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Shear Stress Activates eNOS at the Endothelial Apical Surface Through β1 Containing Integrins and Caveolae

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Abstract—There is now a large body of evidence demonstrating that fluid mechanical forces generated by blood flowing through the vasculature play a direct role in regulating endothelial cell structure and function. Integrin receptors that localize to the basal surface of the endothelium participate in both outside-in and inside-out signaling events that influence endothelial gene expression and morphology in response to flow. Our analyses of apical plasma membranes derived from cultured bovine aortic endothelial cells revealed that integrins are also expressed on this cell surface. Here, we tested whether these integrins participate in mechanotransduction events that are known to occur on the endothelial cell luminal/apical membrane. We found that apically expressed β1 integrins are rapidly activated in response to acute shear stress. Blockade of β1 integrin activation attenuated a shear-induced signaling cascade involving Src-family kinase, PI3-kinase, Akt and eNOS on this cell surface. In addition, β1 integrin activation and associated signaling events were dependent on the structural integrity of caveolae but not the actin cytoskeleton. Taken together, these data indicate that endothelial responses to shear stress are mediated by spatially distinct pools of integrins.

Keywords—Mechanotransduction, Caveolin, Hemodynamic.

INTRODUCTION

Endothelial cells are interposed between the circulating blood and underlying components that comprise the blood vessel wall. This anatomical arrangement subjects the endothelium to fluid mechanical forces generated by flowing blood, such as shear stress. As a result, these cells have a well-developed capacity to rapidly sense and respond to this hemodynamic force. Thus, shear stress is considered an essential regulator of endothelial phenotype and by extension, a mediator of vascular function.

There appears to be several cellular elements within the endothelium that are important for detection and/or conversion of shear stress into biological signals. They include structural components such as the glycocalyx,[30] primary cilia,[20] the cytoskeleton[13] and caveolae,[25,26] receptors such as VEGFR2,[7] Tie-2,[10] P2X4[36] and Bradykinin B2[4] and adhesive proteins including integrins[8] and PECAM-1.[33] Recent evidence suggests that associations made between these as well as other cellular constituents are mechanistically important for mechano-signal propagation which govern endothelial adaptive responses to flow.[35] Of these, we recently described a novel mechano-signaling complex composed of caveolae, its structural protein caveolin-1 and the β1 integrin subunit in regulation of endothelial morphology induced by shear stress.[37]

Spatially, integrin expression is concentrated on the basal surface of the endothelial cell membrane. Here, shear stress alters that conformation of various low affinity integrins allowing them to bind to extracellular matrix proteins and induce signal transduction events that modulate endothelial cell phenotype.[8] Interestingly, several studies demonstrate that fibronectin or RDG-coated beads applied to the apical surface of cultured endothelial cells elicits rapid mechanotransduction responses following mechanical displacement of the integrin-engaged beads.[15,34] Considering our past studies demonstrating that the luminal/apical endothelial surface participates in mechanosignaling *via* caveolae and[24–26] that integrin mechanotransduction is linked to caveolae/caveolin-1,[22,23] we hypothesize that shear-activated β1 integrins are not limited to the basal endothelial cell surface but also present on the endothelial luminal/apical membrane. This concept is supported by the findings presented here showing that β1 integrins localized on the endothelial apical surface are sensitive to shear stress and participate in

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mechanotransduction processes in association with caveolae.

EXPERIMENTAL PROCEEDURES

Antibodies and Reagents

All general buffers and reagents were purchase from either Fisher Scientific or Sigma, unless noted otherwise. Cytochalasin D was purchased from Calbiochem. The following primary antibodies were obtained from commercial sources: IgG (mAb), anti-caveolin-1 (mAb and pAb), anti-paxillin (mAb) and anti-fibronectin were from BD Bioscience; β1 integrin blocking antibody, JB1A, and HUTS 21 and HUTS 4 monoclonal antibodies (mAb) were from Chemicon; β1 integrin (mAb), anti-FAK (pAb) and antibody against Ser1179 of eNOS were from Millipore. All other primary antibodies were purchased from Cell Signaling. Rabbit anti-mouse antibody was from Bethyl. Horse-radish peroxidase conjugated anti-rabbit and anti-mouse secondary antibodies was supplied by Amersham.

Cell Culture

Bovine Aortic Endothelial Cells (BAEC) were purchased from VEC Technologies, Inc. (Rensselaer, NY). Cells were cultured in MCDB-131 medium supplemented with 10% Fetal Bovine Serum (Atlanta Biologicals), 0.05 mg/mL gentamycin sulfate (Cambrex Biosciences) and maintained at 37 °C, 97% humidity and 5% carbon dioxide. All experiments were performed using cells between passages 5 and 8.

