D_{\text{passive}} - D_{\text{constriction}})} \cdot 100\% \quad (D = \text{diameter in } \mu\text{m})$$

At the end of the experiment, intraluminal flow was stopped and a constriction returned. Agonist-induced endothelium-dependent vasodilator responses were evaluated by constructing a cumulative concentration-response curve (CRC) for acetylcholine (ACh, 0.01-10 μ M). Sensitivity ($\text{pD}_2 = -\log [\text{EC}_{50}]$) and the maximal response to the agonist (E_{max}) were determined by sigmoidal curve fitting of individual curves (Graphpad PrismTM 2.01 software).

FID was tested in the presence of either the NO synthase inhibitor N^ω-nitro-L-arginine (NLA, 30 μ mol/L), the bradykinin B₂ receptor antagonist HOE 140 (0.1 μ mol/L), or the combination of both drugs. For each FID experiment a different uterine artery segment was used.

Drugs

U46619 was purchased from Calbiochem (Darmstadt, Germany). Acetylcholine was purchased from Serva Feinbiochemica (Heidelberg, Germany). L-phenylephrine, norepinephrine, N^ω-nitro-L-arginine, sodium nitroprusside and HOE 140 were purchased from Sigma Chemical (St Louis, USA).

Statistical Analysis

Results are shown as means $\pm$ SEM. Significance of the differences between the different groups was determined by ANOVA. Means were compared by paired *t* test or student-Newman Keuls test. *P* < 0.05 was considered to denote statistical significance.

Results

Uterine Artery Structure

Uterine artery passive diameter at an intraluminal pressure of 80 mmHg is shown in Table 5.1. As observed previously,²²⁴ passive lumen diameters of uterine arteries of nonpregnant t-ACE^{-/-} mice are significantly wider compared to their wild-type counterparts. Pregnancy resulted in a significant outward remodeling of the uterine arteries in both strains, confirming our previous studies.^{223,224} This increase in internal diameter tended to be smaller in t-ACE^{-/-} mice compared to t-ACE^{+/+} mice ($\Delta 96 \mu\text{m}$ in t-ACE^{-/-} versus $\Delta 129 \mu\text{m}$ in t-ACE^{+/+}). Medial cross-sectional area of uterine arteries increased to the same extent in t-ACE^{+/+} (from 2.5 ± 0.3 to $5.5 \pm 0.6 \times 10^3 \mu\text{m}^2$) and t-ACE^{-/-} (from 2.9 ± 0.3 to $5.4 \pm 0.3 \times 10^3 \mu\text{m}^2$) mice. At 7 days postpartum, uterine artery structure had regressed to pre-pregnant values in both strains (Table 5.1).

Table 5.1. Lumen diameter (LD) and constrictor responses to phenylephrine (Phe) in uterine arteries of nonpregnant (NP), late- pregnant (LP) and 7 days post-partum (PP) wild-type (t-ACE^{+/+}) and tissue angiotensin-converting enzyme deficient (t-ACE^{-/-}) mice.

strain	state	Passive LD at 80 mmHg (μm)	LD in the presence of 1 $\mu\text{mol/L}$ Phe	constrictor response to 1 $\mu\text{mol/L}$ Phe (μm)	constrictor response to 1 $\mu\text{mol/L}$ Phe (%)
t-ACE ^{+/+}	NP	264 ± 5	110 ± 6	154 ± 6	58 ± 2
	LP	$393 \pm 10^*$	$156 \pm 10^*$	$237 \pm 10^*$	60 ± 2
	PP	300 ± 29	138 ± 25	162 ± 27	55 ± 7
t-ACE ^{-/-}	NP	$284 \pm 10 \dagger$	125 ± 8	159 ± 8	56 ± 2
	LP	$380 \pm 17^*$	$171 \pm 10^*$	$209 \pm 13^*$	55 ± 3
	PP	279 ± 33	179 ± 20	100 ± 20	45 ± 5

Values are shown as means $\pm$ SEM. * $P < 0.05$ versus NP, $\dagger P < 0.05$ versus t-ACE^{+/+}.

Uterine Artery Vasoconstriction

Phenylephrine (1 $\mu\text{mol/L}$) induced comparable constrictions in uterine arteries of t-ACE^{-/-} and t-ACE^{+/+} mice (Table 5.1). Absolute vasoconstriction (μm) in response to phenylephrine (1 $\mu\text{mol/L}$) was larger in uterine arteries of late pregnant mice compared to nonpregnant and postpartum mice, but relative vasoconstriction (%) was not modified by pregnancy (Table 5.1).

Dilator Responses to Acetylcholine

Responses to ACh (pD_2 and E_{max}) did not differ between uterine arteries of nonpregnant t-ACE^{-/-} and t-ACE^{+/+} mice (6.63 ± 0.09 and $96 \pm 1\%$ versus 6.78 ± 0.09 and $96 \pm 1\%$, respectively; Table 5.2). The bradykinin B₂-receptor antagonist HOE-140 (0.1 $\mu\text{mol/L}$) caused a rightward shift of the concentration-response curve and a significant reduction in the maximal vasodilatation (E_{max}) in uterine arteries of t-ACE^{-/-} mice (Figure 5.1A). This

latter effect was significantly larger in $t\text{-ACE}^{-/-}$ mice ($\Delta E_{\max}$ 14%) than in $t\text{-ACE}^{+/+}$ mice ($\Delta E_{\max}$ = 5%; Table 5.2). The nitric oxide synthase inhibitor nitro-L-arginine (NLA) reduced the responses to a larger extent by causing a significant blunting of the maximal response to ACh in uterine arteries of both strains (Table 5.2). The combination of HOE 140 and NLA had no additional inhibitory effect on the ACh-induced vasodilatation in uterine arteries of nonpregnant mice (Table 5.2).

The sensitivity and amplitude of ACh-induced vasodilations were similar in uterine arteries of late-pregnant mice of both strains, and did not differ in values from nonpregnant mice (Figure 5.2A and Table 5.2). However, HOE 140 had a marked inhibitory effect on the maximal response and sensitivity to ACh in uterine arteries of late-pregnant $t\text{-ACE}^{-/-}$ mice compared to late-pregnant $t\text{-ACE}^{+/+}$ mice (Figure 5.2A, Table 5.2). NLA had a comparable inhibitory effect in uterine arteries of $t\text{-ACE}^{+/+}$ and $t\text{-ACE}^{-/-}$ mice (Table 5.2). However, the presence of both HOE 140 and NLA seemed to have an additive inhibitory effect on the vasodilator responses to ACh in pregnant mice of both strains (Table 5.2).

Similar to nonpregnant and late-pregnant mice, the sensitivity and amplitude of ACh-induced vasodilations did not differ in uterine arteries of 7-days postpartum mice (Figure 5.3A and Table 5.1). Again, HOE 140 had a marked effect on both the maximal response and sensitivity to ACh in uterine arteries of $t\text{-ACE}^{-/-}$ mice, whereas it had almost no inhibitory effect in uterine arteries of $t\text{-ACE}^{+/+}$ mice (Figure 5.3A, Table 5.2). NLA reduced the responses to ACh to the same extent as observed in uterine arteries of nonpregnant mice (Table 5.2).

Dilator Responses to Shear Stress

Application of a shear stress stimulus (5 dyne/cm²) resulted in a dilatation, which tended to be larger in uterine arteries of $t\text{-ACE}^{-/-}$ mice (Figure 5.1B). HOE 140 reduced this shear stress-induced or flow-induced dilatation (FID) to a greater extent in uterine arteries of $t\text{-ACE}^{-/-}$ ($\Delta 42\%$) than $t\text{-ACE}^{+/+}$ ($\Delta 29\%$) mice (Figure 5.1B). NLA reduced FID to the same extent in uterine arteries of $t\text{-ACE}^{-/-}$ and $t\text{-ACE}^{+/+}$ mice (Figure 5.1B).

At term pregnancy the relative amplitude of FID was comparable in uterine arteries of both strains and comparable to that of nonpregnant mice (Figure 5.2B). The contribution of bradykinin to FID was significantly larger in UAs of $t\text{-ACE}^{-/-}$ as shown by the greater inhibitory effect of HOE 140 (Figure 5.2B). NLA had a far greater inhibitory effect on FID in uterine arteries of $t\text{-ACE}^{+/+}$ mice compared to $t\text{-ACE}^{-/-}$ mice (figure 5.2B).

FID in uterine arteries of postpartum mice was markedly reduced when compared to observations before and after pregnancy. Moreover, neither B₂ receptor antagonism nor NOS inhibition reduced the FID in uterine arteries of $t\text{-ACE}^{-/-}$ and $t\text{-ACE}^{+/+}$ mice at 7 days after pregnancy (Figure 5.3B).

Figure 5.1. Cumulative concentration-response curves of acetylcholine in uterine arteries under control conditions (CON; open symbols), and in the presence of 0.1 $\mu\text{mol/L}$ of the bradykinin B_2 -receptor antagonist HOE 140 (shaded symbols) from nonpregnant $t\text{-ACE}^{+/+}$ (circles) and $t\text{-ACE}^{-/-}$ (squares) mice. For clarity responses in the presence of the nitric oxide synthase blocker N^m -nitro-L-arginine were omitted (see Table 5.2) (A). Dilatation of uterine arteries in response to shear stress (5 dyne/cm^2) under control conditions (open bars), in the presence of HOE 140 (0.1 $\mu\text{mol/L}$; shaded bars), and N^m -nitro-L-arginine (NLA, 30 $\mu\text{mol/L}$; black bars) from nonpregnant $t\text{-ACE}^{+/+}$ and $t\text{-ACE}^{-/-}$ mice (B). Data are shown as means $\pm$ SEM. * $P < 0.05$ versus CON.

Figure 5.2. Cumulative concentration-response curves of acetylcholine in uterine arteries under control conditions (CON; open symbols), and in the presence of 0.1 $\mu\text{mol/L}$ of the bradykinin B_2 -receptor antagonist HOE 140 (shaded symbols) from late pregnant $t\text{-ACE}^{+/+}$ (circles) and $t\text{-ACE}^{-/-}$ (squares) mice. For clarity responses in the presence of the nitric oxide synthase blocker N^m -nitro-L-arginine were omitted (A). Dilatation of uterine arteries in response to shear stress (5 dyne/cm^2) under control conditions (open bars), in the presence of HOE 140 (0.1 $\mu\text{mol/L}$; shaded bars), and N^m -nitro-L-arginine (NLA, 30 $\mu\text{mol/L}$; black bars) from late-pregnant $t\text{-ACE}^{+/+}$ and $t\text{-ACE}^{-/-}$ mice (B). Data are shown as means $\pm$ SEM. * $P < 0.05$ versus CON.

Table 5.2. Sensitivity and maximal vasodilator responses to acetylcholine in uterine arteries of nonpregnant (NP), late pregnant (LP) and 7 days post-partum (PP) wild-type (t-ACE^{+/+}) and tissue angiotensin-converting enzyme deficient (t-ACE^{-/-}) mice.

Condition	parameter	t-ACE ^{+/+}			t-ACE ^{-/-}		
		NP	LP	PP	NP	LP	PP
Control	pD ₂	6.78 ± 0.09	6.47 ± 0.14	7.07 ± 0.09	6.63 ± 0.09	6.78 ± 0.16	6.71 ± 0.08
	E _{max}	96 ± 1	95 ± 1	96 ± 2	96 ± 1	94 ± 3	85 ± 5
0.1 μM HOE 140	pD ₂	6.60 ± 0.19	6.38 ± 0.14	7.13 ± 0.15	6.32 ± 0.19	6.21 ± 0.36	6.24 ± 0.17†
	E _{max}	91 ± 3	84 ± 5	95 ± 5	82 ± 4†	49 ± 8†	54 ± 11†
30 μM NLA	pD ₂	6.48 ± 0.21	6.20 ± 0.16	6.70 ± 0.32	6.70 ± 0.32	6.41 ± 0.14	5.91 ± 0.31
	E _{max}	77 ± 7†	72 ± 10†	81 ± 10	62 ± 14†	74 ± 8†	70 ± 14†
HOE 140 +NLA	pD ₂	6.69 ± 0.10	6.11 ± 0.10	ND	6.21 ± 0.10	5.75 ± 0.23	ND
	E _{max}	69 ± 10†	47 ± 4†	ND	69 ± 11†	32 ± 10†	ND

Values are shown as means±SEM. * *P*<0.05 versus NP, † *P*<0.05 versus control.

Figure 5.3. Cumulative concentration-response curves of acetylcholine in uterine arteries under control conditions (CON; open symbols), and in the presence of 0.1 μmol/L of the bradykinin B₂-receptor antagonist HOE 140 (shaded symbols) from 7-days postpartum t-ACE^{+/+} (circles) and t-ACE^{-/-} (squares) mice. For clarity responses in the presence of the nitric oxide synthase blocker N^ω-nitro-L-arginine were omitted (A). Dilatation of uterine arteries in response to shear stress (5 dyne/cm²) under control conditions (open bars), in the presence of HOE 140 (0.1 μmol/L; shaded bars), and N^ω-nitro-L-arginine (NLA, 30 μmol/L; black bars) from 7-days postpartum t-ACE^{+/+} and t-ACE^{-/-} mice (B). Data are shown as means±SEM.

Discussion

This study demonstrates that bradykinin can be released by the arterial wall via chemical (acetylcholine-induced) and mechanical (shear stress- or flow-induced) stimulation, confirming previous observations of the important role of the kallikrein-kinin system in flow-dependent arterial dilatation, through activation of bradykinin B₂-receptors coupled to endothelial NO release.^{37,223} Moreover, tissue angiotensin-converting enzyme (tissue ACE) was found to mask a contribution of bradykinin to acetylcholine-induced vasodilatation, and the role of bradykinin seemed to be modified during chronically altered local blood flow occurring during and after pregnancy.

We hypothesized that the contribution of bradykinin to flow-induced dilatation would be more pronounced in uterine arteries from t-ACE^{-/-} mice, since angiotensin-converting enzyme degrades bradykinin into smaller inactive peptides. The reason why we used late-pregnant and postpartum mice is twofold. Firstly, in parallel studies we studied the role of the kallikrein-kinin system and the renin-angiotensin system in uterine arterial structural responses to chronically increased blood flow during gestation and to a reduction in local blood flow after birth (7 days after pregnancy).^{223,224} Secondly, in settings of chronically elevated blood flow arterial flow-induced dilatation has been shown to be up-regulated, mainly by endothelium-derived vasoactive agents such as nitric oxide and prostacyclin.⁴⁹²

Responses to acetylcholine were similar in uterine arteries of wild-type and t-ACE^{-/-} mice, indicating that the release of endothelium-derived factors is preserved despite deficiency of tissue ACE. The presence of the bradykinin B₂ receptor antagonist HOE-140 caused a significant reduction in the amplitude of vasodilatation only in uterine arteries of t-ACE^{-/-} mice. This suggests that tissue ACE masks a contribution of bradykinin to acetylcholine-induced vasodilatation.

Uterine arteries responded to shear stress (5 dyne/cm²) by vasodilatation. Flow-induced dilatation tended to be higher in uterine arteries of t-ACE^{-/-} mice compared to their wild-type counterparts and blockade of bradykinin B₂-receptors reduced flow-induced dilatation to a larger extent in uterine arteries of t-ACE^{-/-} mice. This indicates that tissue ACE interferes with the function of shear stress-induced bradykinin release, probably by breakdown of the peptide. These observations furthermore confirm the important role of bradykinin as a shear stress-sensitive endothelial autocrine factor.^{37,223}

Pregnancy is known to increase uterine arterial nitric oxide production in rats⁹⁸ and humans.³⁹¹ Expression of endothelial nitric oxide synthase (eNOS) mRNA and protein are increased in ovine pregnancy,³³⁷ as a result of of estrogen-, vascular endothelial growth factor-, and shear stress-induced upregulation of eNOS.^{521,559,457} In both t-ACE^{+/+} and t-ACE^{-/-} mice, pregnancy was not accompanied by altered relative amplitudes of the dilator responses to acetylcholine. There are conflicting reports in the literature stating that acetylcholine-induced vasodilatation of pre-constricted uterine arteries can either be increased^{390,393,581} or unchanged.^{278,354} Methodological differences may account for the observed inconsistencies. For instance, the experimental set-up of the pressure-myograph (arteriograph) differs from the wire-myograph. Diameter changes are recorded in the arteriograph, whereas contractile forces, dependent on the arterial wall mass, are measured in the wire-myograph. In addition, the level of pre-constriction prior to acetylcholine

administration is not always similar in these studies. However, the nature of the endothelium-dependent dilator responses was clearly altered in pregnant mice. In vessels of late pregnant t-ACE^{+/+} mice, the inhibitory effect of N^ω-nitro-L-arginine on both agonist- and shear stress-induced vasodilatation was larger when compared to responses in nonpregnant t-ACE^{+/+} mice, suggesting that more nitric oxide is released in uterine arteries of late pregnant mice in response to acetylcholine, confirming previous observations by our group.²²³ In uterine arteries from late pregnant t-ACE^{-/-} mice, however, bradykinin seemed to replace the role of nitric oxide, since HOE 140 caused a more than 50% inhibition of the acetylcholine-mediated vasodilatation, which was only marginally reduced by N^ω-nitro-L-arginine. The same trend was observed during shear stress-induced vasodilatation in uterine arteries from late pregnant t-ACE^{-/-} mice. These observations suggest that during late pregnancy tissue ACE is upregulated, reducing the contribution of endogenous bradykinin.

Flow-induced dilatation was markedly reduced in uterine arteries of 7 days postpartum mice, despite normal acetylcholine-induced vasodilatation, suggesting a reduced shear stress sensing by the endothelium in these arteries.²²³ No clear role of bradykinin and nitric oxide were observed in these arteries.

Taken together, we confirm the important role of bradykinin as a vasoactive agent that can be released by the endothelial cells as an autocrine factor via agonist and shear stress. This is more pronounced during deficiency of tissue ACE. During physiologically increased blood flow (at term pregnancy) the role of bradykinin seems to be masked by an upregulation of nitric oxide and ACE in wild-type uterine arteries. This in contrast to t-ACE^{-/-} mice, in which the enhanced bradykinin levels appear to be sufficient for the flow-induced vasodilatation.

Chapter 6

Changes in Vascular Distensibility During Angiotensin-Converting Enzyme Inhibition Involve Bradykinin Type 2 Receptors

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Abstract

Objective. Changes in arterial elasticity and structure occur during cardiovascular diseases and can be modified by angiotensin-converting enzyme (ACE) inhibitors. In the present study we investigated the role of tissue-bound ACE (t-ACE) in the regulation of arterial morpho-mechanical properties.

Methods and Results. Large and small arteries of t-ACE^{-/-} mice were isolated to determine the passive pressure-diameter relationship. We observed that arteries of t-ACE^{-/-} mice exhibit a reduced arterial distensibility compared to t-ACE^{+/+}. This reduced arterial distensibility was also observed after 9 weeks of captopril treatment (80 mg/kg/day). We hypothesized that bradykinin type 2 receptor (B₂) stimulation might be involved in the regulation of arterial elasticity. t-ACE^{-/-} and t-ACE^{+/+} mice were treated with the B₂ antagonist HOE 140 (1 mg/kg/day) for 14 days. After HOE 140 treatment, both the structural and mechanical changes observed in the t-ACE^{-/-} carotid artery were abolished. Although HOE 140 administration increased blood pressure in both groups by approximately 10 mmHg, the pressure difference between the two groups was similar.

Conclusion. Tissue-ACE is involved in the regulation of arterial distensibility. The changes observed in t-ACE^{-/-} mice are not caused by an altered fetal development. Moreover, it is likely that the regulation of arterial distensibility by ACE involves stimulation of the B₂ receptor.

Introduction

In cardiovascular diseases like hypertension,^{316,453,467} myocardial infarction,³⁴ heart failure,^{193,374} and atherosclerosis^{83,342,570} the arterial structure and arterial mechanics are altered. Since several of these alterations are reduced or prevented by angiotensin-converting enzyme (ACE) inhibition^{237,371,316,453} or angiotensin type 1 receptor antagonism,⁴¹⁵ the involvement of the renin angiotensin system (RAS) in the regulation of arterial structure and mechanics has been suggested.

ACE is one of the central enzymes of the RAS and is responsible for the production of angiotensin II. In the cardiovascular system, ACE appears as a circulating enzyme in the blood and as an ecto-enzyme attached to the cell membrane. The importance of its position on the cell membrane for the regulation of blood pressure and renal function has been demonstrated in mice lacking the membrane- or tissue-bound ACE (t-ACE).¹⁴¹ Moreover, lack of t-ACE changed the remodeling of pulmonary arteries during chronic hypoxia.⁵⁶⁸ We hypothesized that ACE and especially t-ACE is involved in the regulation of vascular characteristics. To test this hypothesis we investigated the structure and mechanics of the thoracic aorta, common carotid artery and first-order mesenteric resistance artery of mice exhibiting only the N-terminal part of ACE, which is capable of proteolytic cleavage but incapable of binding to the cell-membrane (t-ACE^{-/-}). Arterial characteristics determined *in vitro* were compared to those of wild-type (t-ACE^{+/+}) mice. To evaluate whether the lack of t-ACE during pre- and postnatal development results in altered adult arterial characteristics, we included a second experimental group. t-ACE^{+/+} mice were treated with the ACE inhibitor captopril from 3 to 12 weeks after birth and their arterial characteristics were compared to arteries from untreated t-ACE^{+/+} mice. ACE is, apart from a prominent component of the RAS, also a component of the kallikrein-kinin system and responsible for the degradation of bradykinin. During ACE inhibition both the production of angiotensin II and the degradation of bradykinin are reduced.

We hypothesized that stimulation of the bradykinin type 2 (B₂) receptor might play an important role in the arterial alterations that occur during ACE inhibition. The involvement of the B₂ receptor in the arterial morpho-mechanical alterations during ACE inhibition was investigated by comparing the arterial structure and elasticity of t-ACE^{+/+} and t-ACE^{-/-} mice with or without chronic (14 days) treatment with the B₂ receptor antagonist HOE 140.

Materials and Methods

Animals

Mice Lacking t-ACE. The generation of mice with a C57Bl6/129/SV genetic background and lacking t-ACE has been described by Esther and colleagues.¹⁴¹ Mice heterozygous for the mutated allele (t-ACE^{+/-}) were bred to obtain mice homozygous for the mutated allele (t-ACE^{-/-}) and their wild-type littermates (t-ACE^{+/+}). To genotype pups, genomic DNA was isolated from the tail using the Qiagen DNA isolation kit (Qiagen, Hilden Germany). Ready-to-go PCR beads (Amersham Pharmacia Biotech) were used for PCR analyses on

genomic DNA. The three primers used are described by Esther and colleagues.¹⁴¹ Age-matched adult (6-to 12-month old) female mice (t-ACE^{+/+} and t-ACE^{-/-}) were used to investigate the effects of t-ACE deficiency on the peripheral arterial structure and mechanics.

Captopril-Treated Mice. Male and female wild-type mice at the age of 3 weeks had access to either tap water or captopril dissolved in tap water (80 mg/kg/day, Sigma-Aldrich, Steinheim, Germany) until they reached the age of 12 weeks. Female mice were used to investigate the effects of postnatal ACE inhibition. Male mice were used to determine conscious blood pressure.

HOE 140-Treated Mice. At the age of 12 weeks mice were anesthetized with ketamine (50 mg/kg i.m.) and xylazine (5 mg/kg s.c.) and osmotic minipumps (Alzet 2002; Alza, Palo Alto, CA, USA) filled with HOE 140 (1 mg/kg/day, Sigma-Aldrich, Steinheim, Germany) dissolved in saline were subcutaneously implanted at the back of the neck. Mice were either untreated or treated with HOE 140 for 14 days.

Blood Pressure Measurement

Conscious blood pressure was determined in untreated, captopril-treated or HOE 140-treated male t-ACE^{+/+} and t-ACE^{-/-} mice. At 4 days before the end of treatment, mice were anaesthetized with pentobarbital (110 mg/kg) and placed on a heating pad to maintain body temperature at 37 °C to implant the catheters. One saline-filled catheter (PE-25) was placed in the abdominal aorta via the femoral artery to record systemic arterial pressure; a second saline-filled catheter (PE-10) was placed in the jugular vein for infusions. Both catheters were subcutaneously tunneled to the neck and fixed.

Four days after surgery, the arterial catheter was connected to a pressure transducer (Micro-Switch, model 156PC 156WL, Honeywell, Amsterdam, the Netherlands). Blood pressure signals were recorded and 10 min of stable recording were used to determine mean arterial pressure (MAP) at rest. To determine ACE inhibition in captopril-treated mice, angiotensin I and II (0-35 ng, dissolved in saline) were cumulatively infused via the venous catheter while measuring the MAP. Each dose was injected at the time of maximal MAP to avoid tachyphylaxis. Dose-response curves for angiotensin I and II were compared to untreated mice. In HOE 140-treated mice, blockade of the B₂ receptor was established via a bolus injection (i.v.) of bradykinin (25 ng)¹⁹ dissolved in 10 µl of sterile water while recording MAP.

Pressure-Diameter Curves

3- to 4-mm segments of first-order mesenteric resistance arteries were isolated. Arteries were transferred to an organ chamber (arteriograph system, Living Systems Instrumentation, Burlington, VT, USA). One end of the segment was mounted onto the proximal glass micropipette. After flushing the artery with calcium-free modified *Krebs-Ringer* buffer (composition in mmol/L: 144 NaCl, 4.7 KCl, 1.2 MgSO₄, 1.2 KH₂PO₄, 14.9 HEPES and 5.5 glucose, pH 7.4) the distal part of the segment was closed with a nylon knot. 10 µmol/L Na-nitroprusside (SNP) was added to the organ chamber to assure maximal vasodilatation. The cannulated arterial segment was pressurized using a feedback-controlled pressure source (Living Systems Instrumentation) and placed under a binocular lens connected to a video camera for recording of arterial diameter. A pressure-diameter

(P-D) relationship was established by recording the diameter during stepwise (10 mmHg) increases in intraluminal pressures from 20 to 140 mmHg. After each step the vessel was allowed to equilibrate until a stable diameter was reached.

A similar procedure was followed for the thoracic aorta and common carotid artery. However, these arteries were only cannulated at one end while the other end was closed (blind sac). Due to the intransparency of the thoracic aorta and carotid artery, outer diameters were recorded. For t-ACE^{-/-} and t-ACE^{+/+} mice, the thoracic aorta, carotid and mesenteric resistance artery were used, while the carotid and mesenteric resistance artery were used from untreated t-ACE^{+/+} and t-ACE^{+/+} mice treated with captopril. In untreated t-ACE^{+/+} and HOE 140 treated t-ACE^{+/+} mice, the P-D relationship of the carotid artery was determined.

Morphometry

Vessels were formalin-fixed at 80 mmHg in the arteriograph. Fixed vessels were embedded in paraffin and cross-sections (4 μ m) were stained with Lawson's solution (Boom, Meppel, the Netherlands) to visualize the internal and external elastic laminae. Video images were made from cross-sections using a Zeiss axioscope and a standard charge-coupled device camera (Sony). Using JAVA software (JAVE 1.21, Jandel Scientific, Corte Madera, CA, USA) the circumference of the internal and external elastic laminae were determined. From these values the media cross-sectional area (mCSA) was calculated. To obtain the elastin/collagen ratio, cross sections were deparaffinised and incubated in phosphomolybdic acid (0.2%) for 5 minutes, followed by incubation with Sirius Red (0.1%) in saturated picric acid solution for 90 minutes. After washing with 0.1 M HCl for 2 minutes, sections were dehydrated and protected with coverslips.¹²⁸ Collagen content was measured at 400X magnification in the aortic media (JAVA 1.21, Jandel Scientific).

Calculation of Parameters

Arterial distensibility or the relative change in arterial lumen volume induced by a given increase in intraluminal pressure was estimated by: $DC = \Delta D_n / ((D_{n-1})/2) \cdot \Delta P$ (DC = distensibility; D_n = outer diameter at a given intraluminal pressure; P = intraluminal pressure).⁸⁵ For the mesenteric resistance artery circumferential wall stress and incremental elastic modulus were also calculated.⁴³⁶ Circumferential wall stress (σ) is related to wall thickness (h) and wall tension (T): $\sigma = T/h$. According to Laplace's Law, T depends upon transmural pressure (P_t) and radius (r): $T = P_t \cdot r$. Incremental elastic modulus (E_{inc}) was calculated according to Bergel³⁸: $E_{inc} = 1.5 \tau_o^2 \tau_i \cdot \Delta P / (r_o^2 - r_i^2) \cdot \Delta r_i$, which derives from $0.75 \cdot D/h \cdot DC$, where r_o = outer radius; r_i = inner radius, and D = outer diameter.

Statistics

Data are expressed as mean $\pm$ SEM. A Student t-test was used to test the impact of the genetic modification, captopril or HOE 140 treatment on blood pressure. The impact of the genetic modification, captopril or HOE 140 treatment was tested with a two-way

ANOVA for repeated measurements in case of the P-D curves. $P < 0.05$ was considered statistically significant.

Results

General Observations

Age-matched adult t-ACE^{-/-} mice (male and female) were significantly lower in body weight compared to t-ACE^{+/+} mice (Table 6.1 and 6.2). Also body weight of young t-ACE^{+/+} (both sexes) mice treated with captopril for 9 weeks was significantly lower than untreated t-ACE^{+/+} mice. In male t-ACE^{-/-} and t-ACE^{+/+} mice treated with HOE 140 for 14 days abolished the difference in body weight.

Hypotension (80 ± 3 versus 110 ± 4 mmHg, Table 6.1) was observed in t-ACE^{-/-} mice. HOE 140 treatment increased MAP with 10 mmHg in both t-ACE^{-/-} and t-ACE^{+/+} mice without changing the blood pressure difference of approximately 30 mmHg between the two groups. Heart rate was unaffected by t-ACE deficiency or antagonist treatment.

Table 6.1. General parameters in male t-ACE^{+/+} and t-ACE^{-/-} mice, untreated or treated with captopril or HOE 140

	t-ACE ^{+/+}	t-ACE ^{-/-}	t-ACE ^{+/+}	t-ACE ^{+/+}	ACE ^{+/+}	t-ACE ^{-/-}
				Captopril	HOE 140	HOE 140
Gender	male	male	male	male	male	male
Age	6-12 mo.	6-12 mo.	12-14 wks	12-14 wks	12-14 wks	12-14 wks
Number	7	7	7	5	6	5
Body weight (g)	30 ± 1	26 ± 2 *	28 ± 1	33 ± 1 †	26 ± 1	26 ± 1
MAP (mmHg)	110 ± 4	80 ± 3 *	111 ± 2	80 ± 7 †	120 ± 5	87 ± 8 ‡
Heart rate (bpm)	662 ± 14	657 ± 19	550 ± 36	600 ± 48	673 ± 32	612 ± 50

* $P < 0.05$ versus 6-12 mo. t-ACE^{+/+}; † $P < 0.05$ versus 12-14 wks t-ACE^{+/+}; ‡ $P < 0.05$ versus HOE 140-treated t-ACE^{+/+}.

Pressure-Diameter Curves

Pressure-Diameter (P-D) curves and calculated arterial distensibility of the thoracic aorta (TA), common carotid artery (CA), and first-order mesenteric resistance artery (MrA) of adult age-matched female t-ACE^{-/-} and t-ACE^{+/+} mice are shown in Figure 6.1. CA and MrA of young untreated and captopril-treated t-ACE^{+/+} female mice were used to construct P-D curves and to (Figure 6.2). The P-D curves of the TA and the CA show a typical S-shape comparable to the curves described by Bergel obtained in the TA (blind sac).³⁸ The P-D curves of the MrA were constructed in a situation of fixed length and show a typical progressive decrease of distensibility.³⁸ In t-ACE^{-/-} vessels the maximal diameter was reached at a lower pressure compared to t-ACE^{+/+} vessels. This is reflected in the distensibility, which is shifted to the left in the case of the TA and CA, resulting in a significantly lower distensibility between 80 and 120 mmHg. The distensibility of the MrA was consistently lower in t-ACE^{-/-} mice.

After prolonged captopril treatment (80 mg/kg/day) a comparable effect on the MrA distensibility was observed in t-ACE^{-/-} arteries. Figure 6.3 shows the incremental

elastic modulus (E_{inc}) as a function of the circumferential wall stress for both the t-ACE^{-/-} and t-ACE^{+/+} and the untreated t-ACE^{+/+} and captopril-treated t-ACE^{+/+} arteries. E_{inc} was only calculated for the MrA because wall thickness of the TA and CA could not be visualized. In both MrA of t-ACE^{-/-} and captopril-treated mice, E_{inc} was elevated compared to the MrA of t-ACE^{+/+} mice.

A reduced distensibility was not only seen in CA from female mice, but also CA of male t-ACE^{-/-} mice showed a reduced distensibility compared to CA of t-ACE^{+/+} male mice (Figure 6.4). After treating t-ACE^{+/+} mice with HOE 140 (1 mg/kg/day) for 14 days, no difference in the P-D curves could be observed (Figure 6.4A/B). In t-ACE^{-/-} mice, HOE 140 treatment changed the P-D curve of the CA (Figure 6.4A/B) and distensibility curves (Figure 6.4C/D); both curves were shifted towards the t-ACE^{+/+} curves. HOE 140 treatment had no effect on the distensibility curves of the CA of t-ACE^{+/+} mice (Figure 6.4C/D).

Table 6.2. Morphometry of vessels fixed at 80 mmHg

Genotype	Gender	Age	BW (g)	n	media CSA ($\times 10^3 \mu m^2$)	Radius (μm)	Mt (μm)	W:L $\times 100$
<i>Thoracic Aorta</i>								
t-ACE ^{+/+}	female	6-12 mo.	28 $\pm$ 1	6	123 $\pm$ 8	454 $\pm$ 6	41 $\pm$ 2	9.0 $\pm$ 0.5
t-ACE ^{-/-}	female	6-12 mo.	23 $\pm$ 1 *	5	91 $\pm$ 1 *	437 $\pm$ 17	32 $\pm$ 1 *	7.4 $\pm$ 0.6
<i>Carotid Artery</i>								
t-ACE ^{+/+}	female	6-12 mo.	28 $\pm$ 1	5	20 $\pm$ 2	212 $\pm$ 6	14 $\pm$ 1	6.6 $\pm$ 0.3
t-ACE ^{-/-}	female	6-12 mo.	23 $\pm$ 1 *	5	19 $\pm$ 2	209 $\pm$ 5	14 $\pm$ 1	6.6 $\pm$ 0.7
t-ACE ^{+/+} untreated	female	12-14 wks	22 $\pm$ 1	7	17 $\pm$ 1	192 $\pm$ 3	14 $\pm$ 1	7.1 $\pm$ 0.6
t-ACE ^{+/+} captopril	female	12-14 wks	19 $\pm$ 1 **	8	12 $\pm$ 1 **	199 $\pm$ 5	9 $\pm$ 1 **	4.8 $\pm$ 0.6 **
t-ACE ^{+/+}	male	12-14 wks	28 $\pm$ 1	6	20 $\pm$ 1	202 $\pm$ 5	15 $\pm$ 1	7.6 $\pm$ 0.1
t-ACE ^{-/-}	male	12-14 wks	23 $\pm$ 1 *	6	14 $\pm$ 1 *	196 $\pm$ 1	11 $\pm$ 1 *	5.6 $\pm$ 0.3 *
t-ACE ^{+/+} HOE 140	male	12-14 wks	26 $\pm$ 1	7	20 $\pm$ 3	208 $\pm$ 15	15 $\pm$ 1	7.6 $\pm$ 0.1
t-ACE ^{-/-} HOE 140	male	12-14 wks	26 $\pm$ 1	5	17 $\pm$ 1	191 $\pm$ 4	14 $\pm$ 1	7.2 $\pm$ 0.7
<i>Mesenteric Artery</i>								
t-ACE ^{+/+}	female	6-12 mo.	28 $\pm$ 1	7	3.2 $\pm$ 0.3	108 $\pm$ 6	4.6 $\pm$ 0.3	4.3 $\pm$ 0.2
t-ACE ^{-/-}	female	6-12 mo.	23 $\pm$ 1 *	7	4.0 $\pm$ 0.5	129 $\pm$ 8	4.7 $\pm$ 0.4	3.1 $\pm$ 0.5
t-ACE ^{+/+} untreated	female	12-14 wks	22 $\pm$ 1	6	2.4 $\pm$ 0.4	80 $\pm$ 4	4.6 $\pm$ 0.6	5.8 $\pm$ 0.6
t-ACE ^{+/+} captopril	female	12-14 wks	19 $\pm$ 1 **	9	2.3 $\pm$ 0.1	93 $\pm$ 3 **	3.9 $\pm$ 0.1	4.3 $\pm$ 0.2 **

Abbreviations used: BW:body weight; CSA: cross-sectional area; Mt: media thickness; W:L: wall-to-lumen ratio. * $P < 0.05$; ** $P < 0.01$ versus untreated age- and sex-matched controls.

Morphometry

Four parameters of arterial structure were determined and are presented in Table 6.2. In adult female t-ACE^{-/-} mice no significant differences in CA and MrA structure were found compared to t-ACE^{+/+}. In the TA of t-ACE^{-/-} mice, media cross-sectional area (CSA) and medial thickness (Mt) were significantly reduced but did not result in a significantly lower wall-to-lumen ratio (W:L).

Figure 6.1. Relationship between imposed intraluminal pressure and diameter in isolated thoracic aorta (A), carotid artery (C), and mesenteric resistance artery (E) from $t\text{-ACE}^{+/+}$ (open symbols) and $t\text{-ACE}^{-/-}$ (closed symbols) mice. Calculated distensibility for the thoracic aorta (B), carotid artery (D), and mesenteric resistance artery (F). * $P < 0.05$ indicates significant difference between $t\text{-ACE}^{+/+}$ and $t\text{-ACE}^{-/-}$ mice.

Prolonged treatment with captopril resulted in a significant reduction in CSA and Mt in the CA, while the radius was unchanged. The opposite effect was observed in the MrA: the radius was enlarged without changing CSA and Mt, resulting in a reduced W:L in both the CA and MrA. A reduced W:L caused by a smaller CSA and MT without an altered radius was also observed in the CA of young male *t*-ACE^{-/-} mice. After 14 days of HOE 140 treatment the W:L of the CA of male *t*-ACE^{-/-} mice normalized.

Composition of the arterial wall was determined by measuring the collagen and elastin content. Because of the small vessel size reliable values for collagen and elastin percentages could only be obtained from aortic sections. The percentages of collagen (12 ± 1 versus 12 ± 1 %) and elastin (49 ± 2 versus 49 ± 2 %) were similar in TA of *t*-ACE^{-/-} and *t*-ACE^{+/+} mice.

Figure 6.2. Relationship between imposed intraluminal pressure and diameter in isolated carotid arteries (A) and mesenteric resistance arteries (C) from *t*-ACE^{+/+} (open symbols) and captopril-treated *t*-ACE^{+/+} (closed symbols) mice. Calculated distensibility for the carotid artery (B) and mesenteric resistance artery (D). * $P < 0.05$ indicates significant difference between captopril-treated and untreated *t*-ACE^{+/+} mice.

Discussion

In the present study the effects of t-ACE deficiency,¹⁴¹ pharmacological ACE inhibition and antagonism of B₂ receptors on the structure and mechanics of systemic arteries were investigated. t-ACE deficiency reduced distensibility of large and small vessels in male and female mice. Similar effects were obtained in large and small vessels isolated from t-ACE^{+/+} mice treated with captopril for 9 weeks. Since in these animals treatment was started after weaning, these data suggest that the vascular alterations found in t-ACE^{-/-} mice are not due to t-ACE deficiency during fetal development. Whether stimulation of B₂ receptors plays a significant role in the regulation of vascular distensibility was investigated by treating both t-ACE^{-/-} and t-ACE^{+/+} mice with the B₂ receptor antagonist HOE 140 for 14 days. The results show that the reduced distensibility observed in t-ACE^{-/-} mice could be abolished by blocking B₂ receptors.

The pressure-diameter curves of the thoracic aorta, carotid and mesenteric resistance artery of t-ACE^{-/-} were different from those of t-ACE^{+/+} mice. The maximal diameter in t-ACE^{-/-} arteries was reached at a lower pressure compared to t-ACE^{+/+} arteries. This indicates a reduced distensibility in all three artery types. The calculated distensibility curves of the large arteries showed a significant leftward shift leading to a reduction in distensibility between 80-120 mmHg. The distensibility of the mesenteric resistance artery of t-ACE^{-/-} mice was consistently lower than the distensibility of the mesenteric resistance artery of t-ACE^{+/+} mice.

