



LUND UNIVERSITY

Mechanosensing in the vascular wall - the role of cellular microdomains in vascular remodeling

Albinsson, Sebastian

2007

[Link to publication](#)

Citation for published version (APA):

Albinsson, S. (2007). *Mechanosensing in the vascular wall - the role of cellular microdomains in vascular remodeling*. [Doctoral Thesis (compilation), Department of Experimental Medical Science]. Department of Experimental Medical Science, Lund University.

Total number of authors:

1

General rights

Unless other specific re-use rights are stated the following general rights apply:

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: <https://creativecommons.org/licenses/>

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

Mechanosensing in the vascular wall

- the role of cellular microdomains in vascular remodeling

Sebastian Albinsson

Institutionen för Experimentell Medicinsk Vetenskap

Kärlfysiologi

Akademisk avhandling

som med vederbörligt tillstånd av Medicinska fakulteten vid Lunds Universitet för
avläggande av doktorsexamen i Medicinsk Vetenskap kommer att offentligen
försvaras i Segerfalksalen, Biomedicinskt centrum, Sölvegatan 19,
fredagen den 20 april 2007 kl 09.15

Fakultetsopponent

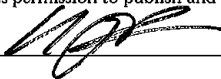
Professor Graeme Nixon
Institute of Medical Sciences
University of Aberdeen

Organization LUND UNIVERSITY Department of Experimental Medical Science	Document name DOCTORAL DISSERTATION	
	Date of issue 2007-04-20	
	Sponsoring organization	
Author(s) Sebastian Albinsson		
Title and subtitle Mechanosensing in the vascular wall - the role of cellular microdomains in vascular remodeling		
<p>Abstract</p> <p>The vascular wall has a remarkable capacity to adapt to external forces exerted by the intraluminal blood pressure and flow. This includes rapid change in contractile tone as well as chronic alteration of vessel structure if the stimulus persists. Mechanical stretch of intact blood vessels ex vivo promotes growth and contractile differentiation. The molecular mechanisms involved are not well defined, but contractile differentiation has been suggested to be mediated by polymerization of the cytoskeletal protein actin. The machinery that signals growth may be assembled in membrane invaginations termed caveolae, and a role for caveolae in mechanosensing has accordingly been proposed. The studies summarized in this thesis aimed to determine the role of these two cellular domains in mechanosensitive signaling in the intact vascular wall using rat or mouse portal veins as well as carotid and small mesenteric arteries.</p> <p>In the portal vein, we found that stretch promotes contractile differentiation via Rho activation and actin polymerization. An intact actin cytoskeleton is required for stretch-induced synthesis of smooth muscle specific marker proteins and for global protein synthesis. We also found that stabilizing actin filaments produced the same effects as stretch on protein synthesis. Stretch dependency of growth and differentiation was maintained in mice lacking caveolin-1 and vascular caveolae. In arteries from these mice, a reduced myogenic tone was observed, which was mainly caused by excessive nitric oxide (NO) production. Blood pressure was however maintained in vivo despite increased NO production. Maintenance of blood pressure in the setting of increased NO production could be due to increased alpha1-adrenergic contraction, hypertrophic remodeling and increased plasma volume.</p> <p>In summary, the data suggest that stretch stimulates polymerization of actin, which is necessary for expression of smooth muscle differentiation markers and growth of the intact vascular wall. On the other hand, stretch-induced growth and differentiation is not dependent on caveolin-1 or caveolae, which may however play a role for contractile responses to mechanical stimuli.</p>		
Key words: smooth muscle, actin, caveolin, growth, differentiation, wall stress, shear stress, hypertension		
Classification system and/or index terms (if any):		
Supplementary bibliographical information:		Language English
ISSN and key title: 1652-8220		ISBN 978-91-85559-35-0
Recipient's notes	Number of pages 145	Price
	Security classification	

Distribution by (name and address)

I, the undersigned, being the copyright owner of the abstract of the above-mentioned dissertation, hereby grant to all reference sources permission to publish and disseminate the abstract of the above-mentioned dissertation.

Signature



Date 2007-03-11

Mechanosensing in the vascular wall

- the role of cellular microdomains in vascular remodeling

Sebastian Albinsson

Department of Experimental Medical Science
Vascular Physiology



LUND UNIVERSITY
Faculty of Medicine

Sebastian Albinsson

Department of Experimental Medical Science

Lund University

BMC D12

221 84 Lund

Sweden

Phone: +46 46 222 44 89

sebastian.albinsson@med.lu.se

Cover image

Front: Mirror image of half of a cross sectioned carotid artery. Caveolin-1 proteins are stained with a fluorescent antibody (red) and elastic laminae in the media are visible by autofluorescence (green). This image was acquired by laser confocal microscopy.

Back: Confocal image of isolated vascular smooth muscle cells stained in red for monomeric actin and green for filamentous actin.

Printed by Media-Tryck, Lund University, Sweden

© Sebastian Albinsson 2007

© The American Physiological Society

© The American Society for Biochemistry and Molecular Biology.

Table of contents

TABLE OF CONTENTS	3
LIST OF PAPERS	4
ABBREVIATIONS	5
ABSTRACT	6
INTRODUCTION	7
THE ADAPTIVE PROPERTIES OF THE VASCULAR WALL.....	7
THE VASCULAR SMOOTH MUSCLE CELL	9
<i>Smooth muscle contraction</i>	10
<i>Smooth muscle differentiation</i>	10
<i>Smooth muscle growth</i>	12
CELLULAR MICRODOMAINS - THE ACTIN CYTOSKELETON.....	13
<i>Agents used to modify actin polymerization</i>	14
<i>Focal adhesions</i>	14
CELLULAR MICRODOMAINS - CAVEOLAE	15
<i>The caveolin-1 knockout mouse</i>	16
MECHANOTRANSDUCTION IN THE VASCULATURE	16
<i>Sensing shear stress</i>	18
<i>Stretch sensing in vascular smooth muscle</i>	18
AIMS OF THE STUDY	20
METHODS FOR STUDYING MECHANOSENSING IN VASCULAR SMOOTH MUSCLE	21
THE PORTAL VEIN MODEL.....	21
MEASURING GROWTH AND DIFFERENTIATION	22
MEASURING ACTIN POLYMERIZATION	23
PRESSURE MYOGRAPHY AND PRESSURIZED CAROTID ARTERIES	23
RESULTS AND DISCUSSION	26
STRETCH-DEPENDENT GROWTH AND DIFFERENTIATION IN PORTAL VEINS - ROLE OF ACTIN POLYMERIZATION (PAPERS I-III)	26
ROLE OF CAVEOLAE IN MECHANOSENSING IN VASCULAR SMOOTH MUSCLE (PAPERS IV-V) ..	30
FINAL REMARKS	32
CONCLUSIONS	35
POPULÄRVETENSKAPLIG SAMMANFATTNING	36
ACKNOWLEDGEMENTS	39
REFERENCES	41

List of papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

- I. Zeidan, A., Nordström, I., Albinsson, S., Malmqvist, U., Swärd, K., and Hellstrand, P. **Stretch-induced contractile differentiation of vascular smooth muscle: sensitivity to actin polymerization inhibitors.** (2003) *Am J Physiol Cell Physiol* **284**, C1387-1396
- II. Albinsson, S., Nordström, I., and Hellstrand, P. **Stretch of the vascular wall induces smooth muscle differentiation by promoting actin polymerization.** (2004) *J Biol Chem* **279**, 34849-34855
- III. Albinsson S and Hellstrand P. **Integration of signal pathways for stretch-dependent growth and differentiation in vascular smooth muscle.** (2007; submitted)
- IV. Albinsson S*, Shakirova Y*, Rippe A, Baumgarten M, Rosengren B-I, Rippe C, Hallmann R, Hellstrand P, Rippe B and Swärd K. **Arterial remodeling in caveolin-1 deficient mice** (2007; submitted) (*These authors contributed equally)
- V. Albinsson S, Nordström I, Swärd K and Hellstrand P. **Differential dependence of stretch and shear stress signaling on caveolin-1 in the vascular wall** (2007; submitted)

Abbreviations

4E-BPs	4E-binding repressor proteins	MLCK	myosin light chain kinase
ADF	actin depolymerizing factor	MMP	matrix metalloproteinases
Cav	caveolin	MNK1	MAP kinase-interacting protein 1
ECM	extracellular matrix	NO	nitric oxide
EDHF	endothelium derived hyper polarizing factor	PDGF	platelet derived growth factor
eIF	eukaryotic initiation factors	PECAM-1	platelet endothelial cell adhesion molecule
eNOS	endothelial nitric oxide synthase	PGI₂	prostacyclin
ERK1/2	extracellular-signal regulated kinase 1 and 2	PI3-kinase	phosphatidylinositol-3-kinase
FAK	focal adhesion kinase	ROCK	Rho- associated kinase
KO	knock out	SM-MHC	smooth muscle myosin heavy chain
LIMK	LIM-kinase	SRF	serum response factor
MABP	mean arterial blood pressure	TCF	ternary complex factors
MBCD	methyl-beta cyclodextrin	TRP	transient receptor potential
mDia	mammalian diaphanous homolog	VE-cadherin	vascular endothelial cell cadherin
MAP-kinase	mitogen activated protein kinase	VEGFR2	vascular endothelial growth factor receptor 2

Abstract

The vascular wall has a remarkable capacity to adapt to mechanical forces exerted by the intraluminal blood pressure and flow. This includes rapid change in contractile tone as well as chronic alteration of vessel structure if the stimulus persists. Stretch of the intact blood vessel wall promotes growth and contractile differentiation. The molecular mechanisms involved are not well defined, but contractile differentiation has been suggested to be mediated by polymerization of the cytoskeletal protein actin. Part of the machinery that signals growth may be assembled in membrane invaginations termed caveolae, and a role for caveolae in mechanosensing has accordingly been proposed. The studies summarized in this thesis aimed to determine the role of these two cellular domains in mechanosensitive signaling in the intact vascular wall using rat or mouse portal veins as well as carotid and small mesenteric arteries.

In the portal vein, we found that stretch promotes contractile differentiation via Rho activation and actin polymerization. An intact actin cytoskeleton is required for stretch-induced synthesis of smooth muscle specific marker proteins and for global protein synthesis. We also found that stabilizing actin filaments produced the same effects as stretch on protein synthesis. Stretch dependency of growth and differentiation was maintained in mice lacking caveolin-1 and vascular caveolae. In arteries from these mice, a reduced myogenic tone was observed, which was mainly caused by excessive nitric oxide (NO) production. Blood pressure was however maintained *in vivo* despite increased NO production. Maintenance of blood pressure in the setting of increased NO production could be due to increased α_1 -adrenergic contraction, hypertrophic remodeling and increased plasma volume.

In summary, the data suggest that stretch stimulates polymerization of actin, which is necessary for expression of smooth muscle differentiation markers and growth of the intact vascular wall. On the other hand, stretch-induced growth and differentiation is not dependent on caveolin-1 or caveolae, which may however play a role for contractile responses to mechanical stimuli.

Introduction

The adaptive properties of the vascular wall

The wall of arteries and veins consists of three layers. The intima, which is the innermost layer composed of endothelial cells that line the lumen, acts as a semipermeable barrier for fluid and other components of the blood. The media, which is mainly composed of smooth muscle cells and extracellular matrix (ECM) proteins, regulates vessel diameter. The outermost layer of the blood vessels is the adventitia, which is a protective layer of fibroblasts and connective tissue. The relative abundance of these components varies throughout the vascular circuit.

The primary function of the vascular smooth muscle is contraction and relaxation and thereby regulation of vessel diameter. This is of major importance in small resistance arteries, which are responsible for the distribution of blood flow to organs. The lumen diameter of small arteries also regulates mean arterial blood pressure (MABP) by controlling the peripheral resistance in the arterial system. This is of relevance in essential hypertension, where increased peripheral resistance caused by chronic narrowing of resistance arteries will cause further elevation of MABP, resulting in an increased risk of cardiac failure, atherosclerosis and stroke.

There are both intrinsic and extrinsic mechanisms regulating vessel diameter. Circulating vasoactive mediators such as angiotensin II and catecholamines, as well as locally released mediators from the autonomic nervous system, confer extrinsic regulation of smooth muscle contraction. Intrinsic regulation of vessel diameter is mainly mediated by two mechanosensitive mechanisms. One is the sensing of shear stress on the endothelium caused by an increase in blood flow. This leads to a release of vasodilators from the endothelium, which relaxes the smooth muscle cells. The other mechanism involves stretch sensing by the smooth muscle cells themselves, resulting in narrowing of the vessel lumen. The latter mechanism is referred to as myogenic tone. An increase in transmural pressure or internal diameter causes an increased wall tension according to the law of Laplace (Fig 1).

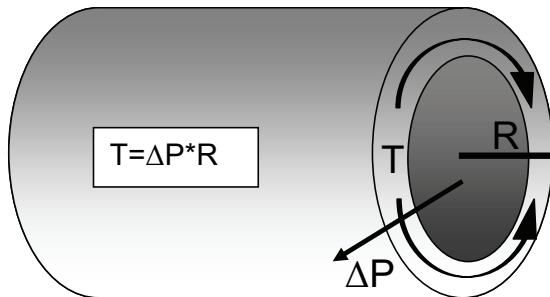


Figure 1 Laplace's law describes the relationship between wall tension (T), the transmural pressure difference (ΔP) and vessel radius (R).

An increase in wall tension will also increase wall stress, which is the force per unit cross sectional area. The vascular wall has two ways to reduce wall stress back towards the normal level. One is to decrease the radius of the vessel, which is accomplished by myogenic constriction in a matter of seconds to minutes after an increase in transmural pressure. The other mechanism is provided by remodeling, a chronic change in wall geometry. The different types of remodeling are illustrated in Fig 2. In hypertensive patients, an inward remodeling with an increased wall to lumen ratio is often observed in small resistance arteries (74)

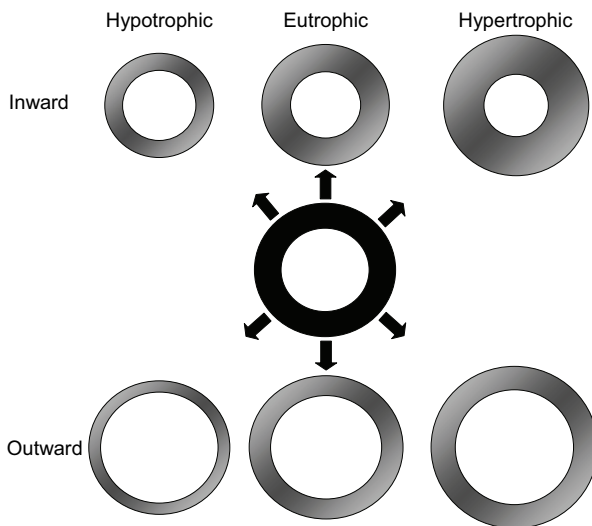


Figure 2. The change in lumen diameter and wall thickness determines the mode of vascular remodeling. (Adapted from (75)).

