PECAM-1 Mediates NO-Dependent Dilation of Arterioles to High Temporal Gradients of Shear Stress

Zsolt Bagi, John A. Frangos, Jiunn-Chern Yeh, Charles R. White, Gabor Kaley, Akos Koller

Objective—In response to changes in wall shear stress (WSS) the vascular endothelium releases several factors, among others nitric oxide. On the basis of studies of endothelial cells in culture, suggesting that platelet endothelial cell adhesion molecule-1 (PECAM-1) is specifically involved in sensing and coupling high temporal gradients of fluid shear stress with activation of eNOS, we hypothesized that dilations of isolated skeletal muscle arterioles from PECAM-1 knockout mice (PECAM-KO) will be reduced to rapid increases in WSS elicited by increases in perfusate flow.

Methods and Results—Small and large step increases in flow resulted in substantial dilations in arterioles of WT mice (45±4%), but they were markedly reduced in arterioles of PECAM-KO mice (22±5%). The initial slope of dilations, when WSS increased rapidly, was greater in vessels of WT than those of PECAM-KO mice (slopes: 0.378 and 0.094, respectively), whereas the second phase of dilations, when flow/shear stress was steady, was similar in the 2 groups (slopes: 0.085 and 0.094, respectively). Inhibition of eNOS significantly reduced the initial phase of dilations in arterioles from WT, but not from those of PECAM-KO mice. The calcium ionophore A23187 elicited similar NO-mediated dilation in both WT and PECAM-KO mice.

Conclusions—In isolated arterioles of PECAM-KO mice activation of eNOS and consequent dilation by agonists is maintained, but the dilation to high temporal gradients of wall shear stress elicited by increases in perfusate flow is reduced. Thus, we propose that PECAM-1 plays an important role in the ability of the endothelium to sense and couple high temporal gradients of wall shear stress to NO-mediated arteriolar dilation during sudden changes in blood flow in vivo. **(*Arterioscler Thromb Vasc Biol*. 2005;25:1590-1595.)**

Key Words: PECAM-1 ■ arteriole ■ endothelium ■ flow-induced dilation ■ nitric oxide

The vascular endothelium is uniquely situated to act as the signal transduction interface between hemodynamic forces and the underlying vascular smooth muscle. Increases in shear stress have long been shown to result in vasodilatation mediated, in part, by the endothelium-dependent release of nitric oxide (NO).[1–5] Since endothelium-derived NO was first identified and characterized, fluid shear stress has been established as the most potent stimulus that regulates the activity of endothelial NO synthase (eNOS).[6] We have shown previously that stepwise increases in perfusate flow, via increases in wall shear stress, elicit substantial dilations of isolated arterioles.[3–5] Recent in vitro studies in cultured endothelial cells showed that the sudden onset of fluid flow induces a burst of NO production, which seemed to be related more to the magnitude of temporal gradient of shear stress than to its absolute value,[6,7] suggesting that the vascular endothelium is able to discriminate between temporal gradients of shear stress as also proposed by others.[7a] The molecular basis of shear stress–induced mechanochemical signal transduction, and the endothelium's ability to discriminate between high temporal gradients and steady values of shear stress, remains largely unclear, and has not yet been demonstrated in intact vessels. Given that shear stress does not involve a traditional receptor–ligand interaction, identification of the molecule(s) responsible for sensing shear stress and converting it into dilation has been problematic. It has been suggested that the lipid bilayer itself may act as a flow sensing receptor.[8,9] Fluid shear stress is a frictional force that acts on the apical surface of the endothelial cell layer and induces tensile stress across the endothelial cell membrane, which in turn resists the imposed shear stress with tension. Analysis of shear stress distribution in the endothelial cell membrane shows that tension is greatest within the cell–cell junctions.[10] When an endothelial monolayer is exposed to increasing levels of fluid shear stress, cell–cell junction–associated proteins have been shown to undergo a corresponding reorganization, whereas the integrity of the endothelial monolayer remains unaffected.[11]

A number of membrane associated proteins, such as platelet endothelial cell adhesion molecule-1 (PECAM-1), are specifically localized to cell–cell junctions. In cultured endothelial cells, PECAM-1 is diffusely distributed in the

Original received November 25, 2004; final version accepted May 2, 2005.

From the Department of Physiology (Z.B., G.K., A.K.), New York Medical College, Valhalla, NY; the La Jolla Bioengineering Institute (J.A.F., J.-C.Y., C.R.W.), La Jolla, Calif; and the Department of Pathophysiology (Z.B., A.K.), Semmelweis University, Budapest, Hungary.

J.A.F. and A.K. served as co-senior authors.