Since t-ACE^{-/-} mice are lacking t-ACE during the critical phase of fetal development, in which many RAS components, including ACE, are highly expressed,^{331,486} the reduced vascular distensibility might be the result of an altered fetal development. To investigate this hypothesis, t-ACE^{+/+} mice were treated with captopril from 3 to 12 weeks of age and their vascular distensibility was compared to untreated t-ACE^{+/+} mice.

Figure 6.3. Calculated incremental elastic modulus (E_{inc}) of the mesenteric resistance artery plotted against the circumferential wall stress (CWS) determined in t-ACE^{+/+} (open symbols) and t-ACE^{-/-} (closed symbols) mice (A). Calculated incremental elastic modulus (E_{inc}) of the mesenteric resistance artery plotted against the circumferential wall stress (CWS) determined in captopril-treated (closed symbols) and untreated (open symbols) t-ACE^{+/+} mice (B).

Effectiveness of the captopril treatment is demonstrated by blood pressure reduction. Moreover, an inhibited blood pressure increase after the infusion of angiotensin I was observed in $t\text{-ACE}^{-/-}$ mice, while the pressor response to angiotensin II remained unchanged (data not shown). After prolonged captopril treatment, a comparable reduction in distensibility was observed in the carotid and mesenteric resistance artery as observed in $t\text{-ACE}^{-/-}$ mice. These results suggest that the reduced vascular distensibility observed in $t\text{-ACE}^{-/-}$ mice is not due to an altered fetal development.

Figure 6.4. Relationship between imposed intraluminal pressure and diameter in isolated carotid arteries from $t\text{-ACE}^{+/+}$ (open symbols) and $t\text{-ACE}^{-/-}$ (closed symbols) mice (**A**). Relationship between imposed intraluminal pressure and diameter in isolated carotid arteries from $t\text{-ACE}^{+/+}$ (open symbols) and $t\text{-ACE}^{-/-}$ (closed symbols) HOE 140-treated mice (**B**). Calculated distensibility for carotid arteries from $t\text{-ACE}^{+/+}$ (open symbols) and $t\text{-ACE}^{-/-}$ (closed symbols) mice (**C**). Calculated distensibility for carotid arteries from $t\text{-ACE}^{+/+}$ (open symbols) and $t\text{-ACE}^{-/-}$ (closed symbols) HOE 140-treated mice (**D**). * $P < 0.05$ indicates significant difference between $t\text{-ACE}^{+/+}$ and (untreated) $t\text{-ACE}^{-/-}$ mice.

Since the elastic properties of the arteries are tested *in vitro* without flow or vascular tone, the decreased distensibility is directly related to the vessel wall composition.^{38,467} The elastic properties of maximally relaxed arteries depend on vessel wall components and wall geometry. Morphometric analysis of arteries from adult female t-ACE^{-/-} mice showed that only the medial CSA, Mt, and W:L of the thoracic aorta were decreased, while the morphometry of the carotid artery and mesenteric resistance artery was unaltered. In the captopril-treated female mice, medial CSA, Mt, and W:L were reduced in the carotid artery, while the radius of the mesenteric resistance artery was significantly wider compared to the untreated artery, resulting in a reduced W:L.

By calculating the incremental elastic modulus (E_{inc}), or the relationship between strain and stress, changes in the vessel wall morphometry, which affect vascular distensibility, are taken into account. An increased E_{inc} was observed in both t-ACE^{-/-} and captopril-treated mesenteric resistance arteries. Although wall thickness of the carotid artery and thoracic aorta could not be visualized, it was determined after fixation at 80 mmHg. Medial CSA and Mt of the thoracic aorta were reduced in the absence of t-ACE. The morphometry of the carotid artery was similar in both t-ACE^{-/-} and t-ACE^{+/+} female mice. In captopril-treated mice and male t-ACE^{-/-} mice, the carotid artery medial CSA and Mt (and hence Wt) were reduced without a change in the radius. In all arteries distensibility (DC) was reduced between 80 and 120 mmHg. When DC is decreased and Wt is either equal or reduced, $E_{inc} (D/WT \cdot DC)$ is increased. This implies that E_{inc} is increased in all tested arteries of t-ACE^{-/-} mice compared to arteries of t-ACE^{+/+} mice and in the captopril-treated carotid and mesenteric resistance arteries compared to the untreated carotid and mesenteric resistance artery. This increased E_{inc} reflects an increase in arterial stiffness.

These results suggest that ACE inhibition induces arterial stiffness, which seems to be paradoxical when compared to previous studies. Under several pathological circumstances such as hypertension,^{319,467} myocardial infarction,³⁴ and atherosclerosis,⁸³ ACE inhibition enhances vascular compliance. The first remark that should be made by comparing the results of the present study with previous observations is that the majority of these studies were performed in a situation of cardiovascular disease, in which elevated RAS activity is likely. In the present study, the effects of either t-ACE deficiency or prolonged ACE inhibition on the arterial compliance were investigated without obvious signs of cardiovascular disease. Notable are the studies by Baumbach and colleagues on cerebral arterioles in which they demonstrate that overexpression of human renin and angiotensinogen induces hypertrophy and an increased distensibility of these vessels.²⁹ The opposite was demonstrated in spontaneously hypertensive rats: hypertension decreased arterial stiffness, which could be abolished by the administration of an ACE inhibitor.²⁰⁶ These results are comparable to the increased arterial stiffness observed in the present study. A second remark concerns the fact that *in vitro* distensibility could not directly be extrapolated to *in vivo* distensibility.⁴⁶⁷ *In vivo*, at least two additional factors should be taken into account: the operating pressure and the vascular tone,^{467,468} both influenced by angiotensin II and bradykinin.

To investigate the role of the B₂ receptor in the vascular distensibility changes observed during ACE inhibition, the pressure-diameter curves of the carotid artery of untreated and HOE 140-treated male t-ACE^{-/-} and t-ACE^{+/+} mice were compared. As in

female t-ACE^{-/-} mice, the pressure-diameter curve of the carotid artery of male t-ACE^{-/-} mice reached its maximal diameter at a lower pressure than the carotid artery from t-ACE^{+/+} mice. Thus, in both male and female t-ACE^{-/-} mice, vascular distensibility is reduced between 80 and 120 mmHg. A decreased distensibility accompanied by a reduced wall thickness while the diameter was unaltered will lead to an increased E_{inc} . Reduced carotid artery wall thickness in t-ACE^{-/-} mice was not observed in female t-ACE^{-/-} mice. This discrepancy might be due to the difference in age. Male t-ACE^{-/-} and t-ACE^{+/+} mice were treated with HOE 140. A bolus injection of bradykinin (25 ng),¹⁹ which reduced blood pressure by approximately 30 mmHg in untreated mice, did not affect blood pressure in HOE 140-treated t-ACE^{-/-} and t-ACE^{+/+} mice (data not shown), which indicates that the B₂ receptors were effectively blocked. After 14 days of treatment with HOE 140 the difference in the pressure-diameter and distensibility curves of carotid arteries from t-ACE^{-/-} and t-ACE^{+/+} mice was no longer observed. The distensibility curve of carotid arteries from t-ACE^{-/-} mice was significantly shifted towards the distensibility curves of their wild-type counterparts. Morphometric analysis of carotid arteries from the treated t-ACE^{-/-} and t-ACE^{+/+} mice revealed that the vascular geometry of the treated vessels was similar. Normalization of the vascular geometry could be related to the normalization in body weight after HOE 140 treatment. Alterations in body weight and glucose metabolism during ACE inhibition have been described previously and the effects on insulin and glucose metabolism have been attributed to the elevated bradykinin levels.^{216,217} After HOE 140 treatment, both the reduction in carotid artery distensibility and wall thickness were restored. Thus, the increased E_{inc} observed in arteries of t-ACE^{-/-} mice can be abolished by 14 days treatment with HOE 140.

As in young rats, the blood pressure is affected by HOE 140 treatment resulting in an increase of approximately 10 mmHg compared to the blood pressure values for untreated t-ACE^{-/-} and t-ACE^{+/+} mice.³³⁴ The blood pressure difference of approximately 30 mmHg between untreated t-ACE^{-/-} and t-ACE^{+/+} mice remained unaffected by 14 days of HOE 140 treatment. Despite maintenance of this pressure difference arterial distensibility was restored by HOE 140, which suggests that the increased arterial stiffness observed in t-ACE^{-/-} mice is not directly related to the blood pressure.

In the present study, the vascular distensibility was measured *in vitro* without flow or vascular tone. Therefore, alterations in distensibility are directly related to the vessel wall composition.⁴⁶⁷ Collagen and elastin densities were similar in aortas from t-ACE^{-/-} and t-ACE^{+/+} mice, suggesting that the reduced distensibility found in t-ACE^{-/-} mice is not due to a change in the elastin to collagen ratio. An alternative explanation for the reduced vascular distensibility might be found in the cell-matrix cross-bridges. For instance, cell-matrix cross-bridges between fibronectin and the $\alpha_5\beta_1$ integrin receptor are responsible for the formation of a network between all wall components.⁴⁵ Both angiotensin II and bradykinin are able to influence the vascular fibrinolytic balance.^{60,61,570}

In summary, ACE inhibition, either genetically- or pharmacologically-induced, results in arterial stiffness in mice. These arterial changes can be abolished by B₂ receptor antagonism, which suggests that the B₂ receptor is involved in the increased arterial stiffness observed during ACE inhibition. However, the exact mechanism remains still unclear. Remarkable is that HOE 140 administration only affected carotid artery

distensibility of t-ACE^{-/-} and not t-ACE^{+/+} mice, suggesting that B₂ receptor antagonism does not affect arterial distensibility in general but only during reduced ACE activity.

Chapter 7

Tissue Angiotensin-Converting Enzyme in Imposed and Physiological Flow-Related Arterial Remodeling in Mice

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Abstract

Objective. To test whether membrane-bound angiotensin-converting enzyme (t-ACE) is involved in arterial remodeling, we applied unilateral carotid artery ligation and studied uterine arteries before, during and after pregnancy in t-ACE^{-/-} and t-ACE^{+/+} mice.

Results. In carotid arteries of t-ACE^{-/-} mice, blood pressure, outer diameter and medial cross-sectional area (mCSA) were reduced, while blood flow and the number of medial cells were not modified. In the ligated carotid artery, mCSA and the number of medial cells were increased while outer D and distensibility were reduced. These changes were significantly less pronounced in t-ACE^{-/-} than t-ACE^{+/+} mice. In the contralateral carotid artery, blood flow and outer diameter were comparably increased in the absence of significant alterations of mCSA or the number of medial cells in both strains.

In uterine arteries of t-ACE^{-/-} mice, internal diameter was larger and mCSA was unaltered. At term pregnancy, internal diameter and mCSA of the uterine artery were reversibly increased. Structural changes of the uterine artery during and after pregnancy were comparable in both strains.

Conclusion. Tissue-ACE contributes to arterial structure and remodeling. It plays a major role in hyperplastic inward remodeling of the carotid artery imposed by blood flow cessation, but is not essential for outward hypertrophic and subsequent inward hypotrophic remodeling of the uterine artery during and after pregnancy.

Introduction

Many components of the renin-angiotensin system (RAS) are localized in tissues indicating the existence of a local RAS.^{132,226} Angiotensin-converting enzyme (ACE) is present in all major organs and blood vessels.²³² This tissue bound ACE plays a major role in the local production of angiotensin II (Ang II)¹⁴⁴ and in the local degradation of bradykinin.³²²

Ang II modulates vasomotor tone,⁸ cell growth and apoptosis,^{41,428} cell migration and extracellular matrix deposition,^{241,330} and stimulates the production of other vasoconstrictor- and growth factors.^{82,159} It plays important roles in the functional and structural integrity of the arterial wall and in the pathogenesis of cardiovascular diseases.^{104,478} Experimental and clinical studies with ACE inhibitors and angiotensin type 1 (AT₁) receptor antagonists consistently documented beneficial effects of these agents in treating and preventing cardiovascular diseases such as hypertension and atherosclerosis.^{36,423,481} Some of the beneficial effects of ACE inhibitors can be attributed to reduced breakdown of bradykinin. Antiproliferative effects of kinins via B₂-receptors were demonstrated in arterial injury models using both ACE inhibitors and B₂-receptor antagonists.^{138,437,528}

Pressure and flow determine circumferential wall stress (CWS) and wall shear stress (WSS) in the arterial system. On an acute basis they modulate arterial smooth muscle tone: an increase in transmural pressure triggers a myogenic contractile response;²⁷⁰ an increase in flow induces an endothelium-dependent vasodilatation.⁵⁰⁷ On a chronic basis, negative feedback control of CWS and WSS is achieved by modulation of arterial wall thickness and arterial lumen diameter, respectively.^{21,434} The mechanisms of this arterial remodeling largely remain to be established.^{479,547,555} They might involve Ang II that is produced within the arterial wall in response to an increase in CWS.²¹ Furthermore, there is increasing evidence that elevated WSS releases bradykinin from the endothelium.^{37,223}

Arterial remodeling in response to altered blood flow is often investigated using surgery to shunt flow between adjacent arteries.^{122,173,282,293,300,434,463,479,547,555} For instance, upon unilateral ligation of a carotid artery, the arterial blood flow is acutely and persistently abolished in the ipsilateral artery and doubled in the contralateral artery. Thereafter the lumen diameter and wall mass of the vessels are modified and a neointima may develop.³⁰⁰ This approach is increasingly used in the mouse to investigate the molecular mechanisms that participate in the structural responses of large elastic arteries to an increase and to a decrease in blood flow.^{122,173,282,293,300,463,479} Also physiological challenges may be considered. During pregnancy, blood flow through the uterine circulation increases substantially and reversibly.¹⁶⁰ In the mouse, the uterine vasculature undergoes luminal expansion and an increase in wall mass,²²³ to accommodate this increase in uterine blood flow. The relationships between imposed, physiological and pathological arterial remodeling remain incompletely understood. Furthermore, while structural consequences of increased transmural pressure differ between large elastic and small muscular arteries,^{9,377,439} regional heterogeneity of flow-related arterial remodeling has been little investigated.

Two strains of ACE deficient mice have been generated, one that completely lacks somatic ACE¹⁴⁰ and one that lacks the carboxy-terminal membrane-spanning anchor

region.¹⁴¹ In the latter tissue ACE deficient (t-ACE^{-/-}) mice, blood pressure is reduced^{2,141} and the structural and mechanical properties of the aorta, carotid artery and mesenteric resistance artery, are modified.²

In the present study, we hypothesized that membrane-bound ACE plays a pivotal role during flow-induced arterial remodeling. To test this hypothesis we used two models of flow-related arterial remodeling. We applied unilateral carotid artery ligations³⁰⁰ and studied uterine arteries before, during and after pregnancy²²³ in wild-type and t-ACE^{-/-} mice.

Methods

Animals

The generation of mice with C57Bl6/SV genetic background and lacking tissue-bound angiotensin converting enzyme (t-ACE^{-/-}) has been described by Esther and colleagues.¹⁴⁰ Mice heterozygous for the mutated ACE allele (t-ACE^{+/-}) were mated to obtain mice homozygous for the mutated allele (t-ACE^{-/-}) and their wild type littermates (t-ACE^{+/+}). All animals were housed in groups of 2 to 4 and had free access to standard food (SRMA-1210; Hope Farms, Woerden, the Netherlands) and tap water. All experiments were conducted according to institutional guidelines. Genotyping was performed as previously described.²

Blood Pressure and Heart Rate

Male mice were anaesthetised with pentobarbital (10 mg/kg) and fixed on a heating-pad to control body temperature at 37°C. A saline filled catheter (PE 25) was inserted into the abdominal aorta via the femoral artery and connected to a pressure transducer (Microswitch, model 156PC 156 WL, Honeywell Inc. Amsterdam, the Netherlands). Blood pressure signals were recorded and 10 minutes of stable blood pressure recordings were used to calculate the mean arterial blood pressure and heart rate.

Unilateral Carotid Artery Ligation

At 3 to 4 months of age, male t-ACE^{+/+} (n=12) and t-ACE^{-/-} (n=12) mice were anaesthetised with ketamine and xylazine (100 and 10 mg/kg SC). Body temperature was maintained at 37 °C by a thermostatically controlled heating platform. Both common carotid arteries were exposed through a midline incision in the neck. In half of all the animals (experimental animals), the left common carotid artery was ligated with 5.0 surgical suture at the carotid bifurcation as described by Kumar and Lindner.³⁰⁰ One of the t-ACE^{+/+} and two of the t-ACE^{-/-} mice died within 24 hours after the surgery. In sham-operated t-ACE^{+/+} and t-ACE^{-/-} mice, right and left carotid arteries were exposed but not ligated. The animals were allowed to recover for 4 weeks after sham surgery or unilateral carotid artery ligation.

Carotid Artery Blood Flow Measurements

Four weeks after initial surgery the animals were again anesthetized with ketamine and xylazine. Blood flow in the left and right carotid artery was recorded for t-ACE^{+/+} and t-

ACE^{-/-} mice that had undergone either sham surgery or unilateral carotid artery ligation. We used a transit-time ultrasonic flow probe (0.5 mm V series, Transonic Systems) that was mounted on a micromanipulator and positioned halfway between the aortic arch and the carotid artery bifurcation. Mean blood flow was obtained by averaging during a 5-minute period for the left and right carotid artery of each animal.⁴⁷⁹

Pregnancy-Induced Uterine Artery Remodeling

Female mice (age 4 to 6 months) were used to study uterine arterial remodeling, comparing t-ACE^{-/-} with t-ACE^{+/+}. In view of the infertility of male t-ACE^{-/-} mice,¹⁴⁰ male t-ACE^{+/+} mice were used for mating virgin (in the remainder of this article referred to as nonpregnant) t-ACE^{+/+} and t-ACE^{-/-} mice. Consequently, fetuses grown in t-ACE^{-/-} mothers had the t-ACE^{+/+} genotype, and fetuses grown in t-ACE^{+/+} mothers had the t-ACE^{+/+} genotype. Uterine arteries were investigated before pregnancy (NP), at late pregnancy (LP; day 18-19) and at 7 days postpartum (PP).

Pressure-Diameter Curves

Carotid artery segments (5 mm long, halfway between the aortic arch and the carotid bifurcation) and uterine artery segments (3 mm long, halfway between the ovaries and the vagina) were isolated and transferred into an organ chamber (arteriograph system, Living System Instrumentation, Burlington, VT, USA). The chamber was filled with calcium-free HEPES buffer containing 10 μmol/L sodium nitroprusside to assure maximal vasodilatation. Due to the intransparency of the carotid artery, outer diameters were recorded in the carotid artery, and internal diameters for the uterine artery. A pressure-diameter relationship was established by recording the diameter during stepwise (10 mmHg) increases in intraluminal pressures from 20 to 150 mmHg. After each step the vessel was allowed to equilibrate until a stable diameter was reached. After the experiment, the arteries were fixed at a transmural pressure of 100 mmHg in 4% phosphate-buffered formaldehyde and stored in 70% ethanol.

Morphometry

Fixed vessels were embedded in paraffin and cross-sections (4 μm) were stained with Lawson's solution (Boom, Meppel, the Netherlands) to visualize the internal and external elastic laminae. In the case of carotid arteries the morphometric analysis focussed on the central part of the vessel, halfway between the aortic arch and the carotid bifurcation. Unlike the distal part³⁰⁰ near the ligation, the central part showed no signs of neointima formation or inflammation. Video images were made from cross-sections using a Zeiss axioscope and a standard charge-coupled device camera (Sony). Using JAVA software (Sigma Scan, Jandel Scientific, Corte Madera, CA, USA) the circumference of the internal and external elastic laminae were determined. From these values the media cross-sectional area (CSA) was calculated. The average number of medial nuclear profiles per cross-section was measured by counting the nuclear profiles in the smooth muscle layers of hematoxylin-eosine stained cross-sections (n=3 for each artery and individual mouse). Elastin and collagen were determined in carotid arteries, but not in uterine arteries because of the small size of these vessels. Digital images of Lawson stained cross-sections were made at 400X magnification. Using Leica QWin[®] software the density of elastin was

determined in two areas of the media and averaged. Elastin content was calculated by multiplying elastin density with the medial CSA. To stain collagen, cross sections were deparaffinised and incubate in phosphomolybdic acid (0.2%) for 5 minutes, followed by incubation with Sirius Red (0.1%) in saturated picric acid solution for 90 minutes. After washing with 0.1 M HCl for 2 minutes, sections were dehydrated and protected with coverslips.¹²⁸ The density and content of collagen in the media were quantified as described for elastin.

Physiological Solutions and Drugs

The composition of the Ca^{2+} -free HBSS was (in mmol/L) 144 NaCl, 4.7 KCl, 1.2 MgSO_4 , 1.2 KH_2PO_4 , 14.9 HEPES and 5.5 glucose. The drugs used were pentobarbital sodium (Nembutal®, CEVA SANTE Animale BV, Maassluis, the Netherlands), ketamine, xylazine, and sodium nitroprusside (Sigma Chemical, St Louis, MO).

Statistics

Results are expressed as means $\pm$ SEM. In all experiments, n equals the number of mice and vessels. Statistical significance of differences between sets of data was determined by ANOVA with post-hoc Student-Newman-Keuls test. A P -value of <0.05 was considered to indicate a significant difference.

Results

General Observations

Blood pressure was significantly lower in male $\text{t-ACE}^{-/-}$ mice compared to $\text{t-ACE}^{+/+}$ mice (75 ± 4 mmHg versus 100 ± 5 mmHg), while heart rate was comparable (627 ± 21 bpm versus 662 ± 12 bpm, respectively). Carotid arterial blood flow (mean of left and right carotid artery) did not differ significantly between $\text{t-ACE}^{-/-}$ and $\text{t-ACE}^{+/+}$ (0.39 ± 0.04 mL/min versus 0.40 ± 0.02 mL/min, respectively). In both groups of mice, left carotid artery ligation resulted in an elimination of blood flow in the occluded vessel and in a comparable substantial increase of blood flow in the right carotid artery (0.81 ± 0.11 mL/min in $\text{t-ACE}^{-/-}$ and 0.79 ± 0.07 mL/min in $\text{t-ACE}^{+/+}$).

In female mice, deficiency of tissue ACE did not result in abnormalities during and after pregnancy, as far as bodyweight, fetal and placental weight and the number of fetuses and pups are concerned (Table 7.1). However, cardiac hypertrophy during and after pregnancy was less pronounced in $\text{t-ACE}^{-/-}$ mice compared to $\text{t-ACE}^{+/+}$ mice (Table 7.1).

Arterial Structure of $\text{t-ACE}^{+/+}$ and $\text{t-ACE}^{-/-}$ Mice.

Carotid artery outer diameters were significantly smaller in $\text{t-ACE}^{-/-}$ compared to $\text{t-ACE}^{+/+}$ mice, especially at the highest intraluminal pressure (615 ± 14 μm versus 675 ± 13 μm , respectively, Figure 7.1A). Medial cross-sectional area (CSA) was significantly smaller in carotid artery of $\text{t-ACE}^{-/-}$ mice ($14.1 \pm 0.8 \cdot 10^3$ μm^2) than $\text{t-ACE}^{+/+}$ mice ($20.4 \pm 0.9 \cdot 10^3$ μm^2). The density and content of collagen did not differ, but the density and content of elastin tended to be smaller in carotid artery of $\text{t-ACE}^{-/-}$ than $\text{t-ACE}^{+/+}$ mice (Table 7.2).

In contrast to carotid arteries, uterine arteries of nonpregnant $t\text{-ACE}^{-/-}$ were significantly wider compared to $t\text{-ACE}^{+/+}$ mice ($290 \pm 10 \mu\text{m}$ versus $251 \pm 7 \mu\text{m}$, at 140 mmHg, Figure 7.1B), despite comparable media CSA (Figure 7.1B, insert).

Carotid Artery Structural Changes in Response to Altered Blood Flow

The hyperperfused right carotid artery, displayed a significant increase in outer diameter (Figure 7.2). At high transmural pressure the diameter was increased in both strains. At low transmural pressure the diameter was significantly increased in $t\text{-ACE}^{-/-}$ but not $t\text{-ACE}^{+/+}$ mice. Medial collagen, elastin and CSA were not significantly altered in the right carotid artery of $t\text{-ACE}^{-/-}$ and $t\text{-ACE}^{+/+}$ mice after four weeks exposure to elevated blood flow (Figure 7.3 and Table 7.2).

Table 7.1. General characteristics of nonpregnant, late pregnant and 7-days postpartum wild-type ($t\text{-ACE}^{+/+}$) and tissue angiotensin-converting enzyme deficient ($t\text{-ACE}^{-/-}$) mice.

mice	state	BW (g)	HW (mg)	FW (mg)	PW (mg)	n fetuses	n pups
$t\text{-ACE}^{+/+}$	NP	24 ± 1	125 ± 6				
	LP	$38 \pm 1^*$	$148 \pm 9^*$	765 ± 91	111 ± 4	8 ± 1	
	PP	$29 \pm 2^*$	$162 \pm 7^*$				5 ± 1
$t\text{-ACE}^{-/-}$	NP	23 ± 1	113 ± 7				
	LP	$33 \pm 1^*$	$116 \pm 7^\dagger$	895 ± 110	99 ± 4	7 ± 0	
	PP	$27 \pm 1^*$	$141 \pm 9^*$				6 ± 1

Abbreviations used: NP: nonpregnant; LP: late pregnant; PP: postpartum; BW: body weight; HW: heart weight; FW: fetus weight (without placenta), PW: placental weight. All wet weights. $^*P < 0.05$ compared with NP mice, $^\dagger P < 0.05$ compared with $t\text{-ACE}^{+/+}$ LP. Values are expressed as means $\pm$ SEM.

Figure 7.1. Relations between distending intraluminal pressure and outer diameter in isolated carotid arteries of $t\text{-ACE}^{+/+}$ (open symbols) and $t\text{-ACE}^{-/-}$ (filled symbols) mice. *Insert:* media CSA of isolated carotid arteries of $t\text{-ACE}^{+/+}$ (open bars) and $t\text{-ACE}^{-/-}$ (filled bars) male mice (A). Relations between distending intraluminal pressure and inner diameter in isolated uterine arteries of nonpregnant $t\text{-ACE}^{+/+}$ (open symbols) and $t\text{-ACE}^{-/-}$ (filled symbols) mice. *Insert:* media CSA of isolated uterine arteries of nonpregnant $t\text{-ACE}^{+/+}$ (open bars) and $t\text{-ACE}^{-/-}$ (filled bars) mice (B). Values are means $\pm$ SEM. $^* P < 0.05$ versus $t\text{-ACE}^{+/+}$.

Figure 7.2. Effects of unilateral carotid artery ligation on relations between distending intraluminal pressure and outer carotid artery diameter in $t\text{-ACE}^{+/+}$ (A) and $t\text{-ACE}^{-/-}$ (B) mice. Sham: sham-operated; NF: no-flow (ligated) vessels; HF: high flow (hyperperfused) vessels. Values are means $\pm$ SEM. # $P < 0.05$ vs Sham.

Ligation of the left carotid artery resulted in a significant reduction of the outer diameter (Figure 7.2) accompanied by an increase in medial CSA (Figure 7.3) and a dramatic reduction in distensibility of the ligated vessel. The diameter reduction was more pronounced in $t\text{-ACE}^{+/+}$ mice (from 671 ± 13 to 516 ± 25 μm ; 23% reduction) than in $t\text{-ACE}^{-/-}$ mice (from 625 ± 16 to 515 ± 45 μm ; 18% reduction). Also the medial hypertrophy was more pronounced in $t\text{-ACE}^{+/+}$ mice (from 20.4 ± 0.9 to $34.8 \pm 4.2 \times 10^3$ μm^2 , representing a 71% increase) than $t\text{-ACE}^{-/-}$ mice (from 14.1 ± 0.8 to $18.1 \pm 2.3 \times 10^3$ μm^2 , representing a 28% increase) and involved a large increase in the number of medial cells in $t\text{-ACE}^{+/+}$ but not $t\text{-ACE}^{-/-}$ mice (Figure 7.3 lower panel).

In the media of the ligated left carotid artery of both $t\text{-ACE}^{-/-}$ and $t\text{-ACE}^{+/+}$ mice, staining for the monocyte/macrophage marker ED1 was rare and all cells stained for smooth muscle α -actin (not shown). Collagen and elastin densities were not altered in the ligated vessels, but due to the higher medial mass in the ligated carotid artery of $t\text{-ACE}^{+/+}$ mice, elastin content was significantly increased (Table 7.2).

Uterine Artery Structure During and After Pregnancy

During late pregnancy maximal lumen diameter of uterine arteries increased significantly in $t\text{-ACE}^{+/+}$ (from 251 ± 7 to 384 ± 14 μm) and $t\text{-ACE}^{-/-}$ mice (from 290 ± 10 to 384 ± 16 μm , Figure 7.4). Medial CSA increased significantly and comparably during pregnancy in UA of both $t\text{-ACE}^{+/+}$ (from 2.6 ± 0.3 to $5.2 \pm 0.8 \times 10^3$ μm^2) and $t\text{-ACE}^{-/-}$ mice (from 2.9 ± 0.3 to $5.1 \pm 0.4 \times 10^3$ μm^2 , Figure 7.5, upper panels). In neither strain was the medial hypertrophy accompanied by a significant change in the number of nuclear profiles per medial cross section (Figure 7.5, lower panels). By 7 days postpartum, most of the structural changes in the uterine artery were reversed (Figure 7.4 and 7.5). Maximal lumen diameter had partially regressed to pre-pregnant values in both $t\text{-ACE}^{+/+}$ mice (from 384 ± 14 to 290 ± 21 μm) and $t\text{-ACE}^{-/-}$ mice (from 384 ± 16 to 301 ± 17 μm). Medial CSA regressed to nonpregnant values in both strains (Figure 7.5).

Table 7.2. Effect of unilateral artery ligation on media CSA, collagen and elastin in carotid arteries from t-ACE^{+/+} and t-ACE^{-/-} mice.

	Strain	Unit	Normal Flow	High Flow	No Flow
media CSA	t-ACE ^{+/+}	10 ³ μm ²	20.4 ± 0.9	19.4 ± 0.8	34.8 ± 4.2 *
	t-ACE ^{-/-}	10 ³ μm ²	14.1 ± 0.8	17.8 ± 2.4	18.1 ± 2.3
Collagen	t-ACE ^{+/+}	%	39.1 ± 6.4	33.3 ± 8.2	20.5 ± 2.1
		10 ³ μm ²	7.3 ± 1.3	6.4 ± 1.8	6.9 ± 1.8
	t-ACE ^{-/-}	%	46.7 ± 7.1	31.5 ± 8.0	32.8 ± 3.8
		10 ³ μm ²	7.2 ± 1.0	5.5 ± 2.1	6.8 ± 1.9
Elastin	t-ACE ^{+/+}	%	37.4 ± 1.5	35.7 ± 5.5	31.3 ± 5.4
		10 ³ μm ²	6.0 ± 0.6	6.8 ± 0.8	10.0 ± 1.0 *
	t-ACE ^{-/-}	%	31.3 ± 4.9	35.3 ± 4.8	27.7 ± 3.1
		10 ³ μm ²	4.9 ± 0.5	6.0 ± 0.7	5.4 ± 0.7

The data are expressed as percentage (%) and surface (10³ μm²) of media area and are given as mean values ± SEM. * *P* < 0.01 versus normal flow (sham-operated carotid artery) and high flow (right carotid artery).

Figure 7.3. Effects of unilateral carotid artery ligation on medial CSA (upper panel) and the number of medial cells per cross-section (lower panel) of hyperperfused (A and C) and ligated (B and D) carotid arteries in t-ACE^{+/+} and t-ACE^{-/-} mice. Sham: sham-operated; NF: no-flow (ligated) vessels; HF: high flow (hyperperfused) vessels. Values are means ± SEM. # *P* < 0.05 versus sham, * *P* < 0.05 versus t-ACE^{+/+}.

Figure 7.4. Effects of pregnancy on the relations between distending intraluminal pressure and inner diameter in isolated uterine arteries of nonpregnant (NP), late pregnant (LP) and postpartum (PP) t-ACE^{+/+} (A) and t-ACE^{-/-} (B) mice. # $P < 0.05$ versus NP and PP. Values are means $\pm$ SEM.

Discussion

According to our experimental findings, membrane-bound ACE participates in the control of arterial structure. The role of the enzyme differs between imposed and physiological arterial structural changes.

In line with the pressor and hypertrophic effects of angiotensin II,^{41,104,479} t-ACE deficient mice displayed lower blood pressure, reduced carotid artery medial mass and reduced pregnancy-related cardiac hypertrophy. Furthermore, as previously reported,² the carotid artery was narrower but more distensible despite unaltered carotid arterial blood flow. Maintenance of carotid artery blood flow, despite reduced blood pressure implies reduced cerebrovascular resistance. This is largely due to blunted vasomotor tone and an increased structural diameter of resistance arteries as observed in the mesenteric,² and uterine arterial bed (this study). We previously observed that chronic pharmacological ACE inhibition in wild-type mice resulted in comparable alterations of carotid artery structure and mechanics.² Combined, these observations indicate that t-ACE plays a role in the establishment and maintenance of the balance between local hemodynamic and mechanical forces, on the one hand, and the structural and mechanical properties of large elastic arteries, on the other hand. This role of t-ACE is largely similar in male and female mice.² Direct trophic and indirect, *e.g.* pressure-related, influences of angiotensin II or bradykinin, may be involved. Because a 2-week treatment with a B₂ receptor antagonist blunts the difference in carotid artery mass and distensibility, but not the difference in blood pressure between t-ACE^{-/-} and t-ACE^{+/+} mice,² primarily bradykinin seems to be involved.

Figure 7.5. Effects of pregnancy on medial CSA (upper panel) and the number of medial cells per cross-section (lower panel) of uterine arteries in late pregnant (**A** and **C**) and postpartum (**B** and **D**) *t-ACE*^{+/+} and *t-ACE*^{-/-} mice. Medial CSA was determined by morphometry on vessels fixed in vitro at 80 mmHg. Arteries of nonpregnant (NP) mice are indicated by open bars, and arteries of late pregnant (LP) or postpartum (PP) mice are indicated by filled bars. Values are means $\pm$ SEM. # $P < 0.05$ versus NP, * $P < 0.05$ versus *t-ACE*^{+/+}.

In contrast to large elastic arteries, but similar to small muscular mesenteric arteries,² uterine arteries of *t-ACE*^{-/-} displayed a larger lumen diameter despite a comparable medial surface area. This regionality is reminiscent of the hypertrophy and inward eutrophic remodeling that was described for large elastic and small muscular arteries in hypertension, respectively.^{377,439} The molecular and structural basis of this regional heterogeneity is largely unknown. It was proposed that negative feedback control of local mechanical forces can be achieved to a larger extent in muscular arteries than in elastic arteries by alterations in vasomotor tone, i.e. by myogenic tone and flow-induced vasodilatation.^{156,157,270,507}

Local mechanical forces that govern arterial structure include circumferential wall stress (CWS) and wall shear stress (WSS).^{270,507} Chronic changes of these forces result in

arterial remodeling.^{122,173,223,282,293,300,434,463,479,547,555} These arterial adaptive structural responses involve partly interrelated alterations in (a) arterial wall mass, (b) mechanical properties of the arterial wall material and (c) arterial lumen diameter. Because CWS and WSS can stimulate intra-arterial production of angiotensin II and bradykinin, respectively,^{21,37,223} we compared arterial remodeling in large and small muscular arteries of t-ACE^{-/-} and t-ACE^{+/+} mice. In the mouse carotid artery ligation model no differences have been observed between males and females.²⁹³

The increase in contralateral blood flow after unilateral carotid artery ligation, was comparable in t-ACE^{-/-} and t-ACE^{+/+} mice. Following 4-weeks exposure to doubled blood flow, arterial diameter was increased while arterial wall mass and collagen and elastin content were not altered. Although t-ACE was not essential for this outward arterial remodeling, distinct ultrastructural alterations might have contributed to the expansion of the vessel. In the absence of t-ACE which has been shown to be a determinant of large artery stiffness,² not only the maximal diameter but also the diameters at intermediate pressures were increased.

More marked differences were observed between ligated arteries of t-ACE^{-/-} and t-ACE^{+/+}. In ligated carotid arteries of t-ACE^{+/+} mice, outer diameter and arterial distensibility were reduced while medial cross sectional area and medial cell number were increased. The marked stiffening of the arterial wall proceeded without a change in collagen and elastin density; rather the elastin content significantly increased. Changes in diameter, medial cross sectional area and -cell number were less pronounced in t-ACE^{-/-} than t-ACE^{+/+} mice. These findings are in line with previously reported angiotensin II-induced stimulation of vascular smooth muscle cell proliferation and fibronectin production.^{21,104,241} They do, however, raise questions about the stimuli for intra-arterial production of mediators. CWS, a reported stimulus for the local RAS,²¹ is likely to drop in the ligated artery as a result of reduced flow-induced vasodilatation and proceeding wall thickening. WSS, a stimulator of the production of the other major ACE substrate bradykinin^{37,223} and one of its major effectors nitric oxide⁶⁹ is at least initially markedly reduced in the ligated artery. Despite these unknowns, t-ACE seems to be involved in the structural response of large elastic arteries to a moderate increase and a marked decrease in blood flow.

In both t-ACE^{-/-} and t-ACE^{+/+} mice, the increase in uterine blood flow during pregnancy was sufficient to support the survival and growth of a comparable number of fetuses. Diameter and medial mass of the uterine artery increased substantially during pregnancy possibly as an adaptation to the increasing WSS and CWS. In t-ACE^{-/-} mice, the diameter increase was somewhat smaller than in t-ACE^{+/+} mice. We recently reported that outward remodeling of the uterine artery during pregnancy is not altered in tissue kallikrein-deficient mice.²²³ Consequently, angiotensin II rather than bradykinin seems to be involved in the widening of the vessel. We can, however, not fully exclude (i) that uterine arterial blood flow increased to a lesser extent in pregnant t-ACE^{-/-} than t-ACE^{+/+} mice, and (ii) that less remodeling of the vessel was required, because it was already relatively expanded before pregnancy. In contrast to the diameter, the increase in medial mass was comparable in t-ACE^{-/-} and t-ACE^{+/+} mice. Because the number of nuclear profiles per medial section did not change, the wall hypertrophy is primarily due to medial

cell growth. Unlike for the hyperplasia in the no-flow carotid artery, t-ACE^{-/-} is not essential for cellular hypertrophy in the uterine artery.

By 7 days postpartum, uterine artery medial surface area and diameter at mean arterial pressure did no longer differ from pre-pregnancy values. This illustrates the dynamic and plastic nature of the vascular bed. The reversal of the remodeling was obtained by a reduction of medial cell size. We previously reported that also tissue-kallikrein deficiency did not impair reversal of uterine artery remodeling.²²³ Consequently, intramural production of neither angiotensin II nor bradykinin seem to be essential for the adjustments of uterine arterial wall mass and lumen diameter to the marked reduction in blood flow.

Not only the role of t-ACE but also the nature of the remodeling differed in carotid and uterine arteries. Hyperperfusion led to expansion of both vessels but this involved wall and cellular hypertrophy in the uterine artery and not in the carotid artery. Hypoperfusion resulted in a reduction of lumen diameter in both vessels. This was accompanied by wall hypertrophy and hyperplasia in the carotid artery while the number of medial cells was not modified and their size probably reduced in the uterine artery. These regional differences seem not only due to the different extents and kinetics of the imposed and physiological blood flow changes. In rat and mouse small muscular mesenteric arteries, a doubling of blood flow leads to outward hypertrophic remodeling and a marked reduction of blood flow leads to inward hypotrophic remodeling.^{71,434,555} Unlike in the uterine artery, these two types of mesenteric artery remodeling are accompanied by medial cell proliferation and apoptosis⁷¹ leading to a gain and a loss of smooth muscle cells in the high flow and the low flow arteries, respectively. Whether the role of t-ACE is more prominent in the remodeling of large elastic than in small muscular arteries, or more prominent in hyperplastic than in hypertrophic remodeling, awaits further experimentation.

The regional heterogeneity and diversity of the role of t-ACE in arterial structural changes, which we observed in an elastic artery and a pre-existing collateral artery such as the uterine artery, are in line with earlier findings. Angiotensin II and bradykinin stimulate and inhibit the proliferation of medial and neointimal smooth muscle cells in carotid arteries, respectively.^{104,138,528} Yet, angiotensin II can promote shunting of blood flow through pre-existing collateral channels⁶ and has been reported to promote and inhibit angiogenesis at low and high concentrations, respectively.⁵⁷⁶ The former mechanism, along with the proangiogenic effects of bradykinin⁵⁰¹ seems to account for the beneficial effects on capillary density and blood flow observed with inhibitors of t-ACE such as quinaprilate in peripheral and coronary ischemia.¹⁴²

Despite its roles in the morphogenesis and remodeling of vascular beds and in spermatogenesis,¹⁴⁰ t-ACE (this study) and even both membrane-bound and circulating ACE³⁵⁸ are not essential for optimal pregnancy outcome in the mouse. The various maternal systemic and local vascular alterations, including extensive remodeling and angiogenesis in the uterine vascular bed, can proceed in the absence of ACE-derived angiotensin II and do not require local production of bradykinin.²²³ Alternative biosynthetic pathways and various other growth factors and angiogenic stimuli might be upregulated during this physiological response on which there is considerable biological pressure. Recent genetic observations indicate that ACE might rather be detrimental

during human pregnancy.³⁵⁸ The ACE I/D polymorphism affects besides ACE activity, uteroplacental and umbilical blood flows and the recurrence of an adverse pregnancy outcome, including intrauterine growth retardation, in women with a history of preeclampsia.

