Smoothelin-B deficiency results in reduced arterial contractility, hypertension, and cardiac hypertrophy in mice

Citation for published version (APA):

Document status and date:
Published: 01/01/2008

DOI:
10.1161/CIRCULATIONAHA.107.743690

Document Version:
Publisher's PDF, also known as Version of record

Document license:
Taverne

Please check the document version of this publication:

- A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
- The final author version and the galley proof are versions of the publication after peer review.
- The final published version features the final layout of the paper including the volume, issue and page numbers.

Link to publication

General rights
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.

If the publication is distributed under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license above, please follow below link for the End User Agreement:
www.umlib.nl/taverne-license

Take down policy
If you believe that this document breaches copyright please contact us at:
repository@maastrichtuniversity.nl
providing details and we will investigate your claim.

Download date: 21 Oct. 2023
Hypertension

Smoothelin-B Deficiency Results in Reduced Arterial Contractility, Hypertension, and Cardiac Hypertrophy in Mice

Sander S. Rensen, PhD; Petra M. Niessen, PhD; Jan M. van Deursen, PhD; Ben J. Janssen, PhD; Edwin Heijman, MS; Evelien Hermeling, MS; Merlijn Meens, MS; Natascha Lie, MD; Marion J. Gijbels, PhD; Gustav J. Strijkers, PhD; Pieter A. Doevendans, MD, PhD; Marten H. Hofker, PhD; Jo G.R. De Mey, PhD; Guillaume J. van Eys, PhD

Background—Smoothelins are actin-binding proteins that are abundantly expressed in healthy visceral (smoothelin-A) and vascular (smoothelin-B) smooth muscle. Their expression is strongly associated with the contractile phenotype of smooth muscle cells. Analysis of mice lacking both smoothelins (Smtn-A/B−/− mice) previously revealed a critical role for smoothelin-A in intestinal smooth muscle contraction. Here, we report on the generation and cardiovascular phenotype of mice lacking only smoothelin-B (Smtn-B−/−).

Methods and Results—Myograph studies revealed that the contractile capacity of the saphenous and femoral arteries was strongly reduced in Smtn-B−/− mice, regardless of the contractile agonist used to trigger contraction. Arteries from Smtn-A/B−/− compound mutant mice exhibited a similar contractile deficit. Smtn-B−/− arteries had a normal architecture and expressed normal levels of other smooth muscle cell–specific genes, including smooth muscle myosin heavy chain, α-smooth muscle actin, and smooth muscle-calponin. Decreased contractility of Smtn-B−/− arteries was paradoxically accompanied by increased mean arterial pressure (20 mm Hg) and concomitant cardiac hypertrophy despite normal parasympathetic and sympathetic tone in Smtn-B−/− mice. Magnetic resonance imaging experiments revealed that cardiac function was not changed, whereas distension of the proximal aorta during the cardiac cycle was increased in Smtn-B−/− mice. However, isobaric pulse wave velocity and pulse pressure measurements indicated normal aortic distensibility.

Conclusions—Collectively, our results identify smoothelins as key determinants of arterial smooth muscle contractility and cardiovascular performance. Studies on mutations in the Smtn gene or alterations in smoothelin levels in connection to hypertension in humans are warranted.

Key Words: hypertension ■ hypertrophy ■ muscle contraction ■ muscle, smooth ■ vascular resistance

Hypertension is a common condition in Western countries, affecting ≈27% of the population worldwide. It is a major risk factor for the development of life-threatening conditions such as coronary heart disease and stroke. However, the cause of increased blood pressure is unknown in most patients.1 Smooth muscle contractility is one of the primary determinants of vascular resistance, thereby contributing significantly to the maintenance of a physiological blood pressure. Accordingly, molecular defects in the regulation or mechanics of arterial smooth muscle contraction generally cause profound cardiovascular phenotypes. For example, α-smooth muscle actin (α-SMA) knockout mice display impaired vascular contractility and reduced blood flow.2 Likewise, smooth muscle myosin heavy chain (SM-MHC)-B knockouts show a significant decrease in maximal shortening velocity of vascular smooth muscle,3 and SM-calponin–deficient mice have impaired mean arterial pressure (MAP) regulation.4