Similar to pressure, chronic flow loading of arteries is known to give rise to a sustained change in vessel geometry. This is referred to as flow-induced vascular remodeling and is characterized by an increased lumen diameter with increased flow, and vice versa. In a seminal paper it was demonstrated that inward remodeling in response to reduced flow is endothelium-dependent (58). Subsequent work has shown that nitric oxide (NO) and the endothelial nitric oxide synthase (eNOS) are necessary for vascular remodeling in both directions (112, 141).

In some situations, the vascular wall adapts to mechanical forces by formation of a so-called neointima. This is characterized by smooth muscle cells that proliferate and migrate from the media to the intimal layer of the vessel wall (80). Neointima formation typically occurs after balloon angioplasty (restenosis) and in vein grafts after by-pass surgery. Eventually, neointima formation may lead to a complete obstruction of the vessel, which is a major clinical problem as 40-50% of vein grafts are occluded and in need of re-operation after 5-10 years (120). From experimental animal models it is clear that severe changes in pressure/distension or flow contribute to neointima formation (57, 115). Thus, the vascular wall responds to changes in flow (shear stress) and pressure (wall stress) by chronically altering its structure. This is of relevance in various vascular disease states including hypertension, atherosclerosis and restenosis. However, the mechanisms by which mechanical stimuli alters vessel structure are not well understood.

The vascular smooth muscle cell

Normally, vascular smooth muscle cells are contractile (differentiated), quiescent and do not migrate. The vascular smooth muscle cells have an ability to adapt to environmental influences, which involve altered interactions with surrounding cells or matrix proteins, growth factors, cytokines, injury and mechanical forces. This adaptation requires complex changes in cell phenotype and may lead to changes in contractility as well as hypertrophy (increase in cell mass), proliferation (increase in cell number) and migration. Phenotypic modulation is most evident as smooth muscle cells are removed from their tissue environment and placed in cell culture where unrestrained proliferation and migration can occur. These cells de-differentiate to a less contractile and more proliferative phenotype and are commonly termed "synthetic" smooth muscle cells (11). Although this situation is unparalleled *in vivo*, some of the characteristics of these cells are observed in pathological situations following vascular injury (103).

Smooth muscle cells are known to produce and secrete growth factors and enzymes, which are essential for adaptation to the environment. For example, proliferation of smooth muscle cells requires breakdown of the basement membrane that surrounds each cell, and this is accomplished by secretion of matrix metalloproteinases (MMP:s) (79). Furthermore, stretch-induced growth of vascular smooth muscle is partly dependent on autocrine or paracrine signaling by angiotensin II and endothelin-1 (I)(152). However, additional mechanisms are likely to be involved in the regulation of stretch-dependent growth. We have suggested a role for the actin cytoskeleton in both growth and differentiation of vascular smooth muscle (I, II, III, IV).

Smooth muscle contraction

The mechanisms of smooth muscle contraction are only briefly described in this section as the main focus of this work concerns the mechanisms of smooth muscle growth and differentiation. In contrast to striated muscle, smooth muscle contraction is slow and economical. Smooth muscle contraction is achieved by interaction between actin and myosin filaments. In addition, other actin binding proteins such as calponin and caldesmon are part of the contractile and regulatory machinery (49). Myosin is composed of heavy (MHC) and light chains (MLC) and serves as the motor of smooth muscle contraction. When the 20 kDa MLC is phosphorylated by the calcium-calmodulin dependent myosin light chain kinase (MLCK), the cyclical interaction of actin and myosin (cross-bridge cycling) is initiated and the muscle contracts. MLCK and myosin light chain phosphatase (MLCP) are the main regulators of smooth muscle contractility via their effect on MLC phosphorylation, and the activity of these enzymes determines the sensitivity of smooth muscle contraction to intracellular calcium. One important mechanism for calcium sensitization is activation of the Rho pathway which inhibits MLCP via Rho-associated kinase (ROCK) (143).

Smooth muscle differentiation

The contractile differentiation of smooth muscle cells can be identified functionally by contractile ability, morphologically by the presence of dense bodies and myofibrils, and biochemically by the expression of proteins that are known as differentiation marker proteins or smooth muscle specific proteins. These are typically contractile or cytoskeletal proteins, such as smooth muscle myosin heavy chain (SM-MHC), smooth muscle alpha-actin, SM22, tropomyosin, desmin and calponin (86). The genes encoding smooth muscle specific proteins are under the control of the transcription factor serum response factor (SRF) (71).

Paradoxically, as the name indicates, SRF also regulates genes involved in growth and proliferation after serum stimulation. The specificity of SRF in the regulation of smooth muscle specific genes depends on co-factors such as myocardin and the myocardin related transcription factor, MAL/MKL1 (20, 21). Another subset of co-factors, named ternary complex factors (TCF), are responsible for control of genes involved in growth and proliferation and may compete for SRF to control smooth muscle gene expression (Fig 3) (144). All smooth muscle specific genes that are regulated by SRF contain one or more CArG boxes (for CC(A/T rich)GG) in the promoter region, which serve as binding sites for SRF and are crucial for SRF dependent gene transcription (71).

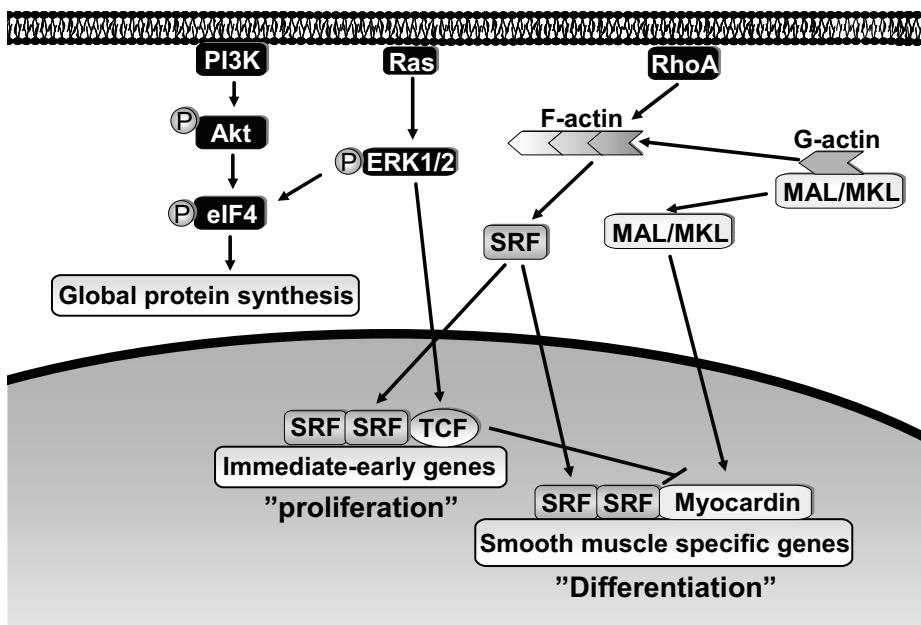


Figure 3 Simplified illustration of signaling mechanisms suggested to be involved in the regulation of growth and differentiation.

An interesting model for the regulation of SRF and MAL/MKL1 by actin polymerization has recently emerged (39, 67, 95, 128). SRF and MAL/MKL1 have been shown to interact with monomeric G-actin, an interaction that inhibits translocation of these transcription factors from the cytosol to the nucleus (65, 72). Polymerization of actin into filamentous F-actin

reduces the pool of free G-actin and thus releases inhibition on SRF and MAL/MKL1. Only a small pool of the total G-actin is involved in this mechanism since most G-actin in the cell is sequestered by actin binding proteins such as profilin and β -thymosin, which prevents interaction with MAL/MKL1 (94). Following actin polymerization, the increase in mRNA expression of smooth muscle specific genes results in an enhanced synthesis of proteins encoding these genes and thus a relative increase in the contents of contractile marker proteins.

Smooth muscle growth

Growth of the vascular smooth muscle involves hypertrophy and/or proliferation. Nearly all growth factors activate the mitogen activated protein kinase (MAP-kinase)-extracellular signal regulated kinase 1 and 2 (ERK1/2) pathway. ERK1/2 is then able to enter the nucleus and phosphorylate TCF:s, which bind to SRF and activate the transcription of immediate-early genes such as *c-fos* and *c-jun* (98) (Fig 3). These are inducible transcription factors that promote the transcription of genes involved in smooth muscle growth. The discrimination between hypertrophy and proliferation is not well understood. However, in cultured smooth muscle cells, thrombin has been shown to stimulate hypertrophy while platelet derived growth factor (PDGF) stimulated proliferation and this was associated with a differential expression of immediate early genes (111).

Normally, smooth muscle cells in the intact vasculature are quiescent and differentiated. However, under certain circumstances, such as after vascular injury or in vein grafts subjected to arterial pressure, smooth muscle cells transiently de-differentiate and proliferate forming a neointima (83, 123). In hypertensive subjects, growth is likely to occur by hypertrophy of the smooth muscle, which may be associated with polyploidy of smooth muscle cells as observed in arteries of hypertensive rats (48). Hypertrophy is a consequence of increased intracellular protein, regulated by the rate of global (overall) protein synthesis and degradation, and increased water content. We have previously shown that stretch-dependent growth of vascular smooth muscle is associated with an increase in protein synthesis and cell size (155).

Protein synthesis requires translation of mRNA into amino acid sequences, which form the protein. Translation of mRNA by ribosomes is under control of eukaryotic initiation factors (eIF) and the 70 kDa ribosomal protein S6 kinase (p70^{S6K}) (40) (23). The initiation factors are regulated in several ways, including (1) transcriptional control i.e. the amount of eIF:s that can be activated, (2) activation via phosphorylation of the subunit eIF4E i.e. via the MAP

kinase-interacting protein 1 (MNK1) and (3) inhibition by binding to 4E-binding repressor proteins (4E-BPs). Two signaling pathways are often implicated in the regulation of protein synthesis and growth of vascular smooth muscle, the MAP-kinase pathway and the phosphatidylinositol-3-kinase (PI3-kinase) pathway (109, 114, 118) (Fig 3). Protein synthesis is likely regulated by these two pathways via eIF activity. MNK1 is a target of ERK1/2 in the MAP-kinase pathway and 4E-BPs are phosphorylated by Akt, a member of the PI3-kinase pathway, which inhibits their binding to eIF4E (40).

The S6 ribosomal protein is phosphorylated at multiple sites for initiation of mRNA translation (47). The major kinase for S6 phosphorylation *in vivo* is the p70^{S6K}, which is activated by phosphorylation at an autoinhibitory domain (23). Inhibition of either the MAP-kinase or the PI3-kinase pathway has been shown to prevent angiotensin II induced phosphorylation of p70^{S6K} in vascular smooth muscle cells (23, 24). Thus, both ERK1/2 and Akt are essential in the regulation of global protein synthesis by both eIF and p70^{S6K}.

The PI3-kinase signaling pathway is also suggested to be involved in contractile differentiation of smooth muscle cells, but it has been difficult to establish the role of PI3-kinase in this process (43, 45, 73, 125, 127). MAP kinase signaling is widely considered to be a repressor of smooth muscle differentiation, but these results originate from studies on isolated smooth muscle cells in culture (54, 99, 144) and it is unknown if the same mechanisms are present in intact vascular smooth muscle.

Cellular microdomains - the actin cytoskeleton

There are at least six isoforms of actin encoded by six different genes. Depending on their isoelectric point they fall into three classes: α , β or γ . In vertebrates, SMCs primarily express four types of actin, namely muscle α - and γ -actin as well as non-muscle β - and γ -actin (81). The latter isoforms are believed to be constituents of the cellular cytoskeleton while muscle α - and γ -actin are important parts of the contractile machinery in the smooth muscle cells (124). It is likely that all of the four actin isoforms are present in two pools in smooth muscle cells, one in the form of filamentous F-actin and the other in the form of monomeric G-actin.

Actin dynamics is primarily controlled by mediators of the Rho pathway (see Fig 7 in DISCUSSION). This pathway involves activation of ROCK and LIM-kinase (LIMK) with subsequent inhibition of cofilin and stabilization of F-actin (9, 68, 78). Cofilin is a member of the actin depolymerizing factor (ADF)/cofilin protein family (14). ADF/cofilins accelerate

depolymerization of actin filaments from the pointed end by binding preferably to ADP-actin and thereby changing the orientation of actin subunits within the filament. In addition, actin dynamics is regulated by a second Rho-controlled pathway involving the mammalian diaphanous homolog (mDia) proteins. The mDia proteins cooperate with LIMK to promote F-actin accumulation possibly via a mechanism that involves interaction with profilin (77, 146). Profilin binds to G-actin and accelerates the turnover of ADP-G-actin to ATP-G-actin resulting in an increased affinity for G-actin to the barbed end of actin filaments as well as to the G-actin binding protein β -thymosin. This mechanism provides a pool of G-actin that is ready for rapid polymerization (93). Finally, Rho is able to promote nucleation of new actin filaments via activation of the Arp2/3 complex.

Agents used to modify actin polymerization

Cell-permeable agents that modify the state of actin filaments have been used extensively in the past. The marine toxins latrunculin and cytochalasin are the most commonly used depolymerizing agents. Latrunculin forms 1:1 complexes with G-actin monomers and thereby inhibits polymerization (15, 131). At low concentrations (0.25 μ M) cytochalasin caps preexisting filaments at the barbed end and thereby causes net depolymerization. However, cytochalasin also causes dimerization and nucleation of short filaments and at higher concentrations (≥ 2 μ M), this results in a decrease in the free G-actin pool (32). Latrunculin is 10 to 100 times more potent than cytochalasin in disrupting actin filaments (131).

The most common agent used to prevent actin depolymerization is jasplakinolide, which is derived from the marine sponge *Jaspis johnstoni*. Jasplakinolide binds to the same site and with similar affinity to F-actin as phalloidin, a cell impermeable agent commonly used to stain actin filaments (130).

Focal adhesions

The actin cytoskeleton is connected to the plasma membrane and the extracellular matrix via anchoring points called focal adhesions. The focal adhesions are complex microdomains and consist of integrins, which are membrane-spanning proteins that bind extracellular matrix molecules. Integrins are formed by heterodimers of alpha and beta chains and to date 24 different heterodimers are known to exist and at least 11 of them are present in smooth muscle (16, 110). In addition, more than 50 different proteins are known to be associated with integrins in focal adhesions. These proteins can be involved in actin binding, second

messenger signaling to cell growth and proliferation, cell motility, matrix assembly and tissue invasion (110). Several focal adhesion associated proteins including vinculin, paxillin and focal adhesion kinase (FAK), are activated by mechanical stretch (61, 62).

Cellular microdomains - caveolae

Caveolae are 50-100 nm large Ω -shaped membrane invaginations with high contents of cholesterol and sphingolipids. In smooth muscle and endothelial cells they are formed through specific interactions amongst lipids and the caveolin proteins (Cav) (12) (Fig 4). The caveolin proteins have a hairpin structure and bind cholesterol with high affinity. Three distinct caveolin genes exist, two of which are critical for formation of caveolae (Cav-1 and Cav-3). Cav-3 is expressed in skeletal muscle, and mutations in Cav-3 cause muscular dystrophy (12).