In summary, observations in t-ACE^{-/-} mice indicate that membrane-bound ACE plays different roles in the development and maintenance of the structural and mechanical properties of large elastic and small muscular arteries. Moreover, our present findings in t-ACE^{-/-} mice, combined with our earlier findings in tissue kallikrein-deficient mice,²²³ indicate that intra-arterial production of angiotensin II contributes substantially to the hyperplastic remodeling of the ligated carotid artery but not to the hypertrophic and hypotrophic remodeling of the uterine artery during and after pregnancy.

Chapter 8

Uterine Artery Dilatation and Remodeling During Postponed Murine Pregnancy

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Submitted

Abstract

Objective. Aging was reported to reduce endothelium-dependent vasodilatation and outward arterial remodeling. We hypothesize that this (1) also applies to small muscular arteries in the uterine vascular bed and (2) adversely affects reproductive performance. We therefore monitored uterine artery vasomotor responses, structural changes and fetal number before and at the 18th day of first pregnancy in approximately 17 (young) and 58 (middle age) weeks old mice.

Results. Uterine arteries were larger, but norepinephrine-induced constriction and dilatation in response to flow, acetylcholine, and Na-nitroprusside were comparable in middle age compared to young mice. N^ω-nitro-L-arginine (1) reduced dilator responses to acetylcholine in both young and middle age mice, but (2) reduced flow-induced dilatation in young, but not middle age mice. Pregnancy had minimal effects on uterine arterial vasomotor responses in young mice, but enhanced myogenic tone in middle age mice. At term pregnancy, the increase in internal diameter, and in media cross-sectional area and the number of viable fetuses were significantly smaller in middle age than young mice.

Conclusion. Aging modifies shear stress-induced vasodilatation and blunts pregnancy-related remodeling of the uterine artery. This might contribute to fetal growth retardation and mortality during postponed pregnancy.

Introduction

Many epidemiologic and animal studies have shown that a sub-optimal fetal micro-environment can have persistent consequences for health in adulthood. Intra-uterine growth restriction, either via malnutrition or placental insufficiency, is associated with reduced birth weight and increased risk for metabolic and cardiovascular disorders in the adult life.^{23,424}

During mammalian pregnancy uterine blood flow increases dramatically.⁴⁵⁶ Adaptation of the uterine vascular bed to meet the increasing metabolic demands of the growing fetus(es) occurs through vasodilatation,³³⁷ outward remodeling and the development of new vessels (angiogenesis).⁶⁰⁷ These changes depend on the inner endothelial lining of the arterial bed.³¹²

Currently, many women postpone childbearing for socio-economic reasons. Increasing maternal age (>35 years) at first childbirth is an independent risk factor for low birth weight and spontaneous abortion.^{163,399} In rodents such as mice, the number of implanted fetuses is initially comparable between young and middle age animals. However, a large number of fetuses dies during the third trimester of pregnancy when the increase in uterine blood flow should be most pronounced.⁵⁶¹

Aging is an important risk factor for cardiovascular diseases such as ischemic heart disease. Although the mechanisms associated with vascular aging are multi-factorial, one underlying cause may be an age-associated endothelial dysfunction³⁰⁵ leading to (i) reduced bio-availability of endothelium-derived vasodilators²⁵⁸ (ii) impaired flow-induced vasodilatation,¹⁸¹ and (iii) a diminished arterial luminal expansion and increased smooth muscle cell hypertrophy in response to altered blood flow.³⁶¹

Most aging-related research concentrates on >65 years of age in humans or comparable stages of life in experimental animals. It is not clear whether significant vascular changes develop in important vascular beds between young adulthood and the mid-life stage. We hypothesize that flow-induced vasodilatation and arterial remodeling are particularly sensitive to aging and that this contributes to reduced reproductive performance and increased pregnancy-associated complications during postponed pregnancy. We therefore compared for 17 and 58 weeks old mice (i) vasomotor responses of the isolated uterine artery, (ii) pregnancy-induced uterine arterial changes, and (iii) fetal properties at 0.9 gestation.

Materials and Methods

Animals

17 weeks old (further referred to as young mice) and 58 weeks old (further referred to as middle age mice) C57Bl/6J mice with a reported mean life span of 120 weeks⁵¹ were purchased from Charles River, Maastricht, the Netherlands. All animals had free access to food and water. Pregnancy was achieved by mating with an experienced male of the same strain. The recovery of a vaginal sperm plug was considered to correspond to day 1 of pregnancy. At the 18th day of their first pregnancy (0.9 gestation) young and middle age

mice were sacrificed. A group of age-matched virgin mice served as nonpregnant controls. All experiments were conducted according to institutional guidelines.

Tissue Preparation

Mice were sacrificed with an overdose of pentobarbital (i.p.). Reproductive parameters (litter size and viable fetuses) were recorded. Viable fetuses were recognized by their pink appearance and their movement in response to a mechanical stimulus. Isolation of the main uterine artery was performed as described.²²³

Pressure Myograph Experiments

At the beginning of each experiment, the uterine artery segment was incubated for 45 minutes at 80 mmHg. In almost all vessels a basal constriction developed. The integrity of the endothelium was evaluated by assessing the response to acetylcholine (1 $\mu\text{mol/L}$) during phenylephrine (1 $\mu\text{mol/L}$)-induced constriction. Arteries that dilated <60% of passive diameter (see below) were excluded. Pressure-induced (myogenic) tone was determined by submitting the arteries to 10 mmHg pressure increments from 20 to 100 mmHg at 5 min interval in the absence of intraluminal flow. Percentage myogenic tone (% MT) at an intraluminal pressure of 80 mmHg was calculated from the formula:

$$\% \text{ MT} = \frac{(D_{\text{passive}} - D_{\text{active}})}{D_{\text{passive}}} \cdot 100$$

The passive diameter is the diameter obtained in Ca^{2+} -free physiological salt solution containing sodium nitroprusside (10 $\mu\text{mol/L}$) which abolishes smooth muscle tone.

After washing, a myogenic constriction returned. A concentration-response curve (CRC) with norepinephrine (on top of this myogenic tone) was constructed by cumulative extraluminal administration of norepinephrine (NE, 0.01-10 μM) to the bath solution. Sensitivity ($\text{pD}_2 = -\log [\text{EC}_{50}]$) and the maximal response with the agonist (E_{max}) were determined by sigmoidal curve fitting of individual curves (Graphpad Prism™ 2.01 software).

Flow-induced dilatation (FID) was studied by applying an intraluminal flow of 10 $\mu\text{l/min}$ representing a calculated wall shear stress value of 5 dyne/cm^2 at an intraluminal pressure of 80 mmHg. Arteries were constricted either spontaneously via myogenic tone or in combination with the thromboxane analogue U46619 to a level of approximately 50-60% of the passive diameter. Since the diameter obtained before application of shear stress was the same for all vessels, the level of shear stress was also comparable for vessels of young and middle age mice. The internal diameter at which maximal FID occurred was recorded and percentage dilatation was calculated as a percentage of the passive internal diameter. At the end of the experiment, intraluminal flow was stopped and a constriction returned. Agonist-induced endothelium-dependent vasodilator responses were evaluated by constructing a cumulative CRC for acetylcholine (ACh, 0.01-10 μM). Time control experiments were conducted in order to check the stability of the FID responses. Three consecutive FID experiments yielded the same diameter responses to flow under control

conditions. Therefore, experiments were repeated on the same single vessel in the presence of the NO synthase inhibitor N^{ω} -nitro-L-arginine (NLA, 30 $\mu\text{mol/L}$). Similarly, endothelium-independent vasodilator responses were analysed by constructing a cumulative CRC for sodium nitroprusside (SNP, 0.1 nM–100 μM) in the presence of both 30 μM NLA and 10 μM indomethacin to block endogenous production of NO and prostaglandins.

At the end of the experiment, arteries were incubated in a Ca^{2+} -free physiological salt solution containing sodium nitroprusside (10 $\mu\text{mol/L}$) at an intraluminal pressure of 80 mmHg to obtain the passive diameter. A passive pressure-diameter relationship was established by recording the diameter during stepwise (10 mmHg) increases in intraluminal pressures from 20 to 100 mmHg. After each step the vessel was allowed to equilibrate until a stable diameter was reached. Wall thickness was recorded to calculate incremental elastic modulus and circumferential wall stress. After the experiment, the arteries were fixed during 30 minutes at a transmural pressure of 80 mmHg in 4% phosphate-buffered formaldehyde, and stored in 70% ethanol solution.

Calculation of Parameters and Morphometry

Circumferential wall stress (CWS) and incremental elastic modulus (E_m) were calculated as described.²²³ Morphometry of fixed vessels was done as described.²²³

Drugs

U46619 was purchased from Calbiochem, Darmstadt, Germany. Acetylcholine was purchased from Serva Feinbiochemica, Heidelberg, Germany. L-phenylephrine, norepinephrine, N^{ω} -nitro-L-arginine, sodium nitroprusside and indomethacin were purchased from Sigma Chemical, St Louis, USA.

Statistics

Results are expressed as means $\pm$ SEM. In all experiments, n equals the number of mice and vessels. Statistical significance of differences between sets of data was determined by ANOVA with post-hoc Student-Newman-Keuls test. A P -value of <0.05 was considered to indicate a significant difference.

Results

General Observations

Young mice had a significantly lower body weight than middle age mice (Table 8.1). Litter size and the number of viable fetuses in the uterus were significantly higher in young late pregnant mice compared to middle age late pregnant mice (Table 8.1). Mean cardiac and placental weights were comparable for both age groups (Table 8.1), but maternal body weight gain during pregnancy was larger for young than middle age mice.

Uterine Artery Structure of Nonpregnant Mice

Uterine arteries were significantly larger in middle age than young nonpregnant mice as shown in the pressure-diameter curves (Figure 8.1A). Passive internal diameter (at 80

mmHg) was $233 \pm 9 \mu\text{m}$ for young mice and $286 \pm 11 \mu\text{m}$ for middle age mice. Arterial wall elasticity was similar for the two mouse groups as reflected by comparable relationships between incremental elastic modulus and circumferential wall stress (Figure 8.1B). Medial cross-sectional area was significantly larger for uterine arteries of middle age mice ($3.6 \pm 0.4 \times 10^3 \mu\text{m}^2$) compared to young adult mice ($2.4 \pm 0.3 \times 10^3 \mu\text{m}^2$, Figure 8.1A, insert).

Table 8.1. General characteristics and reproductive performance of young and middle age mice.

	Young		Middle Age	
	nonpregnant	late pregnant	nonpregnant	late pregnant
Age (wk)	18 $\pm$ 1	16 $\pm$ 1	63 $\pm$ 3	53 $\pm$ 1
Bodyweight (g)	23 $\pm$ 1	38 $\pm$ 2 *	28 $\pm$ 1	35 $\pm$ 1 *
Litter size (n)	NA	8 $\pm$ 1	NA	4 $\pm$ 1 †
Viable fetuses (n)	NA	8 $\pm$ 1	NA	3 $\pm$ 1 †
Heart weight (mg)	125 $\pm$ 5	140 $\pm$ 7 *	123 $\pm$ 10	163 $\pm$ 11 *
Placental weight (mg)	NA	109 $\pm$ 3	NA	124 $\pm$ 4
Fetal weight (mg)	NA	846 $\pm$ 84	NA	1013 $\pm$ 343

Abbreviations: NA: not applicable. Means $\pm$ SEM; * $P < 0.05$ versus nonpregnant; † $P < 0.05$ versus young

Figure 8.1. Uterine artery structure and elasticity of non-pregnant mice. (A) Pressure-diameter relationships in the absence of smooth muscle tone for nonpregnant young (open squares) and middle age (closed squares) mice. *Insert:* media cross-sectional areas (CSA) for uterine arteries of young (white bars) and middle age (black bars) nonpregnant mice. * $P < 0.05$ versus young mice. (B) Incremental elastic modulus (E_{inc})-circumferential wall stress (CWS) relationships for fully relaxed uterine arteries of nonpregnant young (open squares) and middle age (closed squares) mice. Values are mean $\pm$ SEM.

Vasoconstrictor Responses of Nonpregnant Mice.

Step increases in intraluminal pressure caused a constriction from approximately 60 mmHg in uterine arteries of nonpregnant mice of young and middle age mice (Figure 8.2, upper panel). All arteries of young mice developed a myogenic constriction, whereas only 4 out of 8 arteries of middle age mice responded to intraluminal pressure. Consequently,

percentage myogenic tone was significantly reduced in middle age nonpregnant mice compared to young nonpregnant mice ($19 \pm 7\%$ versus $40 \pm 5\%$, respectively). The maximal vasoconstriction induced by NE was comparable in young and middle age mice ($54 \pm 6\%$ versus $57 \pm 4\%$, respectively, Table 8.2). The sensitivity to the catecholamine did not differ between the two groups (Table 8.2).

Vasodilator Responses of Nonpregnant Mice.

The maximal vasodilatation induced by ACh was comparable in young and middle age mice ($96 \pm 2\%$ versus $95 \pm 2\%$, respectively, Figure 8.3 and Table 8.2). The sensitivity to the endothelium-dependent agonist was similar in uterine arteries of both groups (Table 8.2). In young nonpregnant mice, nitro-L-arginine (NLA; $30\ \mu\text{M}$) significantly reduced the maximal dilator response, but not the sensitivity to ACh (Figure 8.3A; Table 8.2); while in middle age nonpregnant mice NLA markedly reduced the sensitivity to ACh (Figure 8.3B; Table 8.2).

All vessels dilated in response to SNP ($0.1\ \text{nM}$ to $100\ \mu\text{M}$) during preconstriction. Maximal endothelium-independent dilatations and sensitivity to the NO donor were comparable in uterine arteries of young and middle age mice (Table 8.2).

Application of a shear stress of $5\ \text{dyne/cm}^2$ resulted in a dilatation, that was comparable for young and middle age nonpregnant mice (Figure 8.4). Inhibition of nitric oxide synthase with NLA ($30\ \mu\text{M}$) significantly reduced flow-induced dilatation in uterine arteries of young nonpregnant mice, but not in middle age mice (Figure 8.4).

Pregnancy-Related Changes in Young Mice.

In young mice uterine artery internal diameter increased 60% from 233 ± 9 to $375 \pm 16\ \mu\text{m}$ at 0.9 gestation (Figure 8.5A). Arterial elasticity was unaltered during pregnancy (data not shown). Significant medial hypertrophy occurred in the uterine artery at 0.9 gestation; media cross-sectional area increased 150% from 2.4 ± 0.3 to $6.0 \pm 0.7 \times 10^3\ \mu\text{m}^2$ (Figure 8.5A, insert). Myogenic tone, constrictor responses to NE, and dilator responses to ACh and SNP, expressed as percent changes, were all similar in uterine arteries of late pregnant young mice compared to nonpregnant young mice (Figure 8.2, *lower panel*; Table 8.2).

Pregnancy-Related Changes in Middle age Mice.

Uterine artery outward remodeling was significantly reduced in middle age mice compared to the young animals. Passive internal diameter increased from 286 ± 11 to $342 \pm 15\ \mu\text{m}$ at late pregnancy, representing only a 20% increase. (Figure 8.5B). Besides a marked reduction in outward remodeling, medial hypertrophy was substantially reduced in uterine arteries of middle age mice compared to their younger counterparts. Medial cross-sectional area increased only 30% at late pregnancy (from 3.6 ± 0.4 to $4.7 \pm 0.5 \times 10^3\ \mu\text{m}^2$, Figure 8.5B, insert).

Myogenic tone of the uterine artery significantly increased at 0.9 gestation in middle age mice (Figure 8.2, *lower panel*; Table 8.2). Constrictor responses to NE, and dilator responses to ACh and SNP were similar in uterine arteries of middle age late pregnant mice compared to nonpregnant mice (Table 8.2).

Figure 8.2. Pressure-induced myogenic constriction in uterine arteries of nonpregnant mice. (A) Pressure-diameter relationships in the absence of Ca^{2+} (passive, open squares) and in the presence of Ca^{2+} (active, closed squares) for young nonpregnant mice. (B) Pressure-diameter relationships in the absence of Ca^{2+} (passive, open squares) and in the presence of Ca^{2+} (active, closed squares) for middle age nonpregnant mice. (C) Pressure-diameter relationships in the absence of Ca^{2+} (passive, open squares) and in the presence of Ca^{2+} (active, closed squares) for young late pregnant mice. (D) Pressure-diameter relationships in the absence of Ca^{2+} (passive, open squares) and in the presence of Ca^{2+} (active, closed squares) for middle age late pregnant mice. Values are mean $\pm$ SEM.

Figure 8.3. Concentration-response curves (CRC) for acetylcholine in uterine arteries of nonpregnant mice under control conditions (*open squares*) and in the presence of 30 μ M NLA (*closed squares*) for young nonpregnant mice (**A**). CRC under control conditions (*open squares*) and in the presence of 30 μ M NLA (*closed squares*) for middle age nonpregnant mice (**B**). Values are mean $\pm$ SEM.

Table 8.2. Constrictor and dilator properties of uterine arteries from nonpregnant and late pregnant young and middle age mice.

	Young Mice		Middle Age Mice	
	nonpregnant	late pregnant	nonpregnant	late pregnant
% MT	40 $\pm$ 5	42 $\pm$ 5	19 $\pm$ 7 †	54 $\pm$ 3
pD ₂ NE	6.73 $\pm$ 0.06	6.69 $\pm$ 0.14	6.42 $\pm$ 0.22	6.59 $\pm$ 0.20
E _{max} (%) NE	54 $\pm$ 6	58 $\pm$ 4	57 $\pm$ 4	53 $\pm$ 3
pD ₂ ACh	6.84 $\pm$ 0.17	6.29 $\pm$ 0.12	7.04 $\pm$ 0.20	7.22 $\pm$ 0.32 ‡
E _{max} (%) ACh	96 $\pm$ 2	95 $\pm$ 1	95 $\pm$ 2	99 $\pm$ 1
pD ₂ ACh + NLA	6.48 $\pm$ 0.21	6.33 $\pm$ 0.11	6.11 $\pm$ 0.18 *	6.68 $\pm$ 0.38
E _{max} (%) ACh + NLA	77 $\pm$ 7 *	62 $\pm$ 13	82 $\pm$ 4 *	90 $\pm$ 5
pD ₂ SNP	7.33 $\pm$ 0.12	6.91 $\pm$ 0.23	7.00 $\pm$ 0.13	7.02 $\pm$ 0.39
E _{max} (%) SNP	68 $\pm$ 9	74 $\pm$ 8	54 $\pm$ 7	72 $\pm$ 16

Abbreviations used: MT: myogenic tone; NE: norepinephrine; ACh: acetylcholine; NLA: nitro-L-arginine; SNP: sodium nitroprusside. Values are mean $\pm$ SEM. * $P < 0.05$ versus E_{max} (%) ACh; † $P < 0.05$ versus nonpregnant young and late pregnant middle age mice; ‡ $P < 0.05$ versus late pregnant young mice.

Figure 8.4. Flow-induced dilatation for uterine arteries under control conditions (*open bars*) and in the presence of 30 μM NLA (*closed bars*) for young and middle age nonpregnant mice. An initial shear stress of 5 dyne/cm^2 was applied to the vessels by a constant intraluminal pressure. * $P<0.05$ versus NLA. Values are mean $\pm$ SEM.

Figure 8.5. Pregnancy-related uterine artery structural changes. Pressure-diameter relationships in the absence of Ca^{2+} for nonpregnant (*open squares*) and late pregnant (*closed squares*) young mice. *Inserts:* media cross-sectional areas (CSA) for uterine arteries of nonpregnant (white bars) and late pregnant (black bars) young mice. * $P<0.05$ versus young mice (**A**). Pressure-diameter relationships in the absence of Ca^{2+} for nonpregnant (*open squares*) and late pregnant (*closed squares*) middle age mice. *Inserts:* media cross-sectional areas (CSA) for uterine arteries of nonpregnant (white bars) and late pregnant (black bars) middle age mice (**B**). * $P<0.05$ versus NP. Values are mean $\pm$ SEM.

Discussion

In uterine arteries of 58 weeks old mice (1) shear stress-induced NO release and (2) pregnancy-related remodeling were reduced. Altered flow-induced vasodilatation and blunted uterine arteriogenesis were associated with impaired pregnancy outcome.

Using an *in situ* “perfusion fixation method”, Van der Heijden and colleagues demonstrated blunted uterine artery remodeling in 40-weeks old late pregnant mice

compared to 12-weeks old young adult late pregnant mice.⁵⁶¹ They did not determine whether this diminished uterine artery remodeling capacity was due to an age-dependent change in endothelial function or smooth muscle cell activity. In this study we used the pressurized artery method (arteriograph setup) to measure acute arterial function and morpho-mechanical arterial properties in isolated vessels. We measured in young (17 weeks) and middle age (58 weeks) mice: 1) acute uterine artery responses to pressure (myogenic constriction), flow or shear stress (flow-induced dilatation) and agonists (norepinephrine, acetylcholine and sodium nitroprusside), 2) uterine artery remodeling (diameter and medial mass changes) at 0.9 gestation, and 3) pregnancy outcome (number of viable fetuses).

With age, the vascular wall undergoes structural changes such as increased stiffness, medial thickening and luminal diameter enlargement.³⁶⁹ In line with these observations, uterine arteries of middle age nonpregnant mice had wider diameters and greater medial cross-sectional areas compared to uterine arteries of young nonpregnant mice. Uterine arterial elasticity was however comparable between young and middle age mice. Aging-related arterial wall stiffening may require a longer period of time. Vascular aging is associated with functional changes at the level of all three layers of the arterial wall of both large conduit arteries and small resistance arteries.³⁵⁵ Age-related endothelial dysfunction has been characterized by diminished agonist-induced vasodilatation⁵²⁷ and flow-induced dilatation.³⁷⁶ The reduced vasodilatation is possibly due to an altered balance between endothelium-derived relaxing factors (*e.g.* NO) and constricting factors (*e.g.* endoperoxides). Furthermore, elevated oxidative stress plays a critical role in the deleterious effects of aging on the endothelium via enhanced scavenging of NO. Changes in vascular reactivity to constrictor agents may be due to a combination of smooth muscle cell alteration and a reduced vasodilator function of the endothelium.³³² The C57Bl/6J mice, which we studied, have a reported life span of >120 weeks. The mice we studied thus represent young adult and middle age conditions and not geriatric conditions. We found comparable flow (shear stress)-induced uterine artery dilatations in young and middle age mice. However, shear stress-induced NO release was significantly reduced in middle age mice as evidenced by the lack of an inhibitory action of N¹⁰-nitro-L-arginine in uterine arteries of middle age mice. Maintenance of flow-induced dilatation, despite blockade of nitric oxide synthase, suggests compensation by other endothelium-derived relaxing factors. Likely candidates are bradykinin,²²³ prostaglandins,⁵²⁴ and endothelium-derived hyperpolarizing factor (EDHF).^{244,246}

Acetylcholine-induced endothelium-dependent vasodilatations were comparable and were sensitive to N¹⁰-nitro-L-arginine in uterine arteries of both age groups. Also, responses to Na-nitroprusside were comparable in young and middle age mice. Collectively these findings indicate that the signaling cascade leading to the phosphorylation of endothelial nitric oxide synthase (eNOS), which mediates endothelial responses to shear stress,¹⁰⁰ is selectively impaired in uterine arteries of middle age mice. This is in line with the reduced shear stress-induced eNOS phosphorylation that was recently observed in mesenteric resistance arteries of aged rats.⁵²⁵ Also, Hoffman and colleagues reported failure of shear stress to stimulate, phosphorylate and upregulate eNOS in human umbilical vein endothelial cells aged in cell culture.²²⁹

Elevated blood flow, and hence increased wall shear stress, is a major determinant of arterial remodeling.^{311,434} This flow-related arterial remodeling has been demonstrated to be endothelium-dependent.³¹² During pregnancy, blood flow through the uterine circulation increases substantially.⁴⁵⁶ Due to this increase in wall shear stress the uterine artery undergoes a structural increase in diameter, termed outward remodeling, and smooth muscle cell or medial hypertrophy.^{223,408,561} In the present study, we observed in middle age mice a diminished uterine artery remodeling (reduced luminal expansion) at 0.9 gestation. Our observation of blunted flow-induced arterial remodeling with aging is consistent with previous studies in carotid and mesenteric arteries of the rat.^{361,554} The trigger for outward arterial remodeling is most likely increased wall shear stress. Tuttle and colleagues have shown that the initial shear stress stimulus is proportional to the increase in lumen diameter during collateral development in mesenteric arteries in young rats.⁵⁵⁵ Langille and colleagues previously suggested that with increasing age higher shear stress levels are required to trigger remodeling.³¹¹

Controversy exists about the role of nitric oxide and eNOS in vascular remodeling. eNOS is known to be regulated by shear stress in the endothelium¹⁰⁰ and its expression varies with the shear stress level.⁵⁵⁵ An important role for eNOS and NO in flow-related arterial remodeling in large arteries has been suggested by several studies.^{361,546,547} However, in ligated mesenteric resistance arteries, flow-induced remodeling was not suppressed during chronic L-NAME treatment in rats.⁸⁴ This paradox might find its origin in endothelium-derived mediators that are resistant to blockade and deficiency of eNOS and which are more prominent in small muscular arteries than in large elastic conduit vessels. In uterine arteries of middle age mice, mechanisms that are resistant to N^ω-nitro-L-arginine could sustain flow-induced vasodilatation but not pregnancy-related remodeling.

The number of viable fetuses was significantly reduced in middle age term pregnant mice compared to young animals. Van der Heijden and colleagues have shown that at day 11 of murine pregnancy, litter size and the number of viable fetuses was similar in both mature and young mice. Thus, the uterine blood flow rise at this time point may be sufficient to allow normal placentation and early embryo development, however when blood flow demands are high at late pregnancy, the number of viable pups decreases progressively suggesting a causal relationship between blunted flow-related uterine artery remodeling and pregnancy outcome.⁵⁶¹ Retrospective population-based studies indicate a higher prevalence of perinatal complications in pregnant women over 35 years of age,^{163,317,399} which may be related to the increased incidences of chronic hypertension and diabetes mellitus at these ages.^{47,564}

In conclusion, aging blunts pregnancy-related uterine artery remodeling, which appears to contribute to a poorer pregnancy outcome. A selective reduction of shear stress-induced nitric oxide release might be the cause for this diminished outward remodeling.

Chapter 9

Acute and Chronic Adaptive Responses to Altered Blood Flow in Large and Small Arteries

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Abstract

In analogy to arterial hemodynamic responses to chronic pressure alterations, blood vessels have adaptive mechanisms to cope with sustained changes in arterial blood flow. This so called flow-induced arterial remodeling can occur in various physiological events, such as during pregnancy and long-term exercise, and in pathological situations, such as peripheral and coronary occlusive disease and atherosclerosis. Flow-induced remodeling of the arterial wall results in diameter adjustments that correct wall shear stress (WSS) toward normal values. Accordingly, chronic blood flow elevations lead to a widening of the arterial structural lumen diameter, whereas chronic blood flow reductions lead to narrowing of vessel diameter. Arterial responses to flow changes involve two processes: acute vasomotor responses and chronic wall reshaping or remodeling. Both processes are regulated by WSS that is sensed by the endothelium. The majority of studies on flow-related arterial remodeling have focussed on the role of endothelium-derived factors. However, a growing body of evidence indicates that blunted acute vasomotor responses to WSS do not necessarily entail a defect in chronic wall restructuring. Either other mediators are involved, or WSS is transmitted from the cell surface and cytoskeletal filaments to the nucleus, independent from mechano-chemical signaling processes, suggesting that acute and chronic responses to WSS can operate independently from each other.

In this review article we define the processes of flow-related arterial remodeling and focus on animal models representing experimental and physiological settings of chronic blood flow alterations in large and small arteries. We describe the spatial events in flow-related arterial remodeling and address the role of acute vasomotor responses, endothelium-derived factors, and the local renin-angiotensin system in chronic structural remodeling of the arterial wall in response to long-term blood flow changes.

Introduction

It is well established that arteries sense and respond to modification of hemodynamic conditions, such as changes in blood pressure and flow. These mechanical stimuli are sensed as circumferential wall stress (CWS, pressure) and wall shear stress (WSS, flow). CWS is the tangential stress induced by the transmural pressure. It is derived from the law of Laplace:

$$CWS = \frac{TP \cdot r}{Wt}$$

where TP is transmural pressure, r is radius and Wt is wall thickness. On the other hand, wall shear stress is the frictional force of the dragging blood flow. It is defined by the law of Pousseuille:

$$WSS = \frac{4 \cdot \eta \cdot Q}{\pi \cdot r^3}$$

in which η is the blood viscosity, Q is the blood flow velocity and r is the radius of the artery. The responses to these stresses consist of acute and chronic components. Acutely, the vasomotor tone changes (*e.g.* pressure-induced (myogenic) tone and flow-induced vasodilatation), while chronic exposure to mechanical stresses leads to a structural adaptation of the arterial wall, which ultimately restore mechanical stresses towards normal values.

Several remodeling responses can be distinguished (Figure 9.1). The classification proposed in 1996 by Mulvany, and other leaders in the field of hypertension-induced resistance-sized artery remodeling,³⁷⁹ is based on the changes of the structural arterial lumen diameter (inward or outward) and on changes in wall mass (increased: hypertrophic, decreased: hypotrophic, and unchanged: eutrophic). For example, an increase in lumen diameter without a change in medial cross-sectional area, would be denoted as an outward eutrophic remodeling. Note that an eutrophic remodeling can result from either an inward remodeling with an increase in wall thickness or an outward remodeling with a decrease in wall thickness, indicating that a change in wall mass may also involve rearrangement of existing wall material or a balance between cell growth and cell death.

Our reasons to write this review on flow-induced arterial remodeling are two-fold. Firstly, a growing consensus is emerging about arterial remodeling during hypertension. In analogy to this pressure-induced arterial remodeling we want to propose a general concept of flow-related arterial remodeling of small and large arteries. Secondly, the increasing number of studies using genetically modified animals yielded a large body of information

on the role of specific proteins and mediators in acute and chronic adaptive responses of arteries to altered blood flow. From these studies a growing body of evidence is emerging that suggest that optimal acute flow-induced vasodilatation is not required for chronic structural arterial remodeling in response to altered blood flow. This observation inspired us to address the role of endothelium-derived vasoactive factors in arterial diameter adjustments to chronic blood flow changes in animal models.

Figure 9.1. Arterial remodeling events (adapted from³⁷⁹) resulting from exposure to chronic alterations in transmural pressure and/or blood flow. On the vertical axis are lumen diameter changes denoted as either inward (decrease in diameter), outward (increase in diameter) or unchanged. Similarly, on the horizontal axis are wall mass changes termed hypotrophic, eutrophic, and hypertrophic as, respectively, a decreased, unchanged, and increased wall mass.

Lessons Learnt From Hypertension Studies

In hypertension, where arteries are chronically exposed to elevated transmural pressure, different kinds of adaptive arterial remodeling have been demonstrated in large and small arteries (Figure 9.2).^{1,410,416,453} Large arteries have a high content of extracellular matrix components such as collagen and elastin. Their capacity to constrict to (neuro)humoral and mechanical factors is less than 10%. Increased transmural pressure leads to an acute passive distension, which induces an elevation of circumferential wall stress. This signal is sensed, transduced, and eventually causes proliferation of cells and rearrangement of pre-existing wall material, and increased extracellular matrix deposition, thus leading to outward hypertrophy.^{318,378,439} The overall effect is an increase in wall thickness that normalizes CWS as depicted in the upper part of Figure 9.2.

In arterioles, increases in transmural pressure initially induce an active vasoconstriction, which opposes the passive distension. During this myogenic response, the thickness of the arteriolar wall increases and CWS is normalized. Thus the CWS sensor will no longer be activated and the proliferative signaling will not occur.⁹ Because of the rearrangement of dynamic focal-adhesion sites, by which the arterial cells are connected to their matrix, the maintained constriction will ultimately become structural,

constricted either spontaneously via myogenic tone or in combination with the thromboxane analogue U46619 to a level of approximately 55% of the diameter in the absence of smooth muscle tone (passive diameter). Some arteries developed a myogenic tone strong enough such that no U46619 had to be added in order to reach the desired constriction level. Time control experiments were conducted in order to check the stability of the FID responses. Three consecutive FID experiments yielded the same diameter responses to flow under control conditions. Therefore, experiments were performed on the same single vessel under control conditions, in the presence of intra- and extraluminally added N^G -nitro-L-arginine methyl ester (L-NAME, 100 $\mu\text{mol/L}$), and repeated a third time in the presence of both L-NAME (100 $\mu\text{mol/L}$) and indomethacin (10 $\mu\text{mol/L}$). Experiments in the presence of the B_2 receptor antagonist HOE-140 (0.1 $\mu\text{mol/L}$) were performed on a different set of $TK^{+/+}$ mice. Concentrations of L-NAME, indomethacin and HOE-140 were selected on the basis of the literature.

At the end of the experiment, arteries were incubated with a Ca^{2+} -free physiological salt solution containing sodium nitroprusside (0.1 mmol/L), and the pressure steps (10–140 mmHg) were repeated to obtain the pressure-diameter relationship in the absence of smooth muscle tone.

Figure 4.1. Experimental scheme to assess flow-induced vasodilatation of isolated pre-constricted uterine arteries. Abbreviations used: ACh: acetylcholine; SNP: sodium nitroprusside.

Pressure and diameter changes were recorded using the Biopac data acquisition system (MP100; Biopac, La Jolla, CA), and analyzed using Acqknowledge® software (Biopac). To determine the maximal lumen diameter, pressure-diameter curves were analyzed by a third-order polynomial curve fitting [$y = A + Bx + Cx^2 + Dx^3$] of individual curves using Graphpad Prism™ 2.01 software.

Myogenic tone was calculated using the formula: $\% \text{ MT} = ((\text{passive diameter} - \text{measured diameter}) / \text{passive diameter}) \times 100$. Flow-induced dilatation was expressed as percentage dilatation of U46619-induced and myogenic tone.¹⁹¹

leading to inward eutrophic remodeling (Figure 9.2, lower part).¹⁷ Small arteries ($\varnothing$: 100–300 μm) possess a weak myogenic reactivity and consequently display an intermediate type of remodeling.

In this concept, CWS is the sensed and controlled variable. In large arteries the hypertrophy normalizes the elevated CWS, and in arterioles this is accomplished by means of the myogenic tone. The extent to which an artery can constrict in general, and in response to an increase in CWS in particular, then determines the type of the adaptive structural response. Whether vasoconstrictors can trigger hypertrophy and how arteriolar constriction is ultimately fixed by a reduction of the structural lumen diameter, remain areas of active research.

In an effort to explain the structural adaptation of arteries to long term changes in blood flow, we used this pressure-induced remodeling theory as a starting point.

Hypertrophic and Eutrophic Inward Remodeling in Hypertension.

Elastic artery

Arteriole

Figure 9.2. Hypertrophic and eutrophic inward remodeling in hypertension. In large elastic arteries, possessing poor myogenic tone, increases in transmurial pressure augment circumferential wall stress, ultimately triggering cell proliferative responses and rearrangement of pre-existing wall material, thus leading to outward hypertrophy. As a consequence, wall thickness increases and this will normalize circumferential wall stress. As opposed to large arteries, arterioles have the capacity to constrict to elevations in transmurial pressure (myogenic tone). This pressure-induced constriction increases wall thickness resulting in a normalization of circumferential wall stress. Sustained myogenic tone might lead to rearrangement of wall material into inward eutrophic remodeling by not fully characterized mechanisms. TP: transmurial pressure; CWS: circumferential wall stress; Wt: wall thickness; MC: myogenic constriction; $\varnothing$: structural diameter; $\rightarrow$: consequence; open arrow: stimulation; $\uparrow$: increase; $\downarrow$: decrease; $\perp$: negative feedback regulation.

Flow-Induced Arterial Remodeling: a Definition

Flow-induced arterial remodeling is the process involving arterial adaptations to chronic alterations in flow (shear stress) imposed on the endothelium, vascular smooth muscle cells (VSMCs), adventitia and extracellular matrix composing the arterial wall. The ultimate result is a normalization of both CWS and WSS.

Remodeling of an arterial bed in response to changes in blood flow can occur via two mechanisms: capillary network expansion via the sprouting of new capillaries from existing capillaries (angiogenesis) and enlargement of existing collateral arterioles (arteriogenesis).^{13,259} In this review we will focus on the processes of arteriogenesis or shear stress-induced arterial remodeling, and not on ischemia-induced angiogenesis.

Changes in architecture of the arterial wall to alterations in hemodynamic stimuli occur under physiological as well as under pathological conditions. Situations in which flow-induced arterial remodeling occurs under physiological conditions are pre- and postnatal development, chronic exercise (leading to outgrowth of skeletal muscle circulation and outward remodeling of the muscular feed arteries), and pregnancy (sprouting of fetoplacental arteries and upstream uterine artery remodeling). Certain pathologies such as atherosclerosis, diabetes, and preeclampsia are associated with reduced or inadequate tissue perfusion leading to pathological adaptations of the vessel wall.

Differences between Small and Large Arteries

Elastic and resistance-sized arteries differ in size, composition, function and local environment.⁴⁵⁰ Resistance-sized arteries have a higher density of VSMCs than large elastic arteries, which have more extracellular matrix components such as collagen and elastin. VSMCs may be more susceptible to arterial wall mass changes caused by altered wall stresses. Furthermore, many small arteries are densely innervated with sympathetic nerves, whereas conduit arteries are not. Acute flow-induced dilatation (FID), opposes myogenic tone, and is more relevant and substantial in muscular, thin-walled arteries than in large arteries. For instance, FID in isolated pre-constricted mouse carotid arteries ($\pm 600 \mu\text{m}$) is approximately 30 to 50 μm , representing 5 to 10% of the diameter,^{37,328} whereas pre-constricted isolated small mesenteric arteries ($\pm 150 \mu\text{m}$) can dilate 50 to 60 μm , representing up to 40% of the passive diameter.^{220,353} Furthermore, the relative contribution of endothelium-derived vasoactive mediators regulating vasomotor tone depends largely on the vessel type. Nitric oxide (NO) is a potent vasodilator that is released in response to elevated WSS. In large arteries, such as the carotid artery, flow-induced dilatation is mainly depended on NO release,³⁵³ and to a lesser extent on PGI_2 and endothelium-derived hyperpolarizing factor (EDHF). However, in resistance arteries, the role of NO is less pronounced. In some arterioles, FID depends solely on the production of prostaglandins,²⁹¹ or a combination of prostaglandins and EDHF.⁵²⁴ Furthermore, matrix metalloproteinases (MMPs) are proteases that can degrade extracellular matrix proteins. Since large arteries have a higher content of these extracellular matrix components, the effect of MMPs on vessel remodeling might be different in large arteries compared to small muscular arteries. Taken together, this difference in endothelial function between large and small arteries might impose

differential effects on the modes of flow-induced arterial remodeling, as far as arterial lumen adjustments and arterial wall mass changes are concerned.

Before addressing the putative mediators involved in flow-induced remodeling, we describe some physiological and experimental settings of flow-induced arterial remodeling and focus on the spatial remodeling events of large and small arteries in response to flow alterations in closer detail.

Models of Flow-Induced Arterial Remodeling

Chronic alterations in blood flow occur under physiological conditions such as during exercise and pregnancy (Table 9.1). During treadmill exercise in rats, a significant increase in blood flow (reaching up to 50-fold) to working skeletal muscle develops,¹² resulting in significant morphological changes in the vascular wall and in the structure of arteriolar networks.^{314,488} During pregnancy a drastic rise in uterine blood flow progressively develops.^{160,335,456} In order to accommodate the increase in uterine blood flow, the uterine vasculature undergoes luminal expansion, longitudinal growth and an increase in wall mass.^{107,223,224,195,394,406,408,561} Immediately after giving birth, uterine blood flow declines sharply, this thus represents a model of physiological blood flow reduction.