In Vitro Flow Experiments

A parallel plate flow chamber (Streamer model, Flexcell Corp.) was use to impose shear stress on cultured BAEC, as described in our past studies.[23,37] To reduce background cell signaling contributed by serum growth factors, cells were acclimated for 2 h in ''flow-media'' consisting of MCDB-131 and 1% FBS prior to placement in the chamber. Monolayers were exposed to acute step change in shear stress applied at a magnitude of 10dynes/cm2 for 1–30 min from static conditions. All experiments included a no-flow, static control sample where endothelial monolayers were incubated in flow media for the same time periods outlined above.

Labeling of Shear-Activated β1 Integrins

To detect activated β1 integrin, cells were incubated for 20 min with either HUTS 4 or HUT 21 antibodies (1 μg/mL) following exposure to shear stress. As a positive control for β1 integrin activation, cells were treated with 1 mM Mn^{2+} for 10 min prior to incubation with HUTS antibody. To determine extent of non-specific antibody binding to the endothelial cell surface, monolayers were incubated with iso-type matched mouse IgG. Following antibody binding, monolayers were processed for isolation of apical endothelial plasma membranes followed by Western blotting.

Membrane Raft/Caveolae Disassembly

As detailed in our prior reports,[3,23] BAEC's were incubated in serum free media containing either 10 mM methyl-β-cycodextrin for 30 min or 5 μg/mL filipin for 5 min at 37 °C prior to shear stress

Caveolin-1 siRNA

Similar to our past reports,[3,23] BAEC were transfected with caveolin-1, SMARTpool siRNA or siScrambled control using DharmaFECT-1 (Dharmacon) according to the manufacturer's protocol. Similar to our previous experiments, cells were used 48 h post-transfection.

Disruption of the Actin Cytoskeleton

Prior to subjecting endothelial cells to shear stress, monolayers were pretreated with 0.25 μM of cytochalasin D for 1 h to disrupt the actin cytoskeletal network. At this concentration, cytochalasin D altered the actin network without disruption of cell–cell contacts (data not shown).

Purification of Luminal Plasma Membranes

Apical endothelial cell plasma membranes were isolated using a silica coating procedure, as previously described.[25] Following exposure to shear stress and, in some cases, antibody labeling for activated β1 integrin, monolayers were rinsed with MES-buffered saline (pH 6.0). Cells were incubated with a positively charged colloidal silica solution for 10 min at 4 °C. Subsequent cross-linking of the silica particles by incubation with polyacrylic acid (0.1%) served to create a stable adherent silica pellicle to increase the density of this cell surface. Endothelial cells were collected by gentle scraping and homogenization in a Type AA Teflon pestle/glass homogenizer (10 strokes at 1800 rpm). The homogenates were filtered through 30 μM mesh and filtrate mixed with 102% (wt/vol) Nycodenz (Life Sciences) containing 20 mM KCl. The Nycodenz/homogenate solution was layered over a continuous

55–70% Nycodenz gradient and centrifuged in a Beckman MLS50 rotor at 18,000 rpm for 30 min at 4 °C. The resulting pellet of purified apical plasma membranes (P) was resuspended in 0.5 mL of MES-buffered saline.

Western Blotting

Endothelial cell apical membranes (P) and whole cell homogenates (H) were processed for Western blot analysis as described in our past work.[25] To detect HUTS antibodies, nitrocellulose membranes were probed with an HRP-conjugated rabbit anti-mouse antibody for 1 h. Binding events were detected by enhance chemiluminescence. Autoradiographs were scanned, digitized and band intensities quantified using Image J software.

Statistical Analysis

For each study, data was gathered from at least three independent experiments and pooled according to group. Mean and standard deviation were calculated and differences between groups analyzed with an unpaired two-tailed Student's t test or ANOVA with a *post hoc* Tukey test using STATGRAPHICS 4.0 software (Statistical Graphics Corp). Differences between control and experimental groups were deemed significant at $p < 0.05$.

RESULTS

Shear Stress Activates β1 Integrins on the Endothelial Apical Surface

Integrins are known mechano-signaling elements at the basal surface of the endothelium.[8] While mechanical displacement of integrins located on the endothelial apical surface also results in mechanotransduction,[15,34] it is unknown whether integrins present on this cell surface are activated and function in mechanotransduction associated with physiological stimuli such as hemodynamic shear stress.

