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Impaired Flow-induced Dilation in Mesenteric Resistance Arteries from Mice Lacking Vimentin

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Abstract

The intermediate filament vimentin might play a key role in vascular resistance to mechanical stress. We investigated the responses to pressure (tensile stress) and flow (shear stress) of mesenteric resistance arteries perfused in vitro from vimentin knockout mice. Arteries were isolated from homozygous (Vim<sup>−/−</sup>, n = 14) or heterozygous vimentin-null mice (Vim<sup>+/−</sup>, n = 5) and from wild-type littermates (Vim<sup>++</sup>, n = 9).

Passive arterial diameter (175 ± 15 μm in Vim<sup>++</sup> at 100 mmHg) and myogenic tone were not affected by the absence of vimentin. Flow-induced (0–150 μl/min) dilation (e.g., 19 ± 3 μm dilation at 150 mmHg in Vim<sup>++</sup>+) was significantly attenuated in Vim<sup>−/−</sup> mice (13 ± 2 μm dilation, P < 0.01). Acute blockade of nitric oxide synthesis (N<sup>0</sup>-nitro-L-arginine, 10 μM) significantly decreased flow-induced dilation in both groups, whereas acute blockade of prostaglandin synthesis (indomethacin, 10 μM) had no significant effect. Mean blood pressure, in vivo mesenteric blood flow and diameter, and mesenteric artery media thickness or media to lumen ratio were not affected by the absence of vimentin.

Thus, the absence of vimentin decreased selectively the response of resistance arteries to flow, suggesting a role for vimentin in the mechanotransduction of shear stress. (J. Clin. Invest. 1997. 100:2909–2914.) Key words: shear stress • resistance arteries • nitric oxide • vimentin • mechanotransduction

Introduction

Pressure-induced tone (myogenic tone) is a characteristic of small resistance arteries and of some veins (1–4). It is opposed by flow-induced dilation, in vitro as well as in vivo (5–8). These two mechanical stimuli participate to a constant basal tone in resistance arteries and allow a rapid adaptation to changes in flow and pressure (1–8). Myogenic tone develops upon stretch of the vascular wall; it depends upon the activation of phos-lophilase C and protein kinase C (9, 10) and does not require a high level of intracellular calcium (11). Myogenic tone is mainly independent of endothelial factors (1–3) and is increased in hypertension (12). On the other hand, flow produces shear stress and triggers a dilation (5–8). Flow-induced dilation is in part dependent on the production of nitric oxide (NO) (5, 6, 8, 13, 14) and cyclooxygenase products (14–16) by the endothelium.

Mechanotransduction of shear stress has been shown to involve extracellular matrix and cell structure proteins (8, 17–22). Shear stress is able to alter endothelial cell shape and to align the cells in the direction of flow (8, 18, 23, 24). Changes in actin turnover and depolymerization of F-actin into G-actin are rapid upon shear stress stimulation (21, 25). Finally, the endothelial cytoskeleton has been shown to have a selective role in the mechanotransduction leading to flow-induced NO release (20). Vimentin (Vim), an intermediate filament of class III, is a main cytoskeletal structure protein (26). Vimentin is sensitive to mechanical stresses (23), including shear stress (27).

In this study we tested the hypothesis that the structure protein vimentin might have a role in the mechanotransduction of the signal induced by flow (shear stress) and/or pressure (tensile stress). We used mice lacking vimentin, a model recently established by Colucci-Guyon et al. (28); surprisingly, the animals develop and reproduce without obvious phenotype. We measured pressure (myogenic)-induced tone and flow (shear stress)-induced dilation in mouse mesenteric resistance arteries isolated in vitro. Arteries isolated from homozygous (Vim<sup>−/−</sup>) and heterozygous (Vim<sup>+/−</sup>) mice were compared with arteries isolated from wild-type mice (Vim<sup>++</sup>):

Methods

Mice lacking vimentin. Vimentin-null mice were obtained by targeted inactivation of the vimentin gene in mice, as previously described (28). All experiments were performed on Vim knockout mice in which the endogenous vimentin gene has been disrupted by an in-frame insertion of Escherichia coli β-galactosidase coding sequence into an exon 1 of vimentin gene.