Table 9.1. Studies Investigating Models of Physiological Flow-Related Remodeling in Several Arteries and Species

Artery Type	Species	Model	References
Aorta	Guinea Pig	Pregnancy	276
	Lamb	Perinatal growth	311
	Human	Pregnancy	133
Carotid	Rat	Pregnancy	277
	Guinea Pig	Pregnancy	277
	Ewe	Pregnancy	195
	Human	Exercise	484
Femoral	Guinea Pig	Pregnancy	275
	Human	Exercise	249
Skeletal muscle arterioles	Rat	Exercise	488
Uterine	Mouse	Pregnancy	223,224,561,571
	Rat	Pregnancy	107,394,406,408,517,518
	Guinea Pig	Pregnancy	145,363
	Rabbit	Pregnancy	508
	Pig	Pregnancy	199
	Ewe	Pregnancy	11,168,195,279
	Human	Pregnancy	59

Experimental models in which chronic alterations in arterial blood flow are induced by surgical manipulation, have been applied to a broad range of arteries in several species (Table 9.2). Most experimental animal models to study arterial remodeling in response to chronic elevations in blood flow involve creation of an arteriovenous fistula

(AVF), which can be performed in a number of large arteries such as the femoral artery^{119,282,350,498,539,546,547} and aorta^{33,202,595} in animals such as the baboon, rabbit, rat and mouse. A major advantage of this method is that supraphysiological blood flow levels are obtained in large arteries. For example, an approximate 30-fold increase in iliac artery blood flow was obtained in monkeys⁶⁰¹ and an 11-fold increase in carotid artery blood flow in the rat.⁵³⁹ Another method is the unilateral ligation of the carotid artery, first introduced by Guyton and Hartley,²⁰⁰ which leads to a reduction of blood flow in the ipsilateral (ligated) artery and a elevation in blood flow in the contralateral right carotid artery. Collaterals interconnecting the facial, cranial and cerebral areas, perfused by both carotid arteries form the anatomical basis for this shunting.²⁵ A comparable method was applied in mice by Kumar and Lindner,³⁰⁰ resulting in a complete cessation of blood flow in the ligated common carotid artery and a nearly doubling in blood flow in the contralateral carotid artery. Analogous to the unilateral carotid artery ligation, this surgical approach can be applied to side branches of the superior mesenteric artery, resulting in decreased blood flow in ligated mesenteric arteries and shunting of blood flow through collaterals resulting in flow-loaded mesenteric arteries.^{327,434,555-557} A number of mouse models exist in which flow-related arterial remodeling has been studied after targeted gene deletion, these are summarized in Table 9.3 and will be discussed.

Table 9.2. Studies Investigating Experimental Settings of Flow-Related Remodeling in Several Arteries and Species

Artery Type	Species	Model	References
Aorta	Mouse	AVF	201,202
	Rat	AVF	33,595
Carotid	Dog	AVF	282
	Human	Ligation	257,296
	Mouse	Ligation	65,138,173,187,210,260,299,300,371,381,383,463,479,502,503,522,59
	Rabbit	AVF	119,348,350,546,547
	Rabbit	Ligation	62,90,310-312,498
	Rat	AVF	539
	Rat	Ligation	361,368,580
	Rabbit	AVF	349
Cerebral	Dog	Ligation	73-75,591
Coronary	Rat	Orchidectomy	578
Cremaster muscle arteriole	Dog	Ligation	97,458
	Rabbit	Ligation	13,15,119,259
Femoral	Rat	Ligation	121,329,558
	Mouse	Ligation	326,327
	Rat	Ligation	71,84,434,435,552-527,586
Mesenteric	Pig	AVF	356
Pulmonary	Human	AVF	182,447
Radial			

Abbreviations used: AVF: arterio-venous fistula.

Spatial Events in Flow-Induced Arterial Remodeling

Responses to flow elevations

Acute WSS elevations initially cause a vasodilatation that tends to restore WSS in both large and small arteries (Figure 9.3). Flow-induced vasodilatation is mediated by endothelium-derived mediators such as NO, PGI₂, EDHF, and bradykinin. Sustained elevated blood flow will eventually result in a structural luminal expansion, most likely resulting from rearrangement of existing wall mass leading to a thinner wall. This thinning of the arterial wall will increase the CWS, which triggers growth and proliferation of arterial wall cells. The resulting wall hypertrophy normalizes CWS.^{71,434} Hence, the overall outward hypertrophic response aims at normalization of both WSS and CWS (Figure 9.4).

Table 9.3. Mouse Models in Which Flow-Related Arterial Remodeling has been Studied After Targeted Gene Deletion

Deleted Gene	Artery Type	Effect on Remodeling	Reference
Desmin	Mesenteric	Exaggerated remodeling	327
Dystrophin	Mesenteric	Reduced outward remodeling	326
Vimentin	Carotid	Exaggerated outward remodeling	479
Vitronectin	Carotid	Increased neointima formation	122
eNOS	Carotid	No inward remodeling	463
eNOS	Carotid	Increased neointima formation	598
iNOS	Carotid	Increased inward remodeling	598
nNOS	Carotid	Increased neointima formation	372
ET _B receptor	Carotid	Increased neointima formation	381
MMP-9	Carotid	Reduced neointima formation	173
FGF2	Carotid	Normal responses	522
P-selectin	Carotid	Reduced neointima formation	299
PAI-1	Carotid	Increased neointima formation	122
Osteopontin	Carotid	Reduced inflammatory response	383
Tissue angiotensin-converting enzyme	Carotid	Decreased medial hypertrophy	224
Tissue kallikrein	Uterine	Normal Outward remodeling	223
Tissue angiotensin-converting enzyme	Uterine	Normal Outward remodeling	224

Outward hypertrophic remodeling is observed in both large elastic arteries and in small muscular arteries. Zarins and colleagues showed that an AVF in primates led to hypertrophic outward remodeling,⁶⁰¹ and in rat aortas with an aortacaval fistula, medial cross-sectional area (CSA) increased due to VSMC hypertrophy, despite a decrease in arterial wall thickness.³³ Similar findings were obtained in carotid arteries with an AVF.³⁵⁰ Increases in wall shear stress result in vascular smooth muscle cell gene expression, such as the gene encoding for the nuclear transcription factor *c-fos*,²⁰² initiating processes such as growth and matrix remodeling, which lead to flow-induced enlargement.

Also in small arteries outward hypertrophic remodeling is observed in response to chronic elevations in blood flow.^{71,434,555-557} The hypertrophy was due to SMC proliferation, because SMC number, as determined by a 3D-dissector technique,

increased, and immunohistochemical assessment of proliferating cell nuclear antigen also showed an increase.⁷¹ The increase in wall mass was accompanied by a doubling of the DNA content of these flow-loaded vessels.⁴³⁴ VSMC reactivity to contractile compounds was increased compared to control arteries, due to the larger muscle mass. After 16 days, when outward remodeling was significant, desmin mRNA levels were reduced, indicative of VSMC de-differentiation. Endothelium-(in)dependent dilatations were not modified in these hyperperfused arteries.⁴³⁵ Furthermore, apoptotic cells were detected in the medial layer,⁷¹ suggesting both apoptotic and proliferative responses of the arterial wall. Also adventitial cell proliferation occurred in the flow-loaded arteries,^{434,555} indicating that all three individual layers of the arterial wall seem to be directly or indirectly sensitive to the degree of WSS imposed.

Flow-Induced Vasodilatation.

Figure 9.3. Flow-induced arterial vasodilatation in both small and large arteries. Acute increases in blood flow elevate wall shear stress which stimulates the release of endothelium-derived vasodilator factors and might reduce the release of endothelium-derived contraction factors.

Abbreviations: BF: blood flow; FID: flow-induced dilatation; WSS: wall shear stress; $\rightarrow$: consequence; open arrow: stimulation; $\uparrow$: increase; $\perp$: negative feedback regulation; ET-1: endothelin-1; $O_2^{\bullet-}$: superoxide anion; H_2O_2 : hydrogen peroxide; NO: nitric oxide; PGI_2 : prostacyclin; EDHF: endothelium-derived hyperpolarizing factor; BK: bradykinin.

A few complications arise when interpreting outward hypertrophic remodeling after drastic blood flow increases. Figure 9.4 suggests that sustained FID will lead to a rearrangement of wall material. However, a direct relation between acute FID and rearrangement has not been determined. Another discrepancy is the fact that large arteries have a limited FID that is insufficient to accommodate strong WSS elevations. During pregnancy uterine blood flow can increase 50-fold in rats, demanding a more than two-fold increase in uterine artery lumen diameter to normalize WSS, which is never accomplished by FID. In several situations of endothelial dysfunction, *e.g.* in diabetes and atherosclerosis, flow-induced arterial remodeling can still proceed. These observations suggest that arteries can follow a different strategy enabling a direct coupling between strongly elevated WSS and rearrangement of wall material leading to outward remodeling, that normalizes WSS. Analogous to Figure 9.4, this wall thinning will increase CWS and triggers a hypertrophic response of the arterial wall that normalizes CWS (Figure 9.5).

Responses to chronic flow reductions

In general, chronic reductions in arterial blood flow lead to a reduction in lumen diameter that normalizes WSS (Figure 9.5). Vasomotor tone will shift towards more vasoconstriction, probably due to less FID. In parallel to sustained pressure-induced vasoconstriction, this narrowing of the lumen diameter will become structural, since the diameter can not be restored to control values after application of several vasodilatory compounds 3 days postligation in mesenteric arteries⁴³⁴ and 7 days postligation in carotid arteries.^{62,462} This structural remodeling at day 7 coincided with an increase in cell death as shown in ligated rabbit arteries.⁹⁰ In large arteries, such as the carotid artery, abrupt flow cessation leads to an initial enlargement in the first week post ligation followed by a progressive decrease in lumen area reaching a plateau after 4 weeks.¹⁸⁷ The intima steadily increases throughout these 4 weeks. Formation of a neointima results in a reduction of the lumen diameter, that tends to normalize WSS (Figure 9.5 upper part). Concomitant with a neointima formation, a paradoxal hypertrophy of the media develops. The reason for this medial growth response is currently unclear, but may involve reduced NO levels favouring VSMC growth.

Flow-Induced Vasodilatation → Outward Hypertrophic Remodeling?

Figure 9.4. Sustained flow-induced dilatation resulting in outward hypertrophic remodeling. Chronic vasodilatation caused by increased wall shear stress will result in rearrangement of wall material that leads to a structural lumen diameter enlargement. This outward remodeling will normalize wall shear stress. Due to the decrease in wall thickness, circumferential wall stress increases, which triggers cell proliferation and/or smooth muscle cell hypertrophy. BF: blood flow; WSS: wall shear stress; FID: flow-induced dilatation; ∅: structural diameter; Wt: wall thickness; CWS: circumferential wall stress; →: consequence; open arrow: stimulation; ↑ increase; ↓ decrease; ⊥: negative feedback regulation.

Arterial Responses to Reduced Blood Flow (in small arteries).

Strong BF decreases (in large arteries):

Figure 9.5. Arterial responses to chronic blood flow reductions. *Upper part:* declines in blood flow will lower wall shear stress that inhibit flow-induced dilatation and augment myogenic tone. Acutely, reduced flow-induced dilatation normalizes wall shear stress, and chronically lead to structural lumen narrowing (compare to pressure-induced inward remodeling as shown in Figure 9.2). This inward remodeling decreases circumferential wall stress and triggers hypotrophy of the arterial wall. *Lower part:* strong blood flow decreases result in neointima formation, either as a response to injury or as part of an adaptive strategy to reduce lumen diameter (in order to normalize wall shear stress) and to increase wall thickness (to normalize circumferential wall stress). Abbreviations: BF: blood flow; WSS: wall shear stress; FID: flow-induced dilatation; MC: myogenic constriction; Ø: structural diameter; Wt: wall thickness; CWS: circumferential wall stress; →: consequence; open arrow: stimulation; ↑ increase; ↓ decrease; ⊥ negative feedback regulation.

In general, arterial responses of small arteries to reduced blood flow lead to an inward hypotrophic remodeling (Figure 9.5 lower part). Reduced WSS shifts the balance between FID and myogenic tone in favour of increased vasomotor tone, resulting in a structural lumen narrowing that decreases CWS, hereby inducing hypotrophy. For instance, after giving birth, uterine blood flow decreases rapidly and is restored to normal blood flow levels. Uterine arteries remodel their lumen diameter and wall mass in response to this blood flow decrease. In mice, uterine artery diameter and wall mass returned to nonpregnant values within 7 days postpartum.^{223,224} A few experimentally-induced models of chronic flow reductions have been applied to small arteries (Table 9.2). One of these involves a reduction of the workload of the rat cremaster muscle by unilateral orchidectomy leading to a reduction in blood flow to the cremaster muscle. After 3 weeks, significant inward hypotrophic remodeling of cremaster arterioles and inhibition of growth of arterioles in both size and number were observed.⁵⁷⁸ Furthermore, ligation of mesenteric resistance arteries in the rat and mice also leads to a rapid inward hypotrophic remodeling.^{71,327,434,553,555-557} VSMC contractility of these hypoperfused rat mesenteric resistance arteries was reduced, which was not solely due to the reduced wall mass.⁴³⁵ Like hyperperfused arteries, apoptosis and dedifferentiation of VSMC were

involved in the structural response to chronic flow reductions.⁷¹ *In vitro* cultured rat mesenteric arteries have demonstrated that active tone (e.g. chronic endothelin-1 vasoconstriction) is necessary to induce inward remodeling under static pressurized conditions, because incubating vessels at low pressure (2-5 mmHg) in the absence of tone did not result in permanent inward remodeling.¹⁷ This suggests that not decreased wall tension, but active tone is the trigger for inward remodeling. It remains to be elucidated whether also tone forms the driving force for flow-induced inward remodeling.

The Endothelium as a Flow Sensor

It is now generally believed that the endothelium is crucial in acute^{43,506} and chronic³¹² responses to changes in WSS, which is easily understandable since the endothelial cell monolayer is positioned at the interface between the vessel wall and the flowing blood. Evidence indicating a role of the endothelium as a shear stress sensor emerged from *in vitro* studies of cultured monolayers.^{110,112} In response to sustained steady flows, endothelial cells align and elongate in the direction of flow.^{110,255} This process involves rapid deformation and reorganization of actin-containing stress fibers, as well as other cytoskeletal intermediate filaments at the sites of cell-matrix contact forming the focal adhesion complexes.²¹⁵ The cytoskeleton is believed to play an important role in mechanosensing of fluid shear stress in whole vessels preparations. Shear stress induces rapid deformation of intermediate filaments.²¹⁵ In arterial rings treated with cytochalasin D, phalloidin, or colchicine, flow-induced vasodilatation was attenuated, implicating the importance of microfilaments and microtubules in flow-induced NO release.²⁵⁰ The intermediate filament protein vimentin is a component of the endothelial cytoskeleton.¹⁶⁶ Vimentin-deficient mice show a blunted acute flow-induced dilatation of mesenteric resistance arteries²²⁰ and an impaired mechanical stability, migration and contractile capacity of fibroblasts.¹³⁴ Unilateral left carotid artery ligation in vimentin knockout mice showed less marked constrictive remodeling and more pronounced medial hypertrophy of ligated arteries compared to ligated vessels of wild-type mice, whereas exaggerated outward remodeling of the contralateral arteries was seen in vimentin-deficient mice compared to arteries of wild-type mice.⁴⁷⁹ Similar overadaptive responses were observed in mesenteric arteries of desmin-deficient mice.³²⁷ So, the absence of one of these two main intermediate filaments lead to the same response (an excessive outward remodeling in response to chronic alterations in blood flow), despite blunted acute flow-induced dilatation,^{220,327} indicating that diameter adjustments in response to chronically altered blood flow can still proceed. This raises the question how shear stress induces long-term remodeling in the absence of an apparently optimal mechanosensing. Since intermediate filaments are linked to the nuclear lamina, shear stress may also effect the karyoskeleton. Through interactions between cell surface receptors and cytoplasmic and nuclear intermediate filaments,²⁶⁶ shear stress may directly regulate gene expression independently of biochemical signaling mechanisms such as NO release.^{215,343}

In general, two levels of molecular signaling can be distinguished in response to shear stress. The first is the rapid activation and regulation of proteins or structures in response to acute changes in flow. One of the best studied shear stress-responsive proteins is endothelial nitric oxide synthase (e(c)NOS or NOS3). Stimulation via this

pathway leads to a rapid increase in endothelial NO production leading to flow-induced vasodilatation.^{43,506} Prostaglandines,⁴⁶ bradykinin,^{37,223} and EDHF,^{244,246} as well as vasoconstrictors, such as endothelin-1²⁹⁷ and reactive oxygen species can also be released in response to shear stress elevation.³²⁴ The second response involves activation of specific transcriptional regulatory elements termed shear stress-response elements (SSREs) in the promoters of shear-responsive genes resulting in the regulation of gene expression. SSREs have been identified in the promotor region of several endothelial expressed genes, such as PDGF-A⁴⁴⁸ and MCP-1.⁵⁰⁰ These proteins are important in several pathophysiological processes of arterial remodeling, indicating that the endothelium not only senses and transduces shear stress, but also initiates gene transcription and arterial remodeling.⁴⁶⁰

Because mechanotransduction is only one aspect of flow-induced arterial remodeling, a detailed outline of the intracellular molecular transduction signaling would be beyond the scope of this review, which focuses on structural alterations in the entire vessel wall. Therefore, the reader is referred to some excellent reviews on signal transduction^{110,318,499,544} and gene expression in response to fluid shear stress.⁵⁴² In the next section we will discuss the role of endothelium-derived vasoactive mediators in more detail.

Role of Endothelium-Derived Mediators

Langille and O'Donnell demonstrated that the typical inward remodeling that develops in response to the prolonged decrease in carotid artery blood flow due to ligation did not occur when the endothelium had been removed.³¹² Other studies confirmed the importance of the presence of the endothelium during flow-related arterial remodeling.^{264,539} The mechanisms by which the endothelium controls flow-induced remodeling are not fully understood. Langille and Bendeck addressed the role of prostanooids, endothelin, and the local RAS in constrictive flow-induced arterial remodeling. However, none of these mediators seemed to affect the remodeling, suggesting that a novel vasoactive substance initiated the adaptive response to reduced blood flow.³¹¹ In this section we address the contribution of endothelium-derived factors on acute and long-term arterial diameter adjustments in response to altered blood flow in closer detail.

Nitric Oxide

Elevations in fluid shear stress increases the activity and expression of eNOS in cultured endothelial cells,¹¹³ whereas low or oscillatory shear stresses do the opposite.⁶⁰⁶ *In vivo* situations of increased blood flow have been shown to be accompanied by increased eNOS expression and NO release.^{361,384,492,523} A direct role of NO in arterial remodeling *in vivo* has not been firmly established. Suppression of NO synthesis by treating rabbits with nonhypertensive doses of the NOS inhibitor N^o-nitro-L-arginine methyl ester (L-NAME) inhibited flow-induced outward remodeling and failed to normalize WSS values in carotid arteries.⁵⁴⁷ A similar observation was made in rats,²⁰¹ suggesting that NO plays a role in the remodeling process in large arteries. However, studies in eNOS-deficient mice only

showed a role for NO in constrictive remodeling, whereas the outward remodeling was comparable between eNOS-deficient mice and wild-type mice.⁴⁶³ An explanation for this discrepancy might be the extent of the flow increase in the two models. A threshold value of WSS elevation to initiate remodeling has been postulated by Brownlee and Langille, since they observed that a 60% increase in BF did not cause any outward remodeling two months after creating a left-to-right carotid anastomosis in adult rabbits.^{62,310} In the eNOS-deficient mice, the increase in carotid artery blood flow was only 50% (below the threshold value of 60%), compared to 3.2 to 7-fold increase in blood flow after applying a left-to-right carotid anastomosis in adult rabbits.

The role of NO in flow-induced remodeling of resistance-sized arteries is controversial. Although eNOS expression in small arteries is correlated with WSS,^{326,555} pharmacological inhibition of eNOS by L-NAME does not prevent flow-related arterial and arteriolar remodeling.⁸⁴ This suggests that other mediators than NO can play a role in flow-related arterial remodeling and can compensate for the loss of NO. Indeed, studies in eNOS-deficient mice have observed compensation for the loss of NO production. Acute vasodilatation in isolated skeletal muscle arterioles was mediated solely by EDHF in female eNOS deficient mice,²⁴⁴ whereas this was mediated by prostaglandins in male eNOS deficient mice.⁵²⁴ Neuronal NOS (nNOS) can also become upregulated in the endothelium in coronary arteries of male eNOS deficient mice, and NO derived from nNOS together with prostaglandins maintain flow-induced dilatation in these mice.²⁴⁵ These data suggest gender and tissue specific compensation of the loss of one vasodilator and might explain why in some arteries, such as the carotid artery, chronic pharmacological inhibition of NOS will not be compensated by alternative endothelium-derived mediators, and in others, such as the mesenteric artery, upregulation by EDHF and/or prostaglandins can occur. However, whether this explains the difference in arterial remodeling in response to chronic changes in blood flow remains to be established.

The unilateral carotid artery ligation method applied to mice deficient in eNOS demonstrated a critical role for endogenous NO as a negative regulator of VSMC proliferation in response to a decrease in blood flow.⁴⁶³ These mice displayed a hyperplastic response of the carotid wall and a greater neointima formation compared to arteries of wild-type mice. *In vivo* gene transfer of eNOS has been shown to significantly inhibit neointima formation after balloon angioplasty,⁵⁷⁴ suggesting a vasculoprotective role of eNOS in the remodeling of hypoperfused arteries. Neuronal NOS (nNOS) deficient mice also exhibited constrictive remodeling caused by flow disruption and accelerated neointima formation, suggesting that nNOS suppresses atherosclerotic lesion formation *in vivo*.³⁷²

Degradation and synthesis of extracellular matrix (ECM) proteins are vital processes during arterial remodeling. In order for vascular cells to expand and migrate matrix reshaping is necessary. NO seems to participate in MMP activation.⁵⁴⁶ Flow-induced MMP activation is increased after flow-induced arterial enlargement of the rabbit carotid artery.⁵⁴⁶ A significant increase in MMP9 expression was measured in the ligated arteries that coincided with cell migration and the initial luminal enlargement,¹⁸⁷ suggesting that MMP activation is crucial in flow-induced arterial enlargement. Further evidence for MMPs to play an essential role in flow-induced remodeling comes from studies using tissue inhibitors of MMPs,³ targeted disruption of MMP-9¹⁷³ and microarray analysis.⁵⁸⁶

After unilateral carotid artery ligation, less intimal proliferation is observed and therefore a larger luminal area in MMP-9 deficient mice.¹⁷³ Furthermore, absence of MMP-9 leads to increased collagen content, and thus increased stiffness of the arterial wall.¹⁷³ Inhibition of certain MMPs may have beneficial effects in that it leads to inward remodeling, hereby strengthening the arterial wall and leading to more stable atherosclerotic plaques. Excellent reviews are available that describe the role of MMPs in vascular remodeling and atherogenesis in more detail.^{172,418}

Renin-Angiotensin System and Kallikrein Kinin System

In vitro experiments have shown that Ang II induces growth responses in cultured rat carotid arteries.⁴⁸⁰ Also, mechanical forces such as pressure and flow, CWS and WSS respectively, stimulated local synthesis of Ang II, which in turn induces protein synthesis expression of extracellular matrix proteins, such as fibronectin and collagen, via the AT₁R.²⁰⁻²² These data imply that the trophic effects of Ang II are mediated by angiotensin type 1 receptor (AT₁R) actions. The progression of neointimal thickening induced by Ang II was mediated via AT₁Rs, and not AT₂Rs, in injured rat carotid arteries.⁵⁶⁵

Chronic blood flow elevations created by placing a clip over the abdominal aorta, caused a reduction in vascular ACE activity by 40%, suggesting that shear stress alters ACE gene and protein expression *in vivo*.⁴⁵¹ Chronic alveolar hypoxia induces pulmonary hypertension and hypertrophy of small muscular pulmonary arteries.⁴⁴² This pulmonary vascular remodeling is attenuated in tissue ACE-deficient mice, suggesting that local production of Ang II by ACE plays an important role in the pathogenesis of pulmonary vascular remodeling during chronic hypoxia.⁵⁶⁸ Pharmacological inhibition of ACE has been shown to suppress pulmonary vascular neointimal formation⁴⁰¹ and the role of ACE inhibitors in the regression of the pressure-induced inward hypertrophic remodeling of resistance arteries has been firmly established (for a review see⁹²). Since ACE inhibitors also block the degradation of kinins, such as bradykinin,⁵⁸⁷ the beneficial effects of ACE inhibitors might be mediated by enhanced levels of kinins. Bradykinin modulates vascular tone by stimulating vascular endothelial B₂ receptors and the subsequent release of NO and prostacyclin (PGI₂).⁶⁹ Bergaya and colleagues, demonstrated the involvement of kinins in acute FID of carotid arteries in studies involving tissue kallikrein deficient mice.³⁷ We observed that pregnancy-related uterine artery remodeling was normal despite blunted FID of uterine arteries in these same knockout mice.²²³ ACE inhibition markedly reduced the neointimal formation after blood flow cessation in mouse carotid arteries.¹³⁸ This inhibition of intimal hyperplasia was significantly blunted after bradykinin receptor inhibition.¹⁴⁷ Furthermore, gene transfer of human tissue kallikrein, the kinin-forming enzyme in the endothelium, inhibited neointima formation after cessation of carotid artery blood flow in mice.¹³⁷ These beneficial actions were mediated via B₂ receptor signaling.¹³⁷ These results demonstrate a role for kinins in suppressing VSMC growth and migration, either directly or indirectly via NO, but do not show a role for kinins in arterial lumen adjustments in response to chronically altered blood flow.

Endothelin 1

Cultured endothelial cells exposed to physiological levels of shear stress cause an early transient upregulation of ET-1 gene expression followed by a sustained suppression.³⁴⁰

ET-1 production is enhanced during elevated CWS, causing increased active tone in resistance arteries during hypertension, promoting VSMC growth and inward remodeling.³¹⁵ ET_B receptor deficient mice developed increased neointima formation after cessation of carotid artery blood flow.³⁸¹ Blockade of ET_A and ET_B receptors reduced the hypertrophic remodeling of rat arteries with experimentally-induced hypertension.³²⁰ This stimulatory effect of ET-1 on DNA synthesis by VSMCs is attributed to the activation of epidermal growth factor receptor²⁶¹ and suppression of endothelial cell apoptosis.⁴⁹⁷ These observations demonstrate that ET-1 exerts its favourable inhibitory growth actions via ET_B receptors in response to reduced WSS, whereas it promotes growth pathways in response to increased CWS.

Growth Factors

In culture, endothelial cells exposed to shear stress respond by transient increases in growth factors such as platelet-derived growth factor (PDGF) A and B^{238,339,400} and basic fibroblast growth factor (FGF).⁴⁷¹ PDGF is a potent mitogen for VSMC *in vitro* and promotes VSMC migration and proliferation.²⁶⁷ In culture, PDGF-A and B chains can both be induced by shear stress²³⁸ and a shear-response element has been found in the promoter sequence of the PDGF-A and B genes.⁴⁴⁸ However during increased blood flow PDGF-B is downregulated.³³⁹ PDGF ligands are upregulated in response to blood flow reductions³⁶⁸ and downregulated in response to elevations in blood flow.⁵⁵²

Basic FGF is an endothelium-derived autocrine growth factor⁴⁷¹ that has high binding affinity for the extracellular matrix. It is upregulated *in vivo* during flow elevations where it may account for the increased endothelial cell density³⁴⁸ and downregulated in response to flow reductions, where it might explain the decreased endothelial cell density.³¹⁰ Fluid shear stress also induces FGF2 mRNA expression,³³⁹ and its expression increases during flow-induced outward remodeling.⁵⁰³ Treatment with FGF2 antibodies significantly inhibited luminal narrowing after carotid artery ligation,⁶⁵ whereas no differences in remodeling were observed in FGF2 knockout mice compared to wild-type mice.⁵⁵² Reasons for this discrepancy might be the difference in method used (cessation versus residual blood flow) or compensation for the loss of FGF2 by other FGFs.

Transforming growth factor-beta (TGF- β) is a potent regulator of the cell cycle in smooth muscle and endothelial cells.^{510,540} In culture, fluid shear stress induced endothelial TGF- β 1 production.⁴⁰⁰ TGF- β 1 and β 3 expression in the aorta was associated with endothelial and VSMC proliferation which preceded the outward remodeling in these flow-loaded aortas.⁵⁹⁵

Effect of Aging on Flow-Induced Arterial Remodeling

From the above it is clear that alterations in flow-induced dilatation or production of endothelium-derived vasoactive compounds has little effect on chronic flow-related arterial remodeling. In sharp contrast, arterial remodeling seems to be very sensitive to age-related arterial changes. Aging progressively results in a reduced endothelial regulation of vasomotor tone,^{301,307,355} a blunted reactivity of VSMC to vasoactive compounds,⁵⁴⁹ differences in protein expression⁵⁵⁴ and changes in ECM turnover and composition.³⁴⁷

Furthermore, aging has been shown to increase the level of reactive oxygen species such as superoxide anions and peroxynitrite.^{103,562} These compounds interact with many signaling pathways that control the expression of adhesion molecules and those that regulate cell proliferation, migration and angiogenesis⁵⁹² and which lead to a reduced bioavailability of endothelial-derived NO.^{198,548,603} Suppression of NO leads to significant increases in ET-1 production.²⁸ These processes create a milieu that predisposes to hypertension, atherosclerotic lesion formation and impairment of flow-induced arterial remodeling.⁴⁸ Studies of carotid artery remodeling in the rabbit^{310,311} and rat³⁶¹ have demonstrated blunting of flow-induced arterial remodeling with aging. Miyashiro and colleagues observed that juvenile rats experienced more outward remodeling in response to a $\pm 46\%$ increase in carotid artery blood flow for a 4-week period than in adult rats.³⁶¹ Brownlee and Langille observed that a 60% increase in BF did not cause any outward remodeling two months after creating a left-to-right carotid anastomosis in adult rabbits, whereas significant outward remodeling in response to a 47% increase in blood flow was visible in juvenile rats,⁶² after which these authors proposed the “threshold hypothesis”. This decreased ability in remodeling might result from a decreased capacity to sense WSS, which could elevate the threshold value to initiate a remodeling response.

Similar to large arteries, flow-loaded mesenteric arteries of mature rats display absence of flow-induced luminal expansion, whereas a normal outward remodeling occurred in juvenile animals.⁵⁵⁴ Medial hypertrophy was present in both juvenile and mature animals, suggesting that absence of luminal expansion does not entail lack of wall remodeling. In other words, WSS elevations were detected by the endothelium and the underlying signaling, resulting in endothelial and VSMC proliferation, is intact in mature animals. The absence of outward remodeling of arteries of mature rats was not due to an altered eNOS expression profile compared to younger rats.⁵⁵⁴ This means that the shear stress-induced regulation of eNOS expression is not impaired by aging and does not account for the impaired remodeling response.

Conclusions

Analogous to vascular adaptations to pressure, arteries have strategies to adapt to long-term alterations in blood flow. Vasoactive mediators that are released by shear stress result in acute vasodilatation or vasoconstriction in order to normalize wall shear stress. Chronic responses to altered wall shear stress involve more long-term molecular events such as gene and protein expression, extracellular matrix re-scaffolding, cell proliferation and apoptosis, that ultimately lead to wall restructuring that normalize circumferential wall stress and wall shear stress. In this review we have addressed the issue whether acute and chronic responses to WSS are associated with each other. A number of studies have studied both acute and chronic responses of arteries to altered blood flow (Table 9.4). Studies using animal models with reduced availability of vasorelaxing compounds or impaired mechanosensing have demonstrated that flow-induced arterial enlargement can still proceed despite blunted acute flow-induced dilatation, suggesting that blunted acute vasomotor responses to WSS do not necessarily entail a defect in chronic wall restructuring. In an effort to explain these observations we have proposed several remodeling strategies of large and small arteries in response to altered blood flow. When

flow-induced vasodilatation is unable to restore chronic wall shear stress values, rearrangement of wall material leading to outward remodeling is initiated by these augmented stresses on the endothelium (Figure 9.6). The exact mechanotransduction and biochemical signaling leading to this outward remodeling are not fully understood. Either other mediators are involved, or WSS is transmitted from the cell surface and cytoskeletal filaments to the nucleus, independent from mechano-chemical signaling processes, suggesting that acute and chronic responses to WSS can operate independently from each other as suggested by Maniotis and colleagues.³⁴³

In both small and large arteries, responses to chronic reductions in blood flow lead to inward remodeling. Predominately in large arteries, chronic large declines in blood flow promote neointimal growth, triggered as part of an adaptive strategy, that reduces lumen diameter and increases wall shear stress towards normal values. Nitric oxide mediates its favourable actions in reducing the intimal growth via antiproliferative and antimigratory effects on vascular smooth muscle cells.

The beneficial actions of ACE inhibition are indirectly related to nitric oxide (by increasing the half-life of bradykinin) and directly related in reducing the trophic actions of Ang II via AT₁ receptors. These actions of vasoactive mediators such as nitric oxide, bradykinin, Ang II, endothelin-1 and reactive oxygen species mostly affect arterial wall mass changes rather than arterial diameter adjustments.

Aging and absence or inhibition of certain MMPs seem to be the only factors that cause defects in arterial diameter adjustments in response to altered blood flow. Arterial diameter adaptations involve ECM re-scaffolding mediated by MMPs and cell migration and cell turnover, that seem to be functional in situations of inappropriate endothelial function, suggesting that acute and chronic responses to altered wall shear stress can operate independently from each other.

Future Perspectives

Flow-related arterial remodeling has never been demonstrated *in vitro*, strongly arguing that the interplay of endothelial cells and blood-born cells, such as monocytes and macrophages, is of importance in reshaping arteries in response to chronic alterations in blood flow. Therefore, much attention will be focussed on physiological and experimental settings of flow-induced remodeling such as occurring during and after pregnancy and arteriovenous shunting. Much information will be extracted from micro-array and proteomics analysis. Given the fact that more and more flow-related arterial remodeling is studied in mice, the use of genetically-altered mice will be of much benefit in understanding the pathways that are involved in flow-induced arterial remodeling. This information is of help in the development of specific pharmacological inhibition and other therapeutic strategies for the treatment of cardiovascular diseases, including atherosclerosis, hypertension, diabetes, preeclampsia, vein graft disease, and restenosis after angioplasty.

Table 9.4. Models studying acute (vasodilatation) and chronic (outward remodeling) arterial adjustments in response to elevations in flow.

Mediator	Model	Artery	Species	Vasodilatation	BF Change	Outward Remodeling	Reference
Desmin	Ligation	Mesenteric	Mouse	reduced	normal	normal	327
Dystrophin	Ligation	Mesenteric	Mouse	reduced	reduced	reduced	326,328
Vimentin	None	Mesenteric	Mouse	reduced	normal	ND	220
Vimentin	Ligation	Carotid	Mouse	ND	reduced	increased	479
Tissue ACE	pregnancy	Uterine	Mouse	normal	increased	normal	224
Tissue kallikrein	None	Carotid	Mouse	reduced	normal	normal	223
Tissue kallikrein	pregnancy	Uterine	Mouse	reduced	increased	normal	223
eNOS	Ligation	Carotid	Mouse	ND	ND	normal	463
NOS (NO)	Chronic L-NAME	Carotid	Rabbit	ND	reduced	reduced	547
NOS (NO)	Chronic L-NAME	Mesenteric	Rat	ND	reduced	normal	84
Unknown	Aging	Mesenteric	Rat	ND	normal	reduced	554
Unknown	Aging	Carotid	Rat	ND	normal	reduced	361
Unknown	Aging	Carotid	Rabbit	ND	normal	reduced	62
Unknown	Aging	Uterine	Mouse	normal/reduced NO release	ND	reduced	Ch. 8

Abbreviations used: BF: blood flow; ND: not determined

Flow-Induced Vasodilatation and Flow-Induced Outward Hypertrophic Remodeling.

Strong BF increases:

Figure 9.6. Flow-induced outward hypertrophic remodeling independent of flow-induced dilatation. *Upper part:* wall shear stress values can be normalized when the amplitude of the blood flow increase can be buffered by flow-induced dilatation. *Lower part:* however, when blood flow demand is large, such as during pregnancy in the uterine vascular bed, another strategy has to be followed. Via as yet undefined mechanisms, large increases in wall shear stress can result directly in rearrangement of wall material that enlarge the lumen diameter and normalize wall shear stress. The decrease in wall thickness rises circumferential wall stress that triggers cell proliferation and/or smooth muscle cell hypertrophy, hereby normalizing circumferential wall stress (*lower part*). BF: blood flow; WSS: wall shear stress; FID: flow-induced dilatation; $\varnothing$: structural diameter; W_t : wall thickness; CWS: circumferential wall stress; $\rightarrow$: consequence; open arrow: stimulation; $\uparrow$: increase; $\uparrow\uparrow$: large increase; $\downarrow$: decrease; $\perp$: negative feedback regulation.

Chapter 10

General Discussion

Two important hormonal systems, the renin-angiotensin system (RAS) and the kallikrein-kinin system (KKS), which are regarded as opposing enzymatic strategies in blood pressure regulation, were subject of this thesis. A major determinant of the RAS is angiotensin-converting enzyme (ACE), which can operate as a circulating enzyme and as an enzyme bound to the cell membrane, *i.e.* tissue ACE or t-ACE. A major determinant of the KKS is kallikrein, which functions as a circulating enzyme (plasma kallikrein) and as a tissue-bound enzyme (tissue kallikrein, TK). The most important mediators of the RAS and KKS are angiotensin II and bradykinin, respectively. In this thesis we evaluated the roles of these two enzymes and their effector peptides in acute and chronic responses of both small and large arteries to altered blood flow (shear stress). We studied these by using genetically-altered mice being deficient in either t-ACE or TK.

Morphological and Mechanical Properties of Arteries from t-ACE^{-/-} and TK^{-/-} mice

Carotid arteries of TK^{-/-} mice were significantly smaller compared to TK^{+/+} mice, whereas uterine artery structure was similar in both strains, suggesting that the KKS participates in the morphogenesis of large elastic arteries (chapter 3 and 4). The importance of t-ACE for the maturation of arterial structure and elasticity was demonstrated in chapter 6. Large and small arteries of t-ACE^{-/-} mice exhibited a reduced arterial distensibility compared to wild-type mice. This increased arterial stiffness was not caused by an altered fetal development, since chronic ACE inhibition (captopril) during postnatal development (starting 3 weeks after birth until 12 weeks of age) decreased arterial distensibility in arteries from wild-type mice. We hypothesized that bradykinin B₂ receptor stimulation might be involved in the regulation of arterial elasticity. After chronic (14-days) HOE 140 treatment, both the structural and mechanical changes in the t-ACE^{-/-} carotid artery were abolished, which suggests that the increased arterial stiffness in t-ACE^{-/-} mice was not related to the blood pressure, but rather involved B₂ receptors.

Role of Angiotensin II and Bradykinin in Acute Arterial Responses to Shear Stress

Bergaya and colleagues showed that carotid arteries of TK^{-/-} mice had a blunted shear stress-induced dilatation, because of a lack of bradykinin generation by TK in response to shear stress or flow.³⁷ Several studies have shown that angiotensin II can lead to the production of bradykinin,^{189,263,330,493,588} that overexpression of angiotensin type 2 (AT₂) receptors can lead to increased bradykinin production,⁵⁵¹ and that AT₂ receptors are involved in flow-induced vasodilatation.³⁵³ In chapter 2, we showed that functional AT₂ receptors, but not AT₁ receptors, play a key role in flow-induced dilatation of carotid arteries. Furthermore, B₂ receptor antagonism in carotid arteries of wild-type mice had a comparable, non-additive effect, suggesting that these actions act in sequence rather than in synergy. Experiments by Katada and Majima provided evidence that the formation of angiotensin II preceded that of bradykinin release.²⁸³ These observations emphasize the interplay of the RAS and the KKS in the regulation of vasomotor tone, and hence arterial blood pressure, by counterbalancing, via endothelium-derived bradykinin formation, the powerful vasoconstrictor actions of angiotensin II.⁵⁶³ It is obvious that ACE, being the angiotensin II-generating and bradykinin-degrading enzyme, plays a central role in these systems. In chapter 4 we confirm the key role of TK in flow-induced dilatation in resistance arteries (uterine artery). That bradykinin can also be released upon chemical

stimulation (acetylcholine) was shown in chapter 5, in which pre-constricted uterine arteries of t-ACE^{-/-} mice have a blunted dilatation in response to acetylcholine when bradykinin B₂ receptors were blocked.

Mammalian pregnancy is known to increase uterine arterial nitric oxide production.^{98,391} Expression of endothelial nitric oxide synthase (eNOS) mRNA and eNOS protein is increased during pregnancy,³³⁷ probably because of estrogen- and vascular endothelial growth factor-induced upregulation of eNOS,^{521,559} hereby increasing uterine blood flow.⁴⁵⁷ We observed an enhanced release of nitric oxide in uterine arteries of late pregnant wild-type and TK^{-/-} mice (chapter 4 and 5). This upregulation was less obvious in uterine arteries of late pregnant t-ACE^{-/-} mice, where the contribution of bradykinin was more pronounced compared to their wild-type counterparts, suggesting that bradykinin can compensate for nitric oxide during settings of enhanced vasodilator activity (chapter 5).