Correspondence to Akos Koller, MD, PhD, Department of Physiology, New York Medical College, Valhalla, NY 10595. E-mail koller@nymc.edu

Arterioscler Thromb Vasc Biol. **is available at http://www.atvbaha.org** **DOI: 10.1161/01.ATV.0000170136.71970.5f**

plasma membrane of solitary cells, but once a cell–cell contact is made, it accumulates at the contact site. In endothelial cells forming a confluent monolayer, it is exclusively localized along the cell–cell border.[12] When confluent endothelial cells are exposed to physiological levels of fluid shear stress, PECAM-1 has been shown to be tyrosine-phosphorylated.[13] Recent in vitro studies from our laboratory and those from other groups have demonstrated that eNOS is complexed with PECAM-1 at the cell–cell junctions, and thus it is likely to be involved in the modulation of eNOS activity.[14,15] Yet, it is not known whether or not PECAM-1 is involved in the discrimination of various patterns of shear stress and leads to sufficient release of NO to result in arteriolar dilation.

On the basis of the aforementioned, we hypothesized that PECAM-1 is involved in the NO-mediation of shear stress–induced dilation of isolated arterioles and also in the ability of the endothelium to sense high temporal gradients of shear stress. Thus, flow/shear stress–induced dilations of isolated skeletal muscle arterioles of PECAM-1 knockout (PECAM-KO) and wild-type (WT) mice were compared.

Methods

Animals

Twelve-week-old male PECAM-KO (n=10) and wild-type (WT; n=13) mice were used (Jackson Labs, Bar Harbor, Me). Animals were fed standard chow and given tap water freely. Mice were housed in our animal care facility. The Animal Care and Use Committee at New York Medical College approved all protocols.

Isolation of Skeletal Muscle Arterioles

Using microsurgery instruments and an operating microscope, a branch of the gracilis muscle artery (≈0.5 mm in length) running intramuscularly was isolated, cannulated, and pressurized as described previously,[4] using a pressure servo control system (Living Systems Instrumentation). The internal arteriolar diameter at the midpoint of the arteriolar segment was measured by videomicroscopy with a microangiometer (Texas Instruments), and changes in arteriolar diameter and intraluminal pressure were continuously recorded with the Biopac-MP100 system (Biopac Systems Inc).

Experimental Protocols

Flow/Shear Stress–Induced Arteriolar Responses

Arteriolar responses were obtained in response to stepwise increases in intraluminal flow (0 to 12 μL/min). In this protocol, each flow rate was maintained for five minutes to allow the vessel to reach a steady state diameter, and then the maximal diameter changes were measured. Changes in diameter of arterioles in response to a 1-step increase in intraluminal flow (from 0 to 12 μL/min for 5 minutes) were also measured. Isolated arterioles were then incubated with the NO synthase inhibitor L-NAME (10^{-4} mol/L, for 20 minutes), and flow-induced responses were obtained again.

Agonist-Induced Arteriolar Responses

Changes in arteriolar diameter were measured to cumulative doses of the calcium ionophore, A23187 (10^{-9} to 10^{-7} mol/L).[16] Also, acetylcholine (ACh, 10^{-6} mol/L) and norepinephrine (NE, 10^{-6} mol/L) were used to test the viability of endothelium and smooth muscle of arterioles.

Preparation of Lysates From Mouse Aorta

Mouse aorta were dissected and briefly rinsed in ice-cold PBS. Three hundred microliters of lysis buffer (50 mmol/L Tris, pH 7.5, 150 mmol/L NaCl, 1% Triton ×100, 60 mmol/L octylglucoside, 2 mmol/L sodium vanadate, and protease inhibitor cocktail from Roche) were added to tissues and homogenized with a Dounce homogenizer. Samples were then incubated on ice for 30 minutes and centrifuged at 14 000*g* for 20 minutes. Supernatants were collected and protein amounts were determined by BCA protein assay (Pierce) using bovine serum albumin as standard. Equal amount of proteins were separated on SDS-PAGE and transferred to polyvinylidene fluoride (PVDF) membranes. Immunoblot analysis was carried out as described previously.[15] Antibodies used for detection (anti–PECAM-1 IgG, anti-eNOS IgG, anti–caveolin-1 IgG, and anti-Actin IgG) were obtained from Santa Cruz Biotechnology.

Body Weight, Basal Diameters, and ACh- and NE-Induced Dilations of Isolated Gracilis Muscle Arterioles of Mice

	WT (n=13)	PECAM-KO (n=10)
Body weight, g	32±2	32±1
Passive diameter, μm	130±5	124±4
Active diameter, μm	92±7	86±10
Dilation to ACh, 10^{-6} mol/L (%)	88±6	79±9
Constriction to NE, 10^{-6} mol/L (%)	37±8	35±7

Values are means and SEM.

