Caveolin-1 regulates shear stress-dependent activation of extracellular signal-regulated kinase

HEONYONG PARK,1 YOUNG-MI GO,1 RITESH DARJI,1 JONG-WHAN CHOI,1 MICHAEL P. LISANTI,2 MATTHEW C. MALAND,1 AND HANJONG J. O1

1Department of Pathology, University of Alabama at Birmingham, Birmingham, Alabama 35294; and 2Department of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, New York 10461

Caveolin-1 regulates shear stress-dependent activation of extracellular signal-regulated kinase. Am J Physiol Heart Circ Physiol 278: H1285–H1293, 2000.—Fluid shear stress activates a member of the mitogen-activated protein (MAP) kinase family, extracellular signal-regulated kinase (ERK), by mechanisms dependent on cholesterol in the plasma membrane in bovine aortic endothelial cells (BAEC). Caveolae are microdomains of the plasma membrane that are enriched with cholesterol, caveolin, and signaling molecules. We hypothesized that caveolin-1 regulates shear activation of ERK. Because caveolin-1 is not exposed to the outside, cells were minimally permeabilized by Triton X-100 (0.01%) to deliver a neutralizing, polyclonal caveolin-1 antibody (pCav-1) inside the cells. pCav-1 then bound to caveolin-1 and inhibited shear activation of ERK but not c-jun NH2-terminal kinase. Epitope mapping studies showed that pCav-1 binds to caveolin-1 at two regions (residues 1–21 and 61–101). When the recombinant proteins containing the epitopes fused to glutathione-S-transferase (GST-Cav1–21 or GST-Cav61–101) were preincubated with pCav-1, only GST-Cav61–101 reversed the inhibitory effect of the antibody on shear activation of ERK. Other antibodies, including m2234, which binds to caveolin-1 residues 1–21, had no effect on shear activation of ERK. Caveolin-1 residues 61–101 contain the scaffolding and oligomerization domains, suggesting that binding of pCav-1 to these regions likely disrupts the clustering of caveolin-1 or its interaction with signaling molecules involved in the shear-sensitive ERK pathway. We suggest that caveola-like domains play a critical role in the mechanosensing and/or mechanosignal transduction of the ERK pathway.

blood flow; vascular biology; atherosclerosis

VASCULAR ENDOTHELIAL CELLS recognize shear stress through unknown mechanosensing system(s) and respond both acutely and chronically by producing autocrine and paracrine factors (7). Through these endothermal responses, shear stress controls vascular tone, vessel wall remodeling, binding of blood cells to endothelium, and hemostasis (7). Shear stress selectively and differentially regulates expression of many genes that are important in the pathophysiology of vessel wall function (see Refs. 4 and 9 for reviews). Furthermore, a conserved cis-acting shear stress-response element has been identified in many shear-sensitive genes including platelet-derived growth factor-B, intracellular adhesion molecule-1, tissue plasminogen activator, and transforming growth factor β-1, suggesting its broad implication in shear-dependent regulation of gene expression (4, 9, 20, 29). An additional cis-acting element, the phorbol ester 12-O-tetradecanoxyphorbol 13-acetate-responsive element, has been found in the monocyte chemotactant protein-1 gene (37). Shear stress also transiently activates transcription factors [nuclear factor-κB (NF-κB), AP-1, and early growth response-1] and immediate-early response genes (c-fos, c-jun, and c-myc) that are involved in the regulation of shear-dependent gene expression (13, 20, 21, 36, 37). Induction of specific genes by shear stress in endothelial cells is mediated by activation of mechanosensitive signaling pathways that include at least three members of the mitogen-activated protein (MAP) kinase family, extracellular signal-regulated kinase (ERK1/2), c-jun NH2-terminal kinase (JNK), and Big MAP kinase 1 (also called ERK5) (2, 18, 23, 43, 47). MAP kinases are important signaling components linking extracellular stimuli to cellular responses such as cell growth, death, differentiation, and metabolic regulation (5, 19).