The Vim<sup>−/−</sup> heterozygous mice were originally obtained by crossing a chimeric male (generated from a C57BL/6 blastocyst injected with the mutated ES cells) with C57BL/6 × DBA/2 F<sub>1</sub> females. The colony was then maintained by brother–sister or first cousin mating between heterozygous mice, thus producing Vim<sup>+/−</sup>, Vim<sup>−/−</sup>, and Vim<sup>+/+</sup> mice (as determined by a PCR-based method) at each generation. These animals were generated after two to five generations. Thus, even though the mice used in this study were not fully inbred, they were as closely genetically related as possible; furthermore, the genetic heterogeneity between mice was similar within each group of genotypes at the vimentin locus and between the three groups. There-

1. Abbreviations used in this paper: L-NOARG, N<sup>0</sup>-nitro-L-arginine; NO, nitric oxide; Vim, vimentin.
fore, the differences between the groups are due solely to the effect of the allelic forms of the vimentin gene.

To identify wild-type (Vim\(^{1/1}\)), heterozygous (Vim\(^{1/2}\)), and homozygous (Vim\(^{2/2}\)) mice of different crosses, DNA from mice tail was extracted, and the presence of targeted vimentin alleles was detected using PCR methods, as described previously (29).

**Blood pressure measurement.** Adult male mice (Vim\(^{1/1}\), Vim\(^{1/2}\), Vim\(^{2/2}\), adult 4–5 mo old), coming from the same litter, were anesthetized with chlorobutanol (2.5 mg/kg, i.p.) and ketamine (25 mg/kg, i.p.) and the carotid artery was cannulated (ID 0.6 mm) to measure blood pressure. The cannulae was connected to a pressure transducer (Gould P10EZ; Spectramed, Oxnard, CA) and the signal was displayed on a chart recorder (Gould, Recording Systems Division, Cleveland, OH). The procedure followed in the care and killing of the study animals was in accordance with the European Community standards on the care and use of laboratory animals (Ministère de l’Agriculture, France, authorization No. 00577).

**Mesenteric resistance artery preparation.** Adult mesenteric artery segments, ~100 μm in internal diameter, were isolated and cannulated at both ends and mounted in a video-monitored perfusion system (30, 31). The artery segment was bathed in a 5-ml organ bath containing physiological salt solution of the following composition (mM): 135.0, NaCl; 15.0, NaHCO\(_3\); 4.6, KCl; 1.5, CaCl\(_2\); 1.2, MgSO\(_4\); 11.0, glucose; and 10.0, N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid. The pH was 7.4 and was monitored continuously. The pO\(_2\) was maintained at a value of 160 mmHg and the pCO\(_2\) was maintained at a value of 37 mmHg. The artery was superfused at a rate of 4 ml \(\cdot\) min\(^{-1}\). Perfusion of the artery with a physiological salt solution of the same composition was set at a rate ranging from 0 to 150 μl \(\cdot\) min\(^{-1}\). The pressure in both ends of the artery segment was monitored using pressure transducers (Fig. 1). Flow in the vessel could be generated through the distal pipette with a peristaltic pump. Pressure in the proximal end of the vessel was controlled by a servo perfusion system. When flow was applied and pressure increased, the difference in pressure between distal and proximal ends of the vessel was changed so that pressure could be increased without change in flow. This supposes that the two pipettes oppose the same resistance to flow; therefore, pairs of pipettes were selected to satisfy this prerequisite. In these conditions, the average pressure between distal and proximal pressures can be assumed to be representative of lumen pressure (pressure-flow control system; Living System Instrumentation Inc., Burlington, VT). Arterial diameter was measured and recorded continuously using a video monitoring system (Living System Instrumentation Inc.). Pressure and flow rate could be changed independently. Equilibrium diameter changes were measured in each segment when intraluminal pressure was set at 10, 25, 50, 75, 100, 125, and 150 mmHg.

Flow-induced dilation was studied by increasing flow rate by steps from 0 to 150 ml \(\cdot\) min\(^{-1}\) when intraluminal pressure was 100 mmHg, or flow rate in the artery was either 0 or 100 μl \(\cdot\) min\(^{-1}\), under pressures of 10, 25, 50, 75, 100, 125, or 150 mmHg (31). In these conditions, flow dilation was studied at different flow rates when myogenic tone was maximal (100 mmHg) and at different pressures when flow-induced dilation was supramaximal (100 μl/min). In a preliminary series of experiments, the flow rate of 100 μl \(\cdot\) min\(^{-1}\) was chosen since it represents a supramaximal dilating rate. Maximum dilation was found to occur at flow rate of 70 min\(^{-1}\) and over, which is also comparable to our previous observation in a similar vessel in the rat (31). In addition, flow rate, measured in vivo, in anesthetized mice, was found to be ~100 μl/min in mesenteric artery similar in size to those studied in vitro (see Results).