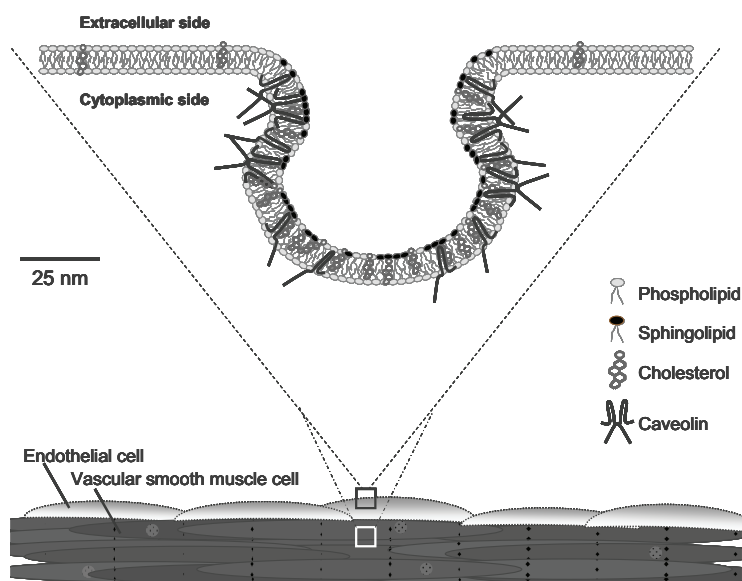


Figure 4 Caveolae are 50-100 nm invaginations in the cell membrane of both endothelial and smooth muscle cells. The structural components of caveolae include phospholipids, sphingolipids, cholesterol and caveolin proteins.

The cells of the vascular wall express primarily Cav-1 and Cav-2 proteins, and since Cav-2 is unstable in the absence of Cav-1, disruption of the *Cav-1* gene leads to loss of caveolae in the

aorta (19, 101). Caveolae are particularly abundant in endothelial, smooth muscle and fat cells and have been ascribed roles in vesicular transport of lipoproteins across the endothelium, in receptor clustering, and in control of cellular signaling (12). In particular, the role of caveolae and caveolins in the regulation of eNOS has been a topic of intense interest. Cav-1 has been shown to interact with and inhibit the activity of eNOS (10, 27, 36-38, 66, 122). Cav-1 is also involved in the regulation of other intracellular signaling events. For example, it is known to be a negative regulator of cell growth by tonic inhibition of the ERK1/2-MAP kinase cascade (25, 35). The Cav-1 scaffolding domain also inhibits the activity of G-proteins, Src-like kinases, and H-Ras (64).

The caveolin-1 knockout mouse

Cav-1 knockout (KO) mice lack caveolae in smooth muscle and endothelium as well as other non-striated muscle tissues (12). Surprisingly, Cav-1 KO mice are viable and fertile although one group reported a reduction of life span (88). Many of the abnormalities found in the Cav-1 KO mice are related to the cardiovascular system. These findings include dysregulated NO production, impaired angiogenesis and endocytosis, and cardiomyopathy (59). Excessive growth of neointimal smooth muscle and cardiac fibroblasts has also been observed, which likely depends on excessive MAP-kinase-ERK1/2 activation in the absence of Cav-1 (13, 44). The effects of Cav-1 ablation in the vasculature may be compensated by different mechanisms and this will be further discussed below.

In addition to vascular defects, Cav-1 KO mice have pulmonary disease with reduced alveolar spaces and thickening of the alveolar wall. Cav-1 KO mice also lack the Cav-2 isoform due to proteasomal breakdown of Cav-2 in the absence of Cav-1. Interestingly, Cav-2 KO mice have a similar lung phenotype, implicating the selective loss of Cav-2 as the primary cause of lung abnormalities also in the Cav-1 KO mice (102).

Mechanotransduction in the vasculature

The sensing of mechanical forces by endothelial and smooth muscle cells has been studied extensively but mainly in the context of isolated cells in culture environment. Cultured endothelial cells can be subjected to shear stress in a flow chamber while isolated smooth muscle cells are cultured on flexible membranes that are mechanically stretched. The advantage of this approach is that in depth studies using advanced molecular tools such as DNA or siRNA transfection are more straightforward in isolated cells than in the intact tissue.

In addition, some techniques require isolated cells, such as patch clamp experiments for measurement of ion channel activity, and microscopic analysis yields far higher resolution in isolated cells than in tissue sections. The main concern with isolated cells, in particular regarding smooth muscle cells, is the fact that the cells are plated on a two-dimensional (2D) surface, which is drastically different from the *in vivo* environment. The surface that the cells are plated on is often coated with an extracellular matrix protein, such as laminin, collagen or fibronectin. Specific integrin heterodimers bind to specific ECM proteins and these integrins are also likely to be involved in the response to mechanical stimuli. It is therefore not surprising that diverse effects of stretch are observed when cells are cultured on various substrates (52, 60, 132, 148). Isolation and culture of smooth muscle as well as endothelial cells causes major effects on the cell differentiation, which is also affected by the choice of ECM protein coating (11, 99, 104, 105, 139). Studies by Thie et al. demonstrate noticeable differences including reduced protein synthesis and proliferation, in smooth muscle cells cultured in a three dimensional (3D) matrix compared to 2D-cultures (135-137). Although 2D and 3D cultures of isolated cells have provided substantial insights into the mechanisms of smooth muscle cell biomechanics and phenotype regulation, the mechanisms of stretch-dependent signaling are probably not identical to those in intact tissue. The use of organ culture of vascular smooth muscle combines the three-dimensional environment of the cells with the advantages of an *in vitro* condition. The term organ culture can be misleading since it implies that organs are cultivated or grown *ex vivo*. The actual definition of the term is that an intact isolated organ is maintained in near physiological conditions in a cell culture environment. This approach has been used previously by us and others to study mechanotransduction in the vasculature (4, 7, 8, 61, 152, 155).

Animal models of hypertension are clearly the most physiological approach to study stretch-dependent effects on the vasculature. Several models, predominantly in rat, are available (91). Results obtained from studies using hypertensive animals can be difficult to interpret due to the systemic effects that inevitably occur when blood pressure is raised in an animal. In particular, sympathetic activity and renin-angiotensin signaling are often affected in these animal models (82, 91), which may be important for vascular effects unrelated to mechanical factors on the vascular wall. Thus, the finding that arteries are remodeled in hypertensive rats cannot be entirely attributed to an increased mechanical stretch of the vessel wall. However, it was recently reported that DOCA-salt hypertensive rats (an inducible model of hypertension) demonstrated hypertrophic remodeling in aorta but not in vena cava, which is subjected to

similar humoral factors but not affected by elevated pressure (147). This suggests that mechanical stress is the driving force for structural changes *in vivo*.

Sensing shear stress

Recent studies suggest that flow is sensed in endothelial cells by a mechanosensory complex consisting of vascular endothelial cell cadherin (VE-cadherin), a junctional protein, platelet endothelial cell adhesion molecule (PECAM)-1, an immunoglobulin family receptor, and vascular endothelial growth factor receptor 2 (VEGFR2) tyrosine kinases (3, 84, 142). The activation of this complex was shown to be upstream of other intracellular events, such as activation of c-Src, PI3-kinase and integrins. These events are essential for activation of eNOS and MAP kinases in endothelial cells in response to shear stress (18, 28, 50, 56). Caveolae have also been implicated in shear stress signaling in endothelial cells (31, 107, 108), which is supported by the localization of VEGFR2 in caveolae (127).

Stretch sensing in vascular smooth muscle

Stretch sensing in smooth muscle is important for both rapid myogenic constriction and long term signaling for remodeling and differentiation. Although studies on stretch sensing in intact vascular smooth muscle are still quite sparse, several mechanisms have been proposed. It is widely accepted that integrins that connect the intracellular actin cytoskeleton to the surrounding ECM have a central role in mechanotransduction in smooth muscle (16, 63). Other factors that have been proposed as mechanosensors are tyrosine kinase and G-protein coupled receptors and ion channels (63). Stretch of the smooth muscle would cause an increased tension on integrins, which is known to initiate activation of integrin associated proteins (92). This effect is dependent on an intact cytoskeleton and will cause clustering of integrins into focal adhesions at the site of tension.

Another possible mechanosensor in smooth muscle is stretch-activated ion channels. Mechanical stretch increases intracellular calcium levels and a role of a transient receptor potential (TRP) cation channel, TRPC6, has recently been reported (129). In this study the tarantula toxin peptide, GsMTx-4, was used to inhibit stretch-induced TRPC6 currents. This peptide has previously been demonstrated to be a specific inhibitor for stretch-activated channels and has interesting therapeutic implications (41, 133).

Stretch sensing is followed by an intracellular response that is complex and rather ill defined. A multitude of mediators are suggested to be involved and their integration into a coherent

picture at this stage may cause more confusion than clarity. (For reviews see (42, 60, 63)). However, in the intact vasculature, stretch/pressure undoubtedly results in an early ERK1/2 phosphorylation and tyrosine phosphorylation of proteins in the 115-140 kDa range (8, 61, 150, 152, 155). The early ERK1/2 phosphorylation caused by stretch correlates with an activation of integrin associated tyrosine kinases FAK and Src (106, 149) This is followed by a reduced but sustained ERK1/2 phosphorylation over several days, which results in increased protein synthesis and smooth muscle growth in veins and large arteries (5, 155). It is important to note that signaling in response to stretch likely involve both direct effects from mechanosensors such as integrins that activate the MAP kinase cascade via FAK as well as indirect effects by autocrine release of receptor agonists such as angiotensin II, endothelin-1 and PDGF (63, 152, 153).

Aims of the study

The objective of this work was to identify mechanisms of mechanotransduction in the vascular wall. The specific aims were:

To investigate the role of the cytoskeleton in stretch-dependent growth and differentiation of smooth muscle.

To clarify the role of Cav-1 in stretch- and flow-dependent signaling

Methods for studying mechanosensing in vascular smooth muscle

The portal vein model

Veins are exposed to lower intraluminal pressure than arteries but show considerable pressure-induced growth, as demonstrated by the hypertrophy, and often rapidly progressing atherosclerosis, of vein grafts exposed to arterial pressure (113). One vessel that has been extensively investigated is the portal vein, in which elevated intraluminal pressure *in vivo* over a few days causes hypertrophy and increased force development (53, 69).

In order to study stretch dependent effects on intact vascular smooth muscle, we have developed an organ culture model for isolated portal veins (155). The portal vein consists predominantly of longitudinal smooth muscle (Fig 5), and longitudinal force thus stretches the smooth muscle cells in the same axis as an increase in pressure would in an artery with circular smooth muscle. For these experiments, the portal veins are dissected free from fat and surrounding tissue and cut at predetermined sites so that all veins have similar lengths. One end of the vein is then attached to a steel hook while the other is attached to a gold weight (Fig 5). We have determined that the optimal length for force development is achieved at a load of approximately 3 mN for mouse portal veins and 6 mN for rat portal veins and the gold weights are adjusted accordingly. This distension approximately represents the physiological condition while the unstretched condition is clearly non-physiological. The portal veins are pre-incubated unstretched for 1 hour and then stretched for 5 min up to 72 hours in culture medium with a low concentration (2 %) of dialyzed fetal calf serum and insulin (10^{-8} M). We have previously observed that stretch under these conditions results in growth of the smooth muscle with a maintained contractile differentiation similar to what is observed at an increased pressure *in vivo* (69, 153).

There are several aspects that make the portal vein a valuable model for studies of stretch-dependent signaling in vascular smooth muscle. 1) The portal vein is a phasic smooth muscle with spontaneous myogenic activity, which is maintained in culture medium (Zeidan A, doctoral thesis, 2003). This is likely to be important for the long term effects of an increased wall stress. 2) Compared to organ cultured pressurized resistance arteries, the portal vein model is far less complicated and provides high reproducibility and throughput. 3) The portal vein is a relatively large vessel and yields sufficient protein for biochemical analysis.

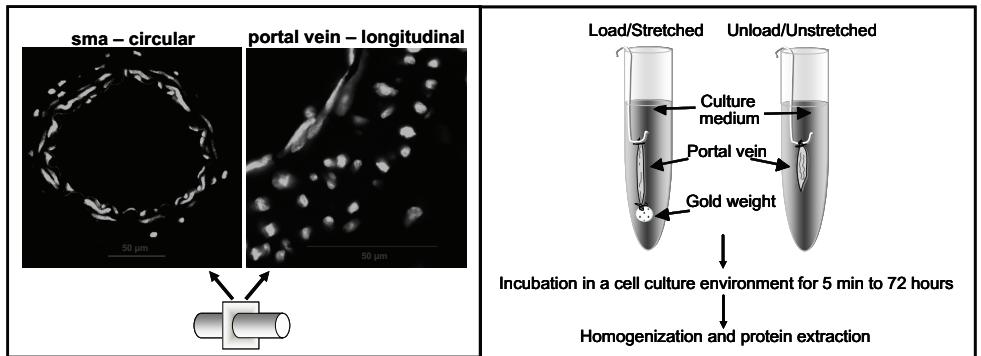


Figure 5. Left: Nuclear staining of small mesenteric artery and portal vein. The small mesenteric artery has a circular smooth muscle layer and the nuclei therefore appear elongated in the cross section. In contrast the portal vein has mainly longitudinal smooth muscle and thus the nuclei are rounded. A thin inner circular smooth muscle layer is visible in the portal vein. Right: Organ culture of the portal veins

Measuring growth and differentiation

Growth of vascular smooth muscle is associated with an increased global protein synthesis, which is dependent on MAP-kinase and PI3-kinase activation (46, 63, 134). Incorporation of radioactively labeled amino acids is a sensitive and reliable way to measure protein synthesis. We have used both [³⁵S]-methionine and L-[4,5-³H]-leucine to determine global protein synthesis. The isotope is present during the final 24 hours of a 72 hour organ culture. The use of L-[4,5-³H]-leucine is convenient but only enables measurement of total protein synthesis in a sample since the ³H-isotope emits very weak beta radiation, which is measured by liquid scintillation counting. Autoradiography with [³⁵S]-methionine is time consuming but offers more detailed analysis of the sample. After homogenization of the tissue, the protein extract is separated by 1D- or 2D-gel electrophoresis. Protein content is analyzed by silver staining while global as well as specific protein synthesis is analyzed by autoradiography. Separate gels can be used in western blotting for analysis of activation of signaling pathways in the same tissue sample. Typically, phosphorylation of cofilin, which is an indicator of Rho activation, and of ERK1/2 and Akt, which regulate global protein synthesis and growth, and of FAK, an indicator of integrin activation, was determined.