To begin to address this question, we compared the protein expression pattern of integrins and integrin-related mechano-signaling molecules between whole cell homogenates (H) and apical plasma membranes (P) purified by colloidal silica technology. Consistent with our past findings,[25] endothelial apical membranes were enriched in caveolin-1 (>10-fold) and eNOS relative to their expression levels in the whole cell (Fig. 1). In addition, both Src-family kinases (SFK) and Akt, while not enriched, localized to this cell surface. In contrast, focal adhesion kinase (FAK) was scantly detected (less than 1% of cell total) in samples of apical membrane isolates. While paxillin was present within the apical membrane compartment, its expression was 65% less then that found in whole cell lysates. These findings indicate that focal adhesion associated proteins are de-enriched in these membrane preparations. More importantly, we found that β1 and β3 integrins are expressed on the endothelial cell apical surface (Fig. 1).

Activation of β1 integrin involves a conformational change in protein structure which exposes epitopes 355–425 in the molecules hybrid domain. The HUTS group of monoclonal antibodies selectively recognize this region and their binding serves as a measure of β1 integrin activation.[14] To test whether β1 integrins present on the endothelial apical surface are capable of confirmation change, cell monolayers were exposed to

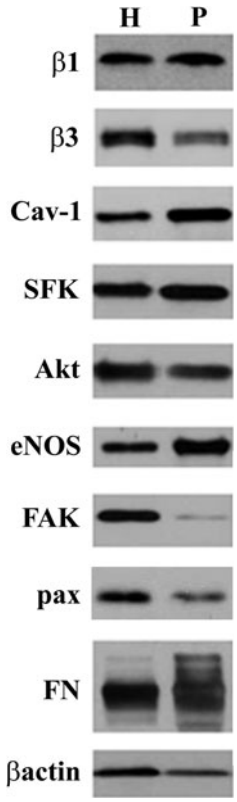

FIGURE 1. β-type integrins are expressed on apical endothelial cell membranes. Apical membranes of BAEC were isolated by colloidal silica method. Proteins from whole cell (H) and purified apical plasma membranes (P) were separated by SDS-PAGE and detected by Western blot. Results show that while β1 and β3 integrins are both present on the endothelial apical cell surface, β1 integrin expression levels were 4-fold greater than expression of β3 integrins. The focal adhesion localized protein, FAK and paxillin (pax), while detected in the P fraction, were de-enriched within apical membranes. Both caveolin-1 and eNOS are significantly enriched at the apical membrane. SFK's, Akt and β-actin also localize, to varying degrees, on this cell surface. The β-type integrin ligand, fibronectin (FN) was also detected in apical membrane isolates. Blots illustrate typical pattern of expression observed from 5 independent experiments.

Mn^{2+} cations, a known stimulus for activation of β-type integrins. Analysis of purified apical membranes showed that both HUTS 21 and HUTS 4 bound to this cell surface following Mn^{2+}-induced activation of integrins (Figs. 2a, 2b). To determine whether shear stress could similarly activate these integrins, apical membrane isolates were probed with a secondary antibody against the HUTS reagents. We observed rapid (1 min) activation β1 integrins present on the endothelial apical membrane which was sustained through 30 min of exposure to shear stress (Figs. 2c, 2d). In similar studies using WOW-1 as a β3 integrin specific activation probe, antibody binding to the apical endothelial membrane could not be detected at this early time point (data not shown). Given the observed pattern of β1 integrin activation in response to shear stress, subsequent experiments were conducted at the earliest time point in an effort to focus on events that initiate mechanotransduction on the endothelial apical surface.

Caveolae Domains and Shear-Induced Activation of β1 Integrins

Several reports indicate that activation of β1 integrins requires the proper membrane concentration of cholesterol, glycosphingolipids [29] and caveolin-1,[17] all