Clinical Perspective p 836

Despite the importance of smooth muscle cell (SMC) contraction for the cardiovascular system, the contractile process itself is still incompletely understood. In particular, the functions of regulatory proteins that are connected to the actin-myosin filaments in vascular SMCs remain poorly defined.5 Candidate thin filament regulatory proteins that have not been studied in this respect are the smoothelins, which are α-SMA–binding proteins...
and mutant (bottom) locus is shown. B, Left, Southern blot analyses of genomic DNA from wild-type and targeted ES cells after SacI digestion. The distance between the SacI sites in the wild-type (top) and mutant (bottom) locus is shown. B, Left; Southern blot analyses of genomic DNA from wild-type and targeted ES cells after SacI digestion with the probe indicated above (in A). The wild-type SacI fragment (WT) is 7.3 kb; the mutant SacI fragment (I) is 6.1 kb. Right, PCR analyses of genomic DNA from Smtn-B+/−, Smtn-B−/− and Smtn-B−/+ mice. The +/+ and −/− PCR fragments are 191 and 240 bp, respectively. C, Q-PCR analysis showing absent smoothelin-B expression in aorta, femoral artery, and jejunum of Smtn-B−/− mice (left; n=6 for each genotype). Q-PCR experiments using primers and probes directed against the part that both smoothelins have in common revealed upregulated expression of either smoothelin-A or an interrupted smoothelin-B transcript in blood vessels but not in jejunum (right; n=6 for each genotype). D, Cross sections of aortas of Smtn-B+/+ and Smtn-B−/+ mice were stained for smoothelin-A and -B (green; ×400 magnification). The cells between the autofluorescent elastic laminae do not stain, showing that smoothelins are not present in vascular SMCs of Smtn-B−/+ mice. Right, Autofluorescence of the elastic laminae is shown in red.

Figure 1. Targeting of the Smtn gene for the generation of mice lacking smoothelin-B. A, Schematic of the targeting strategy. Top, Structure of the Smtn gene. Black boxes indicate smoothelin-B exons; light gray boxes, exons common to smoothelin-A and -B; and white boxes, targeted exons. Middle, Smtn-B−/− targeting vector containing neomycin resistance (NEO) and herpes simplex virus thymidine kinase (TK) genes, both transcribed in the reverse direction to that of the Smtn gene, as indicated by arrows. Bottom, Structure of the targeted Smtn allele and location of the probe used in Southern blot analyses after SacI digestion. The distance between the SacI sites in the wild-type (top) and mutant (bottom) locus is shown. B, Left; Southern blot analyses of genomic DNA from wild-type and targeted ES cells after SacI digestion with the probe indicated above (in A). The wild-type SacI fragment (WT) is 7.3 kb; the mutant SacI fragment (I) is 6.1 kb. Right, PCR analyses of genomic DNA from Smtn-B+/−, Smtn-B−/− and Smtn-B−/+ mice. The +/+ and −/− PCR fragments are 191 and 240 bp, respectively. C, Q-PCR analysis showing absent smoothelin-B expression in aorta, femoral artery, and jejunum of Smtn-B−/− mice (left; n=6 for each genotype). Q-PCR experiments using primers and probes directed against the part that both smoothelins have in common revealed upregulated expression of either smoothelin-A or an interrupted smoothelin-B transcript in blood vessels but not in jejunum (right; n=6 for each genotype). D, Cross sections of aortas of Smtn-B+/+ and Smtn-B−/+ mice were stained for smoothelin-A and -B (green; ×400 magnification). The cells between the autofluorescent elastic laminae do not stain, showing that smoothelins are not present in vascular SMCs of Smtn-B−/+ mice. Right, Autofluorescence of the elastic laminae is shown in red.

proteins specifically and abundantly expressed in contractile SMCs. They are encoded by a single-copy gene that generates 2 major isoforms, both containing a troponin T–like domain. The smaller smoothelin-A isoform is expressed most prominently in visceral SMCs. In contrast, the 110-kDa smoothelin-B, which is encoded by the smoothelin-A exons plus 10 upstream exons, is found only in vascular SMCs. Smoothelin-B expression is particularly high in muscular arteries, whereas expression in elastic arteries is modest.

In recent years, smoothelin-B has been increasingly recognized as an excellent marker of the so-called contractile phenotype of vascular SMCs. Indeed, loss of smoothelin-B expression reliably indicates the disappearance of the contractile SMC phenotype in various vascular disorders ranging from aortic aneurysms to atherosclerosis and restenosis.