Data Analysis

Data are expressed as means±SEM. Flow- and agonist-induced arteriolar responses were expressed in absolute values or as changes in arteriolar diameter as a percentage of the maximal diameter, defined as the passive diameter of the vessel at 80 mm Hg intraluminal pressure obtained in Ca^{2+}-free physiological salt solution (PSS) containing EGTA (ethylene glycol-bis [β-aminoethylether]-N,N,N′,N′-tetraacetic acid, 10^{-3} mol/L) and 10^{-4} mol/L sodium nitroprusside (SNP). Wall shear stress (WSS) values were calculated according to the formula: $WSS=4\eta Q/\pi r^3$, where η is the viscosity of the perfusate (0.007 poise at 37°C), Q is the perfusate flow, and r is the vessel radius. Statistical analyses were performed by 1-way analysis of variance for repeated measures (ANOVA) with Dunnett post test and by the Student *t* test, as appropriate. In certain protocols linear regression analysis was performed using GraphPad Prism version 4.00 for Windows. Slopes of diameter-curves were also calculated and compared with F test. $P<0.05$ was considered statistically significant.

Results

The body weight of WT and PECAM-KO mice was not significantly different. There were no significant differences between active (in the presence of Ca^{2+}) and passive (in Ca^{2+}-free PSS) diameters of skeletal muscle arterioles isolated from the WT and PECAM-KO mice (Table).

Flow-Induced Arteriolar Responses

Intraluminal flow (from 0 to 12 μL/min) was first increased in a stepwise manner,[4] which resulted in corresponding step increases in diameter of both WT and PECAM-KO arterioles as shown by the original traces (Figure 1A). The magnitude of step responses and the maximal response of diameter were, however, significantly reduced in arterioles from PECAM-KO mice (Figure 1A and 1B). The eNOS inhibitor L-NAME significantly reduced dilations to step increases in flow in arterioles of WT, but it did not affect arteriolar responses of PECAM-KO mice (Figure 1B). During the course of these experiments we have observed that in arterioles of WT mice there were rapid increases in diameter at

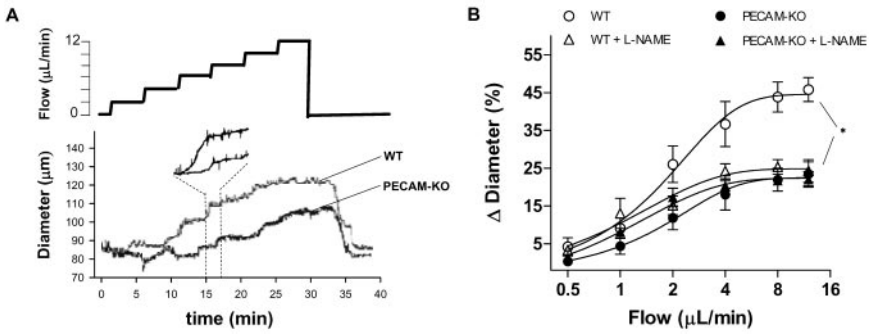

Figure 1. A, Representative record showing diameter of arterioles isolated from WT and PECAM-KO mice in response to stepwise increases in intraluminal flow (from 0 to 12 μL/min). Inset, representative record showing diameter changes of the early phase of the arteriolar response developed to a small-step increase in flow from 2 to 4 μL/min. B, Summary data shows changes in diameter of arterioles of WT and PECAM-KO mice as a function of small-step increases in flow, before and after incubation with L-NAME. Data are mean±SEM. Asterisk indicates significant differences between WT and all other groups.

the onset of flow increases, which were absent in arterioles of PECAM-KO mice (Figure 1A, inset), whereas the later phase of dilations were similar. We hypothesized that the lack of the early phase of the responses is responsible for the reduced overall dilation of PECAM-KO arterioles.

To test this hypothesis, in the next series of experiments flow-induced arteriolar responses were obtained to a 1 large step increase in intraluminal flow (from 0 to 12 μL/min). An original record (Figure 2A) and summary data (Figure 2B) show, 1 large step increase in intraluminal flow resulted in substantial dilations of isolated skeletal muscle arterioles from WT mice, which was greatly reduced in arterioles of PECAM-KO mice. The maximal dilation was significantly reduced in arterioles isolated from PECAM-KO mice (WT, 46±6%; PECAM-KO, 21±5%).