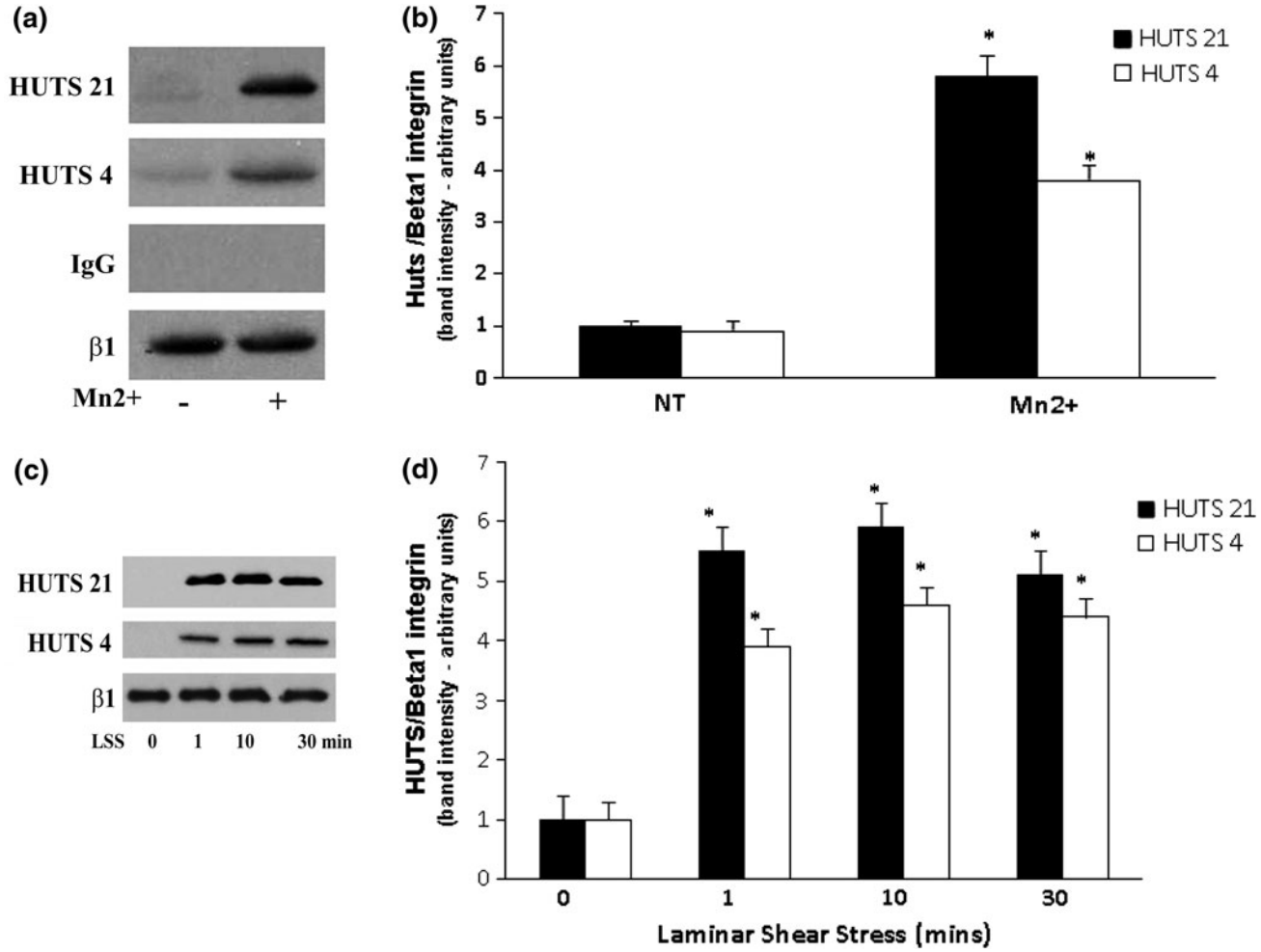

FIGURE 2. **β1 integrins at the endothelial apical surface are activated by shear stress. (a) BAEC's were treated with $MnCl^2$ (1 mM for 10 min) to activate integrins. Cells were incubated with HUTS 21 or HUTS 4 monoclonal antibodies which selectively recognize an activated conformation of β1 integrins. Endothelial apical membranes were isolated and processed for Western blot analysis to determine extent of HUTS antibody binding to this cell surface. Nonspecific antibody binding was evaluated by incubation with isotype-matched IgG. (b) Histographic depiction of Western analysis where * indicates significant enhancement (p value <0.05) over non-treated (NT) samples. (c) BAEC's were subjected to 10 dynes/cm^2 of shear stress (LSS) for indicated time. The cells were incubated with HUTS antibodies followed by colloidal silica purification of apical plasma membranes. Shear-induced activation of β1 integrins were assessed through detection of HUTS antibodies in apical membrane isolates. Western blots are representative of 3–5 independent experiments and (d) is the densitometric quantification of blots for each group at each shear time point. Asterisk (*) indicates significant enhancement (p value <0.05) over static, non-sheared (LSS = 0) control samples. Note that stimulation with $MnCl^2$ or shear stress did not alter β1 integrin levels expressed on the endothelial apical surface.**

components of caveolae. Since caveolae also participate in mechanotransduction processes at the endothelial apical/luminal surface,[24–26] we evaluated that role of caveolae in shear-induced β1 integrin activation at this site. Endothelial cell monolayers were pretreated with either methyl-β-cyclodextrin or caveolin-1 siRNA to disrupt caveolae membrane domains. These treatments, as well as acute exposure to shear stress, did not alter the total amount of β1 integrin expressed within endothelial apical membranes (Figs. 3a, 3b, and 3d). In both cholesterol and caveolin-1 depleted cells, shear-stress induced HUTS 21 binding to the isolated apical membranes was reduced to near baseline levels compared to control samples (Figs. 3a, 3b, and 3d). These data indicate that intact caveolar membranes play a functional role in the process by which shear stress activates β1 integrins at the endothelial apical surface.