Functional studies on smoothelins have been hampered by the rapid downregulation of their expression in vitro and their relative insolubility at physiological ionic concentrations. Therefore, despite its relevance for the characterization of the contractile SMC phenotype, the function of smoothelin-B in vascular SMCs has remained elusive. Recently, however, we showed that smoothelin-A plays a crucial role in intestinal SMC contraction in mice. Smtn-A/B−/− mice, which lack both smoothelin isoforms, develop fatal intestinal problems as a result of drastically decreased intestinal SMC contractility. The severe impact of smoothelin-A deficiency on visceral smooth muscle contraction suggests that smoothelin-B might play an equally important role in vascular smooth muscle. To test this hypothesis, we generated mice lacking only smoothelin-B (Smtn-B−/−) and investigated their cardiovascular physiology. We report that smoothelin-B−/− mice show reduced arterial contractility, which is paradoxically accompanied by elevated MAP because of increased peripheral vascular resistance.

Methods

Generation of Smtn-B−/− Mice

To generate Smtn-B−/− mice, we targeted exons 3 through 7 with a neomycin gene under the control of the thymidine kinase promoter in reverse orientation (Figure 1A). Targeting of embryonic stem cells and germline transmission of the targeted alleles were detected by Southern blotting and polymerase chain reaction (PCR) analysis (Figure 1B; see the online-only Data Supplement for a list of primers). Mice were further backcrossed at least 5 times on C57Bl/6 background before initiation of experiments. Because the mice had a mixed background, we used littermates for experimentation. All animal experiments were approved by the Maastricht University animal ethics committee.

Quantitative Reverse-Transcriptase PCR

Total RNA was extracted from jejunum, hearts, or pooled aortas and femoral arteries from Smtn-B+/+ and Smtn-B−/− mice with Tri-reagent (Sigma-Aldrich, Zwijndrecht, the Netherlands). Reverse
transcription was performed with the iScript cDNA synthesis kit (Biorad, Veenendaal, the Netherlands) and 0.5 or 1 μg RNA. Expression of several transcripts was investigated by quantitative PCR (Q-PCR) with the ABIPrism7700 System (Perkin Elmer, Norwalk, Conn). Applied primers and probes are listed in the supplemental table. The cyclophilin A transcript was used to normalize the amount and quality of the extracted RNA. Smtn-B**−/−** expression levels were set at 1.

**Histology and Immunohistochemistry**

Organs from mice 2 months and 1 year of age were fixed in 3.7% formaldehyde in PBS, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Samples of aorta and femoral artery were snap-frozen and embedded in optical coherence tomography Tissue Tek compound (Sakura, Chicago, Ill). Cryostat sections were blocked with 5% normal donkey serum (Jackson ImmunoResearch, Soham, UK) and stained with a polyclonal antibody raised against 2 smoothelin-specific peptides (described below) and a secondary antibody donkey-anti-rabbit conjugated with FITC (Sigma-Aldrich, St Louis, Mo).

Polyclonal antibody generation was performed by Eurogentec (Seraing, Belgium). Two rabbits were immunized with the smoothelin-B-specific peptide KRFRERQDNKENWL (smoothelin-B residues 52 to 66) and the peptide RQRKRDQDKERERR, which is present in both smoothelin-A and smoothelin-B (smoothelin-A residues 160 to 174, smoothelin-B residues 616 to 628). Synthesized peptides (5 mg) were conjugated to keyhole limpet hemocyanin. The rabbits received 3 booster injections with a 14-day interval. Sera were tested on different tissues for cross-reactivity and smooth muscle specificity.

To determine the staining pattern of the extracellular matrix proteins collagen and elastin, paraffin-embedded cross sections of arteries were stained with Sirius Red and Lawson’s solution. To evaluate α-SMA and SM-MHC expression, sections were incubated with anti-α-SMA (DAKO, Glostrup, Denmark) or anti–SM-MHC antibody (Biomedical Technologies, Stoughton, Mass) diluted 1:200 or 1:40, respectively.

**Vascular Contractility**

Contractility of the thoracic aorta (n = 10), saphenous artery (n = 4), and femoral artery (n = 6) was compared between 10-week-old Smtn-B**−/−** and Smtn-B**+/−** littermates. For the contractility analyses of Smtn-B**+/−** and Smtn-B**−/−** littermates, mice ≈8 weeks of age were used (n = 7 for thoracic aorta and femoral artery). Isolated arteries were mounted in myograph organ baths as previously described. We examined reactivity in response to KCl (40 mmol/L), the thromboxane A2 mimetic U-46619 (0.1 to 100 nmol/L), and the α1-adrenergic agonist phenylephrine (10−6 to 30 μmol/L), all obtained from Sigma-Aldrich. Contractile forces were corrected for vessel segment length and medial thickness and normalized to wild-type values.