Arteries were submitted to the pressure steps with or without intraluminal flow and this was subsequently repeated after addition of either N\(^5\)-nitro-L-arginine (L-NOARG, 10 μM) or indomethacin (10 μM). The concentration of L-NOARG and indomethacin used (10 μM) has an optimal blocking effect in this type of preparation (15, 16, 31, 32). At the end of each experiment, arteries were perfused and superfused with a Ca\(^{2+}\)-free physiological salt solution containing EGTA (2 mM) and sodium nitroprusside (10 μM), and the pressure steps (10–150 mmHg) were repeated to determine the passive diame-

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**Figure 1.** Schematic representation of the experimental setup used to measure the internal diameter of a mouse resistance mesenteric artery in vitro. The resistance mesenteric artery was mounted between two cannulas in a 5-ml organ chamber. The artery was perfused with a physiological salt solution at flow rates ranging from 0 to 150 μl/min. Pressure could be changed from 10 to 150 mmHg under the control of a pressure servo control unit. Diameter and pressure were measured and recorded continuously. Drugs were added to the perfusate and to the superfusate.
ter of the vessel, i.e., in the absence of smooth muscle tone (2, 12, 15, 16, 18, 31). Diameters measured in normal physiological salt solution were considered as diameters under active tone or active diameters (2, 12, 15, 16, 18, 31). Pressure and diameter measurements were collected by a Biopac data acquisition system (MP 100; Biopac, La Jolla, CA), recorded, and analyzed on a Macintosh Quadra computer (Apple Computers, Cupertino, CA) using the Acqknowledge® software (Biopac). Results are given in microns for artery diameters. Myogenic tone was calculated as the percentage of passive diameter (measured diameter/passive diameter × 100) (12, 30, 31). Flow-induced relaxation was expressed as percent dilation of myogenic tone or as increases in diameter induced by flow (microns) as described previously (15, 16, 31).

The integrity of the endothelium was assessed by testing the vasodilator effect of acetylcholine (1 μM) after preconstriction of the mesenteric arteries with phenylephrine (0.01 μM), under an intraluminal pressure of 50 mmHg.

In vivo determination of mesenteric resistance artery flow rate and diameter. In a separate series of experiments, flow and arterial diameter were determined in vivo in mesenteric resistance arteries similar in size and location to those used in the in vitro experiments (as described above). After anesthesia with chlorobutanol (2.5 mg/kg, i.p.) and ketamine (25 mg/kg, i.p.), a medial laparotomy was performed and the last loop of the intestine was exteriorized and placed in a container allowing the superfusion of the preparation. The preparation was irrigated with a physiological salt solution. A 1.5-mm-long segment of a mesenteric resistance artery (second order) was dissected free of fat and connective tissues under a binocular lens (Nachet, Dijon, France). A video camera mounted on a binocular lens allowed recording and analysis of the images of the isolated arterial segment (Microcontrol, Evry, France). Blood flow was measured in the same arterial segment using a small animal blood flow-meter (model T106; Transonic Systems Inc., Ithaca, NY).

Histomorphological study. The morphometric analysis of the mesenteric resistance arteries was performed as described previously (33). Briefly, segments of artery, adjacent to those used in the functional study, were fixed in 10% formaldehyde in saline and sectioned (10-μm-thick sections). Morphometric analysis was performed with an automated image processor (Microvision). The total surface area and area of the lumen were measured. This allowed the calculation of the area of the media (33).

Statistical analysis. Results are expressed as means±SEM. Significance of the differences between the different groups was determined by analysis of variance (one or two factor ANOVA, or ANOVA for consecutive measurements, when appropriate). Means were compared by paired t test or by Bonferroni’s test for multigroup comparisons. P < 0.05 was considered to be significant.

Drugs. N-2-hydroxy-ethylpiperazine-N-2-ethylsulfonic acid, NO-, nitro-L-arginine (L-NAME), L-NOARG, indomethacin, and EGTA were purchased from Sigma Chemical Co. (St. Louis, MO). Other reagents were purchased from Prolabo (Paris, France).

Results

All the experiments described hereafter were performed on animals from intercrosses between Vim+/− mice, which bear a mutated allele of vimentin gene from which bacterial β-galactosidase was expressed in place of vimentin. Mean arterial pressure, measured in the carotid artery, was not significantly different between groups (87±7 mmHg, n = 14 in Vim−/− mice; 92±11 mmHg, n = 5 in Vim+−/− mice; and 90±8 mmHg, n = 9 in Vim++/− mice). Mice body weight was 33±2 g (n = 14) in Vim−/− mice, 37±2 g (n = 5) in Vim−+/− mice, and 37±3 g (n = 9) in Vim++/− mice.