Flow-induced dilatation was markedly reduced in uterine arteries of 7-days postpartum mice, despite normal acetylcholine-mediated vasodilatation, suggesting a reduced shear stress sensing of the endothelium in these arteries (chapter 4 and 5). We have currently no mechanistic explanation for this phenomenon, which might help restore the low uterine blood flow after pregnancy.

Role of Angiotensin II and Bradykinin in Chronic Arterial Responses to Shear Stress

The importance of TK and t-ACE in arterial responses to chronic changes in arterial blood flow was investigated in chapters 3, 4 and 7. Chronic cessation of arterial blood flow resulted in a structural decrease in arterial lumen diameter (inward remodeling) in large conduit arteries and small muscular arteries, confirming numerous other studies. Carotid arteries of TK^{-/-} mice showed a more pronounced inward remodeling in response to a chronic cessation of carotid artery blood flow, whereas carotid arteries of t-ACE^{-/-} showed a reduced inward remodeling compared to their wild-type counterparts (chapter 7), suggesting a protective role of bradykinin in excessive inward remodeling. Inward remodeling was accompanied by a medial hypertrophy and VSMC hyperplasia in carotid arteries of wild-type, TK^{-/-}, but not t-ACE^{-/-} mice. No additional medial hypertrophy was observed in carotid arteries of TK^{-/-} mice. These observations suggest that not bradykinin but angiotensin II, acting as a growth factor^{39-41,480} is responsible for the observed increases in medial wall mass and VSMC number.

In uterine arteries of 7-days postpartum mice, which experience a drastic drop in uterine blood flow after delivery, uterine arterial structure was indistinguishable from that of nonpregnant mice, indicating the dynamic nature of the uterine vascular bed (chapter 4 and 7). Reversal of uterine artery structure was comparable in wild-type, TK^{-/-} and t-ACE^{-/-} mice. Consequently, intramural production of neither angiotensin II nor bradykinin are essential for the adjustments of uterine artery structure to the marked reduction in blood flow occurring after pregnancy.

Arterial responses to chronic elevations in blood flow resulted in an outward eutrophic remodeling in carotid arteries of t-ACE^{-/-} and t-ACE^{+/+} mice (chapter 7). However, no significant remodeling response was observed in carotid arteries of TK^{-/-} and TK^{+/+} mice (chapter 3). The reason for this discrepancy most likely include strain- and age-effects on the remodeling processes.^{210,293,361} In chapters 4 and 7 we studied the

role of TK and t-ACE, respectively, in the pregnancy-related changes of the uterine artery. We observed that TK and t-ACE deficiency did not compromise the outward hypertrophic remodeling of the uterine artery in response to the dramatic uterine blood flow increase.

Overall, these observations indicate that intra-arterial production of angiotensin II contributes substantially to the hyperplastic remodeling of the ligated carotid artery but not to the pregnancy-related uterine artery remodeling during and after pregnancy. Furthermore, they suggest a protective role for bradykinin in inward remodeling of large arteries in response to chronic reductions in blood flow.

Maternal Endothelium and Postponed Pregnancy

The effects of postponed murine pregnancy on acute and chronic uterine artery responses to shear stress were investigated in chapter 8. Endothelium-derived nitric oxide, which modulates arterial vasomotor tone and structure, is involved in the cardiovascular adaptations to pregnancy. Estrogen and shear stress are known to stimulate eNOS phosphorylation. Our findings suggest that by midlife, the effects of shear stress on eNOS are lost, this is in line with human endothelial cells aged in cell culture, where shear stress failed to phosphorylate and upregulate eNOS.²²⁹ Upregulation of alternative endothelium-derived relaxing factors such as prostacyclin and bradykinin compensates during acute flow-induced dilatation,^{246,524} but can not sustain chronic pregnancy-related uterine artery remodeling. This blunts the rise in blood flow during the third trimester of pregnancy, resulting in the observed fetal mortality.

Acute Versus Chronic Arterial Responses to Shear Stress

In chapter 9 we have addressed in detail the issue whether acute and chronic responses to shear stress are interrelated. A number of studies have investigated both acute and chronic responses of arteries to altered blood flow. Overall, these studies suggest that blunted acute vasomotor responses to shear stress do not necessarily entail a defect in chronic wall restructuring. Chronic responses to altered shear stress involve more long-term molecular events such as gene and protein expression, extracellular matrix re-scaffolding, cell proliferation and apoptosis. These processes are most likely unaffected by defects in the cytoskeletal architecture or in the acute release of endothelial-derived relaxing factors. However, defects in the phosphorylation of eNOS and the underlying signaling cascade⁵²⁵ might be detrimental in the process of flow-related arterial remodeling.

Perspectives

It may be worth considering that:

- bradykinin can contribute to the consequences of (pharmacological) modulation of the RAS as is increasingly suggested by experimental and clinical drug-intervention studies.
- flow-induced vasodilatation and -remodeling involve alternative pathways, which raises the possibility that valuable targets for cardiovascular pharmacotherapy remain to be explored. The key to these may be found in the unravelling of the effects of regionality and aging on arterial remodeling.

- Adaptive arterial structural changes differ between pre-existing collateral arteries and large elastic arteries. This opens the possibility for site-selective interventions.
- Effects on arterial remodeling of even middle age are far more marked than those of blockade or deficiency of cardiovascular key enzymes such as TK and t-ACE.

References

1. Aalkjaer C, Haegerty AM, Petersen KK, Swales JD, Mulvany MJ. Evidence for increased media thickness, increased neuronal amine uptake, and depressed excitation-contraction coupling in isolated resistance vessels from essential hypertensives. *Circ Res*. 1987;61:181-186.
2. Aartsen WM, Hilgers RHP, Schiffers PMH, Daemen MJAP, De Mey JGR, Smits JFM. Changes in vascular distensibility during angiotensin-converting enzyme inhibition involve bradykinin type 2 receptors. *J Vasc Res*. 2004;41:18-27.
3. Abbruzzese TA, Guzman RJ, Martin RL, Yee C, Zarins CK, Dalman RL. Matrix metalloproteinase inhibition limits arterial enlargement in a rodent arteriovenous fistula model. *Surgery*. 1998;124:328-335.
4. Abdalla S, Lother H, Abdel-tawab AM, Quirterer U. The angiotensin II AT₂ receptor is an AT₁ receptor antagonist. *J Biol Chem*. 2001;276:39721-39726.
5. Abdalla S, Lother H, el Massiery A, Quirterer U. Increased AT(1) receptor heterodimers in preeclampsia mediate enhanced angiotensin II responsiveness. *Nat Med*. 2001;7:1003-1009.
6. Achard JM, Fournier A, Mazouz H, Caride VJ, Penar PL, Fernandez LA. Protection against ischemia: a physiological function of the renin-angiotensin system. *Biochem Pharmacol*. 2001;62:261-271.
7. Akiyama SK, Olden K, Yamada KM. Fibronectin and integrins in invasion and metastasis. *Cancer Metastasis Rev*. 1995;14:173-189.
8. Alexander RW, Brock TA, Grimbene MA Jr, Rittenhouse SE. Angiotensin increases inositol triphosphate and calcium in vascular smooth muscle. *Hypertension*. 1985;7:447-451.
9. Allen AM, Zhou J, Mendelsohn AO. Localization and function of angiotensin AT₁ receptors. *Am J Hypertens*. 2000;13:31S-38S.
10. Ando J, Tsuboi H, Korenaga R, Takada Y, Toyama-Sorimachi N, Miyasaka M, Kamiya A. Shear stress inhibits adhesion of cultured mouse endothelial cells to lymphocytes by downregulating VCAM-1 expression. *Am J Pathol*. 1994;267:C679-C687.
11. Annibale DJ, Rosenfeld CR, Stull JY, Kamm KE. Protein content and myosin light chain phosphorylation in uterine arteries during pregnancy. *Am J Physiol*. 1990;259:C484-C489.
12. Armstrong RB, Laughlin MH. Exercise blood flow patterns within and among rat muscles after training. *Am J Physiol*. 1984;246:H296-H306.
13. Arras M, Ito WD, Scholz D, Winkler B, Schaper J, and Schaper W. Monocyte activation in angiogenesis and collateral growth in the rabbit hindlimb. *J Clin Invest*. 1998;101:40-50.
14. Ayajiki K, Kindermann M, Hecker M, Fleming I, Busse R. Intracellular pH and tyrosine phosphorylation but not calcium determine shear stress-induced nitric oxide production in native endothelial cells. *Circ Res*. 1996;78:750-758.
15. Baffour R, Berman J, Garb JL, Rhee SW, Kaufman J, Friedman P. Enhanced angiogenesis and growth of collaterals by in vivo administration of recombinant basic fibroblast growth factor in a rabbit model of lower limb ischemia: dose-response effect of basic fibroblast growth factor. *J Vasc Surg*. 1992;16:181-191.
16. Bakker EN, Van der Meulen ET, Spaan JA, Van Bavel E. Organoid culture of cannulated rat resistance arteries: effect of serum factors on vasoactivity and remodeling. *Am J Physiol*. 2000;278:H1233-H1240.
17. Bakker ENTP, Van der Meulen ET, Van den Berg BM, Everts V, Spaan JA, Van Bavel E. Inward remodeling follows chronic vasoconstriction in isolated resistance arteries. *J Vasc Res*. 2002;39:12-20.
18. Bao G, Gohlke P, Qadri F, Unger T. Chronic kinin receptor blockade attenuates the antihypertensive effect of ramipril. *Hypertension*. 1992;20:74-79.
19. Bao G, Qadri F, Stauss B, Stauss H, Gohlke P, Unger T. HOE 140, a new highly potent and long-acting bradykinin antagonist in conscious rats. *Eur J Pharmacol*. 1991;200:179-182.
20. Bardy N, Karillon GJ, Merval R, Samuel J-L, Tedgui A. Differential effects of pressure and flow on DNA and protein synthesis and on fibronectin expression by arteries in a novel organ culture system. *Circ Res*. 1995;77:684-694.
21. Bardy N, Merval R, Benessiano J, Samuel JL, Tedgui A. Pressure and angiotensin II synergistically induce aortic fibronectin expression in organ culture model of rabbit aorta. Evidence for a pressure-induced tissue renin-angiotensin system. *Circ Res*. 1996;79:70-78.
22. Bardy N, Merval R, Samuel JL, Tedgui A. Effects of angiotensin II, pressure and flow on fibronectin expression in a novel organ culture system of perfused, pressurized rabbit aorta. *Circulation*. 1994;90(suppl 1, pt 2):I-515.
23. Barker DJ. Fetal origins of coronary heart disease. *BMJ*. 1995;311:171-174.

24. Barnes MJ, Farndale RW. Collagens and atherosclerosis. *Exp Gerontol.* 1999;34:513-525.
25. Barone FC, Knudsen DJ, Nelson AH, Feuerstein GZ, Willette RN. Mouse strain differences in susceptibility to cerebral ischemia are related to cerebral vascular anatomy. *J Cerebr Blood Flow Metab.* 1993;13:683-692.
26. Barreto-Chaves ML, Ancas I, Krieger JE. Glucocorticoid regulation of angiotensin-converting enzyme in primary culture of adult cardiac fibroblasts. *Am J Physiol.* 2001;280:R25-R32.
27. Barton M, Cosentino F, Brandes RP, Moreau P, Shaw S, Lüscher TF. Anatomic heterogeneity of vascular aging: role of nitric oxide and endothelin. *Hypertension.* 1997;30:817-824.
28. Barton M, Haudenschild CC, D'Uscio LV, Shaw S, Munster K, Lüscher TF. Endothelin ET_A receptor blockade restores NO-mediated endothelial function and inhibits atherosclerosis in apolipoprotein E-deficient mice. *Proc Natl Acad Sci.* 1998;95:14367-14372.
29. Baumbach GL, Sigmund CD, Faraci FM. Cerebral arteriolar structure in mice overexpressing human renin and angiotensinogen. *Hypertension.* 2003;41:50-55.
30. Beasley D, Schwartz JH, Brenner BM. Interleukin 1 induces prolonged L-arginine-dependent cyclic guanosine monophosphate and nitrite production in rat vascular smooth muscle cells. *J Clin Invest.* 1991;87:602-608.
31. Becker RHA, Wiemer G, Linz W. Preservation of endothelial function by ramipril in rabbits on a long-term atherogenic diet. *J Cardiovasc Pharmacol.* 1991;18:S110-S115.
32. Bendeck MP, Langille BL. Rapid accumulation of elastin and collagen in the aortas of sheep in the intermediate perinatal period. *Circ Res.* 1991;69:1165-1169.
33. Ben Driss A, Benessiano J, Poitevin P, Lévy BI, Michel JB. Arterial expansive remodeling induced by high flow rates. *Am. J. Physiol.* 1997;272:H851-H858.
34. Ben Driss A, Himbert C, Poitevin P, Duriez M, Michel JB, Lévy BI. Enalapril improves arterial elastic properties in rats with myocardial infarction. *J Cardiovasc Pharmacol.* 1999;34:102-107.
35. Benetos A, Gavvas H, Stewart JM, Vavrej RJ, Hatnoglou S, Gavvas I. Vasodepressor role of endogenous bradykinin assessed by a bradykinin antagonist. *Hypertension.* 1986;8:971-974.
36. Benetos A, Gautier S, Laffleche A, Topouchian J, Frangin G, Gierd X, Sissman J, Safar ME. Blockade of angiotensin II type 1 receptors : Effect of carotid and radial artery structure and function in hypertensive humans. *J Vasc Res.* 2000;37: 8-15.
37. Bergaya S, Meneton P, Bloch-Fure M, Mathieu E, Alhenc-Gelas F, Lévy BI, Boulanger CM. Decreased flow-dependent dilatation in carotid arteries of tissue kallikrein-knockout mice. *Circ Res.* 2001;88:593-599.
38. Bergel DH. The static elastic properties of the arterial wall. *J Physiol.* 1961;156:445-457.
39. Berk BC. Vascular smooth muscle growth: autocrine growth mechanisms. *Physiol Rev.* 2001;81:999-1030.
40. Berk BC, Rao GN. Angiotensin II-induced vascular smooth muscle cell hypertrophy: PDGF A-chain mediates the increase in cell size. *J Cell Physiol.* 1993;154:368-380.
41. Berk B, Vekshtein V, Gordon H, Tsuda T. Angiotensin II-stimulated protein synthesis in cultured vascular smooth muscle cells. *Hypertension* 1989;13:305-314.
42. Bevan JA, Henrion D. Pharmacological implications of the influence of intraluminal flow on smooth muscle tone. *Annu Rev Pharmacol Toxicol.* 1994;34 :173-190.
43. Bevan JA, Laher I. Pressure and flow-dependent vascular tone. *FASEB J.* 1991;5:2267-2273.
44. Bevan JA, Wellman GC. Intraluminal flow-initiated hyperpolarization shift the membrane potential of arterial smooth muscle toward an intermediate level. *Circ Res.* 1993;73:1188-1192.
45. Bézie Y, Lamazière JMD, Laurent S, Chalande P, Sa Cunha R, Bonnet J, Lacolley P. Fibronectin expression and aortic wall elastic modulus in spontaneously hypertensive rats. *Arterioscler Thromb Vasc Biol.* 1998;18:1027-1034.
46. Bhargyalakshmi A, Frangos JA. Mechanism of shear-induced prostacyclin production in endothelial cells. *Biochem Biophys Res Commun.* 1989;158:31-37.
47. Bianco A, Stone J, Lynch L, Lapinski R, Berkowitz G, Berkowitz RL. Pregnancy outcome at age 40 and older. *Obstet Gynecol.* 1996;87:917-922.
48. Bilato C, Crow MT. Atherosclerosis and the vascular biology of aging. *Aging (Milano).* 1996;8(4):221-234.
49. Bird IM, Zhang L, Magness RR. Possible mechanisms underlying pregnancy-induced changes in uterine artery endothelial function. *Am J Physiol.* 2003;284:R245-R258.
50. Bloomqvist CG, Saltin B. Cardiovascular adaptations to physical training. *Annu Rev Physiol.* 1983;45:988-992.

51. Blüher M, Kahn BB, Kahn RC. Extended longevity in mice lacking the insulin receptor in adipose tissue. *Science*. 2003;299:572-574.
52. Boegehold MA. Heterogeneity of endothelial function within the circulation. *Curr Opin Nephrol Hypertens*. 1998;7:71-78.
53. Bohlen HG. Arteriolar closure mediated by hyperresponsiveness to norepinephrine in hypertensive rats. *Am J Physiol*. 1979;236:H157-H164.
54. Boo YC, Jo H. Flow-dependent regulation of endothelial nitric oxide synthase: role of protein kinases. *Am J Physiol*. 2003;285:C499-C508.
55. Boonen HC, Daemen MJ, Eerdmans PH, Fazzi GE, van Kleef EM, Schiffrers PM, De Mey JG. Mesenteric small artery changes after vasoconstrictor infusion in young rats. *J Cardiovasc Pharmacol*. 1993;22:388-395.
56. Borell U, Fernstrom I, Westman H. Hormonal influences in the uterine arteries: an arteriographic study in the human. *Acta Obstet Gynecol Scand*. 1953;32:271-284.
57. Boulanger CM, Caputo L, Lévy BI. Endothelial AT₁-mediated release of nitric oxide decreases angiotensin II contractions in rat carotid artery. *Hypertension*. 1995;26:752-757.
58. Brayden JE, Nelson MT. Regulation of arterial tone by activation of calcium-dependent potassium channels. *Science*. 1992;256:532-535.
59. Brosens I, Robertson W, Dixon H. The physiological response of the vessels of the placental bed to normal pregnancy. *J Pathol Bact*. 1967;93:569-579.
60. Brown NJ, Gainer JV, Murphey LJ, Vaughan DE. Bradykinin stimulates plasminogen activator release from human forearm vasculature through B₂ receptor-dependent, NO synthase-independent, and cyclooxygenase-independent pathway. *Circulation*. 2000;102:2190-2196.
61. Brown NJ, Gainer JV, Stein CM, Vaughan DE. Bradykinin stimulates tissue plasminogen activator release in human vasculature. *Hypertension*. 1999;33:1431-1435.
62. Brownlee RD, Langille BL. Arterial adaptations to altered blood flow. *Can J Physiol Pharmacol*. 1991;69:978-983.
63. Bruce NW. The effect on fetal development and uteroplacental blood flow of ligating a uterine artery in the rat near term. *Teratology*. 1977;16:327-332.
64. Bruce NW, Gibbs CP. Proceedings: The maternal placental circulation in the conscious rabbit. *J Reprod Fertil*. 1974;36:478.
65. Bryant SR, Bjercke RJ, Erichsen DA, Rege A, Lindner V. Vascular remodeling in response to altered blood flow is mediated by fibroblast growth factor-2. *Circ Res*. 1999;84:323-328.
66. Bucher B, Travo P, Maurent P, Stoclet JC. Vascular smooth muscle cell hypertrophy during maturation in rat thoracic aorta. *Cell Biol Int Rep*. 1982;6:883-892.
67. Burrige K, Fath K, Kelly T, Nuckells G, Turner CE. Focal adhesions: Transmembrane junctions between the extracellular matrix and the cytoskeleton. *Annu Rev Cell Biol*. 1988;4:487-525.
68. Buschmann I, Schaper W. The pathophysiology of the collateral circulation (arteriogenesis). *J Pathol*. 2000;190:338-342.
69. Busse R, Fleming I. Molecular responses of endothelial tissue to kinins. *Diabetes*. 1996;45:S8-S13.
70. Busse R, Mülsch A. Calcium-dependent nitric oxide synthesis in endothelial cytosol is mediated by calmodulin. *FEBS Lett*. 1990;265:133-136.
71. Buus CL, Pourageaud F, Fazzi GE, Janssen G, Mulvany MJ, De Mey JGR. Smooth muscle cell changes during flow-related remodeling of rat mesenteric resistance arteries. *Circ Res*. 2001;89:180-186.
72. Cachefeiro V, Sakakibara T, Nasjletti A. Kinins, nitric oxide, and the hypotensive effect of captopril and ramiprilat in hypertension. *Hypertension*. 1992;19:138-145.
73. Cai WJ, Koltai S, Kocsis E, Scholz D, Kostin S, Luo X, Schaper W, and Schaper J. Remodeling of the adventitia during coronary arteriogenesis. *Am J Physiol*. 2003;284:H31-H40.
74. Cai WJ, Koltai S, Kocsis E, Scholz D, Schaper W, Schaper J. Connexin37, not Cx40 and Cx43, is induced in vascular smooth muscle cells during coronary arteriogenesis. *J Mol Cell Cardiol*. 2001;33:957-967.
75. Cai W, Vosschulte R, Afsah-Hedjri A, Koltai S, Kocsis E, Scholz D, Kostin S, Schaper W, Schaper J. Altered balance between extracellular proteolysis and antiproteolysis is associated with adaptive coronary arteriogenesis. *J Mol Cell Cardiol*. 2000;32:997-1011.
76. Campbell DJ, Kladis A, Duncan AM. Bradykinin peptides in kidney, blood, and other tissues of the rat. *Hypertension*. 1993;21:155-165.

77. Caputo L, Benessiano J, Boulanger CM, Lévy BI. Angiotensin II increases cGMP content via endothelial angiotensin II AT₁ subtype receptors in the rat carotid artery. *Arterioscler Thromb Vasc Biol.* 1995;15:1646-1651.
78. Caputo L, Tedgui A, Lévy BI. Control of carotid vasomotor tone by local RAS in normotensive and spontaneously hypertensive rats: role of endothelium and flow. *Circ Res.* 1995;77:303-309.
79. Carmeliet P. Mechanisms of angiogenesis and arteriogenesis. *Nature Medicine.* 2000;6:389-395.
80. Carretero OA, Scicli AG. Local hormonal factors (intracrine, autocrine, and paracrine) in hypertension. *Hypertension.* 1991;18:158-169.
81. Celermajor DS, Sorensen KE, Spiegelhalter DJ, Georgakopoulos D, Robinson J, Deanfield JE. Aging is associated with endothelial dysfunction in healthy men years before the age-related decline in women. *J Am Coll Cardiol.* 1994;24:471-476.
82. Charchar FJ, Kapuscinski M, Harrap SB. Persistent reduction in renal nerve growth factor mRNA after perindopril treatment of young spontaneously hypertensive rats. *Hypertension* 1998;31:678-683.
83. Charpiot P, Holland PH, Figgi A, Piquet P, Scalbert E, Bodard H, Barlatier A, Latrille V, Trannier P, Mercier C, Luccioni R, Calaf R, Garçon D. ACE inhibition with perindopril and atherogenesis-induced structural and functional changes in minipig arteries. *Arterioscler Thromb.* 1993;13:1125-1138.
84. Ceiler DL, De Mey JGR. Chronic NG-nitro-L-arginine methyl ester treatment does not prevent flow-induced remodeling in mesenteric feed arteries and arcading arterioles. *Arterioscler Thromb Vasc Biol.* 2000;20: 2057-2063.
85. Ceiler DI., Nelissen-Vranken HJMG, Smits JFM, De Mey JGR. Pressure but not angiotensin II-induced increases in wall mass or tone influences static and dynamic aorta mechanics. *J Hypertens.* 1999;17:1109-1116.
86. Chen HI, Chiang IP, Jen CJ. Exercise training increases acetylcholine-stimulated endothelium-derived nitric oxide release in spontaneously hypertensive rats. *J Biomed Sci.* 1996;3:454-460.
87. Chen HI, Li HT. Physical conditioning can modulate endothelium-dependent vasorelaxation in rabbits. *Arterioscler Thromb Vasc Biol.* 1993;13:852-856.
88. Chen L, Xin X, Eckhart AD, Yang N, Faber JE. Regulation of vascular smooth muscle growth by alpha 1-adrenoreceptor subtypes in vitro and in situ. *J Biol Chem.* 1995;270:30980-30988.
89. Cherry P, Furchgott R, Zawadzki J, Jothianandan D. Role of endothelial cells in relaxation of isolated arteries by bradykinin. *Proc Natl Acad Sci. USA.* 1982;79:2106-2110.
90. Cho A, Mitchell L, Koopmans D, Langille BL. Effects of changes in blood flow rate on cell death and cell proliferation in carotid arteries of immature rabbits. *Circ Res.* 1997;81:328-337.
91. Christen T, Bochaton-Piallat ML, Neuville P, Rensen S, Redard M, Van Eys G, Gabbiani G. Cultured porcine coronary artery smooth muscle cells. A new model with advanced differentiation. *Circ Res.* 1999;85:99-107.
92. Chrysant SG. Vascular remodeling: the role of angiotensin-converting inhibitors. *Am Heart J.* 1998;135:S21-S30.
93. Clozel M, Gray GA, Breu V, Löffler BM, Osterwalder R. The endothelin ET_B receptor mediates both vasodilation and vasoconstriction in vivo. *Biochem Biophys Res Commun.* 1992;186:867-873.
94. Cockcroft JR, Sciberras DG, Goldberg MR, Ritter JM. Comparison of angiotensin converting enzyme inhibition with angiotensin II receptor antagonism in the human forearm. *J Cardiovasc Pharmacol.* 1993;22:579-584.
95. Cockell AP, Poston L. Flow-mediated vasodilatation is enhanced in normal pregnancy but reduced in preeclampsia. *Hypertension.* 1997;30:247-251.
96. Colluci-Guyon E, Portier M-M, Dunia I, Paulin S, Pournin S, Babinet C. Mice lacking vimentin develop and reproduce without an obvious phenotype. *Cell.* 1994;79:679-694.
97. Conrad MC, Anderson III JL, Garrett JB Jr. Chronic collateral growth after femoral artery occlusion in the dog. *J Appl Physiol.* 1971;31:550-555.
98. Conrad KP, Vernier KA. Plasma level, urinary excretion, and metabolic production of cGMP during gestation in rats. *Am J Physiol.* 1989;257:R847-R853.
99. Cooke CL, Davidge ST. Pregnancy-induced alterations of vascular function in mouse mesenteric and uterine arteries. *Biol Reprod.* 2003;68:1072-1077.
100. Corson MA, James NL, Latta SE, Nerem RM, Berk BC, Harrison DG. Phosphorylation of endothelial nitric oxide synthase in response to fluid shear stress. *Circ Res.* 1996;79:984-991.

101. Crijns FRL, Wolffenbuttel BHR, De Mey JGR, Struijker-Boudier HAJ. Mechanical properties of mesenteric arteries in diabetic rats: consequences of outward remodeling. *Am J Physiol*. 1999;276:H1672-H1677.
102. Crowe DL, Brown TN. Transcriptional inhibition of matrix metalloproteinase 9 (MMP-9) activity by a c-fos/estrogen receptor fusion protein is mediated by the proximal AP-1 site of the MMP-9 promoter and correlates with reduced tumor cell invasion. *Neoplasia*. 1999;4:368-372.
103. Csiszar A, Ungvar Z, Edwards JG, Kaminski P, Wolin MS, Koller A, Kaley G. Aging-induced phenotypic changes and oxidative stress impair coronary arteriolar function. *Circ Res*. 2002;90:1159-1166.
104. Daemen MJ, Lombardi DM, Bosman FT, Schwartz SM. Angiotensin II induces smooth muscle cell proliferation in the normal and injured rat arterial wall. *Circ Res*. 1991;68:450-456.
105. Danckwardt L, Shimizu I, Bonner G, Rettig R, Unger T. Converting enzyme inhibition in kinin-deficient brown Norway rats. *Hypertension*. 1990;16:429-435.
106. D'Angelo LS. Shear stress induces changes in the morphology and cytoskeleton organization of arterial endothelial cells. *Eur J Vasc Surg*. 1995;9:86-92.
107. D'Angelo G, Osol G. Regional variation in resistance artery diameter responses to alpha-adrenergic stimulation during pregnancy. *Am J Physiol*. 1993;264:H78-H85.
108. Daugherty A, Manning MW, Cassis LA. Angiotensin II promotes atherosclerotic lesions and aneurysms in apolipoprotein E-deficient mice. *J Clin Invest*. 2000;105:1605-1612.
109. Daugherty A, Manning MW, Cassis LA. Antagonism of AT₂ receptors augments angiotensin II-induced abdominal aortic aneurysms and atherosclerosis. *Br J Pharmacol*. 2001;134:865-870.
110. Davies PF. Flow-mediated endothelial mechanotransduction. *Physiol Rev*. 1995;75:519-559.
111. Davies PF, Olesen S-P, Clapham DE, Morrel EM, Schoen FJ. Endothelial communication: state of the art lecture. *Hypertension*. 1988;11:563-572.
112. Davies PF, Tripathi SC. Mechanical stress mechanisms and the cell: an endothelial paradigm. *Circ Res*. 1993;72:239-245.
113. Davis ME, Cai H, Drummond GR, Harrison DG. Shear stress regulates endothelial nitric oxide synthase expression through c-Src by divergent signaling pathways. *Circ Res*. 2001;89:1073-1080.
114. Davis ME, Grumbach IM, Fukui T, Cutchins A, Harrison DG. Shear stress regulates endothelial nitric oxide synthase promoter activity through nuclear factor kappa-B binding. *J Biol Chem*. 2004;279:163-168.
115. Davis MJ. Myogenic response gradient in an arteriolar network. *Am J Physiol*. 1993;264:H2168-H2179.
116. Davis MJ, Donvitz JA, Hood JD. Stretch-activated single-channel and whole cell currents in vascular smooth muscle. *Am J Physiol*. 1992;262:C1083-C1088.
117. Davis MJ, Hill MA. Signaling mechanisms underlying the vascular myogenic response. *Physiol Rev*. 1999;79:387-423.
118. Davis MJ, Meininger GA, Zawieja DC. Stretch-induced increases in intracellular calcium of isolated vascular smooth muscle cells. *Am J Physiol*. 1992;263: H1292-H1299.
119. De Kleijn DPV, Sluijter JPG, Smit J, Velema E, Richard W, Schoneveld AH, Pasterkamp G, Borst C. Furin and membrane type-1 metalloproteinase mRNA levels and activation of metalloproteinase-2 are associated with arterial remodeling. *FEBS Letters* 2001;501:37-41.
120. Delp MD, McAllister RM, Laughlin MH. Exercise training alters endothelium-dependent vasoreactivity of rat abdominal aorta. *J Appl Physiol*. 1993;75:1354-1363.
121. De Mey JG, Van der Heijden H, Janssen G, Fazzi G. Structural and functional remodeling of poststenotic arteries in the rat. *Adv Exp Med Biol*. 1993;346:283-290.
122. De Waard V, Arkenhout EK, Carmeliet P, Lindner V, Pannekoek H. Plasminogen activator inhibitor 1 and vitronectin protect against stenosis in a murine carotid artery ligation model. *Arterioscler Thromb Vasc Biol*. 2002;22:1978-1983.
123. Diamond SL, Eskin SG, McIntire LV. Fluid flow stimulates tissue plasminogen activator secretion by cultured human endothelial cells. *Science*. 1989;243:1483-1485.
124. Dimmeler S, Fleming I, Fissthaler B, Herman C, Busse R, Zeiher AM. Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation. *Nature*. 1999;399:601-605.
125. Dimmeler S, Rippmann V, Weiland U, Haendeler J, Zeiher AM. Angiotensin II induces apoptosis of human endothelial cells: proliferative effect of nitric oxide. *Circ Res*. 1997;81:970-976.
126. Dinunno FA, Jones PP, Seals DR, Tanaka H. Age-associated arterial wall thickening is related to elevations in sympathetic activity in healthy humans. *Am J Physiol*. 2000;278:H1205-H1210.

127. Dinenna FA, Tanaka H, Monahan KD, Clevenger CM, Eskurza I, DeSouza CA, Seals DR. Regular endurance exercise induces expansive arterial remodeling in the trained limbs of healthy men. *J Physiol.* 2001;534:287-295.
128. Dolber PC, Spach MS. Picrosirius red staining of cardiac muscle following phosphomolybdic acid treatment. *Stain Technol.* 1987;62:23-26.
129. Donoghue M, Hsieh F, Baronas E, Godbout K, Gosselin M, Stagliano N, Donovan M, Woolf B, Robison K, Jeyaseelan R, Breitbart RE, Acton S. A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. *Circ Res.* 2000;87:e1-e9.
130. Dull RO, Davies PF. Flow modulation of agonist (ATP) response (Ca^{2+}) coupling in vascular endothelial cells. *Am J Physiol.* 1991;261:H149-H156.
131. Dzau VJ. Vascular wall renin-angiotensin pathway in control of the circulation. A hypothesis. *Am J Med.* 1984;77:31-36.
132. Dzau VJ. Circulating versus local renin-angiotensin system in cardiovascular homeostasis. *Circulation.* 1988;77:4-12.
133. Easterling TR, Benedetti TJ, Schmucker BC, Carlson K, Millard SP. Maternal hemodynamics and aortic diameter in normal and hypertensive pregnancies. *Obstet Gynecol.* 1991;78:1073-1077.
134. Eckes B, Dogic D, Colucci-Guyon E, Wang N, Maniotis A, Ingber D, Merckling A, Langz F, Aumailley M, Delouee A, Koteliansky V, Babinet C, Krieg T. Impaired mechanical stability, migration and contractile capacity in vimentin-deficient fibroblasts. *J Cell Sci.* 1998;111:1897-1907.
135. Egashira K, Inou T, Hirooka Y, Kai H, Sugimachi M, Suzuki S, Kuga T, Urabe Y, Takeshita A. Effects of age on endothelium-dependent vasodilation of resistance coronary artery by acetylcholine in humans. *Circulation.* 1993;88:77-81.
136. Egashira K, Koyanagi M, Kitamoto S, Ni W, Kataoka C, Morishita R, Kaneda Y, Akiyama C, Nishida K, Sueishi K, Takeshita A. Anti-monocyte chemoattractant protein-1 gene therapy inhibits vascular remodeling in rats: blockade of MCP-1 activity after intramuscular transfer of a mutant gene inhibits vascular remodeling induced by chronic blockade of NO synthesis. *FASEB J.* 2000;14:1974-1978.
137. Emanueli C, Salis MB, Chao J, Chao L, Agata J, Lin K-F, Munao A, Straino S, Minasi A, Capogrossi MS, Madeddu P. Adenovirus-mediated human tissue kallikrein gene delivery inhibits neointima formation induced by interruption of blood flow in mice. *Arterioscler Thromb Vasc Biol.* 2000;20:1459-1466.
138. Emanueli C, Salis MB, Figueroa C, Chao J, Chao L, Gaspa L, Capogrossi MC, Madeddu P. Participation of kinins in the captopril-induced inhibition of intimal hyperplasia caused by interruption of carotid blood flow in the mouse. *Br J Pharmacol.* 2000;130:1076-1082.
139. Erdős EG. Angiotensin I converting enzyme and the changes in our concepts through the years: Lewis K. Dahl memorial lecture. *Hypertension.* 1990;16:363-370.
140. Esther CR Jr, Howard TE, Marino EM, Goddard JM, Capecchi MR, Bernstein KE. Mice lacking angiotensin-converting enzyme have low blood pressure, renal pathology, and reduced male fertility. *Lab Invest.* 1996;74:953-965.
141. Esther CR Jr, Marino EM, Howard TE, Machaud A, Corvol P, Capecchi MR, Bernstein KE. The critical role of tissue angiotensin-converting enzyme as revealed by gene targeting in mice. *J Clin Invest.* 1997;99:2375-2385.
142. Fabre JE, Rivard A, Magnier M, Silver M, Isner JM. Tissue inhibition of angiotensin-converting enzyme activity stimulates angiogenesis in vivo. *Circulation.* 1999;99:3043-3049.
143. Falcone JC, Meininger GA. Endothelin mediates a component of the enhanced myogenic responsiveness of arterioles from hypertensive rats. *Microcirculation.* 2000;6:305-313.
144. Falkenhahn M, Gohlke P, Paul M, Stoll M, Unger T. The renin-angiotensin system in the heart and vascular wall: new therapeutic aspects. *J Cardiovasc Pharmacol.* 1994;24:S6-S13.
145. Fallgren B, Bjellin L, Edvinsson L. Effect of pregnancy and sex steroids on alpha-1 adrenoceptor mechanisms in the guinea-pig uterine vascular bed. *Pharmacol Toxicol.* 1988;63:375-381.
146. Faraci FM, Sigmund CD. Vascular biology in genetically altered mice: smaller vessels, bigger insight. *Circ Res.* 1999;85:1214-1225.
147. Farhy RD, Carretero OA, Ho KI, Scicli AG. Role of kinins and nitric oxide and the effects of angiotensin-converting enzyme inhibitors on neointima formation. *Circ Res.* 1993;72:1202-1210.
148. Farhy RD, Ho KI, Carretero OA, Scicli AG. Kinins mediate the antiproliferative effect of ramipril in the rat carotid artery. *Biochem Biophys Res Commun.* 1992;182:283-288.