Measuring actin polymerization

A major challenge in this project was the evaluation of the effect of stretch on actin polymerization in intact smooth muscle. Actin polymerization was measured by estimation of the F/G-actin ratio in stretched and unstretched portal veins. This was done using two different methods. One involves quantification of F- and G-actin cellular fractions by western blotting. The general approach is homogenization of the tissue followed by ultra centrifugation at 100 000 x g to separate the F- actin from G-actin pool. The proteins in the two fractions are then separated by SDS-PAGE and actin is quantitated by western blotting. A detailed protocol for this method was provided by Cytoskeleton Inc. This method has potential drawbacks, e.g. that small actin oligos will not sediment to the F-actin pool and the G-actin pool may therefore be over-estimated. Also, care must be taken to keep the polymerization state steady during the experimental procedure. This is accomplished by homogenization in an F-actin stabilizing buffer which prevents depolymerization of actin filaments, and by carefully controlling the sample temperature. To ensure the reliability of this method we also examined portal veins treated with jasplakinolide, which promotes actin polymerization, and with latrunculin B, an actin depolymerizing agent.

F/G-actin ratio was also assessed using confocal microscopy and fluorescence staining of the two actin pools. DNase1 specifically binds to G-actin in the cytosol and phalloidin is an agent that specifically targets F-actin. The fluorescently conjugated variants FITC-phalloidin and Texas Red-DNase1 were used. Following organ culture the vessels are rapidly fixed in 4% formaldehyde and several transverse sections were mounted on cover slips. The main problem with this method was the variability in staining in different sections of the vein. Approximately 40 measurements from different sections of each portal vein were therefore performed. In total, 180-240 measurements were made for each condition.

Pressure myography and pressurized carotid arteries

Small resistance arteries normally react to an increase in intraluminal pressure by decreasing their diameter to reduce wall stress according to the law of Laplace. This myogenic contraction is an important mechanism for autoregulation of blood flow to different organs. Myogenic tone is counteracted by flow-dependent dilatation, which is a result of the frictional force (shear stress) exerted by flowing blood on the endothelium. Shear stress is determined by the vessel radius, flow velocity and fluid viscosity according to the following formula: $SS=4 \mu Q / \pi r^3$, where μ is the fluid viscosity (in poise), Q is the fluid flow (in milliliters per

second), and r is the radius (in centimeters). Shear stress on the endothelium stimulates the release of several vasodilators such as NO, prostacyclin (PGI₂) and endothelium derived hyperpolarizing factor (EDHF) (145). Physiological levels of arterial shear stress range from 5 -50 dynes/cm² (87).

To measure acute changes in diameter in response to mechanical stimuli the arteries were mounted in a pressure myograph (Fig 6). This consists of an organ bath chamber with two micropipettes on which the artery is cannulated. The micropipettes are connected to a pressure servo system, which regulates intraluminal pressure and flow. The inner and outer vessel diameter is monitored live using a microscope connected to a digital video camera and computer software with edge detection capability. Mesenteric resistance arteries from mice were used in this setup and these displayed a strong and reproducible myogenic constriction to intraluminal pressures of 45-120 mmHg. Flow-induced dilatation was evaluated by stepwise increases of intraluminal flow of 25-125 μ l/min, which corresponded to shear stress values of 4-45 dynes/cm². We mainly used myogenic tone to pre-constrict vessels although in some experiments a low concentration (0.03 μ M) of the α 1-adrenergic agonist cirazoline was used both intra- and extra-luminally. We found that flow caused a washout effect when cirazoline was only applied extraluminally. We also found that it was critical to keep the temperature of the perfusate exactly the same as the superfusate. Slightly lower temperature in the perfusate caused a temperature dependent dilatation and this was prevented by preheating the perfusate through a glass pipette placed in the organ bath chamber.

While mouse mesenteric resistance arteries are ideal for use in the pressure myograph, they are too small for western blot analysis. Typically, 0.5-1 mg of tissue is required for western blot analysis and the weight of one mesenteric artery is around 50 μ g. We therefore developed an experimental setup for pressurization and flow stimulation of mouse carotid arteries. One end of the artery was cannulated on a micropipette while the other was ligated for pressure experiments or left open for flow experiments. This setup did not allow for measurement of diameter but we were able to run up to four parallel carotid arteries simultaneously. After rapid freezing of the arteries, biochemical analysis was performed to evaluate effects on signaling pathways.

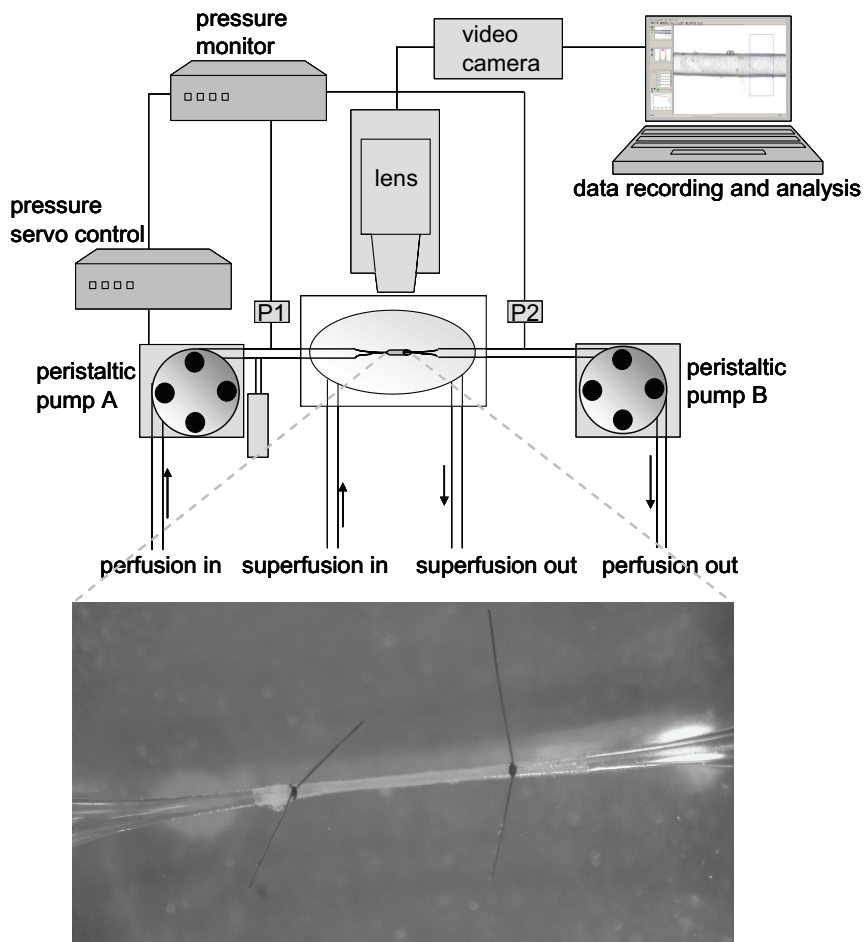


Figure 6 Setup of the pressure myograph. The intraluminal pressure of the mesenteric artery was continuously monitored by two pressure transducers (P1 and P2). These were connected to a servo control system which adjusted intraluminal pressure via a peristaltic pump (A). Intraluminal flow was applied via a second peristaltic pump (B) and the mean pressure of P1 and P2 was maintained by automatic adjustment of pump A. The inner and outer diameter of the vessel was monitored continuously using a microscope connected to a digital video camera and computer software.

Results and discussion

The following section summarizes the findings in papers I-V. Readers are referred to the original papers for details.

Stretch-dependent growth of the portal vein accompanied by a relative increase in contractile force has been observed both *in vivo* and in organ culture (69, 70, 153, 155). The aim of this work was to explain the mechanisms behind these effects and herein I present evidence that suggests a role for the cytoskeleton but not for Cav-1 in stretch dependent growth and differentiation. However, Cav-1 has other effects in the vasculature that will also be discussed.

Stretch-dependent growth and differentiation in portal veins - role of actin polymerization (papers I-III)

In paper I, we found that stretch of the rat portal vein dramatically increased the synthesis of the smooth muscle marker protein SM22 (126). SM22 is an actin binding protein (33) and was recently suggested to be an inhibitor of MMP-9 expression and cell invasion *in vitro* by negative regulation of ERK1/2 phosphorylation (76). We have previously studied the role of SM22 in vascular function using an SM22 knockout mouse (154). We found that these mice had an essentially normal phenotype except for a slight decrease in smooth muscle actin contents and contractile force in response to potassium in mesenteric arteries. At the mRNA level SM22 as well as most other smooth muscle specific genes are regulated by the transcription factor SRF and its cofactors MAL/MKL1 and myocardin. We were intrigued by the role of the actin cytoskeleton as a messenger for contractile differentiation. Studies in isolated cells had shown that depolymerization of actin filaments inhibited smooth muscle specific gene transcription by preventing translocation of SRF and MAL/MKL1 from the cytosol to the nucleus (20, 65, 72). To determine if this effect was present also in intact vascular tissue, we used the actin depolymerizing agents latrunculin B and cytochalasin D on stretched portal veins. Remarkably, depolymerizing the actin filaments inhibited both stretch-dependent increase in growth, ERK1/2 phosphorylation and SM22 synthesis (I). Latrunculin B inhibited the synthesis of SM22 and actin in a concentration dependent manner while cytochalasin D only inhibited the synthesis of these proteins at low concentrations (<1 μ M) and had no effect at higher concentrations. Interestingly, cytochalasin D has been reported to cause dimerization of actin monomers at high ($\geq 2\mu$ M) concentrations (32). This may affect actin binding to SRF and MAL/MKL1. Cytochalasin D may also directly inhibit interaction

by occupation of the MAL/MKL1 binding site on actin, similar to profilin (96). However, high concentrations of cytochalasin D inhibited stretch-dependent growth of the portal vein as measured by the increase in wet weight. Thus, both stretch-dependent differentiation and growth of vascular smooth muscle are sensitive to changes in cytoskeletal structure but it appears that only differentiation is regulated by the intracellular levels of monomeric G-actin.

Actin polymerization is mainly regulated by Rho-dependent mechanisms. In rat portal veins (I) we found that inhibition of Rho also inhibited stretch-dependent growth and contractile differentiation. These results led to the hypothesis that stretch promotes contractile differentiation via Rho-activation and a net increase in actin polymerization. To test this hypothesis I measured Rho-activation using a pulldown assay where activated GTP-bound Rho is precipitated from portal vein protein extracts and analyzed by western blot. The results demonstrated a 2-fold increase in Rho-activation by stretch after 24 hours of organ culture (II). This correlated with a 2-fold increase in cofilin phosphorylation that was abolished by the ROCK inhibitor Y-27632. Active cofilin depolymerizes actin filaments, but this activity is inhibited by phosphorylation. The effect on actin polymerization of a 2-fold increase in activation of the Rho-pathway was difficult to predict. I therefore evaluated the F/G-actin ratio in stretched and unstretched mouse portal veins \pm latrunculin B or jasplakinolide. At 24 hours of organ culture the F/G actin ratio in the vascular wall was significantly increased by approximately 40% in stretched vs. unstretched portal veins. The results also confirmed that latrunculin B and jasplakinolide had the expected effects on the F/G-actin ratio. In the mouse portal vein we identified a number of stretch-sensitive proteins by 2D-gel electrophoresis and western blot, which all turned out to be smooth muscle differentiation markers transcribed from SRF-regulated genes. These proteins include tropomyosin, alpha-actin, desmin, calponin and SM22. By 2D-gel electrophoresis we also confirmed that the synthesis of SM22 and calponin was dependent on ROCK activity in stretched mouse portal veins. We could now confidently state that stretch-dependent contractile differentiation is promoted by a net increase in actin polymerization caused by activation of the Rho/ROCK pathway.

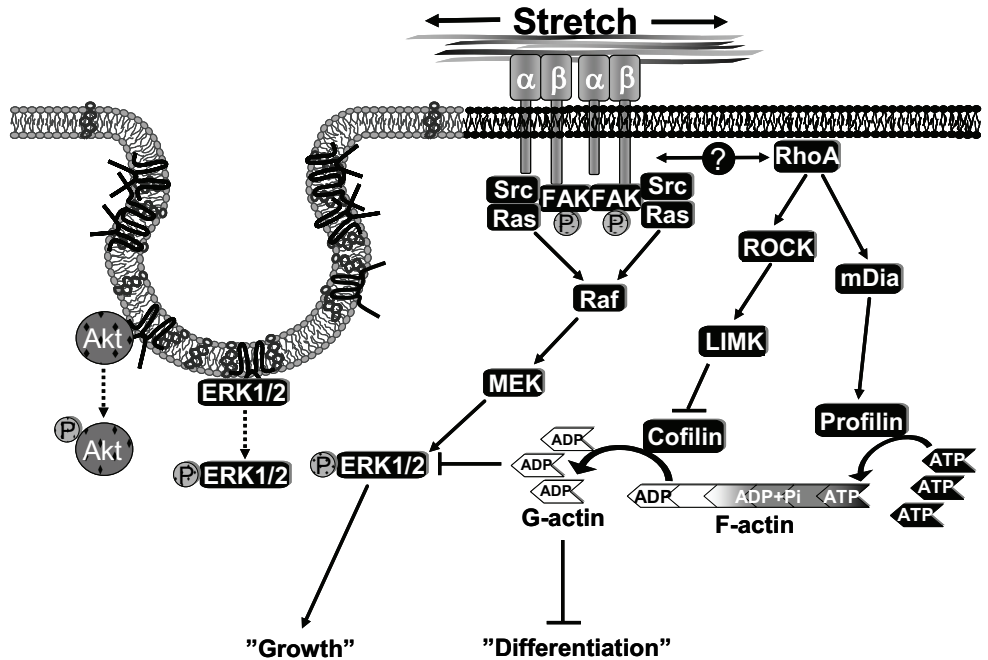


Figure 7 Signaling pathways suggested to be involved in stretch-dependent growth and differentiation in vascular smooth muscle. Stretch induces early FAK and ERK1/2 phosphorylation, probably via integrin activation. The late response to stretch involves Rho activation and actin polymerization. These two pathways promote growth and differentiation, respectively. Growth is also promoted by stretch-independent mechanisms by ERK1/2 and Akt phosphorylation in the absence of caveolin-1.