**Hemodynamics**

Five-month-old male Smtn-B**−/−** (n = 7) and Smtn-B**+/−** (n = 10) mice were instrumented with catheters as described before, and conscious MAP and heart rate (HR) were recorded for 30 to 60 minutes on days 3 and 5 after surgery. In addition, the contribution of several endogenous neurohumoral mechanisms to blood pressure was assessed by the following pharmacological protocol. On day 3, MAP and HR responses were recorded during intravenous injection of phenylephrine (dose-response curve, 0.1 to 10 μg/kg in the presence of atropine [1.2 mg/kg] to block baroreflex-mediated bradycardia) and after administration of the β-blocker metoprolol (2.5 mg/kg). On day 5, MAP and HR responses to the α1 blocker prazosin (0.1 mg/kg) and the α1 blocker yohimbine (1 mg/kg) were recorded.

Pulse-wave velocity and pulse pressure were measured under isoflurane anesthesia by a high-fidelity catheter-tip micromanometer (Mikro-tip 1.4 F SPR-671, Millar Instruments, Houston, Tex) that was inserted via the left carotid artery into the aorta of Smtn-B**−/−** (n = 7) and Smtn-B**+/−** (n = 5) mice.

Because of their physical condition and short lifespan, hemodynamic parameters of Smtn-B**+/−** and Smtn-B**−/−** mice were measured in 6-week-old females under 1% to 2% isoflurane anesthesia via a catheter introduced into the right carotid artery (n = 5 for each genotype).

**Magnetic Resonance Imaging**

Magnetic resonance imaging measurements were performed in eleven 8-month-old male mice of each genotype with a 6.3-T horizontal-bore animal scanner (Bruker BioSpin, Ettlingen, Germany) and a 3-cm-diameter birdcage radiofrequency coil (Rapid Biomedical, Rimpar, Germany). End-diastolic volume, end-systolic volume, stroke volume, ejection fraction, and left ventricular mass (1.05 g/cm3) were calculated from a semiautomated segmentation of the images with the FARM MRV CAAS software provided by Pie Medical Imaging (Maastricht, the Netherlands).

To determine the distension of the descending thoracic aorta during the cardiac cycle, a modified fast low-angle shot sequence was used with an in-plane navigator echo. The distension of the thoracic aorta was measured manually at a fixed position for 7 Smtn-B**+/−** and 5 Smtn-B**−/−** mice. From these data, the relative distension as function of time and the maximum distension were derived.

**Statistical Analysis**

Statistical significance was calculated by 2-tailed Student t tests or repeated-measures 2-way ANOVA as indicated with GraphPad Prism software (version 4.0) and SPSS software (version 15.0, SPSS Inc, Chicago, Ill). Results were considered significantly different at values of P < 0.05. Values are expressed as mean ± SEM. The online-only Data Supplement provides a more detailed Methods section.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

**Results**

**Smtn-B**−/−** Mice Develop Normally, Expressing Smoothelin-A but No Intact Smoothelin-B**

To generate Smtn-B**−/−** mice, we replaced Smtn exons 3 through 7 with a neomycin-resistance cassette (Figure 1A and 1B). This deletion disrupts the smoothelin-B gene while leaving the smoothelin-A promoter intact. Intercrosses of Smtn-B**−/−** mice produced Smtn-B**+/−** offspring at mendelian frequency (Smtn-B**+/−**, 28%; Smtn-B**−/−**, 50%; Smtn-B**+/+**, 22%; n = 506), indicating that loss of smoothelin-B does not interfere with embryonic survival. In contrast to Smtn-A/B**−/−** mice, which die rapidly after birth, Smtn-B**+/−** mice appeared normal, had no gross histological abnormalities in any organ as evaluated by an experienced animal pathologist, and had unaltered intestinal function.

Q-PCR analyses showed an absence of intact smoothelin-B transcripts in the aorta, femoral artery, and jejunum of Smtn-B**−/−** mice (Figure 1C). On the other hand, upregulation of either smoothelin-A or an interrupted smoothelin-B transcript still containing its 3′ smoothelin-A sequence was found in Smtn-B**−/−** blood vessels (Figure 1C). However, smoothelins were not detectable in the aorta of Smtn-B**−/−** mice by immunohistochemical stainings with polyclonal antibodies recognizing both smoothelin-A and smoothelin-B17,22 mice expressed normal, had no gross histological abnormalities in any organ as evaluated by an experienced animal pathologist, and had unaltered intestinal function.