Disruption of the Actin Cytoskeleton Does Not Significantly Alter Shear-Induced Activation of Apical Surface β1 Integrins

During the process of mechanotransduction, the actin cytoskeleton serves as a structural system to transmit forces imparted on one site in a cell to another. Since integrin activation can be achieved *via* a mechanism of inside-out signaling through linkages to actin, we tested whether the integrity of the actin cytoskeleton was necessary for activation of β1 integrins localized to the endothelial apical surface. We found that treatment of endothelial cell cultures with the actin microfilament disrupting agent, cytochalasin D, prior to flow, did not significantly alter shear-induced activation of β1 integrins present on the endothelial apical surface (Figs. 3c, 3d).

At the Apical Endothelial Cell Surface, Shear-Induced β1 Integrin Activation Links to eNOS Phosphorylation

Next, we explored a potential functional consequence for shear-activation of β1 integrins on the endothelial cell apical membrane. We focused on a well described pathway stemming from β1 integrin to SFK/PI3 Kinase/Akt which ultimately phosphorylates/activates eNOS.[1] Consistent with our past findings,[25] shear stress rapidly phosphorylated (Ser 1179) a pool of eNOS that was located on the endothelial apical membranes (Fig. 5). SFK (Ser 416) and Akt (Ser 473)

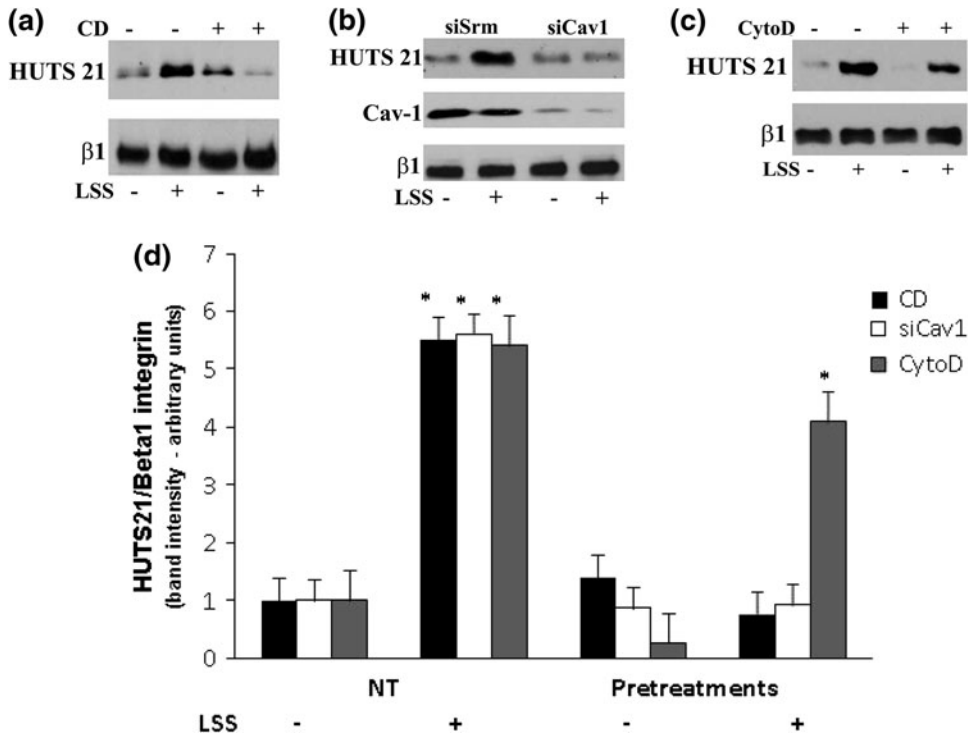

FIGURE 3. Shear-induced activation of apical surface β1 integrins require caveolae but not the actin cytoskeleton. Endothelial cell cultures were pretreated (+) with (a) methyl-β-cycodextrin (CD) as a means of disrupting membrane rafts and caveolae microdomains. (b) Caveolae structures were also abolished through incubation with siRNA directed against caveolin-1(siCav1) or a caveolin-1 scrambled sequence as a control (siSrm). (c) To disrupt the actin network, cells were pretreated with cyotochalasin D (CytoD). In each case, the ability of shear stress (LSS) to convert β1 integrins to an active conformation was assessed by detection of HUTS 21 in apical membrane isolates. In each experiment, β1 integrin expression levels on endothelial apical surface did not vary with pretreatments or shear stress. Blots are illustrative of 4 independent experiments. (d) Histographic presentation of the data where * indicates significant enhancement (p value <0.05) compared to non-sheared (LSS−) group.