Shear stress activates ERK in a rapid and transient manner (maximum by 5 min and returning to basal levels by 30 min of shear exposure), whereas JNK activation occurs over a much slower and prolonged time course (requiring at least 30 min and returning to basal levels after 1 day of shear exposure) (18). Shear stress activates the two MAP kinases by distinct signaling pathways: activation of ERK is mediated by mechanisms involving Gα1,2, protein kinases [Src, focal adhesion kinase (FAK), and protein kinase C-ε], and Ras, whereas JNK activation requires Gβγ, phosphatidylinositol-3-kinase-γ, tyrosine kinases (Src and FAK), and Ras (11, 14, 16, 18, 22, 42). How do endothelial cells ensure the specific activation of each pathway when sharing the same common signaling molecules (e.g., Src, FAK, and Ras)? A likely hypothesis is that spatial compartmentalization of signaling molecules into microdomains provides the mechanism for the differential activation of ERK and JNK. In support of this hypothesis, we (28 and Rizzo et al. (30) have independently...
shown that the cholesterol-sensitive microdomains in the plasma membrane play a critical role in mechano-
sensitive activation of ERK, but not JNK, in bovine
aortic endothelial cells (BAEC) and in perfused rat lung
endothelium (28, 30). At present the underlying molec-
ular mechanisms and morphological understanding by
which cholesterol-sensitive microdomains segregate sig-
alling molecules and elicit selective regulation of the
MAP kinases in response to shear stress are not known.
However, caveolas and caveola-like domains including
“raft” and “glycosphingolipid signaling domains” are
the likely candidates (1, 15, 27, 30, 38).

Caveolas are noncoated micropatches of the plasma
membrane with a variety of shapes (flat, invaginated,
and tubular) and are found in most cell types including
endothelial cells, fibroblasts, smooth muscle cells, and
adipocytes (1, 27). Caveolas serve at least two different
functions: 1) transport of large and small molecules
(transcytosis of macromolecules and potocytosis of ions
and folate) and 2) transmembrane signaling microdo-
 mains (1, 27). Caveolas are enriched with cholesterol,
glycosphingolipids, lipid-anchored signaling molecules,
and caveolin (1, 27).

Caveolin, a 21- to 24-kDa membrane protein, is a
principal component of caveolas and also binds directly
to cholesterol and interacts with such signaling mol-
ecules as G protein α-subunits, Ras, Src family kinases,
and the endothelial form of nitric oxide synthase (eNOS)
(8, 25, 27). Three members (caveolin-1, -2, and -3),
including two isoforms (caveolin-1a and -1b), of the
caveolin gene family in mammalian cells have been
denoted (32, 33, 40). Caveolin-1 is abundantly ex-
pressed in endothelial cells (35), whereas caveolin-3 is
expressed only in muscle cells (40). The tissue distribu-
tion of caveolin-2 has been shown to be similar to that of
caveolin-1 (32). Caveolins are composed of NH2- and
COOH-terminal domains linked by a hairpinlike mem-
brane-spanning domain. Therefore, both the NH2 and
COOH terminals face cytoplasm (see Fig. 1 for a
schematic representation; see also Ref. 27). Caveolin-1
self-oligomerizes or binds caveolin-2 to form homo-
or heterooligomers (27). The NH2-terminal domain of
caveolin-1 contains the oligomerization domain (resi-
dues 61–101) and the scaffolding domain (residues
81–101) (see Fig. 1 and Ref. 39). The oligomerization
domain is the region involved in homooligomerization of
caveolin-1, whereas the signaling molecules present
in caveolas such as Gαi3, Ras, Src, and eNOS bind to the
scaffolding domain of caveolin-1 and are held in the
inactive state (27, 39).

In this study we examined the role of caveolin-1 in
shear stress-dependent activation of ERK by delivering
caveolin antibodies into mildly permeabilized endothe-
lial cells in an attempt to block the shear response.
Moreover, by using the recombinant glutathione-S-
transferase (GST) fusion proteins containing the epi-
topes of the caveolin-1-recognizing caveolin antibodies,
this study determined the critical role of the scaffolding
and oligomerization domains (residues 61–101) of caveo-
lin-1 in shear-dependent activation of ERK.
RESULTS

pCav-1 is delivered into permeabilized BAEC and binds to caveolin-1. When BAEC were incubated for 30 min with pCav-1 in the presence of 0.01% Triton X-100 (minimal permeabilization), the antibody was detected inside virtually all treated cells (Fig. 2, B and C). Delivery of the pCav-1 antibody into the cells required the presence of the detergent, because the antibody was not detected in cells incubated without Triton X-100 (Fig. 2E). When NI-IgG was used as a control in Triton-permeabilized cells, the fluorescent signal was very faint, indicating that the IgG did not bind tightly to the cells and was mostly removed by extensive washing (Fig. 2D). The staining of pCav-1 showed a punctate pattern (Fig. 2C), which is consistent with its reported intracellular localization in the plasma membrane and Golgi apparatus (33). The characteristic punctate staining pattern of pCav-1 was also observed when BAEC were fixed first and subsequently permeabilized with the use of a high concentration of Triton X-100 (0.1%) before cells were incubated with pCav-1 followed by the secondary antibody (Fig. 2A, positive control).