Q-PCR analyses showed an absence of intact smoothelin-B transcripts in the aorta, femoral artery, and jejunum of Smtn-B**−/−** mice (Figure 1C). On the other hand, upregulation of either smoothelin-A or an interrupted smoothelin-B transcript still containing its 3′ smoothelin-A sequence was found in Smtn-B**−/−** blood vessels (Figure 1C). However, smoothelins were not detectable in the aorta of Smtn-B**−/−** mice by immunohistochemical stainings with polyclonal antibodies recognizing both smoothelin-A and smoothelin-B (Figure 1D). Thus, Smtn-B**−/−** mice display a null mutation in vascular tissue and can be used to delineate the role of smoothelin-B in vascular smooth muscle function. As predicted, Smtn-B**−/−** mice displayed normal smoothelin expression in visceral tissues (Figure 1C).

**Normal Contractile Gene Expression and Normal Arterial Structure in Smtn-B−/− Mice**

To study whether levels of important contractile smooth muscle–specific genes were altered by the loss of smoothelin-B, we examined the expression of α-SMA, SM-MHC, and SM-calponin in arteries of 6-month-old mice by...
Q-PCR (n=6 for each genotype). The expression of these components of the SMC contractile machinery was not significantly changed at the mRNA level (Figure 2A). In addition, both α-SMA (Figure 2B) and SM-MHC proteins (S.S.R., unpublished data, 2007) were readily detectable in medial SMCs of Smtn-B−/−/− mice. Moreover, the femoral artery and aorta of Smtn-B−/−/− mice appeared histologically normal and had a similar medial cross-sectional area and vessel radius compared with Smtn-B−/−/− mice (cross-sectional area of aorta, 0.086±0.004 versus 0.075±0.004 mm²; cross-sectional area of femoral artery, 0.0107±0.0005 versus 0.0101±0.0005 mm²; radius of aorta, 367±4 versus 349±10 μm; radius of femoral artery, 145±5 versus 143±5 μm for Smtn-B−/−/− versus Smtn-B−/− mice, respectively). The staining patterns of the extracellular matrix proteins collagen and elastin also were normal in Smtn-B−/−/− mice, showing regular arrangement of elastic fibers and laminae without fragmentation (Figure 2C and 2D). Hence, loss of smoothelin-B does not affect blood vessel architecture or the expression of other major smooth muscle contractile proteins.

Severely Compromised Arterial Contractile Capacity in Smtn-B−/− Mice

To examine the effect of smoothelin-B deficiency on vascular smooth muscle function, we measured contractility of the femoral artery and the saphenous artery, which contain high amounts of smoothelin-B. We also determined contractility of the thoracic aorta, which contains little smoothelin-B.7 Several contractile agonists were applied to isolated vessel segments in a myograph to assess the integrity of different signal transduction pathways that activate SMC contraction. Because sensitivities to the contractile stimuli did not differ significantly between genotypes, only differences between maximal responses are discussed. Maximal contractile responses generated by aortas of Smtn-B−/− mice were attenuated during stimulation with K+, the thromboxane A2 mimetic U46619, or the α1-adrenergic agonist phenylephrine, although the difference with Smtn-B−/− aortas was not significant (Figure 3A). In contrast, maximal contractions produced by both femoral and saphenous arteries of Smtn-B−/− mice were strongly and significantly decreased compared with

Figure 2. Normal contractile gene expression and arterial structure in Smtn-B−/− mice. A, Expression of α-SMA, SM-MHC, and SM-calponin (SM-Calp) was measured by Q-PCR (n=6 for each genotype). Levels were normalized to cyclophilin A expression, and Smtn-B−/−/− levels were set at 1. No significant change in expression levels was found between Smtn-B−/−/− and Smtn-B−/− mice. B, Representative immunohistochemical staining of α-SMA in the aorta (top; ×100 magnification) and femoral artery (bottom; ×200 magnification) of Smtn-B−/− and Smtn-B−/−/− mice showing no differences between the genotypes. C, Representative images of Sirius Red staining of the aorta (top; ×400 magnification) and femoral artery (bottom; ×200 magnification) of Smtn-B−/− and Smtn-B−/− mice showing no major differences in collagen content or distribution. D, Representative images of Lawson’s elastin staining of the aorta (top; ×400 magnification) and femoral artery (bottom; ×200 magnification) of Smtn-B−/− and Smtn-B−/− mice showing regular elastic fibers in both genotypes.
control mice (Figure 3B and 3C). Contractility of the femoral artery in response to phenylephrine was reduced by 50%. Maximal contractile responses of the saphenous artery to all 3 stimuli were reduced by 60% to 70%.