Figure 2C shows changes in calculated wall shear stress as a function of time. In the first couple of seconds wall shear stress suddenly increased when flow was increased from 0 to 12 μL/min, indicating that during this time the arterioles were exposed to high temporal gradients of shear stress (Figure 2C). In WT arterioles wall shear stress significantly decreased after the onset of flow steps, whereas no significant changes in wall shear stress were observed in arterioles of PECAM-KO mice (Figure 2C). The reason for the differences in the changes of

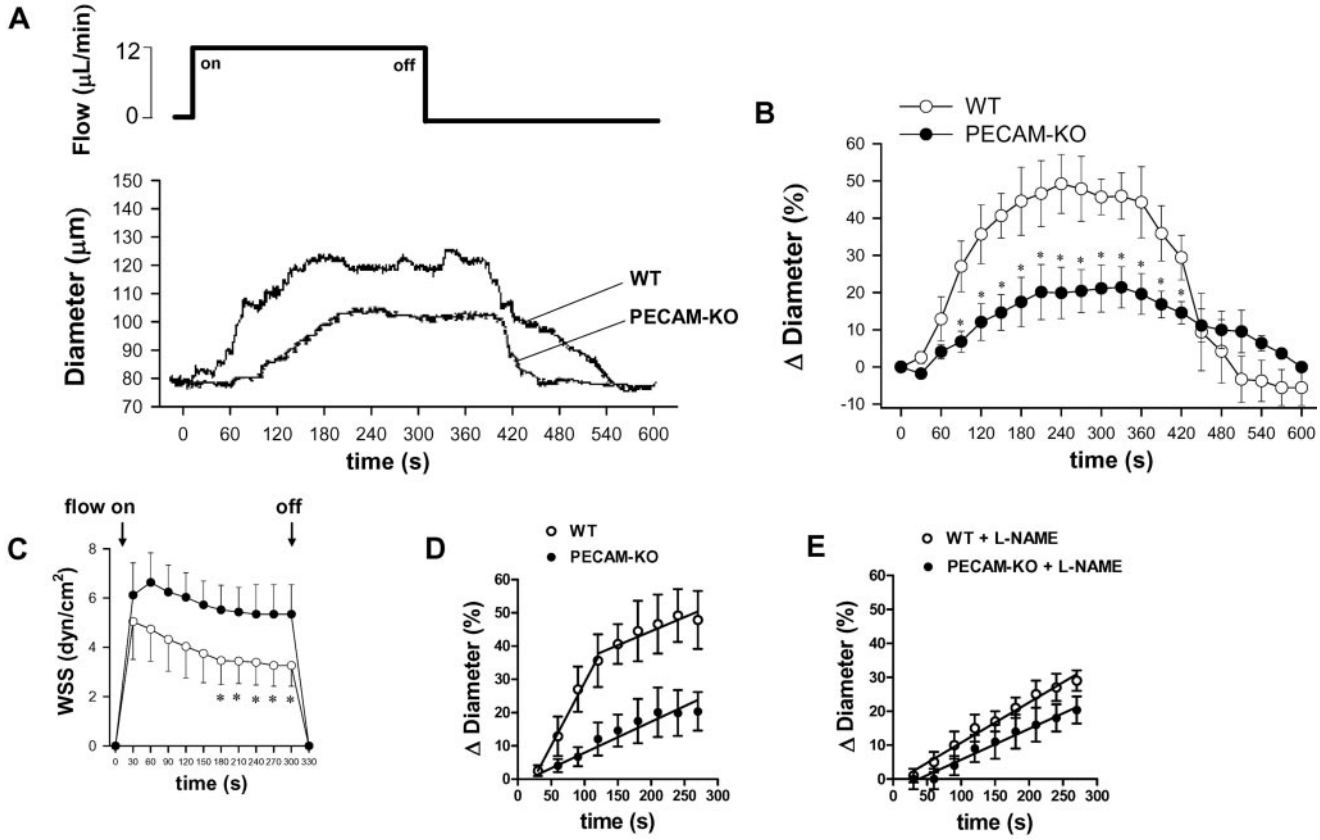

Figure 2. Representative trace of an experimental recording (A) and summary data (B) showing changes in diameter of arterioles isolated from WT and PECAM-KO mice in response to a 1-step rapid increase in intraluminal flow (from 0 to 12 μL/min). Asterisks indicate significant differences from WT animals. C, Calculated WSS as a function of time in arterioles of WT and PECAM-KO mice. Slopes of the initial and late phases of the diameter–time curves obtained from linear regression analysis, before (D) and after (E) incubation with L-NAME. Asterisks indicate significant difference from the maximum shear stress value in PECAM-KO mice (note: no significant differences were observed in WT mice). Data are mean±SEM.