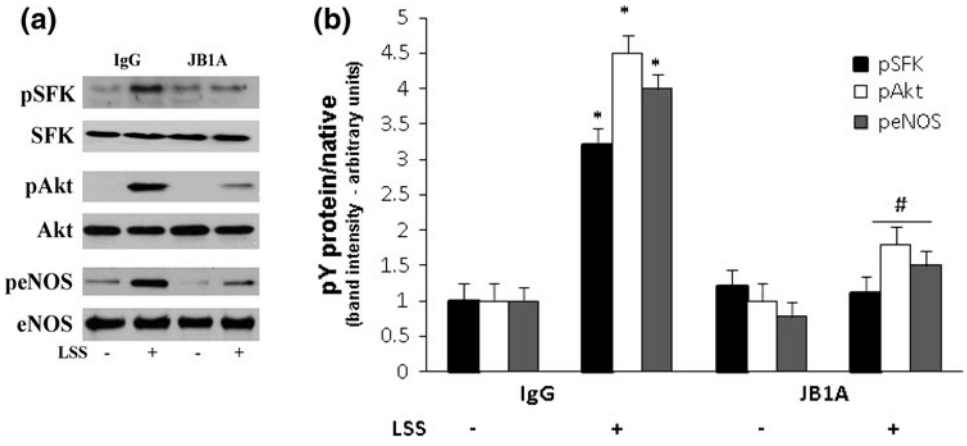

FIGURE 4. Shear-induced phosphorylation of apical surface eNOS is dependent on shear-activation of β1 integrins. (a) BAEC cells were pretreated with β1 integrin blocking (JB1A) or isotype matched control antibody (IgG) and then subjected to acute onset of laminar shear stress (LSS). Apical plasma membranes were isolated and prepared for Western blot analysis. The results show that shear stress induced rapid (2 min) and significant phosphorylation of tyrosine residues at position 416 in Src-family kinases (pSFK), Ser-473 phosporylation in Akt (pAkt) and phosphorylation of eNOS on Ser residue 1179 (peNOS), which strongly correlates with eNOS activation. These shear-induced phosphorylation events were blocked in cells pretreated with JB1A. Western blots are representative of 5 separate experiments. (b) Densitometric quantification of Western blots for each group where * indicates significant enhancement (p value <0.05) compared to non-sheared (LSS−) group and # represents significant (p value <0.05) reduction compared to the IgG-treated and sheared (LSS+) group.

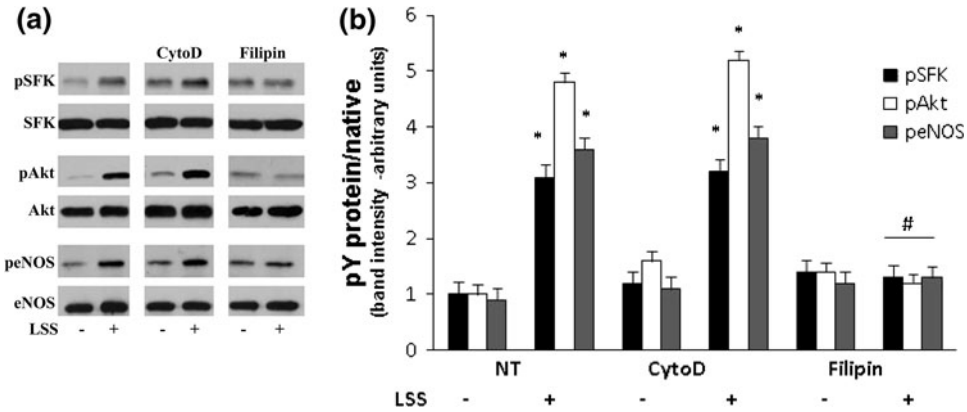

FIGURE 5. Shear-induced mechano-signaling at the endothelial apical surface is attenuated following disruption of membrane rafts/caveolae but not the actin cytoskeleton. (a) BAEC cells were pretreated with filipin to disrupt raft/caveolae domains or cytochalasin D to perturb the actin cytoskeleton. Following exposure to stress, apical plasma membranes were isolated and prepared for Western blot analysis. Ablation of raft/caveolae resulted in attenuation of shear-induced phosphorylation of SFK, Akt and eNOS. In contrast, disrupting the structural integrity of the actin cytoskeleton did not significantly alter the shear-induced phosphorylation events observed on the endothelial apical surface. Western blots are representative of 4–6 separate experiments. (b) Histographic presentation of the data where * indicates significant enhancement (p value <0.05) and # represents significant (p value <0.05) reduction compared to non-sheared (LSS−) group.

