Plaque-prone hemodynamics impair endothelial function in pig carotid arteries

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Plaque-prone hemodynamics impair endothelial function in pig carotid arteries. Am J Physiol Heart Circ Physiol 290: H2320–H2328, 2006. First published January 13, 2006; doi:10.1152/ajpheart.00486.2005.—Hemodynamic forces play an active role in vascular pathologies, particularly in relation to the localization of atherosclerotic lesions. It has been established that low shear stress combined with cyclic reversal of flow direction (oscillatory shear stress) affects the endothelial cells and may lead to an initiation of plaque development. The aim of the study was to analyze the effect of hemodynamic conditions in arterial segments perfused in vitro in the absence of other stimuli. Left common porcine carotid segments were mounted into an ex vivo arterial support system and perfused for 3 days under unidirectional high and low shear stress (6 ± 3 and 0.3 ± 0.1 dyn/cm²) and oscillatory shear stress (0.3 ± 3 dyn/cm²). Bradykinin-induced vasorelaxation was drastically decreased in arteries exposed to oscillatory shear stress compared with unidirectional shear stress. Impaired nitric oxide-mediated vasodilation was correlated to changes in both endothelial nitric oxide synthase (eNOS) gene expression and activation in response to bradykinin treatment. This study determined the flow-mediated effects on native tissue perfused with physiologically relevant flows and supports the hypothesis that oscillatory shear stress is a determinant factor in early stages of atherosclerosis. Indeed, oscillatory shear stress induces an endothelial dysfunction, whereas unidirectional shear stress preserves the function of endothelial cells. Endothelial dysfunction is directly mediated by a downregulation of eNOS gene expression and activation; consequently, a decrease of nitric oxide production and/or bioavailability occurs.

The endothelial NO synthase (eNOS) enzyme is the major source of NO in the vascular endothelium. The activation of this enzyme can be affected by a variety of factors, including availability of cofactors and substrates, as well as subcellular localization and phosphorylation (4, 10–12, 20). Changes in its enzymatic activity are likely to form the basis of altered NO production and, consequently, are one of the earliest biochemical changes preceding endothelial dysfunction (5, 17).

In vitro experiments have shown that unidirectional shear stress stimulated NO production in cultured ECs, and this is mediated by an activation of the eNOS enzyme and an upregulation of eNOS gene expression (2, 8, 19, 31). On the contrary, oscillatory shear stress, mimicking plaque-prone hemodynamics, failed to induce eNOS gene expression (30). Previous studies (18) demonstrated that eNOS activation may be induced by increasing Ser177 phosphorylation, whereas humoral stimuli, such as bradykinin (BK), may activate eNOS by promoting Thr495 dephosphorylation.

Different from numerous studies on cultured ECs, we focused on the effects of shear stress pattern on intact arteries. Hence, cellular and functional adaptation of EC to different flow conditions is studied in a native tissue context.

We submitted porcine arterial segments to unidirectional high and low shear stress and to oscillatory shear stress conditions for 3 days using an ex vivo arterial support system (EVASS). We demonstrated that oscillatory shear stress directly affects endothelial function at least partly through a downregulation of eNOS protein expression and BK-mediated eNOS activation.

MATERIALS AND METHODS

Arterial perfusion system. Segments of left common porcine carotid artery were obtained at the slaughterhouse (from 6-mo-old pigs weighting 120–150 kg). The arteries were harvested and mounted on the perfusion system (Fig. 1A) as previously described in detail (1, 13). The arteries were perfused with medium 199 endothelial basal medium (Amimed), containing 5% fetal bovine serum, 10 mmol/l HEPES, 20 μg/ml gentamicin, 100 U/ml penicillin, 100 U/ml streptomycin, and 0.75 μg/ml amphotericin B (Invitrogen). Medical-grade dextran (8%, 70,000 mol wt; Sigma) was added to increase the medium viscosity to blood viscosity levels (μ ≈ 0.04 Ns/m²). The medium was constantly gassed by 10% CO₂–90% air to better preserve endothelial function. The perfusion chamber containing the arteries was kept in an incubator at 37°C. Perfusion flow was provided by a gear pump system (Ismatec). A function generator producing a 1-Hz sinusoidal flow rate controlled the pump. Flow rate was measured with ultrasonic flowmeters (Transonic System, EMKA Technology). Arterial lumen diameters were measured by using an ultrasonic echotracking device (NIUS, Asulab). Perfusion pressure was set at 70 mmHg.