To estimate what percentage of total caveolin-1 is bound to the pCav-1 antibody in minimally permeabilized cells, its staining intensity was compared with that of the positive control group by quantitative fluorescence microscopy. Assuming that the fluorescence intensity of pCav-1 staining in positive control cells (prefixed and highly permeabilized cells, Fig. 2A) is 100% (total immunoreactive caveolin-1), 6.8 ± 0.4% (n = 99) of total caveolin-1 bound to the antibody in minimally permeabilized cells (compare Fig. 2, A and B). These results show that pCav-1 was delivered inside virtually all minimally permeabilized cells and also suggest that it bound to a fraction of the total caveolin-1.

To confirm the binding of pCav-1 to caveolin-1 in permeabilized cells, BAEC were incubated with the antibody in the presence of Triton for increasing periods of time and washed with PBS. Cell lysates were then incubated with protein A-agarose to retrieve the pCav-1-caveolin-1 complex. Western blot analysis showed that the immune complex contained both the heavy chain of pCav-1 (pCav-1 IgG-H) and caveolin-1 only when cells were incubated with pCav-1 and Triton for 30 min (Fig. 3). Taken together, the results shown in Figs. 2 and 3 confirm that the pCav-1 antibody was delivered to the cells and bound to caveolin-1 in minimally permeabilized BAEC.

pCav-1 inhibits shear stress-dependent activation of ERK, but not JNK, in Triton-permeabilized BAEC. To investigate whether the pCav-1 antibody inhibits shear-dependent activation of ERK, cells were pretreated with the antibody in the presence of Triton before being subjected to shear stress. As we showed previously (28), permeabilization of BAEC by low concentrations of Triton has no significant effect on shear-dependent activation of ERK (Fig. 4, A and C). However, when cells were incubated with pCav-1 in the presence of
Triton (minimally permeabilized), shear stress-dependent activation of ERK was inhibited in an antibody concentration-dependent manner, whereas NI-IgG had no effect (Fig. 4, A and C). The inhibitory effect of pCav-1 on the shear activation of ERK was not observed if cells were not Triton permeabilized (Fig. 4B).

Although pCav-1 appeared to have a small effect in static controls of nonpermeabilized cells in some studies, its effect was not statistically significant (Fig. 4, B and C).

Our previous report (28) showed that removal of cholesterol from the plasma membrane selectively inhibits shear-dependent activation of ERK without affecting that of JNK. This finding suggested that the cholesterol-sensitive, caveolae-like domains specifically mediate the shear-sensitive ERK pathway, but not JNK. This hypothesis was further tested in this study by determining whether pCav-1 inhibits shear-dependent activation of JNK. To this end, we treated BAEC with pCav-1 in the presence of Triton before exposing cells to static conditions or shear stress. Consistent with our previous report (28), Triton permeabilization of BAEC did not alter basal or shear-dependent activation of JNK (Fig. 5). Furthermore, treatment of cells with pCav-1 or NI-IgG had no effect on shear-dependent activation of JNK. Together, the current and previous findings (28) show that shear-

![Fig. 2. Delivery of polyclonal caveolin-1 antibody (pCav-1) into permeabilized bovine aortic endothelial cells (BAEC). A: positive control. BAEC were first fixed in paraformaldehyde and glutaraldehyde, highly permeabilized (0.1% Triton X-100 for 30 min), and blocked for 1 h before being incubated with pCav-1. B–E: unlike control shown in A, cells were not fixed and highly permeabilized before antibody was added. Instead, cells were incubated with 1 µg/ml pCav-1 (B, C, and E) or nonimmune (NI)-IgG (D) in presence (B, C, and D) or absence (E) of 0.01% Triton X-100 for 30 min at 37°C. Cells were then washed in PBS and fixed in paraformaldehyde and glutaraldehyde. pCav-1 and NI-IgG were identified by incubation with Cy3-conjugated goat anti-rabbit IgG for 30 min. Fluorescence micrographs (A and B: original magnification, ×500; C–E: original magnification, ×2,500; C is a higher magnification of B) are representative of at least 3 independent experiments.