As previously shown in other tissues and arteries (28), the absence of vimentin in the mesenteric resistance arteries was confirmed in the present study by immunofluorescence (data not shown).

Blood flow, measured in vivo in mesenteric resistance arteries, was not significantly affected by the absence of vimentin (115±12 μl · min⁻¹, n = 5, in Vim−/−; vs. 125±13 μl · min⁻¹, vs. 125±13 μl · min⁻¹, Figure 2. Typical recordings showing the internal diameter and the luminal pressure measured in resistance mesenteric artery segments isolated from a Vim−/− mouse. Passive diameter (C) was obtained in a Ca²⁺-free physiological salt solution containing EGTA (2 mM) and sodium nitroprusside (10 μM). Active diameter was measured with a flow rate of either 0 μl/min (A) or 100 μl/min (B).
The histomorphometric analysis of the mesenteric resistance arteries showed that the absence of vimentin did not significantly affect the thickness of the media tunica (5.7±1.0 μm, n = 5, in Vim−/−; vs. 6.1±0.4 μm, n = 5 in Vim+/+), nor the media to lumen ratio (0.22±0.04, n = 5, in Vim−/−; vs. 0.23±0.05, n = 5 in Vim+/+).

Fig. 2 shows typical recordings obtained under three different conditions. Step increases in intraluminal pressure from 10 to 50 mmHg induced an increase in artery diameter, whereas further step increases in pressure induced no more increase or a decrease in diameter, reflecting the development of myogenic tone (Fig. 2, traces A and B). In Fig. 2, trace B flow rate was set at 100 μl · min−1 and the artery diameter was higher than in trace A, where no flow was applied. The difference can be considered as flow-induced dilation (15, 16, 31). Passive diameter values obtained in a Ca²⁺-free isotonic salt solution containing EGTA (2 mM) and sodium nitroprusside (10 μM) are shown in Fig. 2 (trace C). In these conditions, no tone was able to develop and each step increase in pressure induced an increase in diameter. The difference between passive diameter and the diameter measured under physiological conditions (with or without flow) was considered as active tone.

Passive diameter values obtained in a Ca²⁺-free physiological salt solution containing EGTA (2 mM) and sodium nitroprusside (10 μM) were not statistically different in all three groups (Fig. 3).

Myogenic tone (active tone in the absence of flow) in mesenteric resistance arteries from Vim−/− mice was not significantly different from myogenic tone in arteries from Vim−/− or Vim+/+ mice (Fig. 4). The addition of L-NOARG (10 μM) and indomethacin (10 μM) induced no significant change in myogenic tone in all three groups (not shown).

Flow-induced relaxation was significantly reduced in mesenteric resistance arteries from Vim−/− mice as compared with arteries from Vim+/− and Vim+/+ mice (Fig. 5). There was no significant difference in flow-induced dilation between arteries from Vim−/− and Vim+/+ mice (Fig. 5). The addition of L-NOARG (10 μM) to the perfusate and to the superfusate significantly decreased flow-induced relaxation in all three groups (Fig. 6). The addition of indomethacin (10 μM) induced no further significant change in flow-induced relaxation in the three groups (Fig. 6). The addition of indomethacin (10 μM) without preliminary addition of L-NOARG (10 μM) induced no significant change in flow-induced dilation in the three studied groups (not shown).

The endothelial function was also assessed by testing the vasodilatory effect of acetylcholine (1 μM) after preconstriction with phenylephrine (0.01 μM). Phenylephrine (0.01 μM, at 50 mmHg) induced a decrease in diameter from 132±14 to 55±9 μm (Vim−/− mice, n = 14). The further addition of acetylcholine (1 μM) induced an increase in diameter from 55±9 to 141±13 μm (Vim−/− mice, n = 14). Phenylephrine (0.01 μM)-induced tone and acetylcholine (1 μM)-induced dilation were not significantly different in Vim−/−, Vim+/+, or in Vim+/− mice (not shown).

**Discussion**

In this study we showed that flow (shear stress)-induced dilation was significantly attenuated in resistance mesenteric arteries isolated from mice lacking vimentin, whereas pressure (tensile stress)-induced tone was not affected. Myogenic tone and flow-dependent vasodilation are mechanisms of major impor-
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tance for the control and adaptation of blood pressure and tissue perfusion under most physiological circumstances.