Groups and sampling. The groups were formed according to shear stress patterns: unidirectional high shear stress (6 ± 3 dyn/cm²), unidirectional low shear stress (0.3 ± 0.1 dyn/cm²), and oscillatory shear stress (0.3 ± 3 dyn/cm²) (Fig. 1B). The oscillatory shear stress pattern had a low mean shear stress value (0.3 dyn/cm²) equal to the

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mean shear stress of the unidirectional low shear stress group but high amplitude of pulsation (± 3 dyn/cm²), which equals the amplitude of pulsation in the unidirectional high shear stress group. Arteries were perfused for 1 and 3 days. At 1 day of perfusion, no differences between the groups were observed; consequently, the results were not shown. Fresh harvested arteries were used as the control group. Six arteries (n = 6) were considered in each group.

ECs function analysis. To determine vascular contractility and endothelial NO-mediated vasorelaxation, arterial rings were cut from the arterial segments and tested before and after each perfusion experiment, according to previous works (6, 23) done on pig carotid arteries. Arterial rings (0.5 cm wide) were mounted in an organ chamber and equilibrated in Krebs solution at 37°C, constantly gassed with a 5% CO₂-95% O₂ mixture. Resting tension was adjusted to 2 g. Arterial rings were precontracted with 90 mmol/l KCl until a constant contraction level was reached. A dose-response curve to norepinephrine (NE, Sigma) was then determined and normalized to contraction, obtained with 90 mmol/l KCl. After being washed extensively, arteries were precontracted with a defined dose of NE to achieve 50 – 60% of contraction obtained with 90 mmol/l KCl and then relaxed by increasing concentrations of BK from 10⁻⁹ to 10⁻⁶ mol/l (Sigma). Dose-response curves to BK were determined. Percentages of BK-induced vasorelaxation were normalized to the precontraction obtained with the defined dose of NE. Endothelium-independent NO-mediated vasorelaxation capacity of the arteries has been tested with the addition of 10⁻⁵ mol/l of sodium nitroprusside (SNP) to the samples. Tension evolution was sampled at a rate of 1 Hz and recorded using of IOX Data software (version 1.7.0; EMKA Technology). Data were analyzed with Datanalysis 1.80.03 (EMKA Technology).

In vitro flow perfusion of isolated ECs. To detect the pathway of BK-mediated eNOS activation, we used an experimental system generating different flow patterns on isolated pig endothelial cells. Briefly, the perfusion system is composed of compliant tubes coated with 20 ng/ml bovine fibronectin and seeded with porcine ECs. Tubes were mounted into specially designed fittings (31). When cells formed a confluent monolayer, they were inserted in a perfusion loop composed of a medium reservoir and a gear pump (Ismatec). Cells were exposed to the same hemodynamic environments as those used for native arteries for a period of 24 h. Pressures mean value was maintained at 100 mmHg. After perfusion, cells were immediately exposed to 10⁻⁷ mol/l of BK for 30 s to induce BK-mediated eNOS activation. Some cells were not stimulated by BK and were kept as control. Proteins were harvested for further analysis.

Extraction and quantification of RNA. Total tissue RNA was extracted from the arterial segments using the Tri-Pure extraction kit (Sigma), following the manufacturer’s instructions. Reverse transcriptase reaction was performed, starting from 5 μg of total RNA in the presence of 0.5 μmol/l of deoxynucleotides mixture (dATP, dCTP, dGTP, and dTTP) (Amersham), 1 μg oligo(dT) (Promega), and 200 U/μl Superscript II (Invitrogen) at 42°C for 1 h. Quantitative real-time PCR was performed on ABI PRISM 7700 (BD Biosciences) using QuantiTect probe PCR kit (Qiagen) consisting of an oligonucleotide...
with a 5′-FAM(6-carboxyfluorescein) and a 3′-TAMRA (6-carboxytetramethylrhodamine) quencher dye. PCR primers and probes sequences were designed with Primer 3 software available on the net (25), synthesized by MWG Biotech Sequences, and were as follows:

1) eNOS forward primer 5′-GTGGAAATCAACCTGGCTGT-3′, backward primer 5′-GACCATCTCCTGGTGGAAGA-3′, and taqman probe 5′-FAM(6-carbobxyfluorescein)TGACCATTGTGGACC-TATCAC-TAMRA(6-carboxytetramethylrhodamine)-3′;