The mechanisms regulating stretch-dependent activation of Rho are still unclear. Integrins that connect the cytoskeleton to the surrounding extracellular matrix are likely to be involved in stretch sensing, and the integrin associated enzyme FAK has been suggested to interact with signaling proteins involved in the Rho-pathway as well as the MAP-kinase and PI3-kinase pathways (110). Stretch-dependent FAK phosphorylation in the portal vein was biphasic with increases in phosphorylation at 5-15 min and at 24-72 hours. I have shown that the late activation of Rho, and subsequent inactivation of cofilin, at 24 hours correlates with an increased FAK phosphorylation at this timepoint (III). The initial peak correlated with the early ERK1/2 phosphorylation, and pressure-induced ERK1/2 phosphorylation has previously been suggested to be mediated by integrin and FAK-dependent mechanisms in intact aorta smooth muscle (61). In that study, integrin-dependent signaling was inhibited with RGD

peptides, which compete with specific integrins for binding sites on ECM proteins. We attempted to inhibit integrin signaling in the portal vein with several cyclic and linear RGD peptides in various concentrations as well as an integrin β 1-blocking antibody with limited success. None of these interventions significantly affected either FAK, Akt or Erk1/2 phosphorylation in stretched portal veins. In the absence of a positive control, which could assure that the peptides actually bound integrins in the smooth muscle, we cannot conclude that different mechanisms are involved in the portal vein vs. aortic smooth muscle. We are currently evaluating mechanosensitive signaling in several vascular beds from smooth muscle specific FAK knock out mice. These experiments should shed some light on the role of integrin signaling in stretch-dependent growth and differentiation.

So far our studies had shown that growth and differentiation in response to stretch requires an intact cytoskeleton and that stretch itself promotes actin polymerization. The final test of our hypothesis that actin polymerization is involved in stretch-dependent signaling was to see if the effects of stretch could be reproduced by stabilization of the actin cytoskeleton. It is likely that the *in vivo* state resembles the stretched phenotype rather than the unstretched and that the majority of the changes observed occur in the unstretched portal vein. I used jasplakinolide to stabilize actin filaments and could show that this agent increases SM22 synthesis (II). The lowest effective concentration (100 nM) also increased the F/G actin ratio in unstretched portal veins approximately to the same extent as stretch. Interestingly, by measurement of the entire lane of the autoradiograph, global protein synthesis was also found to be increased by jasplakinolide. Using inhibitors of the MAP-kinase and PI3-kinase pathway I confirmed that these two pathways were important for global protein synthesis in the portal vein (III). The next step was then to test whether jasplakinolide activated these pathways. This was done by measurements of ERK1/2 and Akt phosphorylation in portal veins incubated with jasplakinolide for 24 hours. Remarkably, jasplakinolide caused a 3-fold activation of ERK1/2 phosphorylation in both stretched and unstretched portal veins but had no effect on Akt phosphorylation. Using an inhibitor of MEK in the MAP-kinase pathway, it was shown that jasplakinolide-induced global protein synthesis is dependent on ERK1/2 phosphorylation. We also found that the stretch-dependency of FAK phosphorylation was eliminated by jasplakinolide. The effect of jasplakinolide on ERK1/2 phosphorylation thus seems to be a direct effect of the actin cytoskeleton on MAP-kinase activation and not mediated through FAK activation. The exact mechanisms behind this effect are not known but a recent report suggests eNOS activity to be regulated by association with actin in endothelial cells (55). It

thus seems as though the state of the actin cytoskeleton may have important effects on several different aspects of cellular signaling.

Role of caveolae in mechanosensing in vascular smooth muscle (papers IV-V)

The discovery and detailed characterization of microdomains, such as caveolae, in the plasma membrane has uncovered new aspects of the complex machinery of the cell. Until recently, the role of caveolae in cellular signaling was often studied using agents that extract cholesterol from the plasma membrane, such as methyl-beta cyclodextrin (MBCD). This treatment disrupts caveolae but also affects other cholesterol-rich domains, i.e. lipid rafts. In recent years additional tools have been developed to study caveolae function, such as Cav-1 siRNA and knock out mice for different isoforms of the caveolin protein. We have previously observed that acute stretch-dependent signaling and growth of the portal vein is dependent on cholesterol in the cell membrane (152). In addition, recent reports suggest a role for caveolae and Cav-1 in mechanosensing in the vascular wall (89, 107, 108, 117, 151). However, as much evidence indicates that mechanical stretch is essential for the differentiation of smooth muscle around hollow organs from mesenchymal stem cells (51) it seems unlikely that mechanosensing is entirely dependent on caveolae in the vasculature since Cav-1 KO mice develop essentially normally.

A detailed characterization of the structure and function of blood vessels from Cav-1 KO mice revealed a basal increase in cross sectional area in all vessels examined including the aorta, mesenteric resistance artery, carotid artery, femoral artery and portal vein (IV-V)(121). We also found that blood pressure was essentially unchanged in Cav-1 KO mice despite an increase in NO production. A potential explanation for this was an increased sensitivity of the smooth muscle for adrenergic agonists and an increased plasma volume (IV). My contribution to this paper was primarily to investigate mechanosensing in small mesenteric arteries using a pressure myograph. This revealed a decreased myogenic tone in Cav-1 KO, which was largely but not entirely dependent on excessive NO production. The mechanism behind the remaining NO-independent effect on myogenic tone is unknown but will be investigated in future studies. Recently published data suggest that, in cerebral arteries, reduced myogenic tone in Cav-1 KO mice is due to reduced depolarization and depolarization-induced calcium influx (1). In small mesenteric arteries, a reduced Rho-activity has been suggested to contribute to the weakened myogenic tone in Cav-1 KO mice (22).

I also found flow-dependent dilatation to be reduced by Cav-1 ablation. However, this effect was only observed when vessels were pre-constricted with myogenic tone alone (IV). It is noteworthy that the role of caveolae in flow-induced dilatation differs depending on the contractile stimulus used. Another surprising finding was that flow-induced dilatation was completely independent of NO. In our hands, flow-induced dilatation in small mesenteric arteries seems to be dependent on EDHF since a combination of the NOS inhibitor, L-NAME and an inhibitor of PGI₂ synthesis, indomethacin, was ineffective in preventing dilatation. In fact, flow-induced dilatation was restored in the Cav-1 KO in the presence of an inhibitor of NO production. Although these results are confusing they are not incompatible with previous findings. NO independent flow-induced relaxations have been demonstrated previously and a similar effect of NO inhibition in the Cav-1 KO was recently reported (151). This may be related to the finding that NO production attenuates the release of EDHF (6). It is also possible that NO accumulates in the vessels of Cav-1 KO mice under no-flow conditions and that the application of flow results in a partial washout of NO, which counteracts the relaxing effect of shear stress (100). In agreement with this hypothesis, I often observed an initial transient contraction in response to flow in Cav-1 KO, but not WT mice or in Cav-1 KO mice treated with L-NAME. From the pressure myograph studies we could conclude that acute mechanotransduction in arterial smooth muscle and endothelium was affected by Cav-1 ablation but that this effect mainly depended on excessive and dysregulated NO production.

We next sought to determine if stretch- and flow dependent signaling for contractile differentiation and growth was affected by loss of caveolae in the Cav-1 KO mouse. For this I used the portal vein model as well as a newly developed experimental setup using mouse carotid arteries for investigation of acute signaling in response to pressure and shear stress. In the portal vein we found that stretch-dependent signaling to growth (ERK1/2 and FAK) and differentiation (cofilin) was maintained in the absence of caveolae (V). In accordance with this, stretch-sensitive global protein synthesis as well as smooth muscle specific protein synthesis was similar in Cav-1 KO and WT mice. However, in agreement with earlier observations we found an increased basal activation of ERK1/2 and Akt in Cav-1 KO mice, which also resulted in an increase in basal global protein synthesis and cross sectional area of the portal vein. In the carotid artery, we found that 1 hour of 120 mmHg intraluminal pressure increased ERK1/2 phosphorylation in both WT and Cav-1 KO mice. Shear stress for 15 minutes did not affect ERK1/2 phosphorylation but increased Akt phosphorylation in WT mice. This has been shown previously and is an important effect for eNOS activation and NO

production (17, 34). In the carotid arteries of Cav-1 KO mice we did not observe any significant flow-dependent Akt phosphorylation. This may be important for the perturbed flow-induced dilatations that we and others have observed in Cav-1 KO mice. A difference in Cav-1 dependency of stretch- versus flow-sensing may explain the difference between our results and that of Sedding et al. who described that mechanosensitive Akt and ERK1/2 phosphorylation was diminished in Cav-1 KO veins subjected to arterial pressure and flow (117).

Final remarks

In this thesis I have described several vascular abnormalities where mechanotransduction in the vasculature and phenotypic modulation of smooth muscle cells may play an important role. For example, atherosclerosis is known to occur at sites with turbulent flow while steady laminar flow is protective against this disease (87, 138, 140). Excessive wall stress and/or flow may cause injury to the vessel wall and result in neointima formation and occlusion of vein grafts or restenosis following balloon angioplasty. Wall stress is also a critical factor in different modes of remodeling, such as seen in hypertension, which will be further discussed in this section.

Almost 50 years after Folkow's classic paper (30), which described how an increased wall to lumen ratio could account for the raised resistance in primary hypertension, the debate about whether remodeling is the chicken or the egg in this disease is still ongoing. It is now more widely believed that changes in fluid homeostasis is the basis of hypertension and that vascular changes represent an adaptation to this in order to prevent overperfusion and hypertension in the microcirculation. However, much is uncertain and as Folkow described it "...any physiologist interested in homeostatic mechanisms can certainly have his tour de force in facing primary hypertension, because it provides fascinating examples of how complex control mechanisms may gradually be pushed beyond normal bounds until deterioration ensues." (29) With this in mind, remodeling of small resistance arteries has to be seen as one of several events in primary hypertension and many factors, other than mechanosensing, may be important for changes in arterial structure.

The majority of experiments in this thesis are based on stretched portal veins in organ culture. The clinical relevance of this model can be questioned although portal hypertension is a clinical problem, often secondary to liver disease. The portal vein is certainly not a resistance vessel and our focus is mainly related to arterial hypertension and vein grafts subjected to

arterial pressure. However, as described in METHODS this model has many advantages compared to other methods used to study stretch-dependent effects in vascular smooth muscle. Since our previous findings using this model correlate well with what is observed in pressurized intact arteries (5, 7, 26, 61), as well as *in vivo* findings in experimental animal models (150), we are convinced that the effects on growth and differentiation reported here are relevant for the early (<72 h) events in arteries subjected to an increase in wall stress.

In patients with primary hypertension, eutrophic, rather than hypertrophic, inward remodeling is the main reason for the increased wall to lumen ratio in small resistance arteries (74). It is important to note that the portal vein in our model has no means to acutely reduce wall stress whereas a resistance artery within minutes after an increase in intraluminal pressure will decrease its diameter and essentially normalize wall stress. Using the pressure myograph, I found that an increase in intraluminal pressure from 95 to 120 mmHg in mouse small mesenteric arteries resulted in a minor increase in wall stress from 4,9 to 5,3 N/cm² in the presence of extracellular calcium. In the absence of calcium, wall stress increased from 11 to 14,3 N/cm² at 95 and 120 mmHg, respectively. It is conceivable that a continuous increased wall stress stimulates growth whereas a persistent myogenic contraction abolishes the growth response but eventually results in an inward remodeling due to rearrangement of the cytoskeleton and ECM proteins (74, 97). At a fixed pressure, the induction of immediate early genes was increased in mesenteric arteries with weak myogenic tone and thus high wall stress compared to arteries with strong myogenic tone and low wall stress (2). Consequently, in the aorta, which has no myogenic tone, a rise in pressure results in hypertrophic growth *in vivo* and an increased protein synthesis in organ culture (5, 85).

The results presented herein clarify the mechanisms involved in the vascular response to wall stress and shows that stretch-dependent growth occurs in smooth muscle cells with a maintained contractile phenotype. Stretch-dependent polymerization of actin filaments is perhaps primarily a protective mechanism allowing the cell to adapt to the increased load. It is yet unclear if this polymerization involves both cytoskeletal and contractile isoforms of actin. However, it is obvious that the actin filaments should not be regarded as merely a structural and contractile component of the cell as both growth and differentiation appear to be regulated by the state of actin.

The discrepancy between results obtained in stretched isolated cells and intact smooth muscle has been mentioned several times in this summary and an apparent example of this is the role

of caveolae in stretch-dependent signaling in vascular smooth muscle. In a recent review, Parton et al. concluded: "The most intriguing property of the caveolin/caveolae system is its involvement in mechanosensing. Many of the apparently unrelated functions of caveolae might be related to the ability of caveolae to sense changes in membrane tension or to sense other changes in the physical properties of the plasma membrane..." (90). However, this is mainly based on findings in isolated smooth muscle cells in 2D culture, which appear to be entirely dependent on Cav-1 expression for stretch sensing (117). In contrast, in the intact vascular wall, stretch-dependent growth and differentiation is not limited by the loss of caveolae. It is thus important to distinguish between mechanosensing in isolated smooth muscle cells and intact smooth muscle tissue since mechanosensing seems to be controlled by different mechanisms in these situations. However, caveolae and Cav-1 have major relevance for vascular function and dysfunction as they are important for NO production and flow sensing in the endothelium and, as demonstrated herein, for contractile responses to adrenergic stimuli. Current research is establishing the importance of caveolae in vascular disease. Caveolin KO mice have facilitated this effort and caveolae are now suggested to play a role in atherosclerosis, remodeling, neointima formation and vascular permeability.

The caveolus is indeed a microdomain, which harbors signaling molecules for a wide variety of cellular functions. However, this makes it difficult to dissect the true function of caveolae as some of the observed effects may be secondary. For example, we found that myogenic tone was severely impaired in the Cav-1 deficient mice. At first we suspected that stretch sensing was dependent on caveolae, since this was suggested by the literature, but it turned out that myogenic tone was almost completely restored when NO synthesis was inhibited. The excessive endothelial NO production is also suspected to mediate other effects observed in the Cav-1 KO mice, such as protection against atherosclerosis (119) and the increased vascular permeability (10, 116). An additional comment regarding results obtained in the Cav-1 KO, as well as other knock out animal models, is that several of the observed effects may be compensatory mechanisms rather than a direct effect of the genetic modification. In paper IV we actually found that several mechanisms in the vasculature resulted in a maintained blood pressure in the Cav-1 KO despite an increased NO production. Some of these, such as the increased plasma volume, may be compensatory, and thus not directly related to loss of caveolae.

Conclusions

Rho activity and an intact cytoskeleton are required for stretch-dependent growth and differentiation in vascular smooth muscle.

Rho activity and cofilin phosphorylation are increased by stretch, which stimulates actin polymerization and smooth muscle specific protein synthesis.

In the absence of stretch, growth and differentiation can be induced by stabilization of actin filaments.

Cav-1 is not required for stretch-dependent contractile differentiation and growth. Although acute stretch- and flow-induced signaling is perturbed, this is mainly secondary to excessive endothelial NO release.

In the absence of Cav-1, increased adrenergic contractile responses, hypertrophic arterial remodeling as well as plasma volume expansion may contribute to blood pressure normalization in the setting of excessive NO production.

Populärvetenskaplig sammanfattning

I denna avhandling undersöks hur mekanisk sträckning påverkar den glatta muskulaturen som omger våra blodkärl. En ökad sträckning av blodkärlen sker till exempel vid högt blodtryck vilket leder till en förändrad struktur av kärlet, så kallad remodelering. Ett annat mekaniskt stimuli utgörs av blodets friktion mot de endotelceller som täcker blodkärlens kontaktyta mot blodet. Liksom sträckning spelar blodflödets egenskaper en stor roll för remodelering av kärlväggen. En situation där både trycket och flödet är kraftigt förändrade är vid by-pass operationer då en ven ibland används för att ersätta en förkalkad artär. Venen utsätts då plötsligt för ett högt tryck och flöde och det är troligt att dessa mekaniska stimuli är viktiga bidragande orsaker till den snabba kärlförträngning, så kallad neointimabildning, som allt för ofta ses vid by-pass kirurgi.