To investigate whether lack of smoothelin-A on top of smoothelin-B deficiency has an additional effect on vascular smooth muscle performance, we tested the same vasoactive compounds on arterial vessels isolated from Smtn-A/B−/− mice. Of note, Smtn-A/B−/− mice had a decreased medial cross-sectional area of the thoracic aorta and femoral artery that was proportional to their smaller body size.17 The reduced smooth muscle volume was corrected for in the contractility analyses. Thoracic aortas of Smtn-A/B−/− mice displayed a more pronounced reduction of maximal responses to phenylephrine (Figure 3D). Femoral arteries of Smtn-A/B−/− mice showed greatly reduced contractility regardless of the type of agonist used to trigger contraction (Figure 3E). In general, the extent of the reductions were comparable to those observed in Smtn-B−/− vessels. Taken together, these data show that smoothelin-B is an important determinant of muscular artery contractility.

Increased MAP and Cardiac Hypertrophy in Smtn-B−/− Mice

The physiological consequences of the reduced arterial contractility in Smtn-B−/− mice were analyzed by measuring several hemodynamic parameters in conscious mice. Surprisingly, basal MAP was significantly higher in Smtn-B−/− mice (20 mm Hg; P<0.01), whereas HR was not different (Figure 4A).

In line with the elevated MAP, Smtn-B−/− mice developed a significantly higher ratio of heart weight to body weight at 8 weeks of age (Figure 4B). Ventricular atrial natriuretic factor (ANF) and brain natriuretic peptide (BNP) levels as measured by Q-PCR were elevated in Smtn-B−/− mice (P<0.05, P<0.008, respectively; n=11 for each genotype). Smtn-B+/+ levels were set at 1. *Statistically significant differences.
months of age (Figure 4B). Morphometric analysis of the hearts of these mice showed that both the left and right ventricular walls were enlarged (left ventricle cross-sectional area, 13.4±0.1 versus 12.9±0.1 mm²; right ventricle cross-sectional area, 5.4±0.6 versus 4.8±0.4 mm² for Smtn-B⁻/⁻ versus Smtn-B⁺/⁺ mice). The number of cardiomyocyte nuclei per 1 mm² was similar (79.8±3.0 versus 79.4±4.1 for Smtn-B⁻/⁻ versus Smtn-B⁺/⁺ littersmates), consistent with a hypertrophic response. To establish whether the increased ratio of heart weight to body weight indeed reflected cardiac hypertrophy, we measured the expression of the cardiac hypertrophy markers atrial natriuretic factor and brain natriuretic peptide in the hearts of 8-month-old mice (n=11 for each genotype). mRNA levels of both natriuretic peptides were elevated in Smtn-B⁻/⁻ mice compared with littermates (Figure 4C).

Increased MAP and higher ratios of heart weight to body weight also were detected in Smtn-A/B⁻/⁻ mice at the young age of only 6 weeks (MAP: 95±5 versus 87±2 mm Hg, P<0.01; ratio of heart weight to body weight: 6.8±0.9 versus 5.8±0.4 mg/g, P<0.001 for Smtn-A/B⁻/⁻ versus Smtn-A/B⁺/⁺ littersmates). Collectively, these results show that smoothelin deficiency results in elevated blood pressure, leading to cardiac hypertrophy.

Normal Blood Pressure Control in Smtn-B⁻/⁻ Mice
To determine whether the increased MAP in Smtn-B⁻/⁻ mice was due to alterations in endogenous blood pressure control mechanisms, we determined MAP and HR after injection of several autonomic nervous system blockers in conscious mice. Smtn-B⁻/⁻ and Smtn-B⁺/⁺ mice displayed similar changes in MAP and HR after administration of atropine or atropine plus metoprolol (Figure 5A and 5B), indicating that cardiac parasympathetic tone and sympathetic tone were comparable. In addition, the MAP response to the α₁-adrenergic blockers prazosin and yohimbine did not differ between Smtn-B⁻/⁻ and Smtn-B⁺/⁺ mice (Figure 5C). Thus, no difference exists in autonomic control that may explain the different MAP between the genotypes. In line with the myograph data, MAP responses to intravenous bolus injections of the α₁-adrenergic agonist phenylephrine were lower in Smtn-B⁻/⁻ mice than in Smtn-B⁺/⁺ mice, although the differences were not significantly different at any dose (Figure 5D and 5E).