![Fig. 3. Binding of pCav-1 to caveolin-1 in permeabilized BAEC. BAEC were treated with pCav-1 (2.5 µg/ml) in presence of 0.01% Triton X-100 for up to 30 min at 37°C. Cells were then washed with PBS and lysed in a buffer containing 1% Triton X-100. Cell lysates were incubated with protein A-agarose to immunoprecipitate the antibody-caveolin-1 complex. Top: immune complexes were resolved by 10% SDS-PAGE, transferred to a polyvinylidene difluoride membrane, cut in half, and analyzed by Western blots with a goat anti-rabbit antibody (top of membrane) to identify pCav-1 delivered into cells. Bottom of membrane was probed with a caveolin-1 antibody (m2297) and subsequently with a goat-anti-mouse antibody. Note that the heavy chain of pCav-1 (IgG-H at ~55 kDa) and caveolin-1 (Cav-1 at 22–24 kDa) were immunoprecipitated only from cells that were incubated for 30 min with antibody and Triton X-100. Results are representative of 3 independent experiments.](http://ajpheart.physiology.org/)
dependent activation of JNK is regulated by mechanisms independent of caveolin-1 and plasma membrane cholesterol.

The specific effect of pCav-1 on shear-dependent activation of ERK. To determine whether caveolin-1 antibodies other than pCav-1 could also block ERK activation in response to shear stress, we screened additional caveolin-1 antibodies. For this study, cells were incubated with three monoclonal antibodies specific to caveolin-1, m2297 (the epitope 61–71 residues, a part of the oligomerization domain), m2234 (the epitope 1–21 residues), and C20B (an antibody raised against the whole molecule), in minimally permeabilized cells as described in Fig. 3. The pCav-1 antibody and NI-IgG were also used as positive and negative controls, respectively. First, the ability of various caveolin-1 antibodies to bind caveolin-1 under our minimally permeabilized conditions for 30 min was determined by immunoprecipitation. For this study, minimally permeabilized and antibody-treated cells were lysed in 60 mM octylglucoside buffer (1 h at 4°C), and protein G-agarose was added to the cell lysates. Immunoprecipitates were subsequently probed by Western blot using the m2297 antibody as shown in Fig. 6A. To aid in quantifying the amount of caveolin-1 immunoprecipitated by each antibody, we directly used an aliquot of the lysate in the Western blot studies. As shown in Fig. 6A, the m2234 and pCav-1 antibodies immunoprecipitated 30 ± 2% (n = 3) and 2.7 ± 0.2% (n = 5) of total caveolin-1, respectively. These results suggest that both m2234 and pCav-1 bind to the native form of caveolin-1 and are consistent with previous reports (26, 33). In contrast, the m2297 and mC20B antibodies and NI-IgG did not immunoprecipitate any significant amount of caveolin-1 from cell lysate (Fig 6A). However, m2297 and mC20B work well in Western blot analysis (see Figs. 3 and 6 as well as unpublished results from Transduction Lab described in DISCUSSION), suggesting that these antibodies recognize denatured, but not native, caveolin-1.