149. Fernandez-Alfonso MS, Martorana PA, Licka I, van Even P, Trobis D, Scholkens BA, Paul M. Early induction of angiotensin I-converting enzyme in rat carotid artery after balloon injury. *Hypertension*. 1997;30:272-277.
150. Ferrario CM, Chappell MC, Dean RH, Iyer SN. Novel angiotensin peptides regulate blood pressure, endothelial function, and natriuresis. *J Am Soc Nephrol*. 1998;9:1716-1722.
151. Fishbein MC, Siegel RJ. 1996. How big are coronary atherosclerotic plaques that rupture? *Circulation*. 1996;94:2662-2666.
152. Fishel RS, Thourani V, Eisenberg SJ, Shai SY, Corson MA, Nabel E, Bernstein KE, Berk BC. Fibroblast growth factor stimulates angiotensin converting enzyme expression in vascular smooth muscle cells. Possible mediator of the response to vascular injury. *J Clin Invest*. 1995;95:377-387.
153. Fitzgerald MD, Tanaka H, Tran ZV, Seals DR. 1997. Age-related declines in maximal aerobic capacity in regularly exercising vs. sedentary women: a meta-analysis. *J Appl Physiol*. 1997;83:160-165.
154. Fleming I, Busse R. Signal transduction of eNOS activation. *Cardiovasc Res*. 1999;43:532-541.
155. Folkow B. A study of the factors influencing the tone of denervated blood vessels performed at various pressures. *Acta Physiol Scand*. 1952;27:99-117.
156. Folkow B. Description of the myogenic hypothesis. *Circ Res* 14-15: I279-I285, 1964.
157. Folkow B. Myogenic mechanisms in the control of systemic resistance. Introduction and historical background. *J Hypertens Suppl*. 1989;7:S1-4.
158. Folkow B, Svanborg A. Physiology of cardiovascular aging. *Physiol Rev*. 1993;73:725-764.
159. Force T, Boverie JV. Growth factors and mitogen-activated protein kinases. *Hypertension* 1998;31:152-161.
160. Ford SP. Control of uterine and ovarian blood flow throughout the estrous cycle and pregnancy of the ewe, sow, and cow. *J. Anim. Sci.* 1979;49:1510-1516.
161. Frangos JA, Eskin SG, McIntire LV, Ives CL. Flow effects on prostacyclin production by cultured human endothelial cells. *Science*. 1985;227:1477-1479.
162. Franklin BA, Kahn JK. Delayed progression of coronary atherosclerosis with intensive risk factor modification: effects of diet, drugs, and exercise. *Sports Med*. 1996;22:306-320.
163. Fretts RC, Schmittiel J, McLean FH, Usher RH, Goldman MB. Increased maternal age and the risk of fetal death. *N Engl J Med*. 1995;333:953-957.
164. Frid MG, Aldashev AA, Dempsey EC, Stenmark KR. Smooth muscle cells isolated from discrete compartments of the mature vascular media exhibit unique phenotypes and distinct growth capabilities. *Circ Res*. 1997;81:940-952.
165. Frisbee JC, Stepp DW. Impaired NO-dependent dilation of skeletal muscle arterioles in hypertensive diabetic obese Zucker rats. *Am J Physiol*. 2001;281:H1304-H1311.
166. Fuchs E, Weber K. Intermediate filaments: structure, dynamics, function, and disease. *Annu Rev Biochem*. 1994;63:345-382.
167. Fujii K, Heistad DD, Faraci FM. Effects of diabetes mellitus on flow-mediated and endothelium-dependent dilatation of the rat basilar artery. *Stroke*. 1992;23:1494-1498.
168. Fuller EO, Galetti PM, Takeuchi T. Major and collateral components of blood flow to pregnant sheep uterus. *Am J Physiol*. 1975;229:279-285.
169. Fulton D, Gratton JP, McCabe TJ, Fontana J, Fujio Y, Walsh K, Franke TF, Papapetropoulos A, Sessa WC. Regulation of endothelium-derived nitric oxide production by the protein kinase Akt. *Nature*. 1999;399:597-601.
170. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature*. 1980;288:373-376.
171. Gallinat S, Busche S, Raizada MK, Summers C. The angiotensin II type 2 receptor: an enigma with multiple variations. *Am J Physiol*. 2000;278:E357-E374.
172. Galis ZS, Khatri JJ. Matrix metalloproteinases in vascular remodeling and atherogenesis: the good, the bad, and the ugly. *Circ Res*. 2002;90:251-262.
173. Galis ZS, Johnson C, Godin D, Magid R, Shipley M, Senior RM, Ivan E. Targeted disruption of the matrix metalloproteinase-9 gene impairs smooth muscle cell migration and geometrical arterial remodeling. *Circ Res*. 2002;91:852-859.
174. Garcia-Cardena G, Fan R, Shah V, Sorrentino R, Cirino G, Papapetropoulos A, Sessa WC. Dynamic activation of endothelial nitric oxide synthase by Hsp90. *Nature*. 1998;392:821-824.
175. Garcia-Duran M, de Frutos T, Diaz-Reccasens J, Garcia-Galves G, Jimenez A, Monton M, Farre J, Sanches de Miguel L, Gonzales-Fernandez F, del Mar Arriero M, Rico L, Garcia R, Cassado S, Lopez-

- Farre A. Estrogen stimulates neuronal nitric oxide synthase protein expression in human neutrophils. *Circ Res.* 1999;85:1020-1026.
176. Gardes J, Baussant T, Corvol P, Ménard J, Alhenc-Gelas F. Effect of bradykinin and kininogens in isolated rat kidney vasoconstricted by angiotensin II. *Am J Physiol.* 1990;258:F1273-F1281.
 177. Garg UC, Hassid A. Nitric oxide-generating vasodilators and 8-bromo-cyclic guanosine monophosphate inhibit mitogenesis and proliferation of cultured rat vascular smooth muscle cells. *J Clin Invest.* 1989;83:1774-1777.
 178. Gasic G, Arenas C, Gasic T, Gasic G. Coagulation factors X, Xa and protein S as potent mitogens of cultured aortic smooth muscle cells. *Proc Natl Acad Sci.* 1992;89:323-328.
 179. Gavras H, Brunner HR. Role of angiotensin and its inhibition in hypertension, ischemic heart disease and heart failure. *Hypertension.* 2001;37:342-345.
 180. Geisterfer AA, Peach MJ, Owens GK. Angiotensin II induces hypertrophy, not hyperplasia, of cultured rat aortic muscle cells. *Circ Res.* 1988;62:749-756.
 181. Gerhard M, Roddy MA, Creager SJ, Creager MA. Aging progressively impairs endothelium-dependent vasodilation in forearm resistance vessels in humans. *Hypertension.* 1996;27:849-853.
 182. Girerd X, London G, Boutouyrie P, Mourad J-J, Safar M, Laurent S. Remodeling of the radial artery in response to a chronic increase in shear stress. *Hypertension.* 1996;27:799-803.
 183. Giudicelli JF, Freslon JL, Glasson S, Richer C. Captopril and hypertension development in the SHR. *Clin Exp Hypertens.* 1980;2:1083-1096.
 184. Glagov S. Intimal hyperplasia, vascular remodeling, and the restenosis problem. *Circulation.* 1994;89:2888-2891.
 185. Glagov S, Giddens DP, Zarins CK. Micro-architecture and composition of artery walls: relationship to location, diameter and the distribution of mechanical stress. *J Hypertens.* 1992;10:S101-S104.
 186. Glagov S, Weisenberg E, Zarins CK, Stankunavicius R, Kolletis GJ. Compensatory enlargement of human atherosclerotic coronary arteries. *N Engl J Med.* 1987;316:1371-1375.
 187. Godin D, Ivan E, Johnson C, Magid R, Galis ZS. Remodeling of carotid artery is associated with increased expression of matrix metalloproteinases in mouse blood flow cessation model. *Circulation.* 2000;102:2861-2866.
 188. Gohlke P, Lamberty V, Kuwer I, Bartenbach S, Schnell A, Unger T. Vascular remodeling in systemic hypertension. *Am J Cardiol.* 1993;24:2E-7E.
 189. Gohlke P, Pees C, Unger T. AT₂ receptor stimulation increases aortic cyclic GMP in SHRPP by a kinin-dependent mechanism. *Hypertension.* 1998;31:349-355.
 190. Goldman RD, Chou YH, Dessev G, Eriksson J, Goldman A, Khuon S, Kohnken R, Lowy M, Miller R, Murphy K, Opal P, Skalli O, Straube K. Dynamic aspects of cytoskeletal and karyoskeletal intermediate filament systems during the cell cycle. *Cold Spring Harbor Symp Quant Biol.* 1991;56:629-642.
 191. Gorny D, Loufrani L, Kubis N, Lévy BI, Henrion D. Chronic hydralazine improves flow (shear stress)-induced endothelium-dependent dilatation in mouse mesenteric resistance arteries in vitro. *Microvascular Research.* 2001;64:127-134.
 192. Gosgnach W, Challah M, Coulet F, Michel JB, Battle T. Shear stress induces angiotensin converting enzyme expression in cultured smooth muscle cells: possible involvement of bFGF. *Cardiovasc Res.* 2000;45:486-492.
 193. Goto K, Fujii K, Onaka U, Abe I, Fujishima M. Renin-angiotensin system blockade improves endothelial dysfunction in hypertension. *Hypertension.* 2000;36:575-580.
 194. Greenhill NS, Stehbens WE. Scanning electron-microscopic study of experimentally induced intimal tears in rabbit arteries. *Atherosclerosis.* 1983;49:119-126.
 195. Griendling KK, Fuller EO. Pregnancy-induced changes in sheep uterine and carotid arteries. *Am J Physiol.* 1985;229:H658-H665.
 196. Griendling KK, Minciri CA, Ollerenshaw JD, Alexander RW. Angiotensin II stimulates NADH and NADPH oxidase activation in cultured vascular smooth muscle cells. *Circ Res.* 1994;74:1141-1148.
 197. Groves P, Kurz S, Hanjürg J, Drezler H. Role of endogenous bradykinin in human coronary vasomotor control. *Circulation.* 1995;92:3424-3430.
 198. Gryglewski RJ, Palmer RMJ, Moncada S. Superoxide anion is involved in the breakdown of endothelium-derived vascular relaxing factor. *Nature.* 1986;320:454-456.
 199. Guenther AE, Conley AJ, Van Orden DE, Farley DP, Ford SP. Structural and mechanical changes of uterine arteries during pregnancy in the pig. *J Anim Sci.* 1988;66:3144-3152.

200. Guyton JR, Hartley CJ. Flow restriction of one carotid artery in juvenile rats inhibits growth of arterial diameter. *Am J Physiol*. 1985;248:H540-H546.
201. Guzman RJ, Abe K, Zarins CK. Flow-induced arterial enlargement is inhibited by suppression of nitric oxide synthase in vivo. *Surgery*. 1997;122:273-280.
202. Guzman RJ, Krystkowiak A, Zarins CK. Early and sustained medial cell activation after aortacaval fistula creation in mice. *J Surg Res*. 2002;108:112-121.
203. Haberl RL, Anneser F, Villringer A, Einhaupl KM. Angiotensin II induces endothelium-dependent vasodilation of rat cerebral arterioles. *Am J Physiol*. 1990;258:H1840-H1846.
204. Hachamovitch R, Wicker P, Capasso JM, Anversa P. Alterations of coronary blood flow and reserve with aging in Fischer 344 rats. *Am J Physiol*. 1989;256:H66-H73.
205. Haefeli WE, Linder L, Luscher TF. Quinaprilat induces arterial vasodilation mediated by nitric oxide in humans. *Hypertension*. 1997;30:912-917.
206. Hajdu MA, Heistad DD, Baumbach GL. Effect of antihypertensive therapy on mechanics of cerebral arterioles in rats. *Hypertension*. 1991;17:308-316.
207. Halpern W, Warshaw DM, Mulvany MJ. Mechanical and morphological properties of arterial resistance vessels in young and old spontaneously hypertensive rats. *Circ Res*. 1979;45:250-259.
208. Hambrecht R, Adams V, Erbs S, Linke A, Krankel N, Shu Y, Baither Y, Gielen S, Thiele H, Gummert JF, Mohr FW, Schuler G. Regular physical activity improves endothelial function in patients with coronary artery disease by increasing phosphorylation of endothelial nitric oxide synthase. *Circulation*. 2003;25:3152-3158.
209. Hanemaaijer R, Koolwijk P, Le Clercq L, De Vree WJ, Van Hinsbergh VW. Regulation of matrix metalloproteinase expression in human vein and microvascular endothelial cells: effects of tumor necrosis factor α , interleukin 1 and phorbol ester. *Biochem J*. 1993;296:803-809.
210. Harmon KJ, Couper LL, Lindner V. Strain-dependent vascular remodeling phenotypes in inbred mice. *Am J Pathol*. 2000;156:1741-1748.
211. Hayashi K, Suzuki H, Saruta T. Segmental differences in angiotensin receptor subtypes in interlobular artery of hydronephrotic rat kidneys. *Am J Physiol*. 1993;265:F881-F885.
212. He H, Venema VJ, Gu X, Venema RC, Marrero MB, Caldwell RB. Vascular endothelial growth factor signals endothelial cell production of nitric oxide and prostacyclin through Flk/KDR activation of s-Src. *J Biol Chem*. 1999;274:25130-25135.
213. Hedera P, Bujdakova P, Traubner P, Pancak J. Stroke risk factors and development of collateral flow in carotid occlusive disease. *Acta Neurol Scand*. 1998;98:182-186.
214. Hein L. Genetic deletion and overexpression of angiotensin II receptors. *J Mol Med*. 1998; 76:756-763.
215. Helmke BP, Goldman RD, Davies PF. Rapid displacement of vimentin intermediate filaments in living endothelial cells exposed to flow. *Circ Res*. 2000;86:745-752.
216. Henriksen EJ, Jacob S, Fogt DL, Dietze GJ. Effect of chronic bradykinin administration on insulin action in an animal model of insulin resistance. *Am J Physiol*. 1998;275:R40-R45.
217. Henriksen EJ, Jacob S, Kinnick TR, Youngblood EB, Schnit MB, Dietze GJ. ACE inhibition and glucose transport in insulin-resistant muscles: Roles of bradykinin and nitric oxide. *Am J Physiol*. 1999;277:R332-R336.
218. Henrion D, Benessiano J, Levy BI. In vitro modulation of a resistance artery diameter by the tissue renin-angiotensin system of a large donor artery. *Circ Res* 1997; 80:189-95.
219. Henrion D, Kubis N, Lévy BI. Physiological and pathophysiological functions of the AT₂ subtype receptor of angiotensin I. From large arteries to the microcirculation. *Hypertension*. 2001;38:1150-1157.
220. Henrion D, Terzi F, Matrougui K, Duriez M, Boulanger CM, Colucci-Guyon E, Babinet C, Briand P, Friedlander G, Poitevin P, Levy BI. Impaired flow-induced dilation in mesenteric resistance arteries from mice lacking vimentin. *J Clin Invest*. 1997;100:2909-2914.
221. Hermsteiner M, Zoltan DR, Doetsch J, Rascher W, Kuenzel W. Human chorionic gonadotropin dilates uterine and mesenteric resistance arteries in pregnant and nonpregnant rats. *Pflügers Arch Eur J Physiol*. 1999;439:186-194.
222. Heymann MA, Iwamoto HS, Rudolph AM. Factors affecting changes in the neonatal systemic circulation. *Annu Rev Physiol*. 1981;43:371-383.
223. Hilgers RHP, Bergaya S, Schiffrs PMH, Meneton P, Boulanger CM, Henrion D, Lévy BI, De Mey JGR. Uterine artery structural and functional changes during pregnancy in tissue kallikrein-deficient mice. *Arterioscler Thromb Vasc Biol*. 2003; 23:1826-1832.

224. Hilgers RHP, Schiffrers PMH, Aartsen WM, Fazzi GE, Smits JFM, De Mey JGR. Tissue angiotensin-converting enzyme in imposed and physiological flow-related arterial remodeling mice. *Arterioscler Thromb Vasc Biol.* 2004; 24:In Press.
225. Hill MA, Larkins RG. Alterations in distribution of cardiac output in experimental diabetes in rats. *Am J Physiol.* 1989;257:H571-H580.
226. Hirsch A, Talsness C, Schunkert H, Paul M, Dzau V. Tissue-specific activation of cardiac angiotensin-converting enzyme in experimental heart failure. *Circ Res.* 1991;69:475-482.
227. Hishikawa K, Nakaki T, Marumo T, Hayashi M, Suzuki H, Kato R, Saruta T. Pressure promotes DNA synthesis in rat cultured vascular smooth muscle cells. *J Clin Invest.* 1994;25:1975-1980.
228. Hobbs AJ. Soluble guanylate cyclase: the forgotten sibling. *Trends Pharmacol Sci.* 1997;18:484-491.
229. Hoffmann J, Haendeler J, Aicher A, Rössig L, Vasa M, Zeiher AM, Dimmeler S. Aging enhances the sensitivity of endothelial cells toward apoptotic stimuli. Important role of nitric oxide. *Circ Res.* 2001;89:709-715.
230. Hojo Y, Ikeda U, Takahashi M, Sakata Y, Takizawa T, Okada K, Saito T, Shimada K. Matrix metalloproteinase-1 expression by interaction between monocytes and vascular endothelial cells. *J Mol Cell Cardiol.* 2000;32(8):1459-1468.
231. Hollander W, Kramsch DM, Farmelant M, Madoff IM. Arterial wall metabolism in experimental hypertension of coarctation of the aorta of short duration. *J Clin Invest.* 1968;47:1221-1229.
232. Hollenberg NK, Fisher NDL, Price DA. Pathways for angiotensin II generation in intact human tissue. Evidence from comparative pharmacological interruption of the renin system. *Hypertension.* 1998;32:387-392.
233. Holman E. Problems in the dynamics of blood flow. I. Condition controlling collateral circulation in the presence of an arteriovenous fistula, following the ligation of an artery. *Surgery.* 1949;26:889-917.
234. Honing ML, Smits P, Morrison PJ, Rabelinh TJ. Bradykinin-induced vasodilation of human forearm resistance vessels is primarily mediated by endothelium-dependent hyperpolarization. *Hypertension.* 2000;35:1314-1318.
235. Hooper NM, Keen J, Pappin DJ, Turner AJ. Pig kidney angiotensin converting enzyme. Purification and characterization of amphipathic and hydrophilic forms of the enzyme establishes C-terminal anchorage to the plasma membrane. *Biochem J.* 1987 247:85-93.
236. Hornig B, Kohler C, Drexler H. Role of bradykinin in mediating vascular effects of ACE-inhibitors in humans. *Circulation.* 1997;95:1115-1118.
237. Hosomi N, Mizushige K, Ohyama H, Takahashi T, Kitadai M, Hatanaka Y, Matsuo H, Kohno M, Koziol JA. Angiotensin-converting enzyme inhibition with enalapril slows progressive intima-media thickening of the common carotid artery in patients with non-insulin-dependent diabetes mellitus. *Stroke.* 2001;32:1539-1545.
238. Hsieh HJ, Li NQ, Frangos JA. Shear stress increases endothelial platelet-derived growth factor mRNA levels. *Am J Physiol.* 1991;260:H642-H646.
239. Hsieh HJ, Li NQ, Frangos JA. Shear-induced platelet-derived growth factor gene expression in human endothelial cells is mediated by protein kinase C. *J Cell Physiol.* 1992;150:552-558.
240. Hsieh HJ, Li NQ, Frangos JA. Pulsatile and steady flow induces c-fos expression in human endothelial cells. *J Cell Physiol.* 1993;154:143-151.
241. Hsueh WA, Do YS, Anderson PW, Law RE. Angiotensin II in cell growth and matrix production. *Adv Exp Med Biol.* 1995;10:220-231.
242. Huang A, Koller A. Both nitric oxide and prostaglandin-mediated responses are impaired in skeletal muscle arterioles of hypertensive rats. *Hypertension.* 1996;14:887-895.
243. Huang A, Koller A. Endothelin and prostaglandin H₂ enhance arteriolar myogenic tone in hypertension. *Hypertension.* 1997;30:1210-1215.
244. Huang A, Sun D, Carroll MA, Jiang H, Smith CJ, Connetta JA, Falck JR, Shesely EG, Koller A, Kaley G. EDHF mediates flow-induced dilation in skeletal muscle arterioles of female eNOS-KO mice. *Am J Physiol.* 2001;280:H2462-H2469.
245. Huang A, Sun D, Shesely EG, Levee EM, Koller A, Kaley G. Neuronal NOS-dependent dilation to flow in coronary arteries of male eNOS-KO mice. *Am J Physiol.* 2001;282:H429-H436.
246. Huang A, Sun D, Smith CJ, Connetta JA, Shesely EG, Koller A, Kaley G. In eNOS knockout mice skeletal muscle arteriolar dilation to acetylcholine is mediated by EDHF. *Am J Physiol.* 2000;278:H762-H768.
247. Hudlicka O. What makes vessels grow? *J Physiol.* 1991;444:1-24.

248. Hull SS Jr, Kaiser L, Jaffe MD, Sparks V Jr. Endothelium-dependent flow-induced dilation of canine femoral and saphenous arteries. *Blood vessels*. 1986;23:183-198.
249. Huonker M, Halle M, Keul J. Structural and functional adaptations of the cardiovascular system by training. *Int J Sports Med*. 1996;17:S164-S172.
250. Hutcheson IR, Griffith TM. Mechanotransduction through the endothelial cytoskeleton: mediation of flow-but not agonist-induced EDRF release. *Br J Pharmacol*. 1996;118:720-726.
251. Hutchinson HG, Hein L, Fujinaga M, Pratt RE. Modulation of vascular development and injury by angiotensin II. *Cardiovasc Res*. 1999;41:689-700.
252. Ichiki T, Labosky PA, Shiota C, Okuyama S, Imagawa Y, Fogo A, Niimura F, Ichikawa I, Hogan BL, Inagami T. Effects on blood pressure and exploratory behaviour of mice lacking angiotensin II type-2 receptor. *Nature*. 1995;377:748-750.
253. Ichihara S, Senbonmatsu T, Price E, Ichiki T, Gaffney FA, Inagami T. Angiotensin II type 2 receptor is essential for left ventricular hypertrophy and cardiac fibrosis in chronic angiotensin II-induced hypertension. *Circulation*. 2001;104:346-351.
254. Ichiki T, Usui M, Kato M, Funakoshi Y, Ito K, Egashira K, Takeshita A. Downregulation of angiotensin II type 1 receptor gene transcription by nitric oxide. *Hypertension*. 1998; 31:342-348.
255. Ingber DE. Integrins as mechanotransduction across the cell surface and through the cytoskeleton. *Science*. 1991;3:841-848.
256. Ingber DE. Mechanochemical switching between growth and differentiation during fibroblast growth factor-stimulated angiogenesis in vitro: role of extracellular matrix. *J Cell Biol*. 1991;109:317-330.
257. Irace C, Gnasso A, Cirillo F, Leonardo G, Ciamei M, Crivaro A, Renzulli A, Cotrufo M. Arterial remodeling of the common carotid artery after aortic valve replacement in patients with aortic stenosis. *Stroke*. 2002;33:2446-2250.
258. Ishida S, Hamasaki S, Kamekou M, Yoshitama T, Nakana F, Yoshikawa A, Kataoka T, Saihara K, Minagoe S, Tei C. Advancing age is associated with diminished vascular remodeling and impaired vasodilation in resistance coronary arteries. *Coron Artery Dis*. 2003;14:443-449.
259. Ito WD, Arras M, Scholz D, Winkler B, Htun P, Schaper W. Angiogenesis but not collateral growth is associated with ischemia after femoral artery ligation. *Am J Physiol*. 1997;273:H1255-H1265.
260. Ivan E, Khatri JJ, Johnson C, Magid R, Godin D, Nandi S, Lessner S, Galis ZS. Expansive arterial remodeling is associated with increased neointimal macrophage foam cell content. *Circulation*. 2002;105:2686-2693.
261. Iwasaki H, Eguchi S, Marumo F, Hirata Y. Endothelin-1 stimulates DNA synthesis of vascular smooth-muscle cells through transactivation of epidermal growth factor receptor. *J Cardiovasc Pharmacol*. 1998;31:S182-S184.
262. Jackson WF. Arteriolar tone is determined by activity of ATP-sensitive potassium channels. *Am J Physiol*. 1993;265:H1797-H1803.
263. Jalowy A, Schulz R, Dörge H, Behrends M, Heusch G. Infarct size reduction by AT₁-receptor blockade through a signal cascade of AT₂-receptor activation, bradykinin and prostaglandins in pigs. *J Am Coll Cardiol*. 1998;32:1787-1796.
264. Jamal A, Bendeck M, Langille BL. Structural changes and recovery of function after arterial injury. *Arterioscler Thromb*. 1992;12:307-317.
265. Janiak P, Libert O, Vilaine JP. Role of the renin-angiotensin system in neointima formation after injury in rabbits. *Hypertension*. 1994;24:671-678.
266. Janmey PA. The cytoskeleton and cell signaling: component localization and mechanical coupling. *Physiol Rev*. 1998;78:763-781.
267. Jawien A, Bowen-Pope DF, Lindner V, Schwartz SM, Clowes AW. Platelet-derived growth factor promotes smooth muscle migration and intimal thickening in a rat model of balloon angioplasty. *J Clin Invest*. 1992;89:507-511.
268. Jin ZG, Ueba H, Tanimoto T, Lungu AO, Frame MD, Berk BC. Ligand-independent activation of vascular endothelial growth factor receptor 2 by fluid shear stress regulates activation of endothelial nitric oxide synthase. *Circ Res*. 2003;93:354-363.
269. Joannides R, Haefeli WE, Linder L, Richard V, Bakkali EH, Thuillez C, Lüscher TF. Nitric oxide is responsible for flow-dependent dilatation of human peripheral conduit arteries in vivo. *Circulation*. 1995;91:1314-1319.
270. Johansson B. Myogenic tone and reactivity: definitions based on muscle physiology. *J Hypertension*. 1989;7:55-58.

271. Johnson PC. The myogenic response. In: *Handbook of Physiology. The Cardiovascular System. Vascular Smooth Muscle*. Bethesda, MD: Am. Physiol. Soc, 1981, sect. 2, vol. II, chap. 15, p. 409-442.
272. Jolly M, Sebire N, Harris J, Robinson S, Regan L. The risks associated with pregnancy in women aged 35 years or older. *Hum Reprod*. 2000;15:2433-2437.
273. Joly GA, Schini VB, Vanhoutte PM. Balloon injury and interleukin-1 b induce nitric oxide synthase activity in rat carotid arteries. *Circ Res*. 1992;71:331-338.
274. Jones GT, Martin BJ, Stehbens WE. Endothelium and elastic tears in the afferent arteries of experimental arteriovenous fistulae in rabbits. *Int J Exp Pathol*. 1992;73:405-416.
275. Jovanovic S, Blagojevic Z, Mrvic V, Nikoloic Z, Jovanovic A. Pregnancy is not associated with altered morphology of the femoral artery. *Hum Reprod*. 1999;14:1885-1889.
276. Jovanovic S, Jovanovic A. Remodeling of guinea-pig aorta during pregnancy: a selective alterations of endothelial cells. *Hum Reprod*. 1997;12:2297-2302.
277. Jovanovic S, Jovanovic A. Pregnancy is associated with hypotrophy of carotid artery endothelial and smooth muscle cells. *Hum Reprod*. 1998;13:1074-1078.
278. Jovanovic A, Jovanovic S, Grbovic L. Endothelium-dependent relaxation in response to acetylcholine in pregnant guinea-pig uterine artery. *Hum Reprod*. 1997;12:1805-1809.
279. Joyce JM, Phernetton TM, Magness RR. Effect of uterine blood flow occlusion on shear stress-mediated nitric oxide production and endothelial nitric oxide synthase expression during ovine pregnancy. *Biol Reprod*. 2002;67:320-326.
280. Ju H, Zou R, Venema VJ, Venema RC. Direct interaction of endothelial nitric oxide synthase and caveolin-1 inhibits synthase activity. *J Biol Chem*. 1997;272:18522-18525.
281. Kamata K, Miyata N, Abiru T, Kasuya Y. Functional changes in vascular smooth muscle and endothelium of arteries during diabetes mellitus. *Life Sci*. 1992;50:1379-1387.
282. Kamiya A, Togawa T. Adaptive regulation of wall shear stress to flow change in the canine carotid artery. *Am J Physiol*. 1980;239:H14-H21.
283. Katada J, Majima M. AT₂ receptor-dependent vasodilation is mediated by activation of vascular kinin generation under flow conditions. *Br J Pharmacol*. 2002;136:484-491.
284. Katoh Y, Periasamy M. Growth and differentiation of smooth muscle cells during vascular development. *Trends Card Med*. 1996;6:100-106.
285. Keidar S, Attias J, Coleman R, Wirth K, Scholkens B, Hayek T. Attenuation of atherosclerosis in apolipoprotein E-deficient mice by ramipril is dissociated from its antihypertensive effect and from potentiation of bradykinin. *J Cardiovasc Pharmacol*. 2000;35:64-72.
286. Kersten JR, Pagel PS, Chilian WM, Warltier DC. Multifactorial basis for coronary co-lateralization: a complex adaptive response to ischemia. *Cardiovasc Res*. 1999;43:44-57.
287. Khachigian IM, Anderson KR, Halnon NJ, Gimbrone MA Jr, Resnick N, Collins T. Egr-1 is activated in endothelial cells exposed to fluid shear stress and interacts with a novel shear-stress-response element in the PDGF A-chain promoter. *Arterioscler Thromb Vasc Biol*. 1997;17:2280-2286.
288. Kimura T, Kaburagi S, Tamura T, Yokoi H, Nakagawa Y, Yokoi H, Hamasaki N, Nosaka H, Nobuyoshi M, Mintz GS, Popma JJ, Leon MB. Remodeling of human coronary arteries undergoing coronary angioplasty or atherectomy. *Circulation*. 1997;96:475-483.
289. Kinoshita T, Sato H, Takino T, Itoh M, Akizawa T, Seiki M. Processing of a precursor of 72-kilodalton type IV collagenase/gelatinase A by a recombinant membrane-type 1 matrix metalloproteinase. *Cancer Res*. 1996;56:2535-2538.
290. Koller A, Huang A, Sun D, Kaley G. Exercise training augments flow-dependent dilation in rat skeletal muscle arterioles. *Circ Res*. 1995;76:544-550.
291. Koller A, Sun D, Kaley G. Role of shear stress and endothelial prostaglandins in flow and viscosity induced dilation of arterioles in vitro. *Circ Res*. 1993;72:1276-1284.
292. Kool M, Struijker-Boudier H, Wijnen J, Hoeks A, Van Bortel L. Effects of diurnal variability and exercise training on properties of large arteries. *J Hypertens*. 1992;10:S49-S52.
293. Korshunov VA, Berk BC. Flow-induced vascular remodeling in the mouse: a model for carotid intima-media thickening. *Arterioscler Thromb Vasc Biol*. 2003;23:1-9.
294. Kramer HJ. The renal kallikrein-kinin system. *Renal Physiol*. 1979;80:107-121.
295. Kregel JH, John SW, Langenbach LL, Hodgin JB, Hagaman JR, Bachman ES, Jennette JC, O'Brien DA, Smithies O. Male-female differences in fertility and blood pressure in ACE-deficient mice. *Nature*. 1995;375:146-148.

296. Kubis N, Checoury A, Tedgui A, Lévy BI. Adaptive common carotid arteries remodeling after unilateral internal carotid artery occlusion in adult patients. *Cardiovasc Res.* 2001;50:597-602.
297. Kuchan MJ, Frangos JA. Shear stress regulates endothelin-1 release via protein kinase C and cGMP in cultured endothelial cells. *Am J Physiol.* 1993; 264:H150-H156.
298. Kuchan MJ, Frangos JA. Role of calcium and calmodulin in flow-induced nitric oxide production in endothelial cells. *Am J Physiol.* 1994;266:C628-C636.
299. Kumar A, Hoover JL, Simmons CA, Lindner V, Shebuski RJ. Remodeling and neointimal formation in the carotid artery of normal and P-selectin-deficient mice. *Circulation.* 1997;96:4333-4342.
300. Kumar A, Lindner V. Remodeling with neointima formation in the mouse carotid artery after cessation of blood flow. *Arterioscler Thromb Vasc Biol.* 1997;17:2238-2244.
301. Küng CF, Lüscher TF. Different mechanisms of endothelial function with aging and hypertension in rat aorta. *Hypertension.* 1995;25:194-200.
302. Kuo L, Davis MJ, Chilian WM. Endothelium-dependent, flow-induced dilation of isolated coronary arterioles. *Am J Physiol.* 1990;259:H1063-H1070.
303. Kuzuya M, Kanda S, Sasaki T, Tamaya-Mori N, Cheng XW, Itoh T, Itohara S, Iguchi A. Deficiency of gelatinase A suppresses smooth muscle cell invasion and development of experimental intimal hyperplasia. *Circulation.* 2003;108:1375-1381.
304. Lacolley P, Chalande P, Boumaza S, Cohuet G, Laurent S, Boutouyrie P, Grimaud JA, Paulin D, Lamaziere JM, Li Z. Mechanical properties and structure of carotid arteries in mice lacking desmin. *Cardiovasc Res.* 2001;51:178-187.
305. Lakatta EG. Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises. Part III: cellular and molecular clues to heart and arterial aging. *Circulation.* 2003;107:490-497.
306. Lan Q, Mercurius KO, Davies PF. Stimulation of transcription factors NF kappa B and AP-1 in endothelial cells subjected to shear stress. *Biochem Biophys Res Commun.* 1994;201:950-956.
307. Lang MG, Noll G, Lüscher TF. Effect of aging and hypertension on contractility of resistance arteries: modulation by endothelial factors. *Am J Physiol.* 1995;269:H837-H844.
308. Langille BL. Hemodynamic factors and vascular wall disease. In: Silver E, ed. *Cardiovascular pathology.* 1991; New York, NY: Churchill Livingstone:131-154.
309. Langille BL. Arterial remodeling: relation to hemodynamics. *Can J Physiol Pharmacol.* 1996;74:834-841.
310. Langille BL, Bendeck MP, Keeley FW. Adaptations of carotid arteries of young and mature rabbits to reduced carotid blood flow. *Am J Physiol.* 1989;256:H931-H939.
311. Langille BL, Brownlee RD, Adamson SL. Perinatal growth in lambs: relation to blood flow changes at birth. *Am J Physiol.* 1990;259:H1247-H1253.
312. Langille BL, O'Donnell F. Reductions in arterial diameter produced by chronic decreases in blood flow are endothelial-dependent. *Science.* 1986;231:405-407.
313. Larsen E, Celi A, Gilbert GE, Furie BC, Erban JK, Bonfanti R, Wagner DD, Furie B. PADGEM protein: a receptor that mediates the interaction of activated platelets with neutrophils and monocytes. *Cell.* 1989;59:305-312.
314. Lash JM, Bohlen HG. Time- and order-dependent changes in functional and NO-mediated dilation during exercise training. *J Appl Physiol.* 1997;82:460-468.
315. Lauth M, Berger MM, Cattaruzza M, Hecker M. Elevated perfusion pressure upregulates endothelin-1 and endothelin B receptor expression in the rabbit carotid artery. *Hypertension.* 2000;35:648-654.
316. Lee RMKW, Berecek KH, Tsopors J, McKenzie R, Triggle CR. Prevention of hypertension and vascular changes by captopril treatment. *Hypertension.* 1991;17:141-150.
317. Lehmann DK, Chism J. Pregnancy outcome in medically complicated and uncomplicated patients aged 40 years or older. *Am J Obstet Gynecol.* 1987;157:738-742.
318. Lehoux S, Tedgui A. Signal transduction of mechanical stresses in the vascular wall. *Hypertension.* 1998;32:338-345.
319. Lévy BI, Michel JB, Salzmann JL, Poitevin P, Devissaguet M, Scalbert ME, Safar ME. Long-term effects of angiotensin-converting enzyme inhibition on the arterial wall of adult spontaneously hypertensive rats. *Am J Cardiol.* 1993;71:8e-16e.
320. Li JS, Turgeon A, Schiffrin EL. Effect of chronic treatment with two different ETA selective endothelin receptor antagonists on blood pressure and small artery structure of deoxycorticosterone acetate (DOCA)-salt hypertensive rats. *Am J Hypertens.* 1998;11:554-562.

321. Li S, Kim M, Hu YL, Jalali S, Schlaepfer DD, Hunter T, Chien S, Shyy JY. Fluid shear stress activation of focal adhesion kinase: linking to mitogen-activated protein kinases. *J Biol Chem.* 1997;272:30455-30462.
322. Linz W, Wiemer G, Gohlke P, Unger T, Schölkens BA. Contribution of kinins to the cardiovascular actions of angiotensin-converting enzyme inhibitors. *Pharmacol Rev.* 1995;47:25-49.
323. Liu YH, Yang XP, Sharov VG, Nass O, Sabbah HN, Peterson E, Carretero OA. Effects of angiotensin-converting enzyme inhibitors and angiotensin II type 1 receptor antagonists in rats with heart failure. Role of kinins and angiotensin II type 2 receptors. *J Clin Invest.* 1997;99:1926-1935.
324. Liu Q, Wiener CM, Flavahan NA. Superoxide and endothelium-dependent constriction to flow in porcine small pulmonary arteries. *Br J Pharmacol.* 1998;124:331-336.
325. Loftus JC, Liddington RC. New insights into integrin-ligand interaction. *J Clin Invest.* 1997;99:2302-2306.
326. Loufrani L, Levy BI, Henrion D. Defect in microvascular adaptation to chronic changes in blood flow lacking the gene encoding for dystrophin. *Circ Res.* 2002;91:1183-1189.
327. Loufrani L, Li Z, Lévy BI, Paulin D, Henrion D. Excessive microvascular adaptation to changes in blood flow in mice lacking gene encoding for desmin. *Arterioscler Thromb Vasc Biol.* 2002;22:1579-1584.
328. Loufrani L, Matrougui K, Gorny D, Duriez M, Blanc I, Lévy BI, Henrion D. Flow (shear stress)-induced endothelium-dependent dilation is altered in mice lacking the gene encoding for dystrophin. *Circulation.* 2001;103:864-870.
329. Lu X, Zhao JB, Wang GR, Gregersen H, Kassab GS. Remodeling of the zero-stress state of femoral arteries in response to flow overload. *Am J Physiol.* 2002;280:H1547-H1559.
330. Lui G, Espinosa E, Oemar BS, Lüscher TF. Bimodal effects of angiotensin II on migration of human and rat smooth muscle cells. Direct stimulation and indirect inhibition via transforming growth factor-beta 1. *Arterioscler Thromb Vasc Biol.* 1997;17:1251-1257.
331. Lumbers ER. Functions of the renin-angiotensin system during development. *Clin Exp Pharmacol Physiol.* 1995;22:499-505.
332. Lüscher TF, Tanner FC. Endothelial regulation of vascular tone and growth. *Am J Hypertension.* 1993; 6:283S-293S.
333. Lyon RT, Runyon-Hass A, Davis HR, Glagov S, Zarins CK. Protection from atherosclerotic lesion formation by reduction of artery wall motion. *J Vasc Surg.* 1987;5:59-67.
334. Madeddu P, Parpaglia PP, Demontis MP, Varoni MV, Fattaccio MC, Anania V, Glorioso N. Early blockade of bradykinin B₂-receptors alters the adult cardiovascular phenotype in rats. *Hypertension.* 1995;25:453-459.
335. Magness RR, Phernetton TM, Zheng J. Systemic and uterine blood flow distribution during prolonged infusion of 17 β -estradiol. *Am J Physiol.* 1998;275:H731-H743.
336. Magness RR, Rosenfeld CR, Hassan A, Shaul PW. Endothelial vasodilator production by uterine and systemic arteries. I. Effects of ANG II on PGI₂ and NO in pregnancy. *Am J Physiol.* 1996;270:H1914-H1923.
337. Magness RR, Shaw CE, Phernetton TM, Zheng J, Bird IM. Endothelial vasodilator production by uterine and systemic arteries. II. Pregnancy effects on NO synthase expression. *Am J Physiol.* 1997;272:H1730-40.
338. Malek AM, Alper SL, Izumo S. Hemodynamic shear stress and its role in atherosclerosis. *JAMA.* 1999;282:2035-2042.
339. Malek AM, Gibbons GH, Dzau VJ, Izumo S. Fluid shear stress differentially modulates expression of genes encoding basic fibroblast growth factor and platelet-derived growth factor B chain in vascular endothelium. *J Clin Invest.* 1993;92:2013-2021.
340. Malek A, Izumo S. Physiological fluid shear stress causes downregulation of endothelin-1 mRNA in bovine aortic endothelium. *Am J Physiol.* 1992;263:C389-C396.
341. Malek AM, Izumo S. Control of endothelial cell gene expression by flow. *J Biomech.* 1995;28:1515-1528.
342. Mancini GBJ, Henry GC, Macaya C, O'Neill BJ, Pucillo AL, Carere RG, Wargovich TJ, Mudra H, Lüscher TF, Klibaner MI, Haber HR, Uprichard ACG, Pepine CJ, Pitt B. Angiotensin-converting enzyme inhibition with quinapril improves endothelial vasomotor dysfunction artery disease. The TREND (Trial on Reversing Endothelial Dysfunction) study. *Circulation.* 1996;94:258-265.
343. Maniotis AJ, Chen CS, Ingber DE. Demonstration of mechanical connections between integrins, cytoskeletal filaments, and nucleoplasm that stabilize nuclear structure. *Proc Natl Acad Sci USA.* 1997;94:848-854.

344. Marceau F, Lussier A, Regoli D, Giroud J. Pharmacology of the kinins: their relevance to tissue injury and inflammation. *Gen Pharmacol*. 1983;14:209-229.
345. Marceau F, Sabourin T, Houle S, Fortin JP, Pétitclerc E, Molinaro G, Adam A. Kinin receptors: functional aspects. *Int Immunopharmacol*. 2002;2:1729-1739.
346. Margolius HS. Kallikreins and kinins. Some unanswered questions about system characteristics and roles in human disease. *Hypertension*. 1995;26:221-229.
347. Marin J. Age-related changes in vascular responses : a review. *Mech Aging Dev*. 1995;79:71-114.
348. Masuda H, Kawamura K, Tohda K, Shozawa T, Sageshima M, Kamiya A. Increase in endothelial cell density before artery enlargement in flow-loaded canine carotid artery. *Arteriosclerosis*. 1989;9:812-823.
349. Masuda H, Sugita A, Zhuang YJ. Pathology of the arteries in the central nervous system with special reference to their dilatation to blood flow. *Neuropathology*. 2000;20:98-103.
350. Masuda H, Zhuang YJ, Singh TM, Kawamura K, Murakami M, Zarins CK, Glagov S. Adaptive remodeling of internal elastic lamina and endothelial lining during flow-induced arterial enlargement. *Arterioscler Thromb Vasc Biol*. 1999;19:2298-2307.
351. Matoba T, Shimokawa H, Kubota H, Morikawa K, Fujiki T, Kunihiro I, Mukay Y, Hirakawa Y, Takeshita A. Hydrogen peroxide is an endothelium-derived hyperpolarizing factor in human mesenteric arteries. *Biochem Biophys Res Commun*. 2002;290:909-913.
352. Matrougui K, Eskildsen-Helmond YE, Fiebeler A, Henrion D, Levy BI, Tedgui A, Mulvany MJ. Angiotensin II stimulates extracellular signal-regulated kinase activity in intact pressurized rat mesenteric resistance arteries. *Hypertension*. 2000;36:617-621.
353. Matrougui K, Loufrani L, Heymes C, Lévy BI, Henrion D. Activation of AT₂ receptors by endogenous angiotensin II is involved in flow-induced dilation in rat resistance arteries. *Hypertension*. 1999;34:659-665.
354. Matsumoto T, Kanamaru K, Sugiyama Y, Murata Y. Endothelium-derived relaxation of the pregnant and nonpregnant canine uterine artery. *J Reprod Med*. 1992;37:529-533.
355. Matz RL, de Sotomayor MA, Schott C, Stoclet JC, Andriantsitohaina R. Vascular bed heterogeneity in age-related endothelial dysfunction with respect to NO and eicosanoids. *Br J Pharmacol*. 2000;131:303-311.
356. Medhora M, Bousamra M, Zhu D, Somberg L, Jacobs ER. Upregulation of collagens detected by gene array in a model of flow-induced pulmonary vascular remodeling. *Am J Physiol*. 2001;282:H414-H422.
357. Meininger GA, Davis, MJ. Cellular mechanisms involved in the vascular myogenic response. *Am. J. Physiol*. 1992;263:H647-H659.
358. Mello G, Parretti E, Gensini F, Sticchi E, Mecacci F, Scarselli G, Genuardi M, Abbate R, Fatini C. Maternal-fetal flow, negative events, and preeclampsia. *Hypertension*. 2003;41:932-937.
359. Meneton P, Bloch-Faure M, Hagege AA, Ruetten H, Huang W, Bergaya S, Ceiler D, Gehring D, Martins I, Salmon G, Boulanger CM, Nussberger N, Crozatier B, Gasc J-M, Heudes D, Bruneval P, Doetschman T, Ménard J, Alhenc-Gelas F. Cardiovascular abnormalities with normal blood pressure in tissue kallikrein-deficient mice. *Proc Natl Acad Sci. U.S.A*. 2001;98:2634-2639.
360. Mintz GS, Popma JJ, Pichard AD, Kent KM, Satler LF, Wong SC, Hong MK, Kovach JA, Leon MB. Arterial remodeling after coronary angioplasty. A serial intravascular ultrasound study. *Circulation*. 1996;94:35-43.
361. Miyashiro JK, Poppa V, Berk BC. Flow-induced vascular remodeling in the rat carotid artery diminishes with age. *Circ Res*. 1997;81:311-319.
362. Miyauchi T, Masaki T. Pathophysiology of endothelin in the cardiovascular system. *Annu Rev Physiol*. 1999;61:391-415.
363. Mione MC, Gabella G. Nerve fibers in the uterine artery increase in number in pregnant guinea pigs. *Neuroreport*. 1991;2:537-540.
364. Mo M, Eskin SG, Schilling WP. Flow-induced changes in Ca²⁺-signaling of vascular endothelial cells: effects of shear stress and ATP. *Am J Physiol*. 1991;260:H1698-H1707.
365. Mombouli JV, Vanhoutte PM. Kinins and endothelium-dependent relaxations to converting enzyme inhibitors in perfused canine arteries. *J Cardiovasc Pharmacol*. 1991;18:926-927.
366. Mombouli JV, Vanhoutte PM. Kinins mediate kallikrein-induced endothelium-dependent relaxations in isolated canine coronary arteries. *Biochem Biophys Res Commun*. 1992;185:693-697.
367. Moncada S, Higgs A. The L-arginine-nitric oxide pathway. *N Engl J Med*. 1993;329:2002-2012.
368. Mondy JS, Lindner V, Miyashiro JK, Berk BC, Dean RH, Geary RL. Platelet-derived growth factor ligand and receptor expression in response to altered to altered blood flow in vivo. *Circ Res*. 1997;81:320-327.