Normal Cardiac Function but Increased Peripheral Vascular Resistance in Smtn-B⁻/⁻ Mice
The distensibility of the large arteries affects central arterial pressure. Therefore, we first investigated whether increased distensibility in Smtn-B⁻/⁻ animals contributes to the elevated
MAP. We measured pulse-wave velocity and pulse pressure in the thoracic aorta. At comparable HRs (573±36 versus 562±38 bpm for Smtn-B−/− and Smtn-B+/+ animals; P=0.80) and comparable MAPs (89±9 versus 86±1 mm Hg for Smtn-B−/− and Smtn-B+/+ animals; P=0.45), no significant difference was found in either pulse-wave velocity or pulse pressure (Figure 6A), demonstrating unchanged aortic distensibility in Smtn-B−/− mice.

MAP is by definition the product of cardiac output and total peripheral vascular resistance. Consequently, the elevated MAP in Smtn-B−/− mice might arise from increases in either or both of these factors. We first analyzed cardiac output by magnetic resonance imaging. Stroke volume, HR, and cardiac output did not differ between Smtn-B−/− and Smtn-B+/+ littermates (Table). Because cardiac output was not increased, the elevated MAP in Smtn-B−/− mice had to be due to increased peripheral vascular resistance. Analysis of the distension of the aorta during the cardiac cycle by magnetic resonance imaging (Figure 6B) revealed increased distension in Smtn-B−/− mice throughout the cardiac cycle (Figure 6C). The maximal distension was almost 2-fold greater for Smtn-B−/− mice (P=0.01; Figure 6D). The lumen diameter of the thoracic aorta at the end-diastolic heart phase was 1.15±0.15 versus 1.05±0.17 mm for the Smtn-B−/− and Smtn-B+/+ mice, respectively. The increased aortic distension despite similar aortic distensibility and comparable cardiac output implies the presence of increased peripheral vascular resistance, which causes elevated MAP in Smtn-B−/− mice.

**Discussion**

The present study was instigated by our recent demonstration that mice lacking both smoothelin isoforms display markedly reduced intestinal smooth muscle contraction. In line with this, we show here that loss of the vascular-specific smoothelin-B protein leads to greatly diminished vascular contractile capacity. Paradoxically, this is accompanied by elevated MAP and cardiac hypertrophy. Because cardiac output, autonomic nervous system activity, and large-artery properties are not altered in smoothelin-B−/− deficient mice, the increased pressure must have its origin in altered microvascular properties. The data obtained in this study provide the first evidence that smoothelin-B is essential for vascular smooth muscle performance.

Reduced contractility of Smtn-B−/− muscular arteries was observed regardless of the signal transduction pathways activated. Together with the binding of smoothelin-B to the contractile filaments, this nonselective reduction of contractility suggests that smoothelin-B plays a role at the core of the
vascular SMC contractile machinery. The mechanism by which smoothelin-B affects smooth muscle contraction needs more study. The cardiovascular phenotype of other knockout mouse models of contractile SMC proteins has been shown to be due to downregulation of other proteins involved in SMC contraction, upregulation of related proteins, or expression of different alternatively spliced contractile proteins.\cite{2,22,23,24,25} We have excluded the possibility that downregulation of the most important SMC contractile genes is responsible for the phenotype of Smtn-B\(^{-/-}\) mice. In addition, smoothelin homologs are not detected by database searches, making it unlikely that upregulation of such proteins can compensate for the loss of smoothelin function. It cannot be completely ruled out, however, that changes in alternative splicing or organization of other contractile proteins occur in Smtn-B\(^{-/-}\) animals.

An important determinant of the amount of contractile force in muscle contraction is the degree of cooperativity between multiple actomyosin cross-bridges. Cooperativity in skeletal muscle is coordinated by tropomyosin and the tropinin proteins.\cite{26} However, troponins are not expressed in SMCs. Instead, smooth muscle tropomyosin interacts with h-caldesmon and SM-calponin, which partly take over the role of troponins.\cite{5} Importantly, smoothelins contain a 37–amino acid sequence that is similar to the tail domain of troponin T.\cite{10} In skeletal muscle, this domain not only is required for troponin T interaction with tropomyosin but also is involved in the activation of actomyosin ATPase.\cite{27} Thus, smoothelins might be part of a smooth muscle tropomyosin-troponin–like system. The diminished contractile potential of vascular smooth muscle of Smtn-B\(^{-/-}\) mice may be due to a lack of cooperativity of contraction, which then would depend on a functional smoothelin-tropomyosin system.