2) vascular endothelial (VE)-cadherin forward primer 5′-CGTGGTGGAAACA-CAAGATG-3′, backward primer 5′-TGTGTACCTGGTCTGGTGAAGA-3′, and taqman probe 5′-FAM(TGACCATTGTGGACC)-3′; and

3) GAPDH forward primer 5′-GGGCATGGAACCATGAGAAGT-3′, a backward primer 5′-GTCTTCTGGGCTCAGTGAT-3′, and taqman probe 5′-FAM(TGGAAGGACTCATGACCACA)-TAMRA-3′. The PCR reaction was performed in the presence of each primer (10 μmol/l), probe (10 μmol/l), ×1 Quantitrect probe mastermix (Qiagen), and cDNA (3 μl). Thermocycling conditions were as follow: 50°C for 2 min and 95°C for 15 min, followed by 50 cycles at 95°C for 30 s, 56°C for 30 s, and 76°C for 30 s, with fluorescent reading at each cycle. All samples were run in triplicate. mRNA quantifications were performed by using the relative standard curve method. Standard curves for eNOS and VE-cadherin mRNAs amplifications were established and used to determine the quantity of the respective mRNA in the samples. eNOS mRNA expression has been normalized to VE-cadherin mRNA quantity. Histograms show the expression of eNOS mRNA in the different groups as a percentage of the quantity expressed in the freshly harvested arterial group (control at 0 days).

Western blot analysis. Protein expression was assessed by using the immunoblot technique. After protein extraction with Brij-35 lysis.
buffer [containing 50 mmol/l Tris (pH 7.5), 1 mol/l NaCl, 2 mol/l urea, 0.1% Brij 35, and 1 protease inhibitor cocktail tablet (Roche)], 20 μg of total protein were electrophoresed and transferred to a nitrocellulose filter (Amersham). Filters were incubated with either rabbit anti-Ser1177 phospho-eNOS, anti-Thr495 phospho-eNOS at a dilution of 1:1,000 (Cell Signalling Technology), mouse anti-eNOS at a dilution of 1:1,000 (BD Biosciences), goat anti-VE-cadherin at a dilution of 1:200 (Santa Cruz Biotechnology), or mouse anti-β-actin at a dilution of 1:5,000 (Cytoskeleton), followed by an incubation with goat anti-rabbit or anti-mouse IgG horseradish peroxidase-linked secondary antibodies at a dilution of 1:1,000 (Amersham). Enhanced chemiluminescence was used for detection (Amersham). Protein expression was quantified with the use of a Kodak Image Station 2000R and Kodak 1D Image Analysis Software. eNOS protein expression levels were calculated as the ratio of eNOS over VE-cadherin (marker of ECs). The rate of eNOS phosphorylation was calculated as the ratio of phospo-eNOS over total eNOS protein level for each sample. The calculated ratios were normalized to the average ratio of phosphorylation measured in freshly harvested arteries at 0 days.

Confocal and electron microscopes of endothelium. For fluorescence microscopy, a cell tracer dye (CellTrace Far Red DDAO-SE, Molecular Probes) was used to identify cell contours. Nuclei were identified using 4',6-diamidino-2-phenylindole dihydrochloride (DAPI, Fluka). Cells were incubated for 60 min in presence of CellTrace, followed by a 15-min incubation in DAPI before direct observation. Images were analyzed with an optical confocal laser microscope (Leica TCS SP2 AOBS).
Tissue segments prepared for electron microscopy were fixed in 2.5% glutaraldehyde in 0.1 mol/l Sorensen’s phosphate buffer (pH 7.4) at 4°C for 2 h, and postfixed with 2% osmium tetroxide (Fluka) in the same buffer at room temperature for 1 h, dehydrated in graded alcohol solutions, and dried over CO2. They were sputter coated with gold (MED 010, Bal-Tec) for observation. Inner artery surface was observed by scanning electron microscope (JEOL 6300) at 5 kV.

**Immunofluorescence.** At the end of the perfusion period, part of the arterial segment was rinsed with 0.9% NaCl, snap-frozen in optimum cutting temperature compound (Tissue-Tek), and stored at −80°C for further analysis. For eNOS staining, serial sections (5 µm) were cut, air-dried, and fixed in 100% acetone for 5 min at −20°C. Sections were incubated successively with 0.1% Triton X-100 in PBS for 10 min and then incubated for 60 min with anti-eNOS (1:200, BD Transduction) in 10% normal goat serum in PBS. The sections were then incubated with a goat anti-mouse IgG fluorescein-conjugated (1:250) or goat anti-mouse IgG Alexa 563-conjugated (1:250) as secondary antibody for 45 min. All steps were performed at room temperature. Sections were examined on a Zeiss Axiovert 135 microscope.