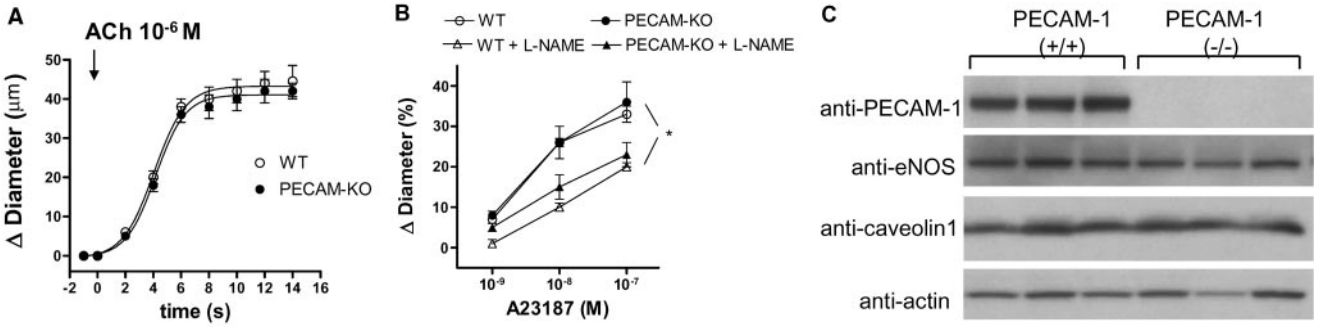

Figure 3. A, ACh (10^{-6} M)-induced changes in diameter of arterioles isolated from WT and PECAM-KO mice as a function of time. B, A23187-induced changes in diameter of arterioles isolated from WT and PECAM-KO mice before and after incubation with L-NAME. Data are mean±SEM. Asterisk indicates significant differences between groups treated with L-NAME and untreated groups. C, Aortic tissue from WT [PECAM-1 (+/+), n=3] and PECAM-KO [PECAM-1 (−/−), n=3] was assayed for PECAM-1, eNOS, caveolin 1, and actin protein levels by Western blots.

shear stress in WT and PECAM-KO arterioles is explained by Figure 2D, in which changes in diameter are depicted.

Figure 2D indicates that in vessels of WT mice 2 distinct phases of response could be identified when arterioles were exposed to a rapid increase in flow/shear stress. The slope of the initial phase of the time–diameter curves (up to the first 120 seconds) was found to be 0.378 (R^2=0.99, $P<0.001$), whereas the slope of the late phase of the time-diameter curves between 120 and 300 seconds was significantly less (slope=0.085, R^2=0.87, $P<0.001$). In contrast, only a single slope was identified in the responses of arterioles isolated from PECAM-KO mice when they were exposed to a rapid increase in flow/shear stress (slope=0.094, R^2=0.92; Figure 2D). There was no significant difference in the slopes of the late phase response of WT and the response of PECAM-KO arterioles (Figure 2D). Furthermore, presence of the eNOS inhibitor L-NAME reduced the initial phase of the diameter–time curve obtained in arterioles of WT, but not those of PECAM-KO mice (Figure 2E).

The Function of Endothelium and Smooth Muscle of PECAM-KO Arterioles

The function of endothelium and smooth muscle in arterioles of WT and PECAM-KO mice was tested with ACh and NE, respectively. ACh elicited dilations with a similar magnitude and time course in arterioles of WT and PECAM-KO mice (Table and Figure 3A). Also, there was no significant difference in the magnitude of NE-induced arteriolar responses between vessels of the two groups of mice (Table).

Agonists-Induced Stimulation of eNOS

Cumulative doses of the calcium ionophore A23187[16] elicited substantial dilations of arterioles of WT mice, responses which were not significantly different in arterioles of PECAM-KO mice (Figure 3B). To evaluate the role of NO in the mediation of A23187-induced dilation, arterioles were incubated with L-NAME and responses were obtained again. Presence of L-NAME significantly reduced the magnitude of A23187-induced dilations of arterioles of WT and PECAM-KO mice in a similar manner (Figure 3B).

Vascular eNOS and Caveolin-1 Content

Western blot analysis for eNOS and caveolin-1 were performed on aortas from both WT (n=3) and PECAM-KO mice (n=3) (Figure 3C). The band intensity from Western blot analysis was quantified by densitometry. The protein quantity of eNOS and caveolin-1 was normalized against the quantity of actin in each sample and is presented as arbitrary units of eNOS/actin and caveolin-1/actin (means±SD, n=3 in each groups). The eNOS/actin was 1.24±0.20 for WT and 1.20±0.07 for PECAM-KO mice. The caveolin-1/actin was 1.79±0.12 for WT and 1.84±0.28 for PECAM-KO mice. Neither eNOS nor caveolin-1 expression level was significantly different between WT and PECAM-KO mice (Figure 3C).

Discussion

The principal findings of the present study are that skeletal muscle arterioles of mice with a genetic ablation of PECAM-1 display an impaired dilation in response to rapid increases in intraluminal flow/shear stress. The diminished sensitivity of arterioles of PECAM-KO mice to high temporal gradients of shear stress was primarily attributable to the reduced NO mediation of dilation, whereas activation of eNOS and dilation by the Ca^{2+} ionophore A23187 were maintained. The total vascular levels of eNOS and caveolae were not different in WT and PECAM-KO mice.