Det finns en funktionell orsak till varför blodkärl som utsätts för ett förhöjt tryck förändrar sin struktur. Sträckningen av varje enskild muskelcell i kärlväggen är beroende av trycket i kärlet, av kärlets diameter och av väggens tjocklek. En mindre diameter eller en tjockare vägg minskar den kraft som varje cell utsätts för vid ett konstant tryck. Artärer med mindre diameter, så kallade resistensartärer, reagerar direkt på ett ökat tryck genom att kontrahera och därmed minska sin diameter. Detta kallas myogen kontraktion och kan räcka för att minska sträckningen av kärlväggen men det leder även till en ytterligare ökad resistens i kärlträdet och därmed ett ännu högre blodtryck. I större artärer och vener som inte har samma förmåga att kontrahera som svar på ett ökat tryck leder sträckning istället till en tillväxt av kärlväggen, antingen genom att varje muskelcell blir större, hypertrofi, och/eller genom att muskelcellerna delar sig, hyperplasi.

De mekanismer som styr de glatta muskelcellernas reaktion på mekaniska stimuli har stor betydelse för förståelsen av kärlsjukdom. Jag har i denna avhandling bland annat undersökt vilken roll de glatta muskelcellernas aktincytoskelett och de små invaginationer i cellmembranet som kallas caveolae spelar för intracellulär signalering vid mekaniska stimuli. Jag har huvudsakligen utnyttjat en försöksmodell där portavenen, som leder venöst blod från tarmarna till levern, från mus eller råtta används för att studera sträckberoende signalering. Portavenerna inkuberas *in vitro* i provrör med cellodlingsmedium i en inkubator som efterliknar miljön *in vivo*. Sträckningen sker genom att en vikt hängs i ena änden av portavenen som är upphängd i en krok (se bild 5 i avhandlingen).

Glatta muskelceller har en förmåga att förändra sin funktion (fenotyp) beroende på yttre omständigheter t.ex. mekaniska stimuli, tillväxtfaktorer och interaktioner med andra celler eller de matrixproteiner som omger cellerna. Normalt är glatta muskelceller i kärlväggen programmerade att genomföra kontraktion och relaxation medan signaler för tillväxt och migration är nedtryckta. En vanlig missuppfattning är att tillväxt hämmar kontraktilitet i glatta muskelceller, något som baseras på tidiga studier utförda på isolerade celler.

Vi har tidigare visat att sträckning av kärlväggen stimulerar både tillväxt och kontraktilitet i glatt muskulatur. Den kontraktila fenotypen definieras bland annat genom cellernas kontraktila förmåga och uttrycket av kontraktila proteiner. I denna avhandling har vi i tre separata studier visat att den kontraktila fenotypen i sträckt glatt muskulatur stimuleras genom en ökning av polymeriseringen av aktin. Detta leder till bildning av aktinfilament som är viktiga för cellens förmåga till kontraktion men bygger delvis även upp det cytoskelett som definierar cellens struktur. Under senare år har cytoskelettet även visats styra regleringen av de gener som definierar den kontraktila fenotypen och jag har kunnat visa att denna mekanism stimuleras av sträckning. För att tillväxt av cellerna ska kunna ske krävs en ökad syntes av cellens samtliga proteiner. Denna globala proteinsyntes ses därför som ett känsligt mått på tillväxtsignalering och vi har kunnat visa att även denna signalväg är beroende av cytoskelettet. Genom att farmakologiskt bryta ner aktinfilament kan vi förhindra effekterna av sträckning och genom att stabilisera aktinfilament kan vi reproducera effekterna av sträckning vad gäller signaler för kontraktil och global proteinsyntes. Flera av dessa fynd är tidigare okända och ger nya inblickar i de mekanismer som styr remodelering och neointimabildning i kärlväggen.

Vi har även studerat sträckkänslig signalering i en mus som saknar proteinet caveolin-1. Detta protein krävs för att bilda de fördjupningar i cellmembranet som kallas caveolae och som är viktiga domäner för intracellulär signalering. Tidigare har dessa domäner misstänkts vara viktiga för sträckkänslig signalering men vi visar att det inte är fallet för tillväxt och kontraktil differentiering. Både den sträckberoende kontraktiliteten och tillväxten är bevarad i portavener från dessa möss. Vi har även undersökt hur artärer från dessa möss reagerar på förändringar i tryck och flöde. Även i artärer verkar den specifika känsligheten för ökat tryck i stort sett vara bevarad i frånvaro av caveolin-1 medan signalering som svar på ökat flöde är delvis förändrad.

Sammanfattningsvis är mekanisk sträckning av kärlväggen ett viktigt stimulus för tillväxt och kontraktil differentiering av glatta muskelceller. Denna process är beroende av en ökad aktinpolymerisering, men oberoende av caveolae. Dessa fynd bidrar till vår kunskap om strukturella adaptiva förändringar i kärlväggen vid hjärt- kärlsjukdom.

Acknowledgements

I wish to express my gratitude to everyone who has helped and supported me through my years as a PhD student. I especially wish to thank:

My supervisor **Per Hellstrand**, for introducing me to the exciting world of portal veins and mechanosensing, for being an outstanding scientist and supervisor, for all your patience, support and encouragement during my PhD, and for always taking the time to share your wisdom with me.

My co-supervisor **Karl Swärd**, for introducing me to the field of caveolae and the outstanding football team "Roth Scandinavia", for your amazing passion for science and your astonishing knowledge. You have truly been a great inspiration for me.

Our lab technician **Ina Nordström**, for always helping out with big and small things, for your endless patience, for all the things you do to keep things running in the lab and to create a joyful atmosphere. A special thanks for all the work you have done in this thesis.

Maria Gomez, for teaching me all about confocal microscopy, for your positive spirit, for all of your good ideas, for being a fun person to be around and for traveling with us to conferences.

Bengt-Olof Nilsson, for all of your help, for all the laughter and for bringing cakes that you "make yourself".

My fellow PhD students **Lisa, Mari, Jenny, Kristina, Malin, Yulia and Daniel**, for all your support in both scientific and other issues. Thank you for your friendship, for keeping up my caffeine dependence by making "fika-breaks" so enjoyable, and for being great traveling partners.

All of the former members of the group, especially **Asad Zeidan**.

The groups of **Lydia Sorokin, Bengt Rippe** and **David Erlinge** for fruitful collaboration.

Magnus Grenegård and **Eva Lindström** for being the best teachers possible, for introducing me to smooth muscle biology, and for all the support and guidance during my master education in science.

All of my friends outside the lab who make my life so much fun and filled with sports, parties, poker games and discussions about everything except science.

My parents, **Olle** and **Jocelyne** and my sisters **Berenice** and **Elisa**, for always being there for me.

References

1. **Adebiyi A, Zhao G, Cheranov SY, Ahmed A, and Jaggar JH.** Caveolin-1 abolishment attenuates the myogenic response in murine cerebral arteries. *Am J Physiol Heart Circ Physiol*, 2006.
2. **Allen SP, Wade SS, and Prewitt RL.** Myogenic Tone Attenuates Pressure-Induced Gene Expression in Isolated Small Arteries. *Hypertension* 30: 203-208, 1997.
3. **Bagi Z, Frangos JA, Yeh JC, White CR, Kaley G, and Koller A.** PECAM-1 mediates NO-dependent dilation of arterioles to high temporal gradients of shear stress. *Arterioscler Thromb Vasc Biol* 25: 1590-1595, 2005.
4. **Bakker EN, Buus CL, Spaan JA, Perree J, Ganga A, Rolf TM, Sorop O, Bramsen LH, Mulvany MJ, and Vanbavel E.** Small artery remodeling depends on tissue-type transglutaminase. *Circ Res* 96: 119-126, 2005.
5. **Bardy N, Karillon GJ, Merval R, Samuel JL, and 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* 77: 684-694, 1995.
6. **Bauersachs J, Popp R, Hecker M, Sauer E, Fleming I, and Busse R.** Nitric oxide attenuates the release of endothelium-derived hyperpolarizing factor. *Circulation* 94: 3341-3347, 1996.
7. **Birukov KG, Bardy N, Lehoux S, Merval R, Shirinsky VP, and Tedgui A.** Intraluminal pressure is essential for the maintenance of smooth muscle caldesmon and filamin content in aortic organ culture. *Arterioscler Thromb Vasc Biol* 18: 922-927, 1998.
8. **Birukov KG, Lehoux S, Birukova AA, Merval R, Tkachuk VA, and Tedgui A.** Increased Pressure Induces Sustained Protein Kinase C-Independent Herbimycin A-Sensitive Activation of Extracellular Signal-Related Kinase 1/2 in the Rabbit Aorta in Organ Culture. *Circ Res* 81: 895-903, 1997.
9. **Bishop AL and Hall A.** Rho GTPases and their effector proteins. *Biochem J* 348 Pt 2: 241-255, 2000.

10. **Bucci M, Gratton JP, Rudic RD, Acevedo L, Roviezzo F, Cirino G, and Sessa WC.** In vivo delivery of the caveolin-1 scaffolding domain inhibits nitric oxide synthesis and reduces inflammation. *Nat Med* 6: 1362-1367, 2000.
11. **Chamley-Campbell J, Campbell GR, and Ross R.** The smooth muscle cell in culture. *Physiol Rev* 59: 1-61, 1979.
12. **Cohen AW, Hnasko R, Schubert W, and Lisanti MP.** Role of caveolae and caveolins in health and disease. *Physiol Rev* 84: 1341-1379, 2004.
13. **Cohen AW, Park DS, Woodman SE, Williams TM, Chandra M, Shirani J, Pereira de Souza A, Kitsis RN, Russell RG, Weiss LM, Tang B, Jelicks LA, Factor SM, Shtutin V, Tanowitz HB, and Lisanti MP.** Caveolin-1 null mice develop cardiac hypertrophy with hyperactivation of p42/44 MAP kinase in cardiac fibroblasts. *Am J Physiol Cell Physiol* 284: C457-474, 2003.
14. **Cooper JA and Schafer DA.** Control of actin assembly and disassembly at filament ends. *Curr Opin Cell Biol* 12: 97-103, 2000.
15. **Coue M, Brenner SL, Spector I, and Korn ED.** Inhibition of actin polymerization by latrunculin A. *FEBS Lett* 213: 316-318, 1987.
16. **Davis MJ, Wu X, Nurkiewicz TR, Kawasaki J, Davis GE, Hill MA, and Meininger GA.** Integrins and mechanotransduction of the vascular myogenic response. *Am J Physiol Heart Circ Physiol* 280: H1427-1433, 2001.
17. **Dimmeler S, Assmus B, Hermann C, Haendeler J, and Zeiher AM.** Fluid shear stress stimulates phosphorylation of Akt in human endothelial cells: involvement in suppression of apoptosis. *Circ Res* 83: 334-341, 1998.
18. **Dimmeler S, Fleming I, Fisslthaler B, Hermann C, Busse R, and Zeiher AM.** Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation. *Nature* 399: 601-605, 1999.
19. **Drab M, Verkade P, Elger M, Kasper M, Lohn M, Lauterbach B, Menne J, Lindschau C, Mende F, Luft FC, Schedl A, Haller H, and Kurzchalia TV.** Loss of caveolae, vascular dysfunction, and pulmonary defects in caveolin-1 gene-disrupted mice. *Science* 293: 2449-2452, 2001.

20. **Du KL, Chen M, Li J, Lepore JJ, Mericko P, and Parmacek MS.** Megakaryoblastic leukemia factor-1 transduces cytoskeletal signals and induces smooth muscle cell differentiation from undifferentiated embryonic stem cells. *J Biol Chem* 279: 17578-17586, 2004.
21. **Du KL, Ip HS, Li J, Chen M, Dandre F, Yu W, Lu MM, Owens GK, and Parmacek MS.** Myocardin is a critical serum response factor cofactor in the transcriptional program regulating smooth muscle cell differentiation. *Mol Cell Biol* 23: 2425-2437, 2003.
22. **Dubroca C, Loyer X, Retailleau K, Loirand G, Pacaud P, Feron O, Balligand JL, Levy BI, Heymes C, and Henrion D.** RhoA activation and interaction with Caveolin-1 are critical for pressure-induced myogenic tone in rat mesenteric resistance arteries. *Cardiovasc Res* 73: 190-197, 2007.
23. **Eguchi S and Inagami T.** Signal transduction of angiotensin II type 1 receptor through receptor tyrosine kinase. *Regul Pept* 91: 13-20, 2000.
24. **Eguchi S, Iwasaki H, Ueno H, Frank GD, Motley ED, Eguchi K, Marumo F, Hirata Y, and Inagami T.** Intracellular signaling of angiotensin II-induced p70 S6 kinase phosphorylation at Ser(411) in vascular smooth muscle cells. Possible requirement of epidermal growth factor receptor, Ras, extracellular signal-regulated kinase, and Akt. *J Biol Chem* 274: 36843-36851, 1999.
25. **Engelman JA, Chu C, Lin A, Jo H, Ikezu T, Okamoto T, Kohtz DS, and Lisanti MP.** Caveolin-mediated regulation of signaling along the p42/44 MAP kinase cascade in vivo. A role for the caveolin-scaffolding domain. *FEBS Lett* 428: 205-211, 1998.
26. **Eskildsen-Helmond YE and Mulvany MJ.** Pressure-induced activation of extracellular signal-regulated kinase 1/2 in small arteries. *Hypertension* 41: 891-897, 2003.
27. **Feron O, Belhassen L, Kobzik L, Smith TW, Kelly RA, and Michel T.** Endothelial nitric oxide synthase targeting to caveolae. Specific interactions with caveolin isoforms in cardiac myocytes and endothelial cells. *J Biol Chem* 271: 22810-22814, 1996.
28. **Fleming I, Fisslthaler B, Dixit M, and Busse R.** Role of PECAM-1 in the shear-stress-induced activation of Akt and the endothelial nitric oxide synthase (eNOS) in endothelial cells. *J Cell Sci* 118: 4103-4111, 2005.