**Statistics.** ANOVA was used to analyze variance between groups. When possible, two-tailed Student’s t-test was performed. A value of P ≤ 0.05 was considered significant.

**RESULTS**

**Effect of shear stress patterns on EC function.** BK-mediated relaxation was detected on freshly harvested arteries and on arterial segments exposed to different types of shear stress. In arteries exposed for 3 days to unidirectional high and low shear stress (6 ± 3 or 0.3 ± 0.1 dyn/cm², respectively), the vasodilation in response to different concentrations of BK (10⁻⁹, 10⁻⁶ mol/l) was maintained at levels comparable with those observed in freshly harvested arteries (Fig. 2A). On the other hand, the endothelial function in arteries exposed to oscillatory shear stress (0.3 ± 3 dyn/cm²) drastically decreased after 3 days of perfusion. In these arteries, BK-derived relaxation decreased by ~65% compared with high shear stress group (P ≤ 0.02). After 1 day of perfusion, no differences were observed between groups, suggesting that such a short period of perfusion was not sufficient to reveal the effect of flow patterns on endothelial function (data not shown). To assess the involvement of NO in the observed BK-stimulated vasorelaxation, arteries were stimulated with BK in the presence of 10⁻⁴ mol/l of Nω-nitro-L-arginine methyl ester (L-NAME). This treatment completely blocked BK-induced vasorelaxation (Fig. 2B). Furthermore, incubating arterial rings with 10⁻⁵ mol/l SNP assessed the endothelium-independent relaxation capacity of the arteries in response of NO. No statistical differences were observed in NO-mediated vasorelaxation among the different types of shear stress groups after 3 days of perfusion (Fig. 2C).

**Preservation of endothelial monolayer.** To assess whether the difference in BK-mediated vasorelaxation was a real effect or an artifact due to a loss of ECs after the perfusion, arterial segments were analyzed by confocal and electronic microscopy in high unidirectional and oscillatory shear stress groups and compared with the freshly harvested arterial group. No differences were observed. ECs formed a homogeneous and confluent monolayer before and after 3 days of perfusion independently from the hemodynamic environments applied (Fig. 3).

**Shear stress-mediated eNOS gene expression and activation in native tissue.** We first assessed that VE-cadherin expression, a specific marker of ECs, was not affected by any shear stress pattern used, allowing us to use this protein to normalize eNOS protein level to the total amount of endothelium-derived proteins contained in each sample (data not shown). Consistent with the data of endothelial function evolution during perfusion, 3 days of culture under oscillatory shear stress led to a 25% decrease in expression of eNOS protein compared with high shear stress (Fig. 4A, P ≤ 0.05). The decrease in eNOS protein level observed in arteries exposed to oscillatory shear stress was also statistically significant compared with freshly harvested arteries, the reduction rate reaching 35% (Fig. 4A, P ≤ 0.05). In contrast, 3 days perfusion under unidirectional shear stress maintained eNOS protein expression at levels comparable to the freshly harvested arterial group.
eNOS expression along the internal lumen of vessels exposed to different flow patterns was analyzed by immunofluorescence. In perfused arteries, a lower eNOS expression in arteries exposed to oscillatory shear stress was observed compared with arteries exposed to unidirectional shear stress (Fig. 5A). We also analyzed eNOS expression on cross sections of freshly harvested arteries at 1 cm from the bifurcation. eNOS expression was not equally distributed along the luminal surface. Indeed, the outer wall of the artery, supposed to be exposed to a perturbed flow in vivo, showed an undetectable expression of eNOS protein (Fig. 5B). The integrity of the endothelial layer was confirmed by DAPI staining and by immunostaining with anti-CD31 antibody (data not shown). Regardless of the shear stress pattern, all groups perfused for 3 days in vitro exhibited a reduction in eNOS mRNA level compared with the freshly harvested arteries. In arteries exposed to high shear stress, eNOS mRNA level was ~50% of that expressed in freshly harvested arteries, but these results were not statistically different. The same results were observed after 1 day of perfusion (data not shown). On the contrary, a significant 70% decrease of eNOS mRNA level was observed in arteries exposed to oscillatory shear stress compared with arteries exposed to unidirectional high shear stress (Fig. 4B, P ≤ 0.04).