Hemodynamic forces undoubtedly play a significant role in maintaining vascular homeostasis. Many of the biochemical pathways transducing changes in fluid shear stress to changes in diameter have been characterized; however, the nature of the mechanoreceptor(s) that sense and transduce shear stress into a biochemical signal is still unclear. Mechanochemical transduction is proposed to occur when membrane-associated signaling proteins are activated by increases in intramolecular mobility.[17] In light of stress analysis showing that tension is greatest within the cell–cell junctions during the rapid onset of fluid flow,[10] one would expect that structures and/or molecules that are sensitive to fluid shear stress are concentrated at the apex of the apical surface of the cell.[18] Interestingly, PECAM-1 is exclusively localized at the cell–cell apposition site in confluent endothelial cells, and it has long been recognized that direct application of mechanical force

results in PECAM-1 tyrosine phosphorylation[19,20] (for review see Fujiwara et al[21]). Thus, in the present study we hypothesized that PECAM-1 is an important molecule in the process, which transforms increases in fluid shear stress to arteriolar dilation. More precisely, we speculated that PECAM-1 is involved specifically in the transduction of the rapid increase in shear stress at the onset of the increase in perfusate flow in intact arterioles.

To test this hypothesis, we have used the PECAM-KO mice that were developmentally indistinguishable from WT controls (Table). This conclusion is further supported by the findings that both endothelial function and vascular smooth muscle responses to pharmacological perturbations were not significantly different between the 2 groups (Figure 3A and 3B; Table).

Flow-induced arteriolar responses were first obtained in response to step increases in shear stress elicited by small steps of perfusate flow increase. At each step the dilations were reduced, and also the maximum response at 12 μL/min was significantly less in PECAM-KO arterioles as compared with vessels of WT mice (Figure 1A and 1B). We noticed that the reduction of arteriolar dilations of PECAM-KO mice to these step increases in flow/shear stress was primarily attributable to the greatly reduced early phase of the response (Figure 1A, inset).

To further examine the role of the early phase of the shear stress–induced response, arteriolar responses were also obtained to a large 1-step increase in perfusate flow. A large 1-step increase in flow elicited a significantly reduced dilation in arterioles of PECAM-KO mice (Figure 2A and 2B). Figure 2C shows that in both groups of vessels the rapid increase in flow elicited high temporal gradients in shear stress (Figure 2C). The high temporal gradient of shear stress elicited dilation in WT arterioles, which consequently resulted in a reduction in shear stress. In contrast, in PECAM-KO arterioles there is a limited regulation of wall shear stress (Figure 2C). This conclusion is further supported by the findings that in WT mice, 2 distinct phases of diameter response were identified when arterioles were exposed to a rapid increase in shear stress (Figure 1D). The single phase seen in the PECAM-KO arteriolar response is similar to the late phase response of WT arterioles, suggesting that the PECAM-KO arterioles lack the mechanotransduction mechanism to respond to high temporal gradients in shear stress, whereas the mechanism required to respond to steady shear stress seems to remain intact.

Collectively, these findings suggest that in intact arterioles PECAM-1 is involved in sensing and transducing high temporal gradients in shear stress to mechanical response, ie, dilation. One can speculate that the PECAM-1 pathway–mediated release of NO may have an important physiological role when blood flow suddenly increases, for example at the beginning of exercise. In this condition, when vessel diameter is still not increased there is a great increase in shear stress as a function of time. High level of wall shear stress increases power dissipation in the circulatory system imposing additional work on the heart. The ability of endothelium to sense sudden increases in shear stress and converting them to dilation, which in a negative feedback manner reduces shear stress, prevents unnecessary energy expenditure in the vascular system[3,5,16]; PECAM-1 seems to be importantly involved in this process. This issue seems to be important because PECAM-1 molecules may have significant relevance to pathologic hemodynamics leading to heart diseases, as human studies have shown that certain single nucleotide polymorphisms in PECAM-1 are linked to an increased risk of coronary artery disease.[22,23]

In cultured endothelial cells we have previously demonstrated that eNOS is complexed with PECAM-1 at the cell–cell junction.[15] Thus, it was logical to hypothesize that ablation of PECAM-1 is associated with a reduced NO release to high temporal gradients of shear stress. Indeed, inhibition of NO synthesis significantly reduced the first phase of dilation in arterioles of WT mice to a rapid increase in flow/shear stress, whereas it did not affect that of arterioles of PECAM-KO mice (Figure 2E). To evaluate the function of eNOS in arterioles of PECAM-KO mice, independent of wall shear stress, responses were also obtained to the calcium ionophore A23187, which is known to activate eNOS by elevation of intracellular Ca^{2+}. We have found that A23187 elicited dilation of arterioles from both groups of animals with the same magnitude (Figure 3B). Also, inhibition of NO synthesis reduced A23187-induced dilations both in arterioles of PECAM-KO and WT mice, suggesting that Ca^{2+}-dependent activation of eNOS and consequent NO release remained intact in PECAM-KO mice.