Next, the effects of each antibody on shear stress-dependent activation of ERK were determined. As shown in Fig. 6B, only pCav-1 inhibited shear activation of ERK, whereas m2297, m2234, mC20B, and NI-IgG did not have any significant effect on either the basal or shear-dependent activation of ERK. Because m2297 and mC20B could not immunoprecipitate appreciable amounts of caveolin-1 (Fig. 6A), the effects of these antibodies on shear activation of ERK may be due to their inability to bind caveolin-1 in minimally permeabilized BAEC. However, it is interesting to note the remarkable difference between the two antibodies (m2234 and pCav-1) that are capable of immunoprecipitating caveolin-1. Whereas m2234 bound 30% of total caveolin-1 (Fig. 6A), it did not have any effect on the shear activation of ERK (Fig. 6B). In contrast, pCav-1 immunoprecipitated 3% of total caveolin-1 (Fig. 6A) while inhibiting the shear-dependent activation of ERK by 89% of control (Fig. 6B, compare lane 2 (544 ± 72% of static control) with lane 12 (153 ± 6.9% of static control)).
are recognized by pCav-1 in a nondenatured state. To determine the amino acid residues of caveolin-1 that bind to pCav-1, we used the recombinant GST fusion proteins containing various fragments of caveolin-1 in immunoprecipitation studies. The recombinant GST-caveolins were produced in E. coli and purified by glutathione-agarose, and its purity was determined by protein staining (Fig. 7A). When pCav-1 was added to various GST-caveolins, only the fusion proteins containing either residues 1–21 or 61–101 were immunoprecipitated (Fig. 7B). In contrast, the m2234 antibody immunoprecipitated the fusion proteins containing residues 1–21 but not residues 61–101 (Fig. 7C). The epitope 1–21 that recognized m2234 in a nondenatured state is the same as that previously reported using denatured and reduced caveolin-1 in Western blot analysis (33).

The inhibitory effect of pCav-1 can be prevented by preabsorption with recombinant fusion protein GST-Cav61–101. The study of epitope mapping of pCav-1 (Fig. 7) showed that two epitopes in caveolin-1 bind to pCav-1: 1) one at residues 1–21, which is at the NH2 terminus of the caveolin-1 isoform, and 2) the other at residues 61–101, spanning both the oligomerization domain (residues 61–101) and the scaffolding domain (residues 81–101). To determine whether one or both epitopes could block the effect of pCav-1, we preincubated the antibody with GST-Cav1–21, GST-Cav61–101, or GST alone before adding the mixtures of antibodies and fusion proteins to permeabilized cells. After cells were exposed to static control or shear stress, activation of ERK was measured. As shown in Figs. 4 and 6, treatment of minimally permeabilized cells with pCav-1 inhibited shear-dependent activation of ERK (Fig. 8, compare lanes 1 and 2 with lanes 3 and 4). However, the inhibitory effect of pCav-1 was completely reversed if the antibody was preincubated with GST-Cav61–101 (Fig. 8, compare lanes 3 and 4 with lanes 9 and 10). In contrast, GST-Cav1–21 or GST alone did not reverse the inhibitory effect of the pCav-1 antibody (Fig. 8, lanes 4–7). Although in some studies treatment of cells with GST-Cav61–101 plus pCav-1 appeared to have a small effect on basal ERK activity (Fig. 8, lane 9), this effect was not statistically significant (n = 3). Furthermore, GST-caveolins or GST alone had no effect in the absence of the antibody on control or shear-dependent activation of ERK (Fig. 8, compare lanes 11 and 12 with lanes 13–18). These results show that the peptide 61–101 of caveolin-1 residues 61–101, spanning both the oligomerization domain and the scaffolding domain, is also recognized by pCav-1.

![Image](http://ajpheart.physiology.org/)

**Fig. 6.** Specificity of caveolin-1 antibodies on shear-dependent activation of ERK. BAEC were incubated without (none) or with 2.5 µg/ml caveolin-1 antibodies (m2297, m2234, C20B, or pCav-1) or NI in presence of 0.01% Triton X-100 for 30 min. A: to determine amount of caveolin-1 that can be immunoprecipitated by each antibody, cells were lysed in β-octylglucoside (60 mM), and lysates (200 µg each) were used for immunoprecipitation with protein G-agarose for 1 h at 4°C. Each immunoprecipitate was analyzed by Western blot analysis using an antibody specific to GST (αGST). Proteins were eluted from the protein A- or G-agarose for 1 h. Washed immunoprecipitates (IP) were then analyzed by Western blot analysis with a pERK antibody (pERK1/2) and quantitated densitometrically as described in Fig. 3. Data in bar graph are means ± SE (n = 3). *P < 0.05.

**Fig. 7.** Epitope mapping of caveolin-1 antibodies. Full-length (1–178) and portions (amino acid residue numbers shown above each lane) of caveolin-1 were recombinantly expressed as GST-fusion proteins as described in Fig. 1. A: Coomassie blue staining of purified caveolin fusion proteins. B and C: GST-caveolin fusion proteins were incubated with either pCav-1 (B) or a monoclonal caveolin-1 antibody (m2234) (C) overnight at 4°C and subsequently with Protein A- or G-agarose for 1 h. Washed immunoprecipititates (IP) were then analyzed by Western blot analysis using an antibody specific to GST (αGST). Note that pCav1 recognizes 2 epitopes within caveolin-1 residues 1–21 and 61–101, whereas m2234 binds to 1–21.
caveolin-1 is the essential region that mediates the inhibitory effect of pCav-1 on shear-dependent activation of ERK.