369. Moreau P, d'Uscio LV, Lüscher TF. Structure and reactivity of small arteries in aging. *Cardiovasc Res*. 1998;37:247-253.
370. Mori E, Komori K, Yamaoka T, Tani M, Kataoka C, Takeshita A, Usui M, Egashira K, Sugimachi K. Essential role of monocyte chemoattractant protein-1 in development of restenotic changes (neointimal hyperplasia and constrictive remodeling) after balloon angioplasty in hypercholesterolemic rabbits. *Circulation*. 2002;105:2905-2910.
371. Morishita R, Gibbons GH, Tomita N, Zhang L, Kaneda Y, Ogihara T, Dzau VJ. Antisense oligodeoxynucleotide inhibition of vascular angiotensin-converting enzyme expression attenuates neointimal formation. Evidence for tissue angiotensin-converting enzyme function. *Arterioscler Thromb Vasc Biol*. 2000;20:915-922.
372. Morishita T, Tsutsui M, Shimokawa H, Horiuchi M, Tanimoto A, Suda O, Tasaki H, Huang PL, Sasaguri Y, Yanagihara N, and Nakashima Y. Vasculoprotective roles of neuronal nitric oxide synthase. *FASEB J*. 2002;16:1994-1996.
373. Mueller B, Ruf W. Requirement for binding of catalytically active factor VIIa in tissue factor-dependent experimental metastasis. *J Clin Invest*. 1998;101:1372-1378.
374. Mulder P, Elfertak L, Richard V, Compagnon P, Devaux B, Henry JB, Scalbert E, Desché P, Macé B, Thuillez C. Peripheral artery structure and endothelial function in heart failure effect of ACE inhibition. *Am J Physiol*. 1996;271:H469-H477.
375. Muller DW, Ellis SG, Topol EJ. Experimental models of coronary artery restenosis. *J Am Coll Cardiol*. 1992;19:418-432.
376. Muller-Delp JM, Spier SA, Ramsey MW, Delp MD. Aging impairs endothelium-dependent vasodilation in rat skeletal muscle arterioles. *Am J Physiol*. 2002;283:H1662-H1672.
377. Mulvany MJ. Structure and function of small arteries in hypertension. *J Hypertens Suppl*. 1990;8:S225-S232.
378. Mulvany MJ. Small artery remodeling and significance in the development of hypertension. *News Physiol Sci*. 2002;17:105-109.
379. Mulvany MJ, Baumbach GL, Aalkjaer C, Haegerty AM. Vascular remodeling. *Hypertension*. 1996;28:505-506.
380. Murakami H, Yayama K, Miao RQ, Wang C, Chao L, Chao J. Kallikrein gene delivery inhibits vascular smooth muscle cell growth and neointima formation in the rat artery after balloon angioplasty. *Hypertension*. 1999;34:164-170.
381. Murakoshi N, Miyauchi T, Kakinuma Y, Ohuchi T, Goto K, Yanagisawa M, Yamaguchi I. Vascular endothelin-B receptor system in vivo plays a favourable inhibitory role in vascular remodeling after injury revealed by endothelin-B receptor-knockout mice. *Circulation*. 2002;106:1991-1998.
382. Murrell GA, Jang D, Williams RJ. Nitric oxide activates metalloprotease enzymes in articular cartilage. *Biochem Biophys Res Commun*. 1995;206:15-21.
383. Myers DL, Harmon KJ, Lindner V, Liaw L. Alterations of arterial physiology in osteopontin-null mice. *Arterioscler Thromb Vasc Biol*. 2003;23:1021-1028.
384. Nadaud S, Philippe M, Arnal J-F, Michel J-B, Soubrier F. Sustained increase in aortic endothelial nitric oxide synthase expression in vivo in a model of chronic high flow. *Circ Res*. 1996;79:857-863.
385. Nafilian AJ, Pratt RE, Dzau VJ. Induction of PDGF A-chain and c-myc gene expression by angiotensin II in vascular smooth muscle cells. *J Clin Invest*. 1989;83:1419-1424.
386. Nagel T, Resnick N, Atkinson WJ, Dewey CF Jr, Grimbone MA Jr. Shear stress selectively upregulates intercellular adhesion molecule-1 expression in cultured human vascular endothelial cells. *J Clin Invest*. 1994;94:885-891.
387. Nakae I, Fujita M, Miwa K, Hasegawa K, Kihara Y, Nohara R, Miyamoto S, Ueda K, Tamaki S, Sasayama S. Age-dependent impairment of coronary collateral development in humans. *Heart Vessels*. 2000;15:176-180.
388. Nakajima M, Mukoyama M, Pratt RE, Horiuchi M, Dzau YJ. Cloning of cDNA and analysis of the gene for mouse angiotensin II type 2 receptor. *Biochem Biophys Res Commun*. 1993;197:393-399.
389. Negoro N, Kanayama Y, Haraguchi M, Umetani N, Nishimura M, Konishi Y, Iwai J, Okamura M, Inoue T, Takeda T. Blood pressure regulates platelet-derived growth factor A-chain gene expression in vascular smooth muscle cells in vivo. *J Clin Invest*. 1995;95:1140-1150.
390. Nelson SH, Steinsland OS, Suresh MS, Lee NM. Pregnancy augments nitric oxide-dependent dilator responses to acetylcholine in the human uterine artery. *Hum Reprod*. 1998;13:1361-1367.

391. Nelson SH, Steinsland OS, Wang Y, Yallampalli C, Dong YL, Sanchez JM. Increased nitric oxide synthase activity and expression in the human uterine artery during pregnancy. *Circ Res.* 2000;87:406-11.
392. Ni Y, May V, Braas K, Osol G. Pregnancy augments uteroplacental vascular endothelial growth factor gene expression and vasodilator effects. *Am J Physiol.* 1997;273:H938-H944.
393. Ni Y, Meyer M, Osol G. Gestation increases nitric oxide-mediated vasodilation in rat uterine arteries. *Am J Obstet Gynecol.* 1997;176:856-864.
394. Nienartowicz A, Link S, Moll W. Adaptation of the uterine arcade in rats to pregnancy. *J Dev Physiol.* 1989;12:101-108.
395. Nishida K, Harrison DG, Navas JP, Fisher AA, Dockery SP, Uematsu M, Nerem RM, Alexander RW, Murphy TJ. Molecular cloning and characterization of the constitutive bovine aortic endothelial cell nitric oxide synthase. *J Clin Invest.* 1992;90:2092-2096.
396. Nolly H, Bertini F, Lama MC. Kinin-forming enzymes in vascular tissue. *Adv Exp Med Biol.* 1983;156:399-407.
397. Nolly H, Lama M, Carretero O, Scicli A. The kallikrein-kinin system in blood vessels. *Agents Actions.* 1992;38:1-9.
398. Nolly H, Scicli AG, Scicli G, Carretero OA. Characterization of a kininogenase from rat vascular tissue resembling tissue kallikrein. *Circ Res.* 1985;56:816-821.
399. Nybo Andersen AM, Wohlfahrt J, Christens P, Olsen J, Melbye M. Maternal age and fetal loss: population based register linkage study. *BMJ.* 2000;320:1708-1712.
400. Ohno M, Cooke JP, Dzau VJ, Gibbons GH. Fluid shear stress induces endothelial transforming growth factor beta-1 transcription and production. *J Clin Invest.* 1995;95:1363-1369.
401. Okada K, Bernstein ML, Zhang W, Schuster DP, Botney MD. Angiotensin-converting enzyme inhibition delays pulmonary vascular neointima formation. *Am J Respir Crit Care Med.* 1998;158:939-950.
402. Olesen SP, Clapham DE, Davies PF. Haemodynamic shear stress activates a K⁺ current in vascular endothelial cells. *Nature.* 1988;331:168-170.
403. Oniki T, Iwakami M. Is arterial remodeling truly a compensatory biological reaction? A mechanical deformation hypothesis. *Atherosclerosis.* 1997;132:115-118.
404. Osol G. Myogenic properties of blood vessels in vitro. In: The Resistance Vasculature. 1991; J.A. Bevan, W. Halpern, and M.J. Mulvany, editors. Humana Press. Totowa, NJ. 143-157.
405. Osol G. Mechanotransduction by vascular smooth muscle. *J Vasc Res.* 1995;32:275-292.
406. Osol G, Cipolla M. Pregnancy-induced changes in the three-dimensional mechanical properties of pressurized rat uteroplacental (radial) arteries. *Am J Obstet Gynecol.* 1993;168:268-74.
407. Osol G, Cipolla M. Interaction of myogenic and adrenergic mechanisms in isolated, pressurized uterine radial arteries from late-pregnant and nonpregnant rats. *Am J Physiol.* 1993;168:697-705.
408. Osol G, Cipolla M. Hypertrophic and hyperplastic effects of pregnancy on the rat uterine arterial wall. *Am J Obstet Gynecol.* 1994;171:805-811.
409. Owens GK. Differential effects of antihypertensive drug therapy on vascular smooth muscle cell hypertrophy, hyperplasia and hyperplasia in the spontaneously hypertensive rat. *Circ Res.* 1985;56:525-536.
410. Owens GK. Influence of blood pressure on development of aortic medial smooth muscle hypertrophy in spontaneously hypertensive rats. *Hypertension.* 1987;9:178-187.
411. Owens GK. Regulation of differentiation of vascular smooth muscle cells. *Physiol Rev.* 1995;75:487-517.
412. Owens GK, Schwartz S. Alterations in vascular smooth muscle mass in the spontaneously hypertensive rat. Role of cellular hypertrophy, hyperplasia and hyperplasia. *Circ Res.* 1982;51:280-289.
413. Oza N, Schwartz J, Gould H, Levinsky N. Rat aortic smooth muscle cells in culture express kallikrein-kininogen and bradykinase activity. *J Clin Invest.* 1990;85:597-600.
414. Palmer RM, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature.* 1987;327:524-526.
415. Park JB, Intengan HD, Schiffrin EL. Reduction of resistance artery stiffness by treatment with AT₁-receptor antagonist losartan in essential hypertension. *J Renin Angiotensin Aldosterone Syst.* 2000;1:40-45.
416. Parker SB, Wade SS, Prewitt RL. Pressure mediates angiotensin II-induced arterial hypertrophy and PDGF-A expression. *Hypertension.* 1998;32:452-458.
417. Parsons JT, Parsons SJ. Src family protein tyrosine kinases; cooperating with growth factor and adhesion signaling pathways. *Curr Opin Cell Biol.* 1997;9:187-192.
418. Pasterkamp G, De Kleijn DP, Borst C. Arterial remodeling in atherosclerosis, restenosis and after alteration of blood flow: potential mechanisms and clinical implications. *Cardiovasc Res.* 2000;45:843-852.

419. Pasterkamp G, Schoneveld AH, Van der Wal AC, Haudenschild CC, Clarijs RJ, Becker AE, Hillen B, Borst C. Relation of arterial geometry to luminal narrowing and histological markers for plaque vulnerability: the remodeling paradox. *J Am Coll Cardiol*. 1998;32:655-662.
420. Peeters LLH, Grutters G, Martin CR Jr. Distribution of cardiac output in the unstressed pregnant guinea pig. *Am J Obstet Gynecol*. 1980;153: 417-425.
421. Pei D, Weiss SJ. Furin-dependent intracellular activation of the human stromelysin-3 zymogen. *Nature*. 1995;375:244-247.
422. Peters KG, Vries CD, Williams LT. Vascular endothelial growth factor receptor expression during embryogenesis and tissue repair suggests a role in endothelial differentiation and blood vessel growth. *Proc Natl Acad Sci USA*. 1993;90:8915-8919.
423. Pfeffer M, Braunwald E, Moye L, Basta L, Brown EJ, Cuddy TE, Davis BR, Geltmann EM, Goldman S, Flaker GC, Klein M, Lamas G, Packer M, Rouleau J, Rutherford J, Wertheimer JH, Hawkins CM. Effect of captopril on mortality and morbidity in patients with left ventricular dysfunction after myocardial infarction : results of the survival and ventricular enlargement trial. *N Engl J Med*. 1992; 327:669-677.
424. Phillips DI, Barker DJ, Hales CN, Hirst S, Osmond C. Thinness at birth and insulin resistance in adult life. *Diabetologia*. 1994;37:150-154.
425. Pilz RB, Casteel DE. Regulation of gene expression by cyclic GMP. *Circ Res*. 2003;93:1034-1046.
426. Pohl U, Herlan K, Huang A, Bassenge E. EDRF-mediated shear-induced dilation opposes myogenic vasoconstriction in small rabbit arteries. *Am J Physiol*. 1991;261:H2016-H2023.
427. Pohl U, Holtz J, Busse R, Bassenge E. Crucial role of endothelium in the vasodilation response to increased flow in vivo. *Hypertension Dallas*. 1986;8:37-44.
428. Pollman M, Yamada M, Horiuchi M, Gibbons GH. Vasoactive substances regulate vascular smooth muscle cell apoptosis: countervailing influences of nitric oxide and angiotensin II. *Circ Res*. 1996;79:748-756.
429. Popp R, Fleming I, Busse R. Pulsatile stretch elicits the release of the endothelium-derived hyperpolarizing factor from isolated coronary arteries: a modulator of arterial compliance. *Circ Res*. 1998;82:696-703.
430. Portik-Dobos V, Anstadt MP, Hutchinson J, Bannan M, Ergul A. Evidence for a matrix metalloproteinase induction/activation system in arterial vasculature and decreased synthesis and activation in diabetes. *Diabetes*. 2002;51:3063-3068.
431. Post MJ, Borst C, Pasterkamp G, Haudenschild CC. Arterial remodeling in atherosclerosis and restenosis: a vague concept of a distinct phenomenon. *Atherosclerosis*. 1995;118:S115-S123.
432. Post MJ, de Smet BJ, van der Helm Y, Borst C, Kuntz RE. Arterial remodeling after balloon angioplasty or stenting in an atherosclerotic experimental model. *Circulation*. 1997;96:996-1003.
433. Poston L, Taylor PD. Glaxo/MRS Young Investigator Prize. Endothelium-mediated vascular function in insulin-dependent diabetes mellitus. *Clin Sci (Lond)*. 1995;88:245-255.
434. Pourageaud F, De Mey JGR. Structural properties of rat mesenteric small arteries after 4-wk exposure to elevated or reduced blood flow. *Am J Physiol*. 1997;273:H1699-H1706.
435. Pourageaud F, De Mey JGR. Vasomotor responses in chronically hyperperfused and hypoperfused rat mesenteric arteries. *Am J Physiol*. 1998;274: H1301-H1307.
436. Pourageaud F, Freslon JL. Effects of nitric oxide and sodium nitroprusside on the intrinsic elastic properties of pressurized rat coronary artery. *Fundam Clin Pharmacol*. 1996;10:136-143.
437. Powell JS, Clozel JP, Muller KM, Kuhn H, Hefti F, Hosang M, Baumgartner HR. Inhibition of angiotensin-converting enzyme prevent myointimal proliferation after vascular injury. *Science*. 1989;245:186-188.
438. Prescott MF, Webb RL, Reidy MA. Angiotensin converting enzyme inhibitor versus angiotensin II, AT₁ receptor antagonist. Effects on smooth muscle cell migration and proliferation after balloon catheter injury. *Am J Pathol*. 1991;139:1291-1296.
439. Prewitt RL, Rice DC, Dobrian AD. Adaptation of resistance arteries to increases in pressure. *Microcirculation*. 2002;9:295-304.
440. Price RJ, Skalak TC. Circumferential wall stress as a mechanism for arteriolar rarefaction and proliferation in a network model. *Microvasc Res*. 1994;47:188-202.
441. Rabinovich SE, Lobareva LS, Pashkina TS. Purification and some physico-chemical and enzymatic properties of tissue kallikrein from human urine. *Biokhimiia*. 1990;9:1675-1689.
442. Rabinovitch M, Gamble W, Nadas AS, Miettinen OS, Reid L. Rat pulmonary circulation after chronic hypoxia: hemodynamic and structural features. *Am J Physiol*. 1979;236:H818-H827.

443. Rakugi H, Kim DK, Krieger JE, Wang DS, Dzau VJ, Pratt RE. Induction of angiotensin converting enzyme in the neointima after vascular injury. Possible role in restenosis. *J Clin Invest.* 1994;93:339-346.
444. Rakugi H, Wang DS, Dzau VJ, Pratt RE. Potential importance of tissue angiotensin-converting enzyme inhibition in preventing neointima formation. *Circulation.* 1994;90:449-455.
445. Regoli D, Barabe J. Pharmacology of bradykinin and related kinins. *Pharmacol Rev.* 1980;32:1-46.
446. Reid MR. Abnormal arteriovenous communications, acquired and congenital. III. The effect of abnormal arteriovenous communications on the heart, blood vessels and other structures. *Arch Surg.* 1925;11:25-42.
447. Remuzzi A, Ene-Iordache B, Mosconi L, Bruno S, Arghileri A, Antiga L, Remuzzi G. Radial artery wall shear stress evaluation in patients with arteriovenous fistula for hemodialysis access. *Biorheology.* 2003;40:423-430.
448. Resnick N, Collins T, Atkinson W, Bonthon DT, Dewey CF Jr., Gimbrone MA Jr. Platelet-derived growth factor B chain promoter contains a cis-acting fluid shear stress-response element. *Proc Natl Acad Sci.* 1993;90:4591-4595.
449. Resnick N, Yahav H, Khachigian LM, Collins T, Anderson KR, Dewey CF Jr, Gimbrone MA Jr. Endothelial gene regulation by laminar shear stress. *Adv Exp Med Biol.* 1997;430:155-164.
450. Rhodin JAG. Architecture of the vessel wall. In: Bohr DF, Somlyo AP, Sparks HV Jr, eds. *Handbook of Physiology. Section 2: The Cardiovascular System, Vol II.* Bethesda, Md: American Physiology Society; 1980;1-31.
451. Rieder MJ, Carmona R, Krieger JE, Pritchard KA Jr, Greene AS. Suppression of angiotensin-converting enzyme expression and activity by shear stress. *Circ Res.* 1997;80:312-319.
452. Riezboos J, Vleeming W, Beems RB, Van Amsterdam JGC, Meijer GW, De Wildt DJ, Porsius AJ, Wemer J. Comparison of the antiatherogenic effects of isradipine and ramipril in cholesterol-fed rabbits: II. effect on regression of atherosclerosis and restoration of endothelial dysfunction. *J Cardiovasc Pharmacol.* 1994;23:424-431.
453. Rizzoni D, Porteri E, Piccoli A, Castellano M, Bettoni G, Muescan ML, Pasini G, Guelfi D, Mulvany MJ, Rosci EA. Effects of losartan and enalapril on small artery structure in hypertensive rats. *Hypertension.* 1998;32:305-310.
454. Rosenberg AA, Koehler RC, Jones MD Jr. Distribution of cardiac output in fetal and neonatal lambs with acute respiratory acidosis. *Pediat Res.* 1984;18:731-735.
455. Rosenfeld CR. Distribution of cardiac output in ovine pregnancy. *Am J Physiol.* 1977;232:231-235.
456. Rosenfeld CR. Changes in uterine blood flow during pregnancy. In: *the Uterine Circulation*, CR Rosenfeld, Editor, Ithaca NY. 1989;135-156.
457. Rosenfeld CR, Cox BE, Roy T, Magness RR. Nitric oxide contributes to estrogen-induced vasodilatation of the ovine uterine circulation. *J Clin Invest.* 1996;98:2158-2166.
458. Rosenthal SL, Guyton AC. Hemodynamics of collateral vasodilatation following femoral artery occlusion in anesthetized dogs. *Circ Res.* 1968;23:239-248.
459. Rovainen CM, Wang DB, Woolsey TA. Strobe epi-illumination of fluorescent beads indicates similar velocities and wall shear rates in brain arterioles of newborn and adult mice. *Microvasc Res.* 1992;43:235-239.
460. Rubanyi GM. The role of the endothelium in cardiovascular homeostasis and diseases. *J Cardiovasc Pharmacol.* 1993;4:S1-S14.
461. Rubanyi GM, Romero JC, Vanhoutte PM. Flow-induced release of endothelium-derived relaxing factor. *Am J Physiol.* 1986;250:H1145-H1149.
462. Rudic RD, Bucci M, Fulton D, Segal SS, Sessa WC. Temporal events underlying arterial remodeling after chronic flow reduction in mice: correlation of structural changes with a deficit in basal nitric oxide synthesis. *Circ Res.* 2000;86:1160-1166.
463. Rudic RD, Shesely EG, Maeda N, Smithies O, Segal SS, Sessa WC. Direct evidence for the importance of endothelium-derived nitric oxide in vascular remodeling. *J Clin Invest.* 1998;101:731-736.
464. Rudolph AM. Distribution and regulation of blood flow in the fetal and neonatal lamb. *Circ Res.* 1985;57:811-819.
465. Sadoshima J. Versatility of the angiotensin II type I receptor. *Circ Res.* 1998;82:1352-1355.
466. Safar ME. Carotid artery stiffness with applications to cardiovascular pharmacology. *Gen Pharmacol.* 1996;27:1293-1302.
467. Safar ME, Bortel LMAB van, Struijker-Boudier HAJ. Resistance and conduit arteries following converting enzyme inhibition in hypertension. *J Vasc Res.* 1996;34:67-81.

468. Safar ME, Lévy BI. The effect of hypertension and ACE inhibition on arterial structure and compliance. *Clin Exp Pharmacol Physiol*. 1992;19:29-32.
469. Sakakibara T, Hintze TH, Nasjletti A. Determinants of kinin release in isolated rat hindquarters. *Am J Physiol*. 1998;43:R120-R125.
470. Sarkar R, Meinberg EG, Stanley JC, Gordon D, Webb RC. Nitric oxide reversibly inhibits the migration of cultured vascular smooth muscle cells. *Circ Res*. 1996;78:225-230.
471. Sato Y, Rifkin DB. Autocrine activities of basic fibroblast growth factor: regulation of endothelial cell movement, plasminogen activator synthesis, and DNA synthesis. *J Cell Biol*. 1988;107:1199-1205.
472. Scalbert E, Levy B, Desche P, Devissaguet M, Safar ME. Vascular effects of perindopril: from experimental to clinical investigation. *J Cardiovasc Pharmacol*. 1991;18:S25-S32.
473. Schaper W. New paradigms for collateral vessel growth. *Basic Res Cardiol*. 1993;88:193-198.
474. Schaper W, De Brabander M, Lewi P. DNA synthesis and mitoses in coronary collateral vessels of the dog. *Circ Res*. 1971;28:671-679.
475. Schaper W, Ito WD. Molecular mechanisms of coronary collateral vessel growth. *Circ Res*. 1996;79:911-919.
476. Scheuer DA, Perrone MH. Angiotensin type 2 receptors mediate depressor phase of biphasic pressure response to angiotensin. *Am J Physiol*. 1993;264:R917-R923.
477. Scheuer J, Tipton CM. Cardiovascular adaptations to training. *Annu Rev Physiol*. 1977;39:221-231.
478. Schieffer B, Schieffer E, Hilfiker-Kleiner D, Hilfiker A, Kovanen PT, Kaartinen M, Nussberger J, Harringer W, Drexler H. Expression of angiotensin II and interleukin 6 in human coronary atherosclerotic plaques. *Circulation* 2000;101:1372-1378.
479. Schiffers PMH, Henrion D, Boulanger CM, Colucci-Guyon E, Langa-Vuves F, Van Essen H, Fazzi GE, Lévy BI, De Mey JGR. Altered flow-induced arterial remodeling in vimentin-deficient mice. *Arterioscler Thromb Vasc Biol*. 2000;20:611-616.
480. Schiffers PMH, Struyker-Boudier HAJ, De Mey JGR. Effects of angiotensin II and angiotensin-converting enzyme inhibitors on contractile and growth responses in isolated carotid arteries of the rat. *Basic Res Cardiol*. 1991;86:83-89.
481. Schiffrin EL, Deng LY, Laroche P. Effects of a beta blocker or a converting enzyme inhibitor on resistance arteries in essential hypertension. *Hypertension* 1994;23:83-91.
482. Schlaepfer DD, Hunter T. Integrin signaling and tyrosine phosphorylation: just the FAKs? *Trends Cell Biol*. 1998;8:151-157.
483. Schmaier A, Kuo A, Lundberg D, Murray S, Cines D. The expression of high molecular weight kininogen in human umbilical vein endothelial cells. *J Biol Chem*. 1988;263:16327-16333.
484. Schmidt-Trucksäss A, Schmid A, Brunner C, Scherer N, Zäch G, Keul J, Huonker M. Arterial properties of the carotid artery and femoral artery in endurance-trained and paraplegic subjects. *J Appl Physiol*. 2000;89:1956-1963.
485. Scholz D, Cai WJ, Schaper W. Arteriogenesis, a new concept of vascular adaptation in occlusive disease. *Angiogenesis*. 2001;4:247-257.
486. Schütz S, Le Moullec JM, Corvol P, Gasc JM. Early expression of all the renin-angiotensin system in human development. *Am J Pathol*. 1996;149:2067-2079.
487. Segal SS. Cell-to-cell communication coordinates blood flow control. *Hypertension*. 1994;23:1113-1120.
488. Segal SS, Kurjaka DT, Caston AL. Endurance training increases arterial wall thickness in rats. *J Appl Physiol*. 1993;74:722-726.
489. Senbonmatsu T, Ichihara S, Price E, Gaffney FA, Inagami T. Evidence for angiotensin II type 2 receptor-mediated cardiac myocyte enlargement during in vivo pressure overload. *J Clin Invest*. 2000;106:R25-R29.
490. Senior RM, Griffin GL, Fliszar CJ, Shapiro SD, Goldberg GI, Welgus HG. Human 92- and 72-kilodalton type IV collagenases are elastases. *J Biol Chem*. 1991;266:7870-7875.
491. Sessa WC. The nitric oxide synthase family of proteins. *J Vasc Res*. 1994;31:131-143.
492. Sessa WC, Pritchard K, Seyedi J, Wang J, Hintze TH. Chronic exercise in dogs increases coronary vascular nitric oxide production and endothelial cell nitric oxide synthase gene expression. *Circ Res*. 1994;74:349-353.
493. Seyedi N, Xu X, Nasjletti A, Hintze TH. Coronary kinin generation mediates nitric oxide release after angiotensin receptor stimulation. *Hypertension*. 1995;26:164-170.
494. Shen J, Lusinskas FW, Connolly A, Dewey CF Jr, Gimbrone MA Jr. Fluid shear stress modulates systolic free calcium in vascular endothelial cells. *Am J Physiol*. 1992;262:C384-390.

495. Shenk WG, Martin JW, Leslie MB, Portin BA. The regional hemodynamics of chronic experimental arteriovenous fistulas. *Surg Gynecol Obstet.* 1960;110:44-50.
496. Shi Y, O'Brien JE, Ala-Kokko L, Chung W, Mannion JD, Zalewski A. Origin of extracellular matrix synthesis during coronary repair. *Circulation.* 1997;95:997-1006.
497. Shichiri M, Marumo F, Hirata Y. Endothelin-B receptor-mediated suppression of endothelial apoptosis. *J Cardiovasc Pharmacol.* 1998;31:S126-S127.
498. Sho E, Sho M, Singh TM, Xu C, Zarins CK, Masuda H. Blood flow decrease induces apoptosis of endothelial cells in previously dilated arteries resulting from chronic high blood flow. *Arterioscler Thromb Vasc Biol.* 2001;21:1139-1145.
499. Shyy JY, Chien S. Role of integrins in endothelial mechanosensing of shear stress. *Circ Res.* 2002;91:769-775.
500. Shyy JY, Lin MC, Han J, Hu Y, Pettrime M, Chien S. The cis-acting phorbol ester "12-O-tetradecanoylphorbol 13-acetate"-response element is involved in shear stress-induced monocyte chemotactic protein 1 gene expression. *Proc Natl Acad Sci USA.* 1995;92:8069-8073.
501. Silvestre JS, Bergaya S, Tamarat R, Duriez M, Boulanger CM, Levy BI. Proangiogenic effect of angiotensin-converting enzyme inhibition is mediated by the bradykinin B(2) receptor pathway. *Circ Res.* 2001;89:678-683.
502. Singh R, Pan S, Mueske CS, Witt T, Kleppe LS, Peterson TE, Slobodova A, Chang JY, Caplice NM, Simari RD. Role for tissue factor pathway in murine model of vascular remodeling. *Circ Res.* 2001;89:71-76.
503. Singh T, Abe K, Sasaki T, Zhuang Y, Masuda H, Zarins C. Basic fibroblast growth factor expression precedes flow-induced arterial enlargement. *J Surg Res.* 1998;77:165-173.
504. Siragy HM, Inagami T, Ichiki T, Carey RM. Sustained hypersensitivity to angiotensin II and its mechanism in mice lacking the subtype-2 (AT₂) angiotensin receptor. *Proc Natl Acad Sci USA.* 1999;99:6506-6510.
505. Small JV, Furst DO, Thornell LE. The cytoskeletal lattice of muscle cells. *Eur J Biochem.* 1992;208:559-572.
506. Smiesko V, Johnson PC. The arterial lumen is controlled by flow-related shear stress. *News Physiol Sci.* 1993;8:34-38.
507. Smiesko V, Lang DJ, Johnson PC. Dilator response of rat mesenteric arcading arterioles to increased blood flow velocity. *Am J Physiol.* 1989;257:H1958-H1965.
508. Smith JC, Walters WA, Spong JE. Effects of pregnancy on the diameter of the uterine arteries in the rabbit. *Obstet Gynecol.* 1968;31:788-794.
509. Song K, Kurobe Y, Kanehara H, Wada T, Inada Y, Nishikawa K, Miyazaki M. Mapping of angiotensin II receptor subtypes in peripheral tissues of spontaneously hypertensive rats by autoradiography. *Clin Exp Pharmacol Physiol Suppl.* 1995;22:S17-S19.
510. Song RH, Kocharyan HK, Fortunato JA, Glagov S, Bassiouny HS. Increased flow and shear stress enhance in vivo transforming growth factor-beta 1 after experimental arterial injury. *Arterioscler Thromb Vasc Biol.* 2000;20:923-930.
511. Sonntag WE, Lynch CD, Cooney PT, Hutchins PM. Decreases in cerebral microvasculature with age are associated with the decline in growth hormone and insulin-like growth factor I. *Endocrinology.* 1997;138:3515-3520.
512. Southgate KM, Davies M, Booth RF, Newby AC. Involvement of extracellular matrix-degrading metalloproteinases in rabbit aortic smooth-muscle cell proliferation. *Biochem J.* 1992;288:93-99.
513. Stassen FRM, Raat NJH, Brouwers-Ceiler DL, Fazzi GE, Smits JFM, De Mey JGR. Angiotensin II induces media hypertrophy and hyperreactivity in mesenteric but not epigastric small arteries of the rat. *J Vasc Res.* 1997;34:289-297.
514. Stein J, Congbalay R, Karsh D, Osgood R, Ferris T. The effect of bradykinin on proximal tubular sodium reabsorption in the dog: evidence for functional nephron heterogeneity. *J Clin Invest.* 1972;51:1709-1721.
515. Sterpetti AV, Cavallaro A, Cavallari N, Allegrucci P, Tamburelli A, Agosta F, Bartoli S. Factors influencing the rupture of abdominal aortic aneurysms. *Surg Gynecol Obstet.* 1991;173:175-178.
516. Stevenson E, Davy K, Seals D. Hemostatic, metabolic, and androgenic risk factors for coronary heart disease in physically active and less active postmenopausal women. *Arterioscler Thromb Vasc Biol.* 1995;15:669-677.

517. St-Louis J, Paré H, Sicotte B, Brochu M. Increased reactivity of rat uterine arcuate artery throughout gestation and postpartum. *Am J Physiol*. 1997;273:H1148-H1153.
518. St-Louis J, Sicotte B, Bédard S, Brochu M. Blockade of angiotensin receptor subtypes in arcuate uterine artery of pregnant and postpartum rats. *Hypertension*. 2001;38:1017-1023.
519. Stone PH, Coskun AU, Kinley S, Clark ME, Sonka M, Wahle A, Ilegbusi OJ, Yeghiazarians Y, Popma JJ, Orav J, Kuntz RE, Feldman CL. Effect of endothelial shear stress on the progression of coronary artery disease, vascular remodeling, and in-stent restenosis in humans. In vivo 6-month follow-up study. *Circulation*. 2003;108:438-444.
521. Stormont JM, Meyer M, Osol G. Estrogen augments the vasodilatory effects of vascular endothelial growth factor in the uterine circulation of the rat. *Am J Obstet Gynecol*. 2000;183(2):449-453.
521. Su EJ, Lombardi DM, Siegal J, Schwartz SM. Angiotensin II induces vascular smooth muscle cell replication independent of blood pressure. *Hypertension*. 1998;31:1331-1337.
522. Sullivan CJ, Hoying JB. Flow-dependent remodeling in the carotid artery of fibroblast growth factor-2 knockout mice. *Arterioscler Thromb Vasc Biol*. 2002;22:1100-1105.
523. Sun D, Huang A, Koller A, Kaley G. Short term daily exercise activity enhances endothelial NO synthesis in skeletal muscle arterioles of rats. *J Appl Physiol*. 1994;76:2241-2247.
524. Sun D, Huang A, Smith CJ, Stackpole CJ, Connetta JA, Shesely EG, Koller A, Kaley G. Enhanced release of prostaglandins contributes to flow-induced arteriolar dilation in eNOS knockout mice. *Circ Res*. 1999;85:288-293.
525. Sun D, Huang A, Yan EH, Wu Z, Yan C, Kaminski PM, Oury TD, Wolin MS, Kaley G. Reduced release of nitric oxide to shear stress in mesenteric arteries of aged rats. *Am J Physiol*. 2004;*In Press*.
526. Svensson A. Hypertension in pregnancy: state of the art lecture. *J Hypertens*. 1985;3:S395-S403.
527. Taddei S, Virdis A, Ghiadoni L, Salvetti G, Bernini G, Magagna A, Salvetti A. Age-related reduction of NO availability and oxidative stress in humans. *Hypertension*. 2001;38:274-279.
528. Taguchi J, Abe J, Okazaki H, Ochiai M, Ohno M, Takuwa Y, Kurokawa K. Angiotensin converting enzyme inhibitors or Dup753 prevent neointimal formation following balloon injury with single topical or multiple systemic application. *Biochem Biophys Res Commun*. 1993;196:969-974.
529. Tai LK, Okuda M, Abe J, Yan C, Berk BC. Fluid shear stress activates proline-rich tyrosine kinase via reactive oxygen species-dependent pathway. *Arterioscler Thromb Vasc Biol*. 2002;22:1790-1796.
530. Tanaka M, Tsuchida S, Imai T, Fuji N, Miyazaki H, Ichiki T, Naruse M, Inagami T. Vascular response to angiotensin II is exaggerated through an upregulation of AT₁ receptor in AT₂ knockout mice. *Biochem Biophys Res Commun*. 1999;258:194-198.
531. Taniyama Y, Griendling KK. Reactive oxygen species in the vasculature: molecular and cellular mechanisms. *Hypertension*. 2003;42:1075-1081.
532. Tamura K, Chen YE, Lopez-Illasaca M, Daviet L, Tamura N, Ishigami T, Akishita M, Takasaki I, Tokita Y, Pratt RE, Horiuchi M, Dzau VJ, Umemura S. Molecular mechanism of fibronectin gene activation by cyclic stretch in vascular smooth muscle cells. *J Biol Chem*. 2000;275:34619-34627.
533. Thaler I, Manor D, Itskovitz J, Rottem S, Levit N, Timor-Tritsch I, Brandes JM. Changes in uterine blood flow during human pregnancy. *Am J Obstet Gynecol*. 1990;162:121-125.
534. Thiemermann C. Biosynthesis and interaction of endothelium-derived vasoactive mediators. *Eicosanoids*. 1991;4:187-202.
535. Thoma R. Untersuchungen über die histogenese und histomechanik des Gefasssystems (Enke, Snuttgart); 1893; A.F.W. Hughes, *J. Anat*. 72,1 (1937).
536. Thorin E, Huang PL, Fishman MC, and Bevan JA. Nitric oxide inhibits alpha2-adrenoceptor-mediated endothelium-dependent vasodilation. *Circ Res*. 1998;82:1323-1329.
537. Thoumine O, Ziegler T, Girard PR, Nerem RM. Elongation of confluent endothelial cells in culture: the importance of fields of force in the associated alterations in their cytoskeletal structure. *Exp Cell Res*. 1995;219:427-441.
538. Tjwa M, Luttun A, Autiero M, Carmeliet P. VEGF and PlGF: two pleiotropic growth factors with distinct roles in development and homeostasis. *Cell Tissue Res*. 2003;314:5-14.
539. Tohda K, Masuda H, Kawamura K, Shozawa T. Difference in dilatation between endothelium-preserved and -desquamated segments in the flow-loaded rat common carotid artery. *Arterioscler Thromb*. 1992;12:519-528.
540. Topper JN. TGF-beta in the cardiovascular system: molecular mechanisms of a context-specific growth factor. *Trends Cardiovasc Med*. 2000;10:132-137.