Constitutive activation rate of eNOS in arterial segments appeared not to be influenced by the different hemodynamic environments applied. Indeed, in all the groups analyzed, the same rate of Ser1177 phosphorylation was observed. The rate of Thr495 phosphorylation was not detectable in any groups (data not shown).

**BK-induced changes in eNOS phosphorylation.** To assess whether flow pattern affects BK signaling pathways, we ana-
analyzed the BK-induced changes in eNOS phosphorylation on isolated pig ECs after 24 h of perfusion. In ECs maintained at static conditions or exposed to high and low shear stress, an increase of BK-stimulated eNOS phosphorylation of Ser^{1177} has been detected compared with unstimulated ECs [Fig. 6A (first line) and B, \( P \leq 0.002 \)]. On the contrary, in arteries exposed to oscillatory shear stress, the increase of eNOS phosphorylation of Ser^{1177} after BK treatment was abrogated (Fig. 6, A and B). Moreover, the highest increase in constitutive and BK-induced eNOS activation was observed in ECs exposed to high shear stress condition. Futhermore, Thr^{495} phosphorylation was observed only in ECs exposed to oscillatory shear stress after a BK treatment (Fig. 6A, second line).

**DISCUSSION**

In the present study, we investigated the effect of different hemodynamic environments on endothelial function in arteries perfused ex vivo. The study was designed to test the hypothesis that oscillatory shear stress, mimicking plaque-prone conditions, may lead to impairment of endothelial function, thus constituting a predisposing factor promoting the early formation of atherosclerotic plaques.

In recent years, the importance of hemodynamic forces on EC function and metabolism has become more and more evident (24). Several studies (9, 14, 26, 30) have demonstrated that ECs exhibit a capacity to discern between different hemodynamic environments. Nevertheless, the mechanisms by which hemodynamic forces predispose or protect against plaque development are not fully understood. The majority of studies (8, 22, 30) addressing the effects of hemodynamics on eNOS gene expression were performed in isolated ECs and have illustrated that dynamic characteristics of flow modulate the production of vasoactive mediators, such as NO and endothelin-1. However, isolated cells studies may miss important aspects deriving from cell matrix as well as the intertypic cellular interactions, which may be essential in determining the cell response to hemodynamic force stimuli. Other studies (4, 28) using animal models addressed directly the functional responses of ECs to different flow patterns, but no precise control on mechanical factors was possible. To overcome such limitations, we investigated the effects of hemodynamics on ECs on arterial segments using a modified EVASS (Fig. 1A) (13).

The evolution of endothelial function before and after exposure to different hemodynamic conditions using an organ chamber system was analyzed. A significant decrease of endothelium-derived vasorelaxation was observed in arteries exposed for 3 days to oscillatory shear stress compared with arteries exposed to unidirectional shear stress (Fig. 2A). Loss of endothelium-dependent vasodilation was attributed to a decreased in NO production. Indeed, in our experimental conditions, BK-induced vasorelaxation has been demonstrated to be completely abrogated by the concomitant use of the L-arginine analog L-NAME (Fig. 2B). Moreover, using NO donors, we demonstrated that the NO-mediated vasorelaxation capacity of the artery was still preserved after 3 days of perfusion (Fig. 2C). The unidirectional high and low shear stress did not affect endothelium-derived vasorelaxation. Moreover, all the perfu-

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**Fig. 6. Detection of BK-induced eNOS phosphorylation of Ser^{1177} and dephosphorylation of Thr^{495}.** Isolated porcine endothelial cells were cultured in tubes and submitted to high SS, low SS, and Osc SS or to static condition for 24 h and treated or not treated with BK at concentration of 10^{-7} mol/l.

**A:** representative Western blot analysis showing phosphorylation of Ser^{1177} and Thr^{495} and total eNOS protein level. BK-mediated eNOS activation (+) and untreated cells (-) are shown.

**B:** ratio of phospho-Ser^{1177} eNOS on eNOS protein level after 24 h has been quantified. Data are expressed as means \( \pm \) SD. *\( P \leq 0.002; n = 4 \) arteries.
sion conditions used preserved a uniformly confluent endothelial monolayer (Fig. 3), demonstrating that the decrease of BK-mediated vasorelaxation could not be attributed to a loss of ECs determined by the experimental procedure. These data suggest that plaque-prone flow modulates endothelium-derived vasodilation properties or capacities by impairing BK-induced NO production and/or bioavailability.