29. **Folkow B.** Physiological aspects of primary hypertension. *Physiol Rev* 62: 347-504, 1982.
30. **Folkow B, Grimby G, and Thulesius O.** Adaptive structural changes of the vascular walls in hypertension and their relation to the control of the peripheral resistance. *Acta Physiol Scand* 44: 255-272, 1958.
31. **Frank PG and Lisanti MP.** Role of caveolin-1 in the regulation of the vascular shear stress response. *J Clin Invest* 116: 1222-1225, 2006.
32. **Franki N, Ding G, Gao Y, and Hays RM.** Effect of cytochalasin D on the actin cytoskeleton of the toad bladder epithelial cell. *Am J Physiol* 263: C995-1000, 1992.
33. **Fu Y, Liu HW, Forsythe SM, Kogut P, McConville JF, Halayko AJ, Camoretti-Mercado B, and Solway J.** Mutagenesis analysis of human SM22: characterization of actin binding. *J Appl Physiol* 89: 1985-1990, 2000.
34. **Fulton D, Gratton JP, McCabe TJ, Fontana J, Fujio Y, Walsh K, Franke TF, Papapetropoulos A, and Sessa WC.** Regulation of endothelium-derived nitric oxide production by the protein kinase Akt. *Nature* 399: 597-601, 1999.
35. **Galbiati F, Volonte D, Engelman JA, Watanabe G, Burk R, Pestell RG, and Lisanti MP.** Targeted downregulation of caveolin-1 is sufficient to drive cell transformation and hyperactivate the p42/44 MAP kinase cascade. *Embo J* 17: 6633-6648, 1998.
36. **Garcia-Cardena G, Fan R, Stern DF, Liu J, and Sessa WC.** Endothelial nitric oxide synthase is regulated by tyrosine phosphorylation and interacts with caveolin-1. *J Biol Chem* 271: 27237-27240, 1996.
37. **Garcia-Cardena G, Martasek P, Masters BS, Skidd PM, Couet J, Li S, Lisanti MP, and Sessa WC.** Dissecting the interaction between nitric oxide synthase (NOS) and caveolin. Functional significance of the nos caveolin binding domain in vivo. *J Biol Chem* 272: 25437-25440, 1997.
38. **Garcia-Cardena G, Oh P, Liu J, Schnitzer JE, and Sessa WC.** Targeting of nitric oxide synthase to endothelial cell caveolae via palmitoylation: implications for nitric oxide signaling. *Proc Natl Acad Sci U S A* 93: 6448-6453, 1996.

39. **Geneste O, Copeland JW, and Treisman R.** LIM kinase and Diaphanous cooperate to regulate serum response factor and actin dynamics. *J Cell Biol* 157: 831-838, 2002.
40. **Gingras AC, Raught B, and Sonenberg N.** eIF4 initiation factors: effectors of mRNA recruitment to ribosomes and regulators of translation. *Annu Rev Biochem* 68: 913-963, 1999.
41. **Gottlieb PA, Suchyna TM, Ostrow LW, and Sachs F.** Mechanosensitive ion channels as drug targets. *Curr Drug Targets CNS Neurol Disord* 3: 287-295, 2004.
42. **Haga JH, Li YS, and Chien S.** Molecular basis of the effects of mechanical stretch on vascular smooth muscle cells. *J Biomech*, 2006.
43. **Halayko AJ, Kartha S, Stelmack GL, McConville J, Tam J, Camoretti-Mercado B, Forsythe SM, Hershenson MB, and Solway J.** Phosphatidylinositol-3 kinase/mammalian target of rapamycin/p70S6K regulates contractile protein accumulation in airway myocyte differentiation. *Am J Respir Cell Mol Biol* 31: 266-275, 2004.
44. **Hassan GS, Jasmin JF, Schubert W, Frank PG, and Lisanti MP.** Caveolin-1 deficiency stimulates neointima formation during vascular injury. *Biochemistry* 43: 8312-8321, 2004.
45. **Hayashi K, Takahashi M, Kimura K, Nishida W, Saga H, and Sobue K.** Changes in the balance of phosphoinositide 3-kinase/protein kinase B (Akt) and the mitogen-activated protein kinases (ERK/p38MAPK) determine a phenotype of visceral and vascular smooth muscle cells. *J Cell Biol* 145: 727-740, 1999.
46. **Hellstrand P and Albinsson S.** Stretch-dependent growth and differentiation in vascular smooth muscle: role of the actin cytoskeleton. *Can J Physiol Pharmacol* 83: 869-875, 2005.
47. **Hershey JW.** Protein phosphorylation controls translation rates. *J Biol Chem* 264: 20823-20826, 1989.
48. **Hixon ML, Muro-Cacho C, Wagner MW, Obejero-Paz C, Millie E, Fujio Y, Kureishi Y, Hassold T, Walsh K, and Gualberto A.** Akt1/PKB upregulation leads to

vascular smooth muscle cell hypertrophy and polyploidization. *J Clin Invest* 106: 1011-1020, 2000.

49. **Horowitz A, Menice CB, Laporte R, and Morgan KG.** Mechanisms of smooth muscle contraction. *Physiol Rev* 76: 967-1003, 1996.
50. **Ishida T, Peterson TE, Kovach NL, and Berk BC.** MAP kinase activation by flow in endothelial cells. Role of beta 1 integrins and tyrosine kinases. *Circ Res* 79: 310-316, 1996.
51. **Jakkaraju S, Zhe X, and Schuger L.** Role of stretch in activation of smooth muscle cell lineage. *Trends Cardiovasc Med* 13: 330-335, 2003.
52. **Jalali S, del Pozo MA, Chen K, Miao H, Li Y, Schwartz MA, Shyy JY, and Chien S.** Integrin-mediated mechanotransduction requires its dynamic interaction with specific extracellular matrix (ECM) ligands. *Proc Natl Acad Sci U S A* 98: 1042-1046, 2001.
53. **Johansson B.** Structural and functional changes in rat portal veins after experimental portal hypertension. *Acta Physiol Scand* 98: 381-383, 1976.
54. **Kawai-Kowase K and Owens GK.** Multiple repressor pathways contribute to phenotypic switching of vascular smooth muscle cells. *Am J Physiol Cell Physiol* 292: C59-69, 2007.
55. **Kondrikov D, Han HR, Block ER, and Su Y.** Growth and density-dependent regulation of NO synthase by the actin cytoskeleton in pulmonary artery endothelial cells. *Am J Physiol Lung Cell Mol Physiol* 290: L41-50, 2006.
56. **Koshida R, Rocic P, Saito S, Kiyooka T, Zhang C, and Chilian WM.** Role of focal adhesion kinase in flow-induced dilation of coronary arterioles. *Arterioscler Thromb Vasc Biol* 25: 2548-2553, 2005.
57. **Kumar A and Lindner V.** Remodeling with neointima formation in the mouse carotid artery after cessation of blood flow. *Arterioscler Thromb Vasc Biol* 17: 2238-2244, 1997.
58. **Langille BL and O'Donnell F.** Reductions in arterial diameter produced by chronic decreases in blood flow are endothelium-dependent. *Science* 231: 405-407, 1986.

59. **Le Lay S and Kurzchalia TV.** Getting rid of caveolins: phenotypes of caveolin-deficient animals. *Biochim Biophys Acta* 1746: 322-333, 2005.
60. **Lehoux S, Castier Y, and Tedgui A.** Molecular mechanisms of the vascular responses to haemodynamic forces. *J Intern Med* 259: 381-392, 2006.
61. **Lehoux S, Esposito B, Merval R, and Tedgui A.** Differential regulation of vascular focal adhesion kinase by steady stretch and pulsatility. *Circulation* 111: 643-649, 2005.
62. **Li C and Xu Q.** Mechanical stress-initiated signal transduction in vascular smooth muscle cells in vitro and in vivo. *Cell Signal*, 2007.
63. **Li C and Xu Q.** Mechanical stress-initiated signal transductions in vascular smooth muscle cells. *Cell Signal* 12: 435-445, 2000.
64. **Li S, Couet J, and Lisanti MP.** Src tyrosine kinases, Galpha subunits, and H-Ras share a common membrane-anchored scaffolding protein, caveolin. Caveolin binding negatively regulates the auto-activation of Src tyrosine kinases. *J Biol Chem* 271: 29182-29190, 1996.
65. **Liu HW, Halayko AJ, Fernandes DJ, Harmon GS, McCauley JA, Kocieniewski P, McConville J, Fu Y, Forsythe SM, Kogut P, Bellam S, Dowell M, Churchill J, Lesso H, Kassiri K, Mitchell RW, Hershenson MB, Camoretti-Mercado B, and Solway J.** The RhoA/Rho kinase pathway regulates nuclear localization of serum response factor. *Am J Respir Cell Mol Biol* 29: 39-47, 2003.
66. **Liu J, Garcia-Cardena G, and Sessa WC.** Palmitoylation of endothelial nitric oxide synthase is necessary for optimal stimulated release of nitric oxide: implications for caveolae localization. *Biochemistry* 35: 13277-13281, 1996.
67. **Mack CP, Somlyo AV, Hautmann M, Somlyo AP, and Owens GK.** Smooth muscle differentiation marker gene expression is regulated by RhoA-mediated actin polymerization. *J Biol Chem* 276: 341-347, 2001.
68. **Maekawa M, Ishizaki T, Boku S, Watanabe N, Fujita A, Iwamatsu A, Obinata T, Ohashi K, Mizuno K, and Narumiya S.** Signaling from Rho to the actin cytoskeleton through protein kinases ROCK and LIM-kinase. *Science* 285: 895-898, 1999.

69. **Malmqvist U and Arner A.** Contractile properties during development of hypertrophy of the smooth muscle in the rat portal vein. *Acta Physiol Scand* 133: 49-61, 1988.
70. **Malmqvist U and Arner A.** Isoform distribution and tissue contents of contractile and cytoskeletal proteins in hypertrophied smooth muscle from rat portal vein. *Circ Res* 66: 832-845, 1990.
71. **Miano JM.** Serum response factor: toggling between disparate programs of gene expression. *J Mol Cell Cardiol* 35: 577-593, 2003.
72. **Miralles F, Posern G, Zaromytidou AI, and Treisman R.** Actin dynamics control SRF activity by regulation of its coactivator MAL. *Cell* 113: 329-342, 2003.
73. **Mourani PM, Garl PJ, Wenzlau JM, Carpenter TC, Stenmark KR, and Weiser-Evans MC.** Unique, highly proliferative growth phenotype expressed by embryonic and neointimal smooth muscle cells is driven by constitutive Akt, mTOR, and p70S6K signaling and is actively repressed by PTEN. *Circulation* 109: 1299-1306, 2004.
74. **Mulvany MJ.** Small artery remodeling and significance in the development of hypertension. *News Physiol Sci* 17: 105-109, 2002.
75. **Mulvany MJ, Baumbach GL, Aalkjaer C, Heagerty AM, Korsgaard N, Schiffrin EL, and Heistad DD.** Vascular remodeling. *Hypertension* 28: 505-506, 1996.
76. **Nair RR, Solway J, and Boyd DD.** Expression cloning identifies transgelin (SM22) as a novel repressor of 92-kDa type IV collagenase (MMP-9) expression. *J Biol Chem* 281: 26424-26436, 2006.
77. **Nakano K, Takaishi K, Kodama A, Mammoto A, Shiozaki H, Monden M, and Takai Y.** Distinct actions and cooperative roles of ROCK and mDia in Rho small G protein-induced reorganization of the actin cytoskeleton in Madin-Darby canine kidney cells. *Mol Biol Cell* 10: 2481-2491, 1999.
78. **Narumiya S, Ishizaki T, and Watanabe N.** Rho effectors and reorganization of actin cytoskeleton. *FEBS Lett* 410: 68-72, 1997.
79. **Newby AC.** Matrix metalloproteinases regulate migration, proliferation, and death of vascular smooth muscle cells by degrading matrix and non-matrix substrates. *Cardiovasc Res* 69: 614-624, 2006.

80. **Newby AC and Zaltsman AB.** Molecular mechanisms in intimal hyperplasia. *J Pathol* 190: 300-309, 2000.
81. **North AJ, Gimona M, Lando Z, and Small JV.** Actin isoform compartments in chicken gizzard smooth muscle cells. *J Cell Sci* 107 (Pt 3): 445-455, 1994.
82. **Ono A, Kuwaki T, Kumada M, and Fujita T.** Differential central modulation of the baroreflex by salt loading in normotensive and spontaneously hypertensive rats. *Hypertension* 29: 808-814, 1997.
83. **Orlandi A, Ehrlich HP, Ropraz P, Spagnoli LG, and Gabbiani G.** Rat aortic smooth muscle cells isolated from different layers and at different times after endothelial denudation show distinct biological features in vitro. *Arterioscler Thromb* 14: 982-989, 1994.
84. **Osawa M, Masuda M, Kusano K, and Fujiwara K.** Evidence for a role of platelet endothelial cell adhesion molecule-1 in endothelial cell mechanosignal transduction: is it a mechanoresponsive molecule? *J Cell Biol* 158: 773-785, 2002.
85. **Owens GK.** Control of hypertrophic versus hyperplastic growth of vascular smooth muscle cells. *Am J Physiol* 257: H1755-1765, 1989.
86. **Owens GK.** Regulation of differentiation of vascular smooth muscle cells. *Physiol Rev* 75: 487-517, 1995.
87. **Papaioannou TG, Karatzis EN, Vavuranakis M, Lekakis JP, and Stefanadis C.** Assessment of vascular wall shear stress and implications for atherosclerotic disease. *Int J Cardiol* 113: 12-18, 2006.
88. **Park DS, Cohen AW, Frank PG, Razani B, Lee H, Williams TM, Chandra M, Shirani J, De Souza AP, Tang B, Jelicks LA, Factor SM, Weiss LM, Tanowitz HB, and Lisanti MP.** Caveolin-1 null (-/-) mice show dramatic reductions in life span. *Biochemistry* 42: 15124-15131, 2003.
89. **Park H, Go YM, Darji R, Choi JW, Lisanti MP, Maland MC, and Jo H.** Caveolin-1 regulates shear stress-dependent activation of extracellular signal-regulated kinase. *Am J Physiol Heart Circ Physiol* 278: H1285-1293, 2000.

90. **Parton RG and Simons K.** The multiple faces of caveolae. *Nat Rev Mol Cell Biol* 8: 185-194, 2007.
91. **Pinto YM, Paul M, and Ganten D.** Lessons from rat models of hypertension: from Goldblatt to genetic engineering. *Cardiovasc Res* 39: 77-88, 1998.
92. **Plopper GE, McNamee HP, Dike LE, Bojanowski K, and Ingber DE.** Convergence of integrin and growth factor receptor signaling pathways within the focal adhesion complex. *Mol Biol Cell* 6: 1349-1365, 1995.
93. **Pollard TD, Blanchoin L, and Mullins RD.** Molecular mechanisms controlling actin filament dynamics in nonmuscle cells. *Annu Rev Biophys Biomol Struct* 29: 545-576, 2000.
94. **Posern G, Miralles F, Guettler S, and Treisman R.** Mutant actins that stabilise F-actin use distinct mechanisms to activate the SRF coactivator MAL. *Embo J* 23: 3973-3983, 2004.
95. **Posern G, Sotiropoulos A, and Treisman R.** Mutant actins demonstrate a role for unpolymerized actin in control of transcription by serum response factor. *Mol Biol Cell* 13: 4167-4178, 2002.
96. **Posern G and Treisman R.** Actin' together: serum response factor, its cofactors and the link to signal transduction. *Trends Cell Biol* 16: 588-596, 2006.
97. **Prewitt RL, Rice DC, and Dobrian AD.** Adaptation of resistance arteries to increases in pressure. *Microcirculation* 9: 295-304, 2002.
98. **Price MA, Rogers AE, and Treisman R.** Comparative analysis of the ternary complex factors Elk-1, SAP-1a and SAP-2 (ERP/NET). *Embo J* 14: 2589-2601, 1995.
99. **Qin H, Ishiwata T, Wang R, Kudo M, Yokoyama M, Naito Z, and Asano G.** Effects of extracellular matrix on phenotype modulation and MAPK transduction of rat aortic smooth muscle cells in vitro. *Exp Mol Pathol* 69: 79-90, 2000.
100. **Rasmussen LE, Vanhoutte PM, Jensen BL, and Skott O.** Continuous flow augments reactivity of rabbit carotid artery by reducing bioavailability of NO despite an increase in release of EDHF. *Am J Physiol Heart Circ Physiol* 291: H1521-1528, 2006.