Altogether, these findings indicate a specific lack of NO release in response to high temporal gradients of shear stress that is responsible for the reduced dilation of arterioles of PECAM-KO mice. On the basis of previous studies one can envision that PECAM-1 may represent a scaffold that localizes signaling molecules to a subcellular site that is subject to the highest tension and tension rates when steps in shear stress are applied. PECAM-1 acts as a scaffold for a number of signaling molecules, such as SH2-containing protein phosphatase, PI3-kinase, β-catenin, and phospholipase C-γ (see reference 24 for review). It has been recently demonstrated that PECAM-1 is a scaffold for eNOS and its binding leads to modulation of eNOS activity,[15] and that there is a complex time-dependent interaction between PECAM-1 and tyrosine kinases.[19,20] The physiological relevance of the PECAM-1–eNOS association is further strengthened by the finding that the eNOS pool localized to the cell–cell junction is enriched 2.5-fold over eNOS pools within the caveolae.[25] Interestingly, caveolae, as another possible shear stress–sensing element on the endothelial plasma membrane, have received considerable interest recently.[26–28] Coimmunoprecipitation and domain-mapping studies have demonstrated a direct interaction of eNOS with caveolin-1, which in part regulates eNOS activity.[29–31] In endothelial cells, chronic exposure to steady values of shear stress has been shown to increase both caveolin and eNOS expression leading to enhanced mechanosensitivity to subsequent step changes in hemodynamic forces.[28] Although we cannot directly ascertain the role of caveolae in our experimental design, endothelial caveolin and eNOS content were not different in WT and PECAM-KO mice (Figure 3C). Given that eNOS localization to the cell–cell junction has also been shown to regulate enzyme activity,[14] our findings

do not support a role for caveolae in the transduction of high temporal gradients in shear stress. We speculate, however, that because the latter phase of shear-induced dilation was similar in arterioles of WT and PECAM-KO mice (Figure 2C), caveolae could still play a role in the transduction of steady values of shear stress.

In conclusion, this study is the first to demonstrate that skeletal muscle arterioles of mice with a genetic ablation of PECAM-1 have impaired NO-mediated dilations to high temporal gradients of wall shear stress. Thus, PECAM-1 seems to play an important role in the ability of the endothelium to sense high temporal gradients of shear stress, which is then coupled with NO-mediated vasodilation.

Acknowledgments

This study was supported by grants from the Hungarian National Scientific Research Fund OTKA (M045186 and T048376), the American Heart Association New York State Affiliate (00–50849T), and the National Institutes of Health (HL-43023, HL-46813, and HL-40696).

References

1. Kamiya A, Togawa T. Adaptive regulation of wall shear stress to flow change in the canine carotid artery. *Am J Physiol*. 1980;239:H14–H21.
2. Fiscus RR. Molecular mechanisms of endothelium-mediated vasodilation. *Semin Thromb Hemost*. 1988;14:12–22.
3. Koller A, Kaley G. Endothelial regulation of wall shear stress and blood flow in skeletal muscle microcirculation. *Am J Physiol*. 1991;260: H862–H268.
4. Koller A, Sun D, Huang A, Kaley G. Corelease of nitric oxide and prostaglandins mediates flow-dependent dilation of rat gracilis muscle arterioles. *Am J Physiol*. 1994;267:H326–H332.
5. Koller A, Kaley G. Shear stress dependent regulation of vascular resistance in health and disease: role of endothelium. *Endothelium*. 1996; 4:247–272.
6. Kuchan MJ, Frangos JA. Role of calcium and calmodulin in flow-induced nitric oxide production in endothelial cells. *Am J Physiol*. 1994;266: C628–C636.
7. Frangos JA, Huang TY, Clark CB. Steady shear and step changes in shear stimulate endothelium via independent mechanisms–superposition of transient and sustained nitric oxide production. *Biochem Biophys Res Commun*. 1996;224:660–665.

7a. Butler PJ, Weinbaum S, Chien S, Lemons DE. Endothelium-dependent, shear-induced vasodilation is rate sensitive. *Microcirulation*. 2000;7: 53–65.