Analysis of eNOS protein expression in these arteries confirmed that, after 3 days of perfusion, unidirectional shear stress better preserved the expression of this protein compared with oscillatory shear stress (Fig. 4A). These data were further confirmed on tissues. eNOS expression was higher in arteries exposed to unidirectional flow compared with oscillatory shear stress (Fig. 5). In vivo relevance of our findings was supported by eNOS immunostaining on a cross section of a branched region. An undetectable expression of eNOS was observed in the outer wall of the carotid sinus (Fig. 5B). This region is supposed to experience disturbed flow in vivo.

The decrease in eNOS protein expression in arteries exposed to oscillatory shear stress was also observed at the mRNA level. In fact, a lower eNOS mRNA level in arteries exposed to oscillatory shear stress compared with high shear stress condition was detected (Fig. 4B). However, a decreased eNOS level of mRNA was found for all perfused arterial groups compared with the freshly harvested arteries. This result may be explained by the fact that in animals, carotids arteries are exposed to higher shear stress values, whereas in our system, due to technical limitations, arteries were submitted to a maximal mean shear stress value of 6 dyn/cm². Furthermore, the decrease in eNOS mRNA correlates with a reduced eNOS protein level and BK-mediated vasorelaxation only in arteries consistently exposed to an oscillatory shear stress. These observations further support the hypothesis that the flow pattern (unidirectional vs. bidirectional) is the main mechanical factor influencing endothelial cell function. Moreover, our results also suggest that unidirectional shear stress may preserve endothelial function though a stabilization of eNOS protein, a mechanism that could be mediated by an inhibition of the ubiquitin-proteasome pathway, recently shown to participate to eNOS gene expression regulation (16, 27). An alternative hypothesis may be that those flow patterns also influence the translation rate of eNOS mRNA (29), which in this case would be maximal in arteries exposed to unidirectional shear stress. These hypotheses are currently under investigation.

In arteries exposed to oscillatory shear stress, the degree of the loss in BK-mediated vasorelaxation was more important that the loss in eNOS gene level. This discrepancy may be due to differences occurring at other steps of the BK-activated signaling pathway or, alternatively, to variations of NO bioavailability, eNOS protein activation, level of L-arginine, or others cofactors involved in eNOS activity (3, 20). Based on results obtained in previous studies (8, 10, 11, 18), we decided to investigate the eNOS phosphorylation, an important mechanism for the posttranslational regulation of the protein activity. We analyzed the effect of shear stress on both constitutive as well as BK-inducible phosphorylation of Ser1177 and Thr495 residues of eNOS protein. Indeed, phosphorylation of these two sites has been shown to correlate respectively with the active and inactive state of the enzyme (18). ECs were exposed for 24 h to either unidirectional or oscillatory shear stress, exactly as with the experiment on arteries. At the end of the experiments, cells were immediately tested for the detection of constitutive eNOS phosphorylation or treated with 10⁻⁷ mol/l BK and further analyzed. No statistical difference in the constitutive Ser1177 phosphorylation rate was observed among different shear stress conditions (Fig. 6). On the contrary, BK-induced phosphorylation of Ser1144 residue was clearly dependent on the flow pattern applied. Indeed, in arteries exposed to static condition and unidirectional shear stress, BK treatment induced a higher level of Ser1177 phosphorylation compared with the constitutive Ser1177 phosphorylation rate (Fig. 6B). On the contrary, phosphorylation of Ser1177 after BK treatment was completely abrogated in cells exposed to oscillatory shear stress (Fig. 6A). Moreover, a phosphorylation of Thr495 residue, which corresponds to the inactive state of the enzyme, was only observed in cells exposed to oscillatory shear stress and further treated with BK (Fig. 6, A and B). These data demonstrate that plaque-prone environment affects BK signaling pathways regulating the activity of eNOS.

In conclusion, oscillatory shear stress was shown to decrease BK-induced vasorelaxation through a downregulation of eNOS gene expression and through impairing eNOS enzyme activation. eNOS gene expression appeared to be a limiting factor for the endothelial function, in particular for the BK-induced vasorelaxation. Our data further support the hypothesis that unidirectional, but not oscillatory, shear stress protects the arterial wall against atherosclerotic plaque development by preserving higher levels of eNOS gene expression retaining a good endothelial function.

To our knowledge this is the first study reporting such effects on isolated arterial segments, thus preserving the possible effects of vascular cell interactions as well as cell-extracellular matrix interactions. It constitutes a novel approach for the ex vivo study of flow-induced arterial function and a physiologically relevant tool in the investigation of role of hemodynamics in the initiation of arterial disease.

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