101. **Razani B, Engelman JA, Wang XB, Schubert W, Zhang XL, Marks CB, Macaluso F, Russell RG, Li M, Pestell RG, Di Vizio D, Hou H, Jr., Kneitz B, Lagaud G, Christ GJ, Edelmann W, and Lisanti MP.** Caveolin-1 null mice are viable but show evidence of hyperproliferative and vascular abnormalities. *J Biol Chem* 276: 38121-38138, 2001.
102. **Razani B, Wang XB, Engelman JA, Battista M, Lagaud G, Zhang XL, Kneitz B, Hou H, Jr., Christ GJ, Edelmann W, and Lisanti MP.** Caveolin-2-deficient mice show evidence of severe pulmonary dysfunction without disruption of caveolae. *Mol Cell Biol* 22: 2329-2344, 2002.
103. **Regan CP, Adam PJ, Madsen CS, and Owens GK.** Molecular mechanisms of decreased smooth muscle differentiation marker expression after vascular injury. *J Clin Invest* 106: 1139-1147, 2000.
104. **Relou IA, Damen CA, van der Schaft DW, Groenewegen G, and Griffioen AW.** Effect of culture conditions on endothelial cell growth and responsiveness. *Tissue Cell* 30: 525-530, 1998.
105. **Reusch P, Wagdy H, Reusch R, Wilson E, and Ives HE.** Mechanical strain increases smooth muscle and decreases nonmuscle myosin expression in rat vascular smooth muscle cells. *Circ Res* 79: 1046-1053, 1996.
106. **Rice DC, Dobrian AD, Schriver SD, and Prewitt RL.** Src autophosphorylation is an early event in pressure-mediated signaling pathways in isolated resistance arteries. *Hypertension* 39: 502-507, 2002.
107. **Rizzo V, McIntosh DP, Oh P, and Schnitzer JE.** In situ flow activates endothelial nitric oxide synthase in luminal caveolae of endothelium with rapid caveolin dissociation and calmodulin association. *J Biol Chem* 273: 34724-34729, 1998.
108. **Rizzo V, Sung A, Oh P, and Schnitzer JE.** Rapid mechanotransduction in situ at the luminal cell surface of vascular endothelium and its caveolae. *J Biol Chem* 273: 26323-26329, 1998.
109. **Rocic P, Govindarajan G, Sabri A, and Lucchesi PA.** A role for PYK2 in regulation of ERK1/2 MAP kinases and PI 3-kinase by ANG II in vascular smooth muscle. *Am J Physiol Cell Physiol* 280: C90-99, 2001.

110. **Romer LH, Birukov KG, and Garcia JG.** Focal adhesions: paradigm for a signaling nexus. *Circ Res* 98: 606-616, 2006.
111. **Rothman A, Wolner B, Button D, and Taylor P.** Immediate-early gene expression in response to hypertrophic and proliferative stimuli in pulmonary arterial smooth muscle cells. *J Biol Chem* 269: 6399-6404, 1994.
112. **Rudic RD, Shesely EG, Maeda N, Smithies O, Segal SS, and Sessa WC.** Direct evidence for the importance of endothelium-derived nitric oxide in vascular remodeling. *J Clin Invest* 101: 731-736, 1998.
113. **Sarjeant JM and Rabinovitch M.** Understanding and treating vein graft atherosclerosis. *Cardiovasc Pathol* 11: 263-271, 2002.
114. **Saward L and Zahradka P.** Angiotensin II activates phosphatidylinositol 3-kinase in vascular smooth muscle cells. *Circ Res* 81: 249-257, 1997.
115. **Schachner T, Laufer G, and Bonatti J.** In vivo (animal) models of vein graft disease. *Eur J Cardiothorac Surg* 30: 451-463, 2006.
116. **Schubert W, Frank PG, Woodman SE, Hyogo H, Cohen DE, Chow CW, and Lisanti MP.** Microvascular hyperpermeability in caveolin-1 (-/-) knock-out mice. Treatment with a specific nitric-oxide synthase inhibitor, L-NAME, restores normal microvascular permeability in Cav-1 null mice. *J Biol Chem* 277: 40091-40098, 2002.
117. **Sedding DG, Hermsen J, Seay U, Eickelberg O, Kummer W, Schwencke C, Strasser RH, Tillmanns H, and Braun-Dullaeus RC.** Caveolin-1 facilitates mechanosensitive protein kinase B (Akt) signaling in vitro and in vivo. *Circ Res* 96: 635-642, 2005.
118. **Servant MJ, Giasson E, and Meloche S.** Inhibition of growth factor-induced protein synthesis by a selective MEK inhibitor in aortic smooth muscle cells. *J Biol Chem* 271: 16047-16052, 1996.
119. **Sessa WC.** Atheroprotection in the absence of "caves": is it the fat, the vessels, or both? *Arterioscler Thromb Vasc Biol* 24: 4-6, 2004.
120. **Shah PJ, Gordon I, Fuller J, Seevanayagam S, Rosalion A, Tatoulis J, Raman JS, and Buxton BF.** Factors affecting saphenous vein graft patency: clinical and

- angiographic study in 1402 symptomatic patients operated on between 1977 and 1999. *J Thorac Cardiovasc Surg* 126: 1972-1977, 2003.
121. **Shakirova Y, Bonnevier J, Albinsson S, Adner M, Rippe B, Broman J, Arner A, and Sward K.** Increased Rho activation and PKC-mediated smooth muscle contractility in the absence of caveolin-1. *Am J Physiol Cell Physiol* 291: C1326-1335, 2006.
122. **Shaul PW, Smart EJ, Robinson LJ, German Z, Yuhanna IS, Ying Y, Anderson RG, and Michel T.** Acylation targets endothelial nitric-oxide synthase to plasmalemmal caveolae. *J Biol Chem* 271: 6518-6522, 1996.
123. **Shi Y, O'Brien JE, Jr., Mannion JD, Morrison RC, Chung W, Fard A, and Zalewski A.** Remodeling of autologous saphenous vein grafts. The role of perivascular myofibroblasts. *Circulation* 95: 2684-2693, 1997.
124. **Small JV and Gimona M.** The cytoskeleton of the vertebrate smooth muscle cell. *Acta Physiol Scand* 164: 341-348, 1998.
125. **Sobue K, Hayashi K, and Nishida W.** Molecular mechanism of phenotypic modulation of smooth muscle cells. *Horm Res* 50 Suppl 2: 15-24, 1998.
126. **Solway J, Seltzer J, Samaha FF, Kim S, Alger LE, Niu Q, Morrissey EE, Ip HS, and Parmacek MS.** Structure and expression of a smooth muscle cell-specific gene, SM22 alpha. *J Biol Chem* 270: 13460-13469, 1995.
127. **Sonveaux P, Martinive P, DeWever J, Batova Z, Daneau G, Pelat M, Ghisdal P, Gregoire V, Dessy C, Balligand JL, and Feron O.** Caveolin-1 expression is critical for vascular endothelial growth factor-induced ischemic hindlimb collateralization and nitric oxide-mediated angiogenesis. *Circ Res* 95: 154-161, 2004.
128. **Sotiropoulos A, Gineitis D, Copeland J, and Treisman R.** Signal-regulated activation of serum response factor is mediated by changes in actin dynamics. *Cell* 98: 159-169, 1999.
129. **Spassova MA, Hewavitharana T, Xu W, Soboloff J, and Gill DL.** A common mechanism underlies stretch activation and receptor activation of TRPC6 channels. *Proc Natl Acad Sci U S A* 103: 16586-16591, 2006.

130. **Spector I, Braet F, Shochet NR, and Bubb MR.** New anti-actin drugs in the study of the organization and function of the actin cytoskeleton. *Microsc Res Tech* 47: 18-37, 1999.
131. **Spector I, Shochet NR, Kashman Y, and Groweiss A.** Latrunculins: novel marine toxins that disrupt microfilament organization in cultured cells. *Science* 219: 493-495, 1983.
132. **Spofford CM and Chilian WM.** The elastin-laminin receptor functions as a mechanotransducer in vascular smooth muscle. *Am J Physiol Heart Circ Physiol* 280: H1354-1360, 2001.
133. **Suchyna TM, Tape SE, Koeppe RE, II, Andersen OS, Sachs F, and Gottlieb PA.** Bilayer-dependent inhibition of mechanosensitive channels by neuroactive peptide enantiomers. *Nature* 430: 235-240, 2004.
134. **Takahashi E and Berk BC.** MAP kinases and vascular smooth muscle function. *Acta Physiol Scand* 164: 611-621, 1998.
135. **Thie M, Harrach B, Schonherr E, Kresse H, Robenek H, and Rauterberg J.** Responsiveness of aortic smooth muscle cells to soluble growth mediators is influenced by cell-matrix contact. *Arterioscler Thromb* 13: 994-1004, 1993.
136. **Thie M, Schlumberger W, Semich R, Rauterberg J, and Robenek H.** Aortic smooth muscle cells in collagen lattice culture: effects on ultrastructure, proliferation and collagen synthesis. *Eur J Cell Biol* 55: 295-304, 1991.
137. **Thie M, Schussler B, Robenek H, and Zidek W.** Differential regulation of protein synthesis in smooth muscle cells from normotensive and spontaneously hypertensive rats by a three-dimensional matrix of type I collagen. *J Hypertens* 10: 77-82, 1992.
138. **Thubrikar MJ and Robicsek F.** Pressure-induced arterial wall stress and atherosclerosis. *Ann Thorac Surg* 59: 1594-1603, 1995.
139. **Thyberg J.** Differentiated properties and proliferation of arterial smooth muscle cells in culture. *Int Rev Cytol* 169: 183-265, 1996.
140. **Traub O and Berk BC.** Laminar shear stress: mechanisms by which endothelial cells transduce an atheroprotective force. *Arterioscler Thromb Vasc Biol* 18: 677-685, 1998.

141. **Tronc F, Wassef M, Esposito B, Henrion D, Glagov S, and Tedgui A.** Role of NO in flow-induced remodeling of the rabbit common carotid artery. *Arterioscler Thromb Vasc Biol* 16: 1256-1262, 1996.
142. **Tzima E, Irani-Tehrani M, Kiosses WB, Dejana E, Schultz DA, Engelhardt B, Cao G, DeLisser H, and Schwartz MA.** A mechanosensory complex that mediates the endothelial cell response to fluid shear stress. *Nature* 437: 426-431, 2005.
143. **Uehata M, Ishizaki T, Satoh H, Ono T, Kawahara T, Morishita T, Tamakawa H, Yamagami K, Inui J, Maekawa M, and Narumiya S.** Calcium sensitization of smooth muscle mediated by a Rho-associated protein kinase in hypertension. *Nature* 389: 990-994, 1997.
144. **Wang Z, Wang DZ, Hockemeyer D, McAnally J, Nordheim A, and Olson EN.** Myocardin and ternary complex factors compete for SRF to control smooth muscle gene expression. *Nature* 428: 185-189, 2004.
145. **Vanhoutte PM, Boulanger CM, and Mombouli JV.** Endothelium-derived relaxing factors and converting enzyme inhibition. *Am J Cardiol* 76: 3E-12E, 1995.
146. **Watanabe N, Kato T, Fujita A, Ishizaki T, and Narumiya S.** Cooperation between mDia1 and ROCK in Rho-induced actin reorganization. *Nat Cell Biol* 1: 136-143, 1999.
147. **Watts SW, Rondelli C, Thakali K, Li X, Uhal B, Pervaiz MH, Watson RE, and Fink GD.** Morphologic and Biochemical Characterization of Remodeling in Aorta and Vena Cava of DOCA-salt hypertensive rats. *Am J Physiol Heart Circ Physiol*, 2007.
148. **Wernig F, Mayr M, and Xu Q.** Mechanical stretch-induced apoptosis in smooth muscle cells is mediated by beta1-integrin signaling pathways. *Hypertension* 41: 903-911, 2003.
149. **Wesselman JPM, Dobrian AD, Schriver SD, and Prewitt RL.** Src Tyrosine Kinases and Extracellular Signal-Regulated Kinase 1/2 Mitogen-Activated Protein Kinases Mediate Pressure-Induced C-Fos Expression in Cannulated Rat Mesenteric Small Arteries. *Hypertension* 37: 955-960, 2001.
150. **Xu Q, Liu Y, Gorospe M, Udelsman R, and Holbrook NJ.** Acute hypertension activates mitogen-activated protein kinases in arterial wall. *J Clin Invest* 97: 508-514, 1996.

151. **Yu J, Bergaya S, Murata T, Alp IF, Bauer MP, Lin MI, Drab M, Kurzchalia TV, Stan RV, and Sessa WC.** Direct evidence for the role of caveolin-1 and caveolae in mechanotransduction and remodeling of blood vessels. *J Clin Invest* 116: 1284-1291, 2006.
152. **Zeidan A, Broman J, Hellstrand P, and Sward K.** Cholesterol Dependence of Vascular ERK1/2 Activation and Growth in Response to Stretch: Role of Endothelin-1. *Arterioscler Thromb Vasc Biol* 23: 1528-1534, 2003.
153. **Zeidan A, Nordstrom I, Albinsson S, Malmqvist U, Sward K, and Hellstrand P.** Stretch-induced contractile differentiation of vascular smooth muscle: sensitivity to actin polymerization inhibitors. *Am J Physiol Cell Physiol* 284: C1387-1396, 2003.
154. **Zeidan A, Sward K, Nordstrom I, Ekblad E, Zhang JC, Parmacek MS, and Hellstrand P.** Ablation of SM22alpha decreases contractility and actin contents of mouse vascular smooth muscle. *FEBS Lett* 562: 141-146, 2004.
155. **Zeidan Aa, Nordstrom I, Dreja K, Malmqvist U, and Hellstrand P.** Stretch-Dependent Modulation of Contractility and Growth in Smooth Muscle of Rat Portal Vein. *Circ Res* 87: 228-234, 2000.