Surprisingly, the reduced contractile capacity of smoothelin-B–deficient muscular arteries ex vivo was accompanied by elevated blood pressure in Smtn-B\(^{-/-}\) mice. However, reduced maximal vascular contractility does not necessarily manifest itself in the MAP. Elevation of mean blood pressure may occur as a result of increased cardiac output, increased total peripheral resistance, or their combination. The magnetic resonance imaging measurements in this study show that cardiac output in the Smtn-B\(^{-/-}\) mice is not changed. Therefore, the peripheral resistance of the vascular tree must be affected by the mutation, which is conceivable considering the significant expression of smoothelin-B in the smaller vessels. The increased aortic distension despite similar cardiac output and similar aortic structure, diameter, and distensibility in Smtn-B\(^{-/-}\) mice supports that increased resistance to blood flow is brought about by the smaller downstream parts of the vascular tree.

It is unlikely that the increased MAP is caused by overactivity of the autonomic nervous system because neither blockade of muscarinic receptors nor blockade of \(\beta_1\)– or \(\alpha_1\)-adrenergic receptors revealed differences in blood pressure response and because HR did not differ between the genotypes either. However, changes in other neurohumoral vasopressor systems that control blood pressure, changes in arterial relaxation properties, or differences in the total number of vessels might contribute to the altered MAP in Smtn-B\(^{-/-}\) mice. In addition, we cannot rule out that smoothelin deficiency might have a stimulatory effect on arteriole contractility.

Overall, the cardiovascular phenotype of Smtn-B\(^{-/-}\) mice is similar to that of patients with established hypertension. They, too, have a normal cardiac output with a hypertrophic heart, accompanied by increased peripheral resistance.\cite{28} Two other observations in this study deserve further comment. First, we found no indications of SMC phenotype changes such as decreased contractile gene expression or altered cell morphology in Smtn-B\(^{-/-}\) mice. Therefore, it is unlikely that smoothelin-B plays a role in the regulation of SMC differentiation, as was previously suggested on the basis of its strict contractile phenotype-specific expression.\cite{23} Second, arterial contractility was similarly reduced in mice lacking both smoothelins and mice lacking only smoothelin-B, indicating that smoothelin-B is the functional smoothelin isoform in vascular smooth muscle.

**Conclusions**

The data in this study show that smoothelin-B deficiency causes a major decline in the contractile performance of vascular smooth muscle. Instead of merely reflecting the SMC contractile phenotype, smoothelins appear to actively participate in the contractile process itself. Mutations in the Smtn gene or alterations in smoothelin levels may therefore contribute to the development of hypertension and concomitant cardiac hypertrophy in humans.

**Acknowledgments**

We would like to thank Agnieszka Strzelecka, Jacques Debets, Gregorio Fazzi, Darren Baker, and our colleagues from the animal facility for expert technical assistance in various aspects of the study.

**Sources of Funding**

This work was supported by grants from the Netherlands Heart Foundation (97.167), Stichting De Gelderfonds, Stichting Simpsons, and The Netherlands Organization for Scientific Research (Dr Rensen); the European Vascular Genomics Network and the EC-FP6-project DiMi, LSBH-CT-2005–512146 (Dr de Mey); and the BSIK program Molecular Imaging of Ischemic Heart Disease (BSIK03035) (E. Heijman and Dr Strijkers). Dr Niesen was supported by a Kootstra fellowship from Maastricht University.

**Disclosures**

None.

**References**


---

**CLINICAL PERSPECTIVE**

The causes of essential hypertension remain largely unknown, although it is commonly accepted that vascular smooth muscle dysfunction is a potential culprit. Improved insight into the mechanics and regulation of smooth muscle contraction may provide additional therapeutic targets to treat pathologies such as hypertension. However, our current understanding of these 2 aspects of smooth muscle function is limited. Here, we introduce smoothelin, a protein specifically expressed in fully differentiated, contractile smooth muscle cells, as a crucial component of the vascular smooth muscle cell contractile apparatus. We demonstrate for the first time that smoothelin is necessary for physiological vascular smooth muscle contraction. Smoothelin deficiency in mice resulted in severely reduced contractile potential, particularly in smaller arteries. Paradoxically, this was accompanied by hypertension and concomitant cardiac hypertrophy. Analyses of differently sized blood vessels indicated that the cause of the hypertension is likely to be downstream of vessels like the saphenous artery and/or mediated by overcompensation of blood pressure regulatory systems like the renin-angiotensin system. Recently, imatinib, a drug used in clinical practice, was shown to specifically promote smoothelin expression in vascular smooth muscle cells. Considering the currently reported data, such an increase in smoothelin concentration not only may indicate a more contractile phenotype of the vascular smooth muscle cell but also may improve vascular smooth muscle contractile potential. The combination of an increased knowledge of smoothelin function and the availability of pharmacological tools that affect smoothelin expression provides interesting opportunities to treat pathologies originating from vascular smooth muscle cell dysfunction.