were similarly phosphorylated on this surface in response to shear stress. In contrast, prohibiting activation of β1 integrins with a β1 integrin specific blocking antibody (JB1A) attenuated shear-induced phosphorylation of SFK's, Akt and eNOS (Figs. 4a, 4b). As observed in our assessment of β1 integrin activation by shear stress, loss of caveolae structure prevented shear-induced phosphorylation events whereas disruption of the actin cytoskeleton had no observable effect on phosphorylation of these signaling molecules by shear stress (Figs. 5a, 5b).

DISCUSSION

Fluid shear stress is a key factor in determining endothelial cell structural and functional phenotype. While the mechanisms responsible for force detection and conversion into biochemical signaling are not completely clear, integrins are known to play an essential, early role in the mechanotransduction process.[8] Both β1 and β3 integrins are rapidly activated by shear stress.[32] Moreover, application of functional blocking antibodies directed towards β3 integrin

attenuated shear-induced MAP kinase and NFκB pathways[2,11] while blocking β1 integrin prevented shear-activation of sterol regulatory element-binding proteins[12] and morphological remodeling of the actin cytoskeleton.[37] As is often the case following integrin activation, focal adhesion complexes localize subsequent mechanotransduction events that link to these distal responses.[11,32]

Considering that activated integrins associate with focal adhesions, the endothelial basal membrane is an expected site for mechanotransduction. Indeed, shear stress selectively activates β3 integrins on this cell surface.[32] However, integrin-dependent mechanotransduction has also been demonstrated at the endothelial apical surface. Using a FRET-based Src-reporter system in HUVEC's, Wang and colleagues[34] demonstrated rapid Src activation following controlled mechanical force imposed on fibronectin-coated beads which engaged apically localized integrins. Similarly, application of stress to integrins *via* pulling of RGD-coated magnetic microbeads bound to the apical surface of cultured capillary endothelial cells resulted in a rapid increase in intracellular calcium.[15] Although these findings show that local force placed on apical surface integrins activate mechano-signaling events, apical integrin responses to physiologically relevant mechanical forces imposed on the endothelium is unknown.

Through our laboratories ability to selectively isolate and analyze endothelial apical membranes, we tested whether integrins present on this cell surface are responsive to shear stress. Our data shows that β1 integrins are expressed at the apical cell surface of BAEC's (Fig. 1) and become active in response to shear stress (Fig. 2). In contrast to β1 integrin activation, we were unable to detect acute activation of β3 integrins on this cell surface (data not shown). As previously mentioned, this finding is consistent with studies demonstrating that shear-activated β3 integrins localize to the basal surface of cultured endothelial cells.[32] Considering our data showing that the expression level of β3 integrin was 4-fold less than that of β1 integrin on endothelial apical membranes (Fig. 1), site specific acute activation of each β integrin subtype may be a function of their relative distribution within the endothelium.

As a consequence of shear-activation, integrins can bind to a variety of matrix protein ligands. In response to shear stress, integrin/matrix interactions are known to occur on the endothelial basal surface. Since fibronectin is a component of the flow media, we probed our endothelial apical membranes for this matrix protein. Our analysis shows that fibronectin is present in the membrane isolates (Fig. 1) indicating that potential binding patterns for β1 integrins at this site. It is worthy to note that matrix protein adherence to the endothelial luminal surface is more commonly observed in pathological settings such as in atherosclerosis and clot formation. Given the detection of fibronectin on the endothelial apical surface and that our flow protocol generates spatiotemporal gradients of shear stress similar to those found in regions of the vasculature prone to athroma, our experimental model may mimic conditions associated with development of vascular pathology. Whether our observations are specific to the culture and flow parameters used here or are operational under "athero-prone" flow profiles will require further testing.