541. Topper JN, Cai J, Falb D, Grimbone MA Jr. Identification of vascular endothelial genes differentially responsive to fluid mechanical stimuli: cyclooxygenase-2, manganese superoxide dismutase, and endothelial cell nitric oxide synthase are up-regulated by steady shear stress. *Proc Natl Acad Sci USA*. 1996;93:10417-10422.
542. Topper JN, Gimbrone MA Jr. Blood flow and vascular gene expression: fluid shear stress as a modulator of endothelial phenotype. *Mol Med Today*. 1999;5:40-46.
543. Touyz RM, Schiffrin EL. Angiotensin II regulates vascular smooth muscle cell pH contraction and growth via tyrosine-kinase dependent signaling pathways. *Hypertension* 1997;30:222-229.
544. Traub O, Berk BC. Laminar shear stress: mechanisms by which endothelial cells transduce an atheroprotective force. *Arterioscler Thromb Vasc Biol*. 1998;18:677-685.
545. Tribe RM, Thomas CR, Poston L. Flow-induced dilatation in isolated resistance arteries from control and streptozotocin-diabetic rats. *Diabetologia*. 1998;41:34-39.
546. Tronc F, Mallat Z, Lehoux S, Wassef M, Esposito B, Tedgui A. Role of matrix metalloproteinases in blood flow-induced arterial enlargement: Interaction with NO. *Arterioscler Thromb Vasc Biol*. 2000;20:e120-e126.
547. Tronc F, Wassef M, Esposito B, Henrion D, Glagov S, Tedgui A. Role of NO on flow-induced remodeling of the rabbit common carotid artery. *Arterioscler Thromb Vasc Biol*. 1996;16:1256-1262.
548. Tschudi MR, Barton M, Bersinger NA, Moreau P, Cosentino F, Noll G, Malinski T, Lüscher TF. Effects of age on kinetics of nitric oxide release in rat aorta and pulmonary artery. *J Clin Invest*. 1996;98:899-905.
549. Tschudi MR, Lüscher TF. Age and hypertension differentially affect coronary contractions to endothelin-1, serotonin, and angiotensins. *Circulation*. 1995;91:2415-2422.
550. Tsuda Y, Okazaki M, Uezono Y, Osajima A, Kato H, Okuda H, Oishi Y, Yashiro A, Nakashima Y. Activation of extracellular signal-regulated kinases is essential for pressure-induced proliferation of vascular smooth muscle cells. *Eur J Pharmacol*. 2002;446:15-24.
551. Tsutsumi Y, Matsubara H, Masaki H, Kurihara H, Murasawa S, Takai S, Miyazaki M, Nozawa Y, Ozono R, Nakagawa K, Miwa T, Kawada N, Mori Y, Shibasaki Y, Tanaka Y, Fujiyama S, Koyama Y, Fujiyama A, Takahashi H, Iwasaki T. Angiotensin II type 2 receptor overexpression activates the vascular kinin system and causes vasodilation. *J Clin Invest*. 1999;104:925-935.
552. Tulis DA, Prewitt RL. Medial and endothelial platelet-derived growth factor A chain expression is regulated by in vivo exposure to elevated flow. *J Vasc Res*. 1998;35:413-420.
553. Tulis DA, Unthank JL, Prewitt RL. Flow-induced remodeling in rat mesenteric vasculature. *Am J Physiol*. 1998;274:H874-H882.
554. Tuttle JL, Hahn TL, Sanders BM, Witzman FA, Miller SJ, Dalsing MC, Unthank JL. Impaired collateral development in mature rats. *Am J Physiol*. 2002;283:H146-H155.
555. Tuttle JL, Nachreiner RD, Bhuller AS, Condict KW, Connors BA, Herring BP, Dalsing MC, Unthank JL. Shear level influences resistance artery remodeling: wall dimensions, cell density, and eNOS expression. *Am J Physiol*. 2001;281:H1380-H1389.
556. Unthank JL, Fath SW, Burkhardt HM, Miller S, Dalsing MC. Wall remodeling during luminal expansion of mesenteric arterial collaterals in the rat. *Circ Res*. 1996;79:1015-1023.
557. Unthank JL, Nixon JC, Burkhardt HM, Fath SW, Dalsing MC. Early collateral and microvascular adaptations to intestinal artery occlusion in the rat. *Am J Physiol*. 271:1996;H914-H923.
558. Unthank JL, Nixon JC, Lash JM. Early adaptations in collateral and microvascular resistances after ligation of the rat femoral artery. *J Appl Physiol*. 1995;70:73-82.
559. Vagnoni KE, Shaw CE, Phernetton TM, Meglin BM, Bird IM, Magness RR. Endothelial vasodilator production by uterine and systemic arteries. III. Ovarian and estrogen effects on NO synthase. *Am J Physiol*. 1998;275:H1845-H1856.
560. Valdes G, Germain AM, Corthorn J, Chacon C, Figueroa CD, Muller-Esterl W. Tissue kallikrein and bradykinin B₂ receptor in human uterus in luteal phase and in early and late gestation. *Endocrine*. 2001;16(3):207-215.
561. Van der Heijden OWH, Essers YPG, Simkens LHJ, Teunissen QGA, Peeters LJJH, De Mey JGR, Van Eys GJJM. Aging blunts remodeling of the uterine artery during murine pregnancy. *In Press* 2004.
562. Van der Loo B, Labugger R, Skepper JN, Bachschmid M, Kilo J, Powell JM, Palacios-Callender M, Erusalimsky JD, Quaschnig T, Malinski T, Gygi D, Ullrich V, Lüscher TF. Enhanced peroxynitrite formation is associated with vascular aging. *J Exp Med*. 2000;192:1731-1744.
563. Vanhoutte PM. Endothelial AT₂-receptors: chicken or egg? *Br J Pharmacol*. 2002;136:481-483.

564. Van Katwijk C, Peeters LL. Clinical aspects of pregnancy after the age of 35 years: a review of the literature. *Human Reprod Update*. 1998;4:185-194.
565. Van Kleef EM, Fingerle J, Daemen MJ. Angiotensin II-induced progression of neointimal thickening in the balloon-injured rat carotid artery is AT₁ receptor mediated. *Arterioscler Thromb Vasc Biol*. 1996;16:857-863.
566. Van Kleef EM, Smits JF, De Mey JG, Cleutjens JP, Lombardi DM, Schwartz SM, Daemen MJ. Alpha 1-adrenoceptor blockade reduces the angiotensin II-induced vascular smooth muscle cell DNA synthesis in the rat thoracic aorta and carotid artery. *Circ Res*. 1992;70:1122-1127.
567. Van Royen N, Pick JJ, Schaper W, Bode C, Buschmann I. Arteriogenesis: mechanisms and modulation of collateral artery development. *J Nucl Cardiol*. 2001;8:687-693.
568. Van Suylen RJ, Aartsen WM, Smits JFM, Daemen MJAP. Dissociation of pulmonary vascular remodeling and right ventricular pressure in tissue angiotensin-converting enzyme-deficient mice under conditions of chronic alveolar hypoxia. *Am J Respir Crit Care Med*. 2001;163:1241-1245.
569. Vassalle C, Lubrano V, Domenici C, L'Abbate A. Influence of chronic aerobic exercise on microcirculatory flow and nitric oxide in humans. *Int J Sports Med*. 2003;24:30-35.
570. Vaughan DE. Angiotensin and vascular fibrinolytic balance. *Am J hypertension*. 2002;15:3S-8S.
571. Veerareddy S, Cooke CLM, Baker PN, Davidge ST. Vascular adaptations to pregnancy in mice: effects on myogenic tone. *Am J Physiol*. 2002;283:H2226-H2233.
572. Venema RC, Sayegh HS, Arnal J-F, Harrison DG. Role of enzyme calmodulin-binding domain in membrane association and phospholipid inhibition of endothelial nitric oxide synthase. *J Biol Chem*. 1995;270:14705-14711.
573. Viswanathan M, Tsutsumi K, Correa FM, Saavedra JM. Changes in expression of angiotensin receptor subtypes in the rat aorta during development. *Biochem Biophys Res Commun*. 1991;179:1361-1367.
574. Von der Leyen HE, Gibbons GH, Morishita R, Lewis NP, Zhang L, Nakajima M, Kaneda Y, Cooke JP, Dzau VJ. Gene therapy inhibiting neointimal vascular lesion: in vivo transfer of endothelial nitric oxide synthase gene. *Proc Natl Acad Sci*. 1995;92:1137-1141.275.
575. Wallenstein S, Zucker CL, Fleiss JL. Some statistical methods useful in circulation research. *Circ Res*. 1980;47:1-9.
576. Walther T, Menrad A, Orzechowski HD, Siemeister G, Paul M, Schirner M. Differential regulation of in vivo angiogenesis by angiotensin II receptors. *FASEB J*. 2003;17:2061-2067.
577. Wang DB, Blocher NC, Spence ME, Rovainen CM, Woolsey TA. Development and remodeling of cerebral blood vessels and their flow in postnatal mice observed with in vivo videomicroscopy. *J Cereb Blood Flow Metab*. 1992;12:935-946.
578. Wang DH, Prewitt RL. Microvascular development during normal growth and reduced blood flow: introduction of a new model. *Am J Physiol*. 1991;260:H1966-H1972.
579. Wang J, Wolin MS, Hintze TH. Chronic exercise enhances endothelium-mediated dilation of epicardial coronary artery in conscious dogs. *Circ Res*. 1993;73:829-838.
580. Ward MR, Tsao PS, Agrotis A, Dille RJ, Jennings GL, Bobik A. Low blood flow after angioplasty augments mechanisms of restenosis. Inward vessel remodeling, cell migration, and activity of genes regulating migration. *Arterioscler Thromb Vasc Biol*. 2001;21:208-213.
581. Weiner C, Martinez E, Lui KZ, Ghodi A, Chestnut D. *In vitro* release of endothelium-derived relaxing factor by acetylcholine is increased during the guinea pig pregnancy. *Am J Obstet Gynecol*. 1989;161:1599-1605.
582. Weiner CP, Lizasoain I, Baylis SA, Knowles RG, Charles IG, Moncada S. Induction of calcium-dependent nitric oxide synthase by sex hormones. *Proc Natl Acad Sci USA*. 1994;91:5212-5216.
583. Wells SM, Langille BL, Adamson SE. In vivo and in vitro mechanical properties of the sheep thoracic aorta in the perinatal period and adulthood. *Am J Physiol*. 1998;274:H1749-H1760.
584. Wesselman JP, De Mey JG. Angiotensin and cytoskeletal proteins: role in vascular remodeling. *Curr Hypertens Rep*. 2002;4:63-70.
585. Wesselman JP, Dobrian AD, Schriver SD, Prewitt RL. Src tyrosine kinases and extracellular signal-related kinase 1/2 mitogen-activated protein kinases mediate pressure-induced c-fos expression in cannulated rat mesenteric small arteries. *Hypertension*. 2001;37:955-960.
586. Wesselman JPM, Evelo CTA, Struijker-Boudier HAJ, De Mey JGR. Differential gene expression induced by modified blood flow in rat mesenteric small arteries in vivo. *In Press*. 2004
587. Wiemer G, Scholkens BA, Becker RHA, Busse R. Ramiprilat enhances endothelial autocoid formation by inhibiting breakdown of endothelium-derived bradykinin. *Hypertension*. 1991;18:558-563.

588. Wiemer G, Schölkens BA, Wagner A, Heitsch H, Linz W. The functional role of angiotensin II subtype AT₂-receptors in endothelial cells and isolated ischemic rat hearts. *J Hypertens Suppl.* 1993;11:S234-S235.
589. Wijnen J, Kuipers H, Kool M, Hoeks A, van Baak M, Struijker-Boudier H, Verstappen F, Van Bortel L. Vessel wall properties of large arteries in trained and sedentary subjects. *Basic Res Cardiol.* 1991;86:25-29.
590. Wilson DD, De Garavilla L, Kuhn W, Togo J, Burch RM, Steranka L. D-Arg [Hyp3DPhe7] bradykinin, a bradykinin antagonist, decreases mortality in a rat model of endotoxic shock. *Circ Shock.* 1989;27:93-101.
591. Wolf C, Cai WJ, Vosschulte R, Kolrai S, Mousavipour D, Scholz D, Afsah-Hedjri A, Schaper W, Schaper J. Vascular remodeling and altered protein expression during growth of coronary collateral arteries. *J Mol Cell Cardiol.* 1998;30:2291-2305.
592. Wolin MS, Gupta SA, Oeckler RA. Superoxide in the vascular system. *J Vasc Res.* 2002;39:191-207.
593. Wong AHY, Kulandavelu S, Whiteley KJ, Qu D, Lowell Langille B, Lee Adamson S. Maternal cardiovascular changes during pregnancy and postpartum in mice. *Am J Physiol.* 2002;282:H918-H925.
594. Xin X, Yang N, Eckhart AD, Faber JE. Alpha1D-adrenergic receptors and mitogen-activated protein kinase mediate increased protein synthesis by arterial smooth muscle. *Mol Pharmacol.* 1997;51:764-775.
595. Xu C, Lee S, Shu C, Masuda H, Zarins CK. Expression of TGF- β 1 and β 3 but not apoptosis factors relates to flow-induced aortic enlargement. *BMC Cardiovasc Disorders.* 2002;2:11-21.
596. Yayama K, Hiroki S, Masaoki T, Hiroshi O. Expression of Low-Molecular-Weight Kininogen in mouse vascular smooth muscle cells. *Biol Pharm Bull.* 1998;21:772-774.
597. Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, Yazaki Y, Goto K, Masaki T. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature.* 1988;332:411-415.
598. Yogo K, Shimokawa H, Funakoshi H, Kandabashi T, Miyata K, Okamoto S, Egashira K, Huang P, Akaike T, Takeshita A. Different vasculoprotective roles of NO synthase isoforms in vascular lesion formation in mice. *Arterioscler Thromb Vasc Biol.* 2000;20:e96-e100.
599. Zahradka P, Wilson D, Saward L, Yau L, Cheung PK. Cellular physiology of angiotensin II receptors in vascular smooth muscle cells, in *Angiotensin II Receptor Blockade: Physiological and Clinical Implications* (Dhall NS, Zahradka P, Dixon I and Beamish R eds) 1998: 41-50, Kluwer Academic Publishers, Norwell, MA.
600. Zalewski A, Shi Y. Vascular myofibroblasts: lessons from coronary repair and remodeling. *Arterioscler Thromb Vasc Biol.* 1997;17:417-422.
601. Zarins CK, Zatina MA, Giddens DP, Ku DN, Glagov S. Shear stress regulation of artery lumen diameter in experimental atherogenesis. *J Vasc Surg.* 1987;5:413-420.
602. Zeiher AM, Drexler H, Wollschlaeger H, Just H. Endothelial dysfunction of the coronary microvasculature is associated with impaired coronary blood flow regulation in patients with early atherosclerosis. *Circulation.* 1991;84:1984-1992.
603. Zeiher AM, Drexler H, Saurbier B, Just H. Endothelium-mediated coronary blood flow modulation in humans: effect of age, atherosclerosis, hypercholesterolemia and hypertension. *J Clin Invest.* 1993;92:652-662.
604. Zhou J, Moeller I, Jenkins T, Chai SY, Allen AM, Mitsuru O, Mendelsohn AO. Mapping tissue angiotensin-converting enzyme and angiotensin AT₁, AT₂ and AT₄ receptors. *J Hypertens.* 1998;16:2027-2037.
605. Ziche M, Morbidelli L, Masini E, Amerini S, Granger HJ, Maggi CA, Geppetti P, Ledda F. Nitric oxide mediates angiogenesis in vivo and endothelial cell growth and migration in vitro promoted by substance P. *J Clin Invest.* 1994;94:2036-2044.
606. Ziegler T, Silacci P, Harrison VJ, Hayoz D. Nitric oxide synthase expression in endothelial cells exposed to mechanical forces. *Hypertension.* 1998;32:351-355.
607. Zygmunt M, Herr F, Münstedt K, Lang U, Liang OD. Angiogenesis and vasculogenesis in pregnancy. *Eur J Obstet Gynecol Reprod Biol.* 2003;110:S10-S18.
608. Zwart AS, Davis EA, Widdop RE. Modulation of AT₁ receptor-mediated contraction of rat uterine artery by AT₂ receptors. *Br J Pharmacol.* 1998;125:1429-1436.

Summary

Blood vessels have the adaptive mechanism to respond to, and normalize changes in hemodynamic stimuli such as pressure and flow. Situations of disturbed hemodynamic stresses occur *in vivo* for instance during pathological disorders such as hypertension and atherosclerosis, but also during physiological processes such as exercise and pregnancy. Endothelial cells are directly in contact with the dragging blood flow and are therefore particularly sensitive to changes in shear stress (flow). In the first chapter we described the acute (flow-induced dilatation) and chronic arterial responses (remodeling) to flow in particular. We focused on the renin-angiotensin system and the kallikrein-kinin system, since the main objective of the present thesis was to determine the role of angiotensin II and bradykinin, two important mediators of both systems, in acute and chronic arterial responses to altered blood flow.

Tissue kallikrein-deficient mice (TK^{-/-}) were studied to analyze the effect of an absence of membrane-bound tissue kallikrein (and hence reduced bradykinin synthesis) on acute vasomotor responses and chronic structural responses to flow in both large conduit arteries (carotid arteries) and small peripheral arteries (uterine arteries). In chapter 2 we studied acute flow-induced dilatation in large conduit arteries such as the carotid artery. We confirmed previous observations that flow-induced dilatation was significantly blunted in carotid arteries of TK^{-/-} mice, demonstrating the key role of TK in acute flow-induced dilatation, by releasing endogenous kinins that act on endothelial bradykinin B₂ receptors. Furthermore, we demonstrated that this response required functional angiotensin type 2 receptors.

The role of the vascular kallikrein-kinin system during arterial remodeling in response to chronic changes in blood flow was analyzed by using a surgical model. In chapter 3 unilateral left carotid artery ligation was performed in both wild-type and TK^{-/-} mice, which resulted in a complete cessation in blood flow in the ligated left carotid artery and a doubling of blood flow in the contralateral right carotid artery. The vascular kallikrein-kinin system seems to protect against excessive inward remodeling, but was not involved in medial wall mass changes in response to chronic cessations in blood flow.

The participation of the kallikrein-kinin system in acute flow-induced dilatation was also analyzed in small resistance-sized arteries. Similarly to responses in the carotid artery, flow-induced dilatation was blunted in uterine arteries of TK^{-/-} mice (chapter 4). Pregnancy, characterized by a drastic rise in uterine blood flow, resulted in an enhanced release of nitric oxide in response to shear stress by uterine arteries of both wild-type and TK^{-/-} mice. The difference in the maximal vasodilator response to shear stress persisted between wild-type and TK^{-/-} mice. Despite this reduced vasodilator response to shear stress in TK^{-/-} mice, pregnancy-induced uterine artery outward hypertrophic remodeling in response to the uterine blood flow increase, was not compromised. Immediately after birth uterine blood flow returns to normal flow values, representing a physiological model of reduced blood flow. We observed that flow-induced dilatation was markedly reduced in uterine arteries of 7-days postpartum mice, and the arterial structure regressed toward pre-pregnant structure, demonstrating the dynamic nature of the uterine vascular bed during and after pregnancy.

Tissue angiotensin-converting enzyme-deficient mice (t-ACE^{-/-}) were studied to analyze the effect of an absence of membrane-bound tissue ACE (and hence reduced angiotensin II synthesis, but enhanced endogenous bradykinin levels) on acute vasomotor responses and chronic structural responses to flow in both carotid and uterine arteries. In chapter 5 we investigated the role of endogenous bradykinin in chronic modulation of endothelium-dependent vascular reactivity that occurs during and after pregnancy. We showed that bradykinin can be released by both chemical (acetylcholine) and mechanical (shear stress) stimulation of uterine arteries of wild-type and t-ACE^{-/-} mice. The contribution of bradykinin in these vasodilator responses was higher in uterine arteries of t-ACE^{-/-} mice, since blockade of B₂ receptors inhibited the response to acetylcholine and shear stress to a greater extent in t-ACE^{-/-} mice. During pregnancy the contribution of nitric oxide to the shear stress-induced vasodilatation was more substantial in uterine arteries of wild-type mice compared to t-ACE^{-/-} mice, whereas the contribution of bradykinin was more pronounced in late pregnant t-ACE^{-/-} mice, demonstrating the modulatory role of endothelium-derived vasoactive factors like bradykinin during flow-induced dilatation of uterine arteries in situations of chronically altered uterine blood flow.

Tissue ACE deficiency resulted in a reduced arterial elasticity (chapter 6). This reduced elasticity originated from B₂ receptor stimulation, since chronic (14 days) blockade of these receptors restored the arterial elasticity in t-ACE^{-/-} mice. These data demonstrate the close relationship between the renin-angiotensin system and the kallikrein-kinin system and their contribution to the maturation of the cardiovascular system.

In chapter 7 we tested whether tissue ACE is involved in arterial remodeling by applying the unilateral carotid artery ligation method and studying uterine arteries before, during and after pregnancy in wild-type and t-ACE^{-/-} mice. In the ligated carotid artery of t-ACE^{-/-} mice, the observed medial hyperplasia, inward remodeling and arterial distensibility were reduced compared to the wild-type counterpart. Thus, tissue ACE plays a major role in suppressing the hyperplastic inward remodeling of the carotid artery imposed by blood flow cessation. Comparing the results of chapter 3 and 7, it seems that angiotensin II is responsible for the vascular smooth muscle cell hyperplasia, whereas bradykinin prevents luminal shrinkage in response to drastic decreases in large artery blood flow. Like tissue kallikrein, tissue ACE is not essential for the outward hypertrophic and subsequent inward hypotrophic remodeling of the uterine artery during and after pregnancy.

In chapter 8 the effects of postponed murine pregnancy on acute and chronic uterine artery responses were investigated. Our findings suggest that at middle age the effects of shear stress on endothelial nitric oxide synthase phosphorylation and subsequent nitric oxide release are lost. Upregulation by other endothelium-derived factors like prostacyclin or endothelium-derived hyperpolarizing factor compensate during acute flow-induced dilatation, but can not sustain chronic pregnancy-induced arterial remodeling. This might explain the reduced number of viable fetuses in the third trimester of postponed murine pregnancy.

From the results obtained in this thesis it is clear that blunted acute arterial responses to shear stress do not necessarily entail a defect in the remodeling response after chronic alterations in shear stress. Strengthened by our observations and others we have written in

chapter 9 a review on the supposed dissociation between acute and chronic arterial responses to shear stress.

In summary, the results described in this thesis confirm the beneficial effects of bradykinin as is increasingly suggested by experimental and clinical drug-intervention studies. Furthermore, acute flow-induced vasodilatation and structural flow-induced arterial remodeling involve alternative mechanisms, which opens new possibilities for the development of new targets for cardiovascular-pharmacotherapy. In addition, adaptive structural arterial adaptations to shear stress differ between large elastic and pre-existing collateral arteries, and the effect of even middle age is more marked than deficiency of cardiovascular key enzymes, such as tissue kallikrein or tissue angiotensin-converting enzyme. This raises new perspectives, such as site-directed interventions, for unraveling the effects of aging and regionality on arterial remodeling.

Samenvatting

Bloedvaten hebben de adaptieve eigenschap om te reageren op veranderingen in hemodynamische stimuli zoals druk en stroming (kortweg flow). Processen van een verstoorde hemodynamische balans kunnen zich *in vivo* voordoen bij pathologische aandoeningen zoals hypertensie en atherosclerose, maar ook tijdens fysiologische processen zoals langdurige lichamelijke inspanning en de zwangerschap. Endotheelcellen zijn in direct contact met het stromende bloed en zijn daardoor bijzonder gevoelig voor veranderingen in afschuifspanning of flow. In hoofdstuk 1 beschrijven we de acute (flow-geïnduceerde vaatverwijding) en chronische responsen (remodelering) op flow. Hierbij hebben we ons gericht op de rol van angiotensine II en bradykinine, twee belangrijke mediators van respectievelijk het renine-angiotensine systeem en het kallikreïne-kinine systeem, bij de acute en chronische arteriële responsen op een veranderde flow.

Weefsel kallikreïne-deficiënte muizen (TK^{-/-}) werden bestudeerd om het effect van de afwezigheid van membraan-gebonden weefsel kallikreïne (en dus een verminderde bradykinine synthese) op acute vasomotor responsen en chronische structurele responsen op flow te analyseren in zowel grote arteriën (carotide arteriën) en kleine perifere arteriën (uteriene arteriën). In hoofdstuk 2 hebben we de acute door flow-geïnduceerde vaatverwijding in de carotide arterie onderzocht. We bevestigden eerdere resultaten dat de door flow-geïnduceerde vaatverwijding significant verlaagd is in carotide arteriën van TK^{-/-} muizen, hetgeen de cruciale rol van weefsel kallikreïne in acute door flow-geïnduceerde vaatverwijding aantoont, via de secretie van endogene kinines die aangrijpen op endotheliale bradykinine B₂ receptoren. Bovendien toonden we aan dat deze reactie afhankelijk is van functionele angiotensine type 2 receptoren.

Een chirurgisch model werd toegepast om de rol van het vasculaire kallikreïne-kinine systeem bij arteriële remodelering tijdens chronische flow veranderingen te bestuderen. In hoofdstuk 3 pasten we unilaterale ligatie van de linker carotide arterie toe op zowel wild-type als TK^{-/-} muizen, hetgeen resulteerde in een complete obstructie van de flow door dit afgebonden bloedvat en een verdubbeling van de flow in de rechter carotide arterie. Het vasculaire kallikreïne-kinine systeem bleek te beschermen tegen overmatige inwaardse remodelering, maar was niet betrokken bij mediale vaatwandmassa veranderingen in respons op chronische flow cessaties.

Een bijdrage van het kallikreïne-kinine systeem in acute flow-geïnduceerde vaatverwijding werd tevens onderzocht in kleine weerstandsarteriën. In analogie met de responsen in de carotide arterie, was de door flow-geïnduceerde vaatverwijding verlaagd in uteriene arteriën van TK^{-/-} muizen (hoofdstuk 4). De zwangerschap, die gekarakteriseerd wordt door een drastische toename in de doorbloeding van het uteriene vaatbed, resulteerde in een verhoogde productie van stikstofmonoxide door de uteriene arterie van wild-type en TK^{-/-} muizen in reactie op een verhoogde afschuifspanning op de endotheelcellen. Het verschil in de maximale vaatverwijdende respons op deze afschuifspanning was onveranderd tussen wild-type en TK^{-/-} muizen. Ondanks deze verminderde vaatverwijdende respons in TK^{-/-} muizen, was de door zwangerschap-geïnduceerde uitwaardse hypertrofe remodelering van de uteriene arterie ten gevolge van de verhoogde doorbloeding van het uteriene vaatbed identiek aan die van wild-type muizen. Kort na de geboorte daalt de doorbloeding van het uteriene vaatbed tot normale

waarden, hetgeen een fysiologisch model voor een verminderde flow simuleert. We toonden aan dat de acute door flow-geïnduceerde vaatverwijding in uteriene arteriën van 7 dagen postpartum muizen drastisch is gereduceerd en dat de vaatwand dimensies van de niet-zwangere toestand terugkeerde.

Weefsel angiotensine-converting enzyme-deficiënte (t-ACE^{-/-}) muizen werden bestudeerd om de gevolgen van het gemis van membraan-gebonden weefsel ACE (en dientengevolge een verlaagde angiotensine II synthese, maar tevens verhoogde endogene bradykinine spiegels) te bepalen op acute vasomotor responsen en chronische structurele responsen op flow in zowel carotide als uteriene arteriën. De rol van endogeen bradykinine bij de chronische modulatie van endotheel-afhankelijke vaatwand reactiviteit, die optreedt tijdens en na de zwangerschap, werd onderzocht in hoofdstuk 5. We lieten zien dat bradykinine kan worden vrijgemaakt door zowel chemische (acetylcholine) als mechanische (afschuifspanning) stimulatie van uteriene arteriën van wild-type en t-ACE^{-/-} muizen. De bijdrage van bradykinine in deze vaatverwijdende responsen was groter in uteriene arteriën van t-ACE^{-/-} muizen, daar farmacologische blokkade van B₂ receptoren de reactie op acetylcholine en de afschuifspanning een groter effect had in t-ACE^{-/-} muizen. Tijdens de zwangerschap bleek de bijdrage van stikstofmonooxide aan de door flow-geïnduceerde vaatverwijding substantiëler in uteriene arteriën van wild-type muizen, terwijl de bijdrage van bradykinine groter was in uteriene arteriën van hoogzwangere t-ACE^{-/-} muizen. Deze bevindingen tonen de modulerende rol aan van vasoactieve factoren, zoals bradykinine, geproduceerd door de endotheelcellaag gedurende de door flow-geïnduceerde vaatverwijding van uteriene arteriën in situaties waarbij de doorbloeding van het uteriene vaatbed chronisch is verhoogd.

Weefsel ACE deficiëntie resulteerde in een verlaagde arteriële elasticiteit (hoofdstuk 6). Deze verminderde elasticiteit bleek B₂ receptor stimulatie afhankelijk te zijn, daar chronische (14 dagen) blokkade van deze receptoren de arteriële elasticiteit in t-ACE^{-/-} muizen herstelde. Deze bevindingen bewijzen de interactie tussen het renine-angiotensine systeem en het kallikreïne-kinine systeem en hun bijdrage aan de maturatie van het cardiovasculaire systeem.

In hoofdstuk 7 hebben we onderzocht of weefsel ACE betrokken is bij de arteriële remodelering door de unilaterale carotide arterie ligatie methode toe te passen en uteriene arteriën te bestuderen vóór, tijdens en na de zwangerschap. In de afgebonden carotide arterie van de weefsel ACE-deficiënte muis, gladde spiercel hyperplasie, inwaardse remodelering en arteriële elasticiteit waren allen verminderd in vergelijking met de carotide arterie van de wild-type muis. Hieruit blijkt dat weefsel ACE een grote rol vervult in het onderdrukken van de hyperplastische inwaardse remodelering van de carotide arterie die blootgesteld is aan lage afschuifspanningen. Uit de bevindingen van hoofdstuk 3 en 7 blijkt dat angiotensine II verantwoordelijk is voor de gladde spiercel hyperplasie, terwijl bradykinine de inkrumping tegengaat in respons op drastische verlagingen van de doorbloeding in grote arteriën. Net als weefsel kallikreïne bleek weefsel ACE niet essentieel voor de uitwaardse hypertrofe en inwaardse hypotrofe remodelering van de uteriene arterie tijdens en na de zwangerschap.

De effecten van de uitgestelde zwangerschap van de muis op de acute en chronische uteriene arteriële responsen waren het onderwerp van hoofdstuk 8. Onze bevindingen suggereren dat op middelbare leeftijd de effecten van de afschuifspanning op de

fosforylering van het endotheliaal stikstofmonoxide synthase en de onderliggende stikstofmonoxide productie verloren zijn. Verhoogde uitscheiding van vasoactieve factoren geproduceerd door het endotheel, zoals prostacycline of “endothelium-derived hyperpolarizing factor” kunnen de acute door flow-geïnduceerde vaatverwijding compenseren, maar garanderen geen optimale door zwangerschap-gerelateerde remodelering van de uterine arterie. Dit kan het verlaagde aantal levensvatbare foetussen tijdens het derde trimester van de uitgestelde zwangerschap bij de muis verklaren.

Uit de resultaten beschreven in dit proefschrift blijkt dat een verlaagde acute arteriële response op flow niet noodzakelijkerwijs een defect van de structurele remodelering inhoudt. Gesterkt door onze bevindingen en die van anderen, hebben we een overzichtsartikel geschreven dat de dissociatie tussen acute en chronische vaatwand responsen op flow nader belicht.

De resultaten in dit proefschrift bevestigen de gunstige arteriële effecten van bradykinine. Verder is gebleken dat acute door flow-geïnduceerde vaatverwijding en chronische door flow-gerelateerde remodelering niet met elkaar geassocieerd zijn en dat hun onderliggende mechanismen via andere signaaltransductie processen verlopen. Dit biedt nieuwe perspectieven voor de ontwikkeling van waardevolle “targets” voor cardiovasculair-farmacologische therapie. Bovendien verschillen adaptieve structurele arteriële veranderingen in respons op flow tussen grote elastische vaten en kleine collaterale vaten, en het effect van zelfs een middelbare leeftijd op de arteriële remodelering is uitgesprokener dan een deficiëntie van een van de cardiovasculaire sleutel enzymen, zoals weefsel kallikreïne en weefsel ACE. Dit opent nieuwe perspectieven waaronder “site-directed” interventies die leiden tot een breder inzicht van de effecten van ouderdom en regionaliteit op de arteriële remodelering.

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Curriculum Vitae

Robertus Hubertus Petronella Hilgers was born on August 7th 1972 in Kerkrade, the Netherlands. After completing VWO-B education at the Sancta Maria College in Kerkrade (1991), he enrolled at the Hogeschool Heerlen, the Netherlands (laboratory education speciality biochemistry). After doing his traineeship at the Biochemistry lab of Prof. dr. Ivan Sadowski at the University of British Columbia, Vancouver, Canada, he decided to continue his stay in beautiful Vancouver. Two years later he returned to the Netherlands. He worked two years in the metal industry (Chroomwerk, Kerkrade) before starting as a research analist in the lab of Prof. dr. Jan Rosing at the Biochemistry department of the University of Maastricht (1998). In May 2000 he switched to the neighbours of the Pharmacology & Toxicology department to start a PhD study in the lab of Prof. dr. Jo De Mey. During this PhD project he worked for six months with the sponsorship of a Marie Curie fellowship at the Institut National de la Santé et de la Recherche Médicale (INSERM) U541, IFR Circulation Lariboisière, Université Paris VII at the supervision of Prof. dr. Bernard Lévy, Dr. Daniel Henrion, and Dr. Chantal Boulanger in Paris, France.

Robertus Hubertus Petronella Hilgers werd geboren op 7 augustus 1972 te Kerkrade, Nederland. Na het behalen van het VWO-B diploma aan het Sancta Maria College te Kerkrade (1991) volgde een inschrijving aan de Hogeschool Heerlen, Nederland (laboratorium opleiding specialisatie). Na een stage in het biochemisch laboratorium van Prof. dr. Ivan Sadowski aan de Universiteit van Brits Columbia, Vancouver, Canada, besloot hij te blijven in het mooie Vancouver. Twee jaar later keerde hij terug naar Nederland, om vervolgens twee jaar in de metaalnijverheid (Chroomwerk, Kerkrade) te werken, vooraleer hij begon als onderzoeksassistent in het lab van Prof. dr. Jan Rosing (Biochemie) van de Universiteit Maastricht (1998). In mei 2000 verhuisde hij naar de burens van Farmacologie & Toxicologie in het lab van Prof. dr. Jo De Mey om te beginnen met het werk beschreven in dit proefschrift. Tijdens zijn promotieonderzoek heeft hij zes maanden gewerkt, met de steun van een Marie Curie Fellowship, aan het Institut National de la Santé et de la Recherche Médicale (INSERM) U541, IFR Circulation Lariboisière, Université Paris VII onder supervisie van Prof. dr. Bernard Lévy, Dr. Daniel Henrion en Dr. Chantal Boulanger in Parijs, Frankrijk.

Grants and Awards

Graduate Student Travel Award of the American Society for Pharmacology and Experimental Therapeutics (ASPET) at Experimental Biology '01, Orlando, Florida, March 31-April 4, 2001.

Pre-doctoral Marie Curie European Fellowship (03/2002-09/2002)

Young Investigator Award European Council for Blood Pressure and Cardiovascular Research (ECCR), Seeheim, Germany, October 10-12, 2003.

List of Published Articles

Hilgers RHP, Bergaya S, Schiffers PMH, Meneton P, Boulanger CM, Henrion D, Lévy BI, De Mey JGR. Uterine Artery Structural and Functional Changes During Pregnancy in Tissue Kallikrein-Deficient Mice. *Arterioscler Thromb Vasc Biol.* 2003;23:1826-1832.

Aartsen WM, **Hilgers RHP**, Schiffers PMH, Daemen MJAP, De Mey JGR, Smits JFM. Changes in Vascular Distensibility During Angiotensin-Converting Enzyme Inhibition Involve Bradykinin Type 2 Receptors. *J Vasc Res.* 2004;41:18-27.

Hilgers RHP, Schiffers PMH, Aartsen WM, Fazzi GE, Smits JFM, De Mey JGR. Tissue Angiotensin-Converting Enzyme in Imposed and Physiological Flow-Related Arterial Remodeling in Mice. *Arterioscler Thromb Vasc Biol.* In Press.

Bergaya S, **Hilgers RHP**, Meneton P, Dong Y, Bloch-Faure M, Inagami T, Alhenc-Gelas F, Lévy BI, Boulanger CM. Flow-Dependent Dilatation Mediated by Endogenous Kinins Requires Angiotensin AT₂ Receptors. *Circ Res.* In Press

List of Submitted Articles

Bergaya S, **Hilgers RHP**, Schiffers PMH, Fazzi GE, Meneton P, Lévy BI, De Mey JGR, Boulanger CM. Role of Tissue Kallikrein During Arterial Remodeling in Response to Altered Blood Flow.

Hilgers RHP, Schiffers PMH, De Mey JGR. Intra-Arterial Bradykinin and Vasodilatation in Relation to Acute and Chronic Changes in Blood Flow.

Hilgers RHP, Schiffers PMH, Fazzi GE, De Mey JGR. Uterine Artery Dilatation and Remodeling During Postponed Murine Pregnancy.

Hilgers RHP, Schiffers PMH, Wesselman JP, De Mey JGR. Acute and Chronic Adaptive Responses to Altered Blood Flow in Large and Small Arteries. (*Review*)

Schulten HM, **Hilgers RHP**, Schiffers PMH, Strzelecka A, Fazzi GE, de Muinck ED, De Mey JGR. Reduced Endothelium-Dependent Relaxations and Impaired Acute Flow-Induced Vasodilatation Do Not Compromise Chronic Flow-Related Arterial Remodeling in Hyperlipidemic Mice.

List of Abstracts

De Mey JG, **Hilgers RH**, Aartsen WM, Schiffers PM, Smits JF. Altered flow-induced remodeling in tissue angiotensin-converting enzyme deficient mice. 8th International Symposium on Mechanisms of Vasodilatation. May 31-June 3, 2001, Boston, Mass., USA. *J Vasc Res.*2001;38(suppl 1):9.

Hilgers RH, Aartsen WM, Schiffers PM, De Mey JGR. Arterial stiffness and flow-related arterial remodeling in mice during and after pregnancy. 6th Annual Meeting of the European Council for Blood Pressure and Cardiovascular Research, Noordwijkerhout, the Netherlands, October 26-28, 2001.

Aartsen W, **Hilgers R**, Fazzi G, Debets J, Bitsch N, Schiffers P, Smits J, De Mey J. Phenotyping of the vasculature in mice lacking tissue bound angiotensin converting enzyme. 6th Annual Meeting of the European Council for Blood Pressure and Cardiovascular Research, Noordwijkerhout, the Netherlands, October 26-28, 2001.

Schulten H, **Hilgers RH**, Schiffers PMH, Fazzi GE, De Muinck ED, De Mey JGR. Hypertrophic flow-induced arterial remodeling increases with age in hyperlipidemic mice. Fourth St-Gerlach Vascular Biology Workshop, January 24-25, 2002.

Hilgers RH, Schulten H, Schiffers PM, Fazzi GE, De Muinck E, Strzelecka A, De Mey JGR. 2002. Impaired agonist- and flow-induced endothelium-dependent responses of carotid arteries in hypercholesterolemic mice. 22nd Meeting of the European Society for Microcirculation, August 28-30, 2002, University of Exeter, Devon, UK. *J Vasc Res.* 2002;39(suppl 1):25.

Van der Heijden OWH, Essers YPG, **Hilgers RH**, Aardenburg R, Courtar DA, Kars ME, Van Breugel NHAM, Spaanderman MEA, De Mey JGR, Hofker MH, Peeters LLH, Van Eys GJJM. Effect of gestation on smoothelin expression in vascular remodeling in murine uterine artery smooth muscle cells. SGI, 2003.

Hilgers R, Bergaya S, Henrion D, Boulanger C, Meneton P, Schiffers P, De Mey JGR. Flow-induced vasodilatation and physiological arteriogenesis. Pivotal role of tissue kallikrein in the former but not the latter. Angiogenesis Conference Leiden, February 13-15, 2003.

Hilgers RHP, Bergaya S, Schiffers PMH, Meneton P, Boulanger CM, Henrion D, Lévy BIL, De Mey JGR. Deficiency of tissue kalikrein blunts flow-induced dilatation but not remodeling in murine uterine arteries. Experimental Biology '03, San Diego, April 11-15, 2003.

Hilgers RHP, Schiffers PMH, Aartsen WM, Smits JFM, De Mey JGR. Flow-Induced Arterial Remodeling in Tissue Angiotensin-I Converting Enzyme Deficient Mice. 8th Annual Meeting of the European Council for Blood Pressure and Cardiovascular Research, Noordwijkerhout, the Netherlands, October 10-12, 2003.

Hilgers R, Bergaya S, Schiffers P, Fazzi G, Smits J, Meneton P, Boulanger C, De Mey J. Angiotensin (Ang), Bradykinin (BK) and Converting Enzyme in Imposed and Physiological Flow-Related Arterial Remodeling. IVBM Toronto, Canada, June 1-5, 2004.

Hilgers RHP, Schiffers PMH, Fazzi GE, De Mey JGR. Uterine artery dilatation and remodeling during postponed murine pregnancy. 8th International Symposium on Resistance Arteries. Angers, France, June 20-23, 2004.

Hilgers RHP, Schiffers PMH, Bergaya S, Boulanger C, De Mey JGR. Roles of agiotensin II and bradykinin in flow-related arterial remodeling in mice. 8th International Symposium on Resistance Arteries. Angers, France, June 20-23, 2004.