**DISCUSSION**

The current study demonstrates that shear stress-dependent activation of ERK is inhibited by an antibody to caveolin-1, pCav-1, in Triton-permeabilized BAEC. In this study we have established a unique method to minimally permeabilize cells using a low concentration of Triton X-100. Using this method, we were able to show that pCav-1 can be delivered to virtually all BAEC and that the antibody bound to 3–7% of total caveolin-1 as determined by immunohistochemistry or immunoprecipitation (Figs. 2, 3, and 6A). Although only a small fraction of caveolin-1 was bound by the pCav-1 antibody, it was enough to inhibit shear stress-dependent activation of ERK by 65–90% of control in >12 different experiments. This finding raises the interesting possibility that endothelial cells may contain different pools of caveolin-1, one of which readily binds to the pCav-1 antibody and plays a crucial role in the mechanosensitive activation of ERK. Furthermore, the mechanisms by which the pCav-1 antibody inhibits shear-dependent activation of ERK involve the amino acid residues 61–101 of caveolin-1. These results suggest that the scaffolding and oligomerization domains (residues 61–101) of caveolin-1 are critical in regulating the mechanosensitive activation of ERK.

Caveolin-1 is composed of 178 amino acids, and its topology is thought to resemble a hairpin-like structure with its membrane-spanning domain (residues 102–134) flanked by the NH₂ terminus (residues 1–101) and COOH terminus (residues 134–178), both of which face the cytoplasm (27). Caveolin-1 forms oligomers through its oligomerization domain (residues 61–101) and the COOH terminus (39) and binds directly with many signaling molecules such as heterotrimeric G proteins, Ras, Src family kinases, and eNOS through the scaffolding domain (residues 81–101) (27). These caveolin-binding signaling molecules have been proposed to form signal transduction units in the inactive state within caveolae-like structures, ready to be activated by specific stimuli (27). The sequestration of preassembled signaling units may provide the mechanisms responsible for signaling specificity and efficiency in response to specific stimuli. Most caveolin-binding signaling proteins contain the two related motifs (ΦXΦXXXΦ) and ΦXXXΦXXΦ, where Φ is the aromatic amino acid Trp, Phe, or Tyr) that bind to the scaffolding domain of caveolin-1 (38).

What is the mechanism by which pCav-1 inhibits activation of ERK in response to shear stress? Although pCav-1 binds to both caveolin-1 epitopes (residues 1–21 and 61–101), only the addition of the competing fusion protein GST-Cav1–61 (27) prevents the inhibitory effect of pCav-1 on shear activation of ERK (Fig. 8). A simple interpretation of this finding is that the binding of pCav-1 to the residues 61–101 of caveolin-1 disrupts clustering of caveolin-1 (oligomerization domain at the residues 61–101) or the interaction between the scaffolding domain (residues 81–101) and signaling molecules (e.g., Gₛ, Src, and Ras) that are upstream regulators of the flow-sensitive ERK pathway. Current literature provides strong support for the possibility that binding of pCav-1 to the scaffolding domain would compete with the caveolin-binding signaling molecules and displace them from caveolin-1. This disruption would then prevent activation of the ERK pathway in response to shear stress. It was recently found that the scaffolding domain is both necessary and sufficient for membrane attachment of caveolin-1 (34). This provides an additional possibility that pCav-1 may disrupt attachment of caveolin-1 to membrane, resulting in its inability to mediate the mechanosensitive activation of ERK pathway. At present, we cannot exclude other possibilities, including the possibility that the binding of the pCav-1 antibody to caveolin-1 interferes with trafficking of cholesterol between caveolae and intracellular compartments such as endoplasmic reticulum (1). The exact mechanisms underlying the specific effect of pCav-1 await further studies.