8. Gudi SRP, Nolan JP, Frangos JA. Modulation of GTPase activity of G proteins by fluid shear stress and phospholipid composition. *Proc Natl Acad Sci U S A*. 1998;95:2515–2519.
9. Katoh K, Masuda M, Kano Y, Jinguji Y, Fujiwara K. Focal adhesion proteins associated with apical stress fibers of human fibroblasts. *Cell Motil Cytoskeleton*. 1995;31:177–195.
10. Liu SQ, Yen M, Fung YC. On measuring the third dimension of cultured endothelial cells in shear flow. *Proc Natl Acad Sci U S A*. 1994;91: 8782–8786.
11. Seebach J, Dieterich P, Luo F, Schillers H, Vestweber D, Oberleithner H, Galla HJ, Schnittler HJ. Endothelial barrier function under laminar fluid shear stress. *Lab Invest*. 2000;80:1819–1831.
12. Albelda SM, Oliver PD, Romer LH, Buck CA. EndoCAM: a novel endothelial cell-cell adhesion molecule. *J Cell Biol*. 1990;110: 1227–1237.
13. Harada N, Masuda M, Fujiwara K. Fluid flow and osmotic stress induce tyrosine phosphorylation of an endothelial cell 128 kDa surface glycoprotein. *Biochem Biophys Res Commun*. 1995;214:69–74.
14. Govers R, Bevers L, de Bree P, Rabelink TJ. Endothelial nitric oxide synthase activity is linked to its presence at cell–cell contacts. *Biochem J*. 2002;361:193–201.
15. Dusserre N, L'Heureux N, Bell KS, Stevens HY, Yeh J, Otte LA, Loufrani L, Frangos JA. PECAM-1 interacts with nitric oxide synthase in human endothelial cells: implication for flow-induced nitric oxide synthase activation. *Arterioscler Thromb Vasc Biol*. 2004;24:1796–1802.
16. Huang A, Koller A. Both nitric oxide and prostaglandin-mediated responses are impaired in skeletal muscle arterioles of hypertensive rats. *J Hypertens*. 1996;14:887–895.
17. Beece D, Eisenstein L, Frauenfelder H, Good D, Marden MC, Reinisch L, Reynolds AH, Sorensen LB, Yue KT. Solvent Viscosity and Protein Dynamics. *Biochemistry*. 1980;19:5147–5157.
18. Barbee KA, Mundel T, Lal R, Davies PF. Subcellular distribution of shear stress at the surface of flow-aligned and nonaligned endothelial monolayers. *Am J Physiol*. 1995;268:H1765–H1772.
19. Osawa M, Masuda M, Harada N, Lopes R, Fujiwara K. Tyrosine phosphorylation of PECAM-1 in mechanically stimulated vascular endothelial cells. *Eur J Cell Biol*. 1997;72:229–237.
20. Kaufman DA, Albelda SM, Sun J, Davies PF. Role of lateral cell-cell border location and extracellular/transmembrane domains in PECAM/CD31 mechanosensation. *Biochem Biophys Res Commun*. 2004; 320:1076–1081.
21. Fujiwara K, Masuda M, Osawa M, Kano Y, Katoh K. Is PECAM-1 a mechanoresponsive molecule? *Cell Struct Funct*. 2001;26:11–17.
22. Sasaoka T, Kimura A, Hohta S, Fukuda N, Kurosawa T, Izumi T. Polymorphisms in the PECAM-1 gene, Asn563Ser and Gly670Arg, associated with myocardial infarction in the Japanese. *Ann N Y Acad Sci*. 2001;947:259–269.
23. Listi F, Candore G, Lio D, Cavallone L, Colonna-Romano G, Caruso M, Hoffmann E, Caruso C. Association between PECAM-1 polymorphisms and acute myocardial infarction: a study in patients from Sicily. *Eur J of Immunogenet*. 2004;31:175–178.
24. Ilan N, Madri JA. PECAM-1: old friend, new partners. *Curr Op Cell Biol*. 2003;15:515–524.
25. Heijnen HF, Waaijenborg S, Crapo JD, Bowler RP, Akkerman JW, Slot JW. Colocalization of eNOS and the catalytic subunit of PKA in endothelial cell junctions: a clue for regulated NO production. *J Histochem Cytochem*. 2004;52:1277–1285.
26. Rizzo V, Sung A, Oh P, Schnitzer JE. Rapid mechanotransduction in situ at the luminal cell surface of vascular endothelium and its caveolae. *J Biol Chem*. 1998;273:26323–26329.
27. Rizzo V, McIntosh DP, Oh P, 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*. 1998;273:34724–34729.
28. Rizzo V, Morton C, DePaola N, Schnitzer JE, Davies PF. Recruitment of endothelial caveolae into mechanotransduction pathways by flow conditioning in vitro. *Am J Physiol Heart Circ Physiol*. 2003;285: H1720–H1729.
29. Garcia-Cardena G, Martasek P, Masters BS, Skidd PM, Couet J, Li S, Lisanti MP, 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*. 1997;272:25437–254340.
30. Michel JB, Feron O, Sacks D, Michel T. Reciprocal regulation of endothelial nitric-oxide synthase by Ca2+- calmodulin and caveolin. *J Biol Chem*. 1997;272:15583–15586.
31. Michel JB, Feron O, Sase K, Prabhakar P, Michel T. Caveolin versus calmodulin. Counterbalancing allosteric modulators of endothelial nitric oxide synthase. *J Biol Chem*. 1997;272:25907–25912.