Experimental evidence suggest that shear-activation of integrins are secondary to upstream events including mechano-signaling initiated at cell–cell adhesion complexes, stimulation of PI3-kinase and induction of integrin clustering *via* force transmission through the cytoskeleton.[8,28] Here, we found that disruption of caveolae organelles also prevented β1 integrin activation initiated by shear stress (Fig. 4), a finding consistent with previous reports showing that intact caveolae were necessary for proper activation and function of β1 integrins.[17,29] While there are several potential mechanisms by which caveolae can mediate shear-activation of integrins, including compartmentation of upstream integrin activators, we tested the role of the actin cytoskeleton in our system due to known associations of the actin network with integrins and caveolae.[19] Our results showed that while disruption of the actin network seemed to attenuate activation of β1 integrins localized on the endothelial apical cell surface (Fig. 4c), the apparent decrease in HUTS 21 binding was not statistically significant compared to cells containing an intact cytoskeleton (Fig. 3d). These findings suggest that elements apart from actin appear to be responsible for initiating mechanotransduction events that lead to activation of β1 integrins at the endothelial cell apical surface. While shear-induced changes in membrane lipids, particularly in caveolae, could be considered as a possible mechanism for regulating β1 integrin activation state, the glycocalyx poses a particularly attractive candidate given its demonstrated association with lipid rafts,[5,6] caveolae domains (our unpublished observations), ability to drive integrin clustering events[21] and its role in modulating shear-induced eNOS activity.[30] Alternatively, a pathway involving β1 integrin and transient receptor potential vanilloid 4 (TRPV4) calcium entry channels can be envisioned based on several recent studies showing that mechanical displacement of RGD bound apical integrins activate rapid calcium signals through mechanosensitive TRPV4 channels[16] and that TRPV4 co-sediments with caveolin-1 in membrane raft fractions.[27] In addition, TRPV4 channels have been shown

to stimulate PI3-Kinase-depdendent activation and binding of β1 integrins in capillary endothelial cells exposed to cyclic strain.[31]

Changes in flow and shear stress activate pathways that stimulate eNOS to produce nitric oxide (NO). Past studies demonstrate that inhibition of integrin signaling using β3 integrin blocking antibodies or RDG peptides attenuate NO-dependent, flow-induced dilation of coronary arterioles.[18] Subsequent studies showed FAK as a key second-messenger in this mechanotransduction pathway.[9] Since FAK is localized to focal adhesions and β3 integrins are mechanosensitive at these sites, these findings indicate that mechanotransduction events responsible for flow-induced dilation occur at the endothelial cell basal surface. Logically, compartmentalizing eNOS mechano-activating machinery on this endothelial cell surface would facilitate vasodilation by placing NO in close proximity to the adjacent vascular smooth muscle on which it acts.

In addition, NO plays a role in preventing platelet and leukocyte aggregation on the endothelial cell surface. Thus, some eNOS would also be expected to target to the endothelial luminal surface. Indeed, our past studies show that endothelial luminal/apical membranes localize eNOS. Furthermore, we demonstrated that this pool of eNOS is rapidly activated by flow/shear stress.[24,25] While the regulation of eNOS is rather complex, our findings that β1 integrin and eNOS are activated on the endothelial apical surface by shear stress prompted us to explored whether a mechanistic connection exists between these proteins. We focused our attention on a well-established kinase cascade that is associated with both integrins and eNOS.[1] We found that pretreating endothelial cells with a β1 integrin blocking antibody significantly attenuated shear-induced phosphorylation of eNOS as well as phosphorylation of its upstream mediators Akt and SFK's (Fig. 4). Similar to our observation of β1 integrin activation by shear stress, ablation of caveolae domains attenuated these signaling events while actin disrupting compounds showed little effect (Fig. 5). The later finding is consistent with the concept that an eNOS-activating mechanotransduction pathway may be localized to the endothelial cell basal surface since flow-induced vasodilation is reduced in experimental systems where various components of the cytoskeleton are functionally lost.[13]

The major finding of this study is the presence of shear-sensitive β1 integrins on the endothelial cell apical surface. These integrins served as an upstream mediator in a mechanotransduction pathway that resulted in the phosphorylation of eNOS. While the actin cytoskeleton did not seem to play a major role in this process, caveolae domains were necessary for shear-induced activation of β1 integrins. Although these findings shed new light on the role of integrins, caveolae and the endothelial cells surface exposed to flow, the precise mechanism by which shear stress activates apically expressed β1 integrins and how loss of caveolae/caveolin-1 influence this process remain unclear. Considering the current findings with our past reports demonstrating that caveolae/caveolin-1 form a complex with β1 integrins on the endothelial basal membrane to regulate morphological remodeling of the cytoskeleton induced by shear stress,[22,23] it appears that localized pools β1 integrins, in association with caveolae, play distinct roles in mechanotransduction processes initiated at each membrane surface.

ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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