Caveolin-1 binds to itself as well as caveolin-2 to form homo- or hetero-oligomers (6, 31). Therefore, the inhibitory effect of pCav-1 on shear activation of ERK may involve caveolin-2 directly or indirectly. It is not clear, however, whether BAEC express caveolin-2. Unlike
caveolin-1, the amino acid sequences of caveolin-2 show species-specific (human vs. canine) differences (31, 32). Two caveolin-2 antibodies are currently available: one is specific for human caveolin-2 (Transduction Lab) and the other for the canine form (31). However, both antibodies failed to recognize caveolin-2 by either immunoprecipitation or Western blot analysis in BAEC, whereas the mouse and canine antibodies recognized caveolin-2 expressed in RSV-3T3 cells (provided as a positive control by Transduction Lab) and Madin-Darby canine kidney cells, respectively (data not shown). Caveolin-2 antibodies recognizing the bovine form are not yet available to our knowledge. Although both caveolin-2 antibodies had no effect on the shear activation of ERK (results not shown), these studies need to be evaluated further when bovine-specific caveolin-2 antibodies become available.

Collectively, our current and previous (28) findings suggest that the cholesterol- and caveolin-sensitive platforms in the plasma membrane such as caveolae-like domains are responsible for mediating the activation of ERK in response to shear stress. These findings have implications in not only the mechanisms responsible for spatial sequestration of signaling units but also the elusive mechanosensor(s) that senses the changes in shear stress. Several candidates have been proposed as the potential mechanosensor, including the caveolae structure itself, G proteins, G protein-coupled receptors, integrins–cytoskeleton (tensility theory), and ion channels (3, 7, 12, 30, 44). It is interesting to note that many of these candidates, including caveolae, G protein-coupled receptors, G proteins, and integrins, have been shown to interact with caveolin-1 (3, 27, 44, 45, 46). Recently integrins, transmembrane glycoproteins that act as adhesion receptors for extracellular matrix, have been reported as caveolin-binding molecules (45, 46). Several lines of evidence provide support for the potential importance of caveolin-integrin interaction in the shear-dependent ERK pathway: 1) integrin-dependent cell adhesion is required for activation of ERK (41), 2) αβ3 and β1-integrins and FAK are involved in the mediation of shear-dependent ERK activation (14, 22), and 3) caveolin-1 binds to β1-integrins and plays a critical role in integrin-dependent activation of ERK and cell adhesion (45, 46).

The minimal permeabilization method developed in this study using a non-cholesterol-specific detergent could be used as a general technique to deliver membrane-impermeable macro- or micromolecules (i.e., peptides, antibodies, oligonucleotides, etc.) inside the cells. This could be especially useful as an alternative to other wildly used membrane-permeabilizing agents such as digitonin and filipin, which work by chelating membrane cholesterol, thereby potentially interfering with caveolae-dependent cellular functions (28).

In summary, we report that the scaffolding and/or oligomerization domain of caveolin-1 plays an essential role in shear stress-dependent activation of ERK. On the basis of our current and previous (28) findings, we propose that the shear-sensitive signaling molecules that regulate the ERK pathway are assembled in caveolae-like domains by binding to the scaffolding domain of caveolin-1 in the inactive state. Changes in shear stress level may then specifically trigger rapid, organized, and compartmentalized signaling cascades to activate ERK, but not other pathways. This spatial compartmentalization model provides a plausible mechanism by which shear stress differentially activates MAP kinases and other mechanosensitive responses in endothelial cells.

We thank Dr. Kai Simons for providing a canine caveolin-2 antibody and Dr. V. Darley-Usmar for critically reading this manuscript.

This work was supported by National Heart, Lung, and Blood Institute First Award HL-53601, National Aeronautics and Space Administration Grant NAG2-1348, American Heart Association (AHA) Grant-in-Aid AL-G-960035, and a University of Alabama Health Services Foundation-General Endowment Fund Grant (to H. J. o); a postdoctoral fellowship from AHA-Southeast Consortium (to H. Park); and the Cancer Institute Grant RO1-C580250. The current study was supported by the Charles E. Culpepper Foundation, the G. Harold and Leila Y. Mathers Charitable Foundation, and the Sidney Kimmel Foundation for Research Cancer (to M. P. Lisanti).

Address for reprint requests and other correspondence: H. J. o, Univ. of Alabama at Birmingham, Dept. of Pathology, G019C Volker Hall, Birmingham, AL 35294 (E-mail: jo@path.uab.edu).

Received 21 July 1999; accepted in final form 24 November 1999.

REFERENCES