We found no change in arterial blood pressure in Vim$^{-/-}$ mice despite the decreased flow-induced dilation observed in their mesenteric arteries. This could suggest that the change in mechanotransduction of shear stress due to the absence of vimentin led to an adaptation and/or to regional rearrangement in hemodynamic responsiveness.

Mouse resistance mesenteric arteries responded to pressure by developing myogenic tone and to flow by a dilation. In mouse mesenteric resistance arteries, myogenic tone developed when intraluminal pressure was raised and was comparable to that described in same size resistance arteries in other species (2, 12, 30–33). Myogenic tone was not significantly modified by either NO or prostaglandin blockade; this is in agreement with previous studies showing that the endothelium is of little importance in pressure-induced tone in small resistance arteries (for review see reference 3).

In contrast, as the NO synthase blocker L-NOARG suppressed flow-induced dilation in mice resistance mesenteric arteries, flow-induced dilation can be assumed to depend mainly on the production of NO. This is consistent with previous observations in other species demonstrating a role for NO in flow-dependent dilation in resistance vessels (5, 7, 15, 16, 34, 35). The proportion of flow-induced dilation depending on the production of NO is variable, reflecting tissue and species specificity, and also differences in experimental conditions (35). In some studies, flow-induced dilation depends also on the production of prostaglandins (14–16, 31). The absence of effect of indomethacin on mouse mesenteric artery diameter in the present study suggests that prostaglandins play a mi-

Figure 5. Flow-induced dilation in resistance mesenteric artery segments isolated from Vim$^{-/-}$ (filled boxes, n = 14), Vim$^{+/+}$ (open circles, n = 5), or Vim$^{+/+}$ (open boxes, n = 9) mice. Flow rate was either set at 100 μl/min and pressure was increased stepwise from 10 to 150 mmHg (bottom); or pressure was set at 100 mmHg and flow was increased stepwise from 0 to 150 μl/min. Flow-induced dilation is given as change in diameter (in microns) due to flow. Data are expressed as means±SEM. *P < 0.01; two factor ANOVA for repeated measures, compared with Vim$^{+/+}$.

Figure 6. Flow-induced dilation in resistance mesenteric artery segments isolated from Vim$^{-/-}$ (n = 14, left), Vim$^{+/+}$ (n = 5, not shown, no significant difference as compared with Vim$^{+/+}$), or Vim$^{+/+}$ (n = 9, right) mice. In each group, artery segments were treated consecutively (intra- and extraluminally) with L-NOARG (10 μM, filled squares) and then with indomethacin (10 μM, open circles). In the control group (open squares), artery segments were treated with the solvent for indomethacin (ethanol 0.1%). L-NOARG (10 μM) was dissolved in physiological salt solution. Data are expressed as means±SEM, n = 8–14 per group. *P < 0.001; two factor ANOVA for repeated measures, compared with control.
nor role in flow-induced dilation in this type of mesenteric artery in mice.

We observed a decrease in flow-induced dilation in mesenteric resistance arteries from mice lacking vimentin. Vimentin is a cytoskeletal structure protein sensitive to shear stress. Shear stress induced in endothelial cells rearrangement of vimentin intermediate filaments with the major axes of the cells (23, 27). Cytoskeletal proteins are thought to participate in the mechanotransduction of shear stress into a biochemical signal (8, 17, 20, 21, 36–38). After F-actin stabilization with phalloidin, shear stress–induced calcium release by endothelial cell sarcoplasmic reticulum is decreased (38), shear stress–induced increase in endothelin-1 mRNA is abolished (21), and shear stress–induced EDRF release is attenuated (20). In all of these previous studies (20, 21, 38), the effect of the F-actin stabilization was specific to shear stress. In our study, we show evidence that another structure protein, i.e., vimentin, might participate in the mechanotransduction of shear stress. That the response to tensile stress (myogenic tone) was not affected by the lack of vimentin suggests that another(other) structure protein(s) is(are) involved in the mechanotransduction of this type of radial force. This difference between shear stress and tensile stress suggests that a low force applied radially might involve a different mechanism/pathway than a force applied longitudinally and/or that vimentin could be involved in one and not in the other.

In conclusion, we found that invalidation of the gene encoding for vimentin decreased the response of mouse mesenteric resistance arteries to flow (shear stress) but not to pressure (tensile stress). Vimentin might have a specific role in the mechanotransduction of shear stress.

References


