ABSTRACT

Endothelial nitric oxide synthase (eNOS) is important for cardiovascular homeostasis, vessel remodeling, and angiogenesis. Given the impact of endothelium-derived nitric oxide (NO) in vascular biology, much work in the past several years has focused on the control of NO synthesis by regulatory proteins that influence its function. Indeed calcium-activated calmodulin is important for regulation of NOS activity. Herein we discuss why other proteins, in addition to calmodulin, are necessary for eNOS regulation and summarize the biology of negative and positive regulators of eNOS function in vitro, in cells, and in blood vessels.

Endothelium-derived nitric oxide (NO), the classic relaxing factor discovered by Furchgott in 1980, is produced by the enzyme endothelial nitric oxide synthase (eNOS). Early observations by pharmacologists studying endothelium-dependent relaxations of blood vessels and the release of relaxing factor by cultured endothelial cells demonstrated that removal of extracellular calcium from media solutions blocked agonist-induced release of NO, suggesting that calcium was important for the release of endothelium-derived NO (Singer and Peach, 1982; Forstermann et al., 1991). Additional studies using broken cell systems documented that calcium removal or antagonism of calmodulin (CaM) with inhibitors blocked the generation of NO and NOS activity, suggesting that eNOS was a calcium-calmodulin-requiring enzyme (Busse and Mulsch, 1990; Forstermann et al., 1991). The requirement for calcium-calmodulin was proven upon purification of eNOS to homogeneity (Pollock et al., 1991) and rationalized by the presence of a calmodulin binding motif in the deduced amino acid sequence of the cloned eNOS cDNA.

In the past four years many laboratories have described proteins other than CaM that may negatively or positively impact eNOS function. Insights into the need for additional regulatory proteins important for NO production from endothelial cells stemmed from observations that eNOS was an N-myristoyl protein (Pollock et al., 1992). N-Myristoylation is important for the subcellular targeting of discrete microdomains of cells, and mutations that block N-myristoylation impede proper subcellular targeting and various aspects of signal transduction. Indeed, expression of a nonacylated form of eNOS did not affect enzymatic activity in broken cell lysates but prevented calcium ionophore-stimulated NO release, arguing that additional mechanisms other than CaM, per se, were important for the fidelity of signal transduction coupling to eNOS (Sakoda et al., 1995; Sessa et al., 1995). In addition, the hypothesis that eNOS had to be localized to proper intracellular membranes to be near to other regulatory proteins (scaffolds, chaperones, kinases) provided the rationale for the discovery of additional protein regulators of eNOS function. Described below are putative regulators of eNOS function that have been shown to inhibit or enhance eNOS activity and NO release (Table 1).

ABBREVIATIONS: NO, nitric oxide; eNOS, endothelial NO synthase; ACh, acetylcholine; B2, bradykinin 2; CaM, calmodulin; Erk, extracellular signal-regulated kinase; GA, geldanamycin; GST, glutathione S-transferase; hs90, heat shock protein 90; ID4, intracellular domain 4; IGF, insulin-like growth factor; NOSIP, nitric oxide synthase interacting protein; PI-3K, phosphatidylinositol 3-kinase; VEGF, vascular endothelial growth factor.
Negative Regulatory Proteins

Caveolin. Caveolin, being the major coat protein of caveolae, has several faces that may influence the biology of proteins that localize to cholesterol-rich plasmalemma caveolae. Indeed caveolin-1 is necessary for the biogenesis of caveolae through an unknown mechanism (Smart et al., 1999). In addition, caveolin-1 can serve as a cholesterol binding protein and traffic cholesterol from the endoplasmic reticulum through the Golgi to the plasma membrane. Finally, caveolin has the capacity to directly interact with other intracellular proteins such as e-Src and H-Ras through amino acids 82–101, the putative scaffolding domain (Smart et al., 1999). Indeed, three groups independently demonstrated that eNOS could directly interact with caveolin-1 or caveolin-3 (Feron et al., 1996; García-Cardeña et al., 1996; Ju et al., 1997). The primary binding region of caveolin-1 for eNOS is within amino acids 60–101 and, to a lesser extent, amino acids 135–178 (Garcia-Cardená et al., 1997; Ju et al., 1997). Furthermore, the caveolin-eNOS immunocomplex is disrupted in the presence of caveolin scaffolding peptides (amino acids 82–101) (Michel et al., 1997b).

eNOS contains a consensus caveolin binding motif (Smart et al., 1999) located within amino acids 350–358. The importance of the caveolin interaction with eNOS has been most reproducibly demonstrated by the effects of caveolin scaffolding peptides and GST-caveolin on NO activity. Incubation of pure eNOS with peptides derived from the scaffolding domains of caveolin-1 and -3 resulted in inhibition of eNOS activity (García-Cardená et al., 1997). In cotransfection experiments, caveolin over-expression in COS-7 cells resulted in a reduction of eNOS activity (Michel et al., 1997b), and a reduction in NO release was also observed (Garcia-Cardená et al., 1997). Furthermore, mutagenesis of the predicted caveolin binding motif within eNOS blocked the ability of caveolin to suppress NO release in these latter experiments (Garcia-Cardená et al., 1997). The reduction of eNOS activity by caveolin peptides, or over-expressed caveolin, is reversed by exogenous addition of calmodulin, suggesting a reciprocal regulation of eNOS by calmodulin, an activator, and caveolin, an inhibitor (Michel et al., 1997a).

Collectively, these overall results suggest that NO production is negatively regulated by interactions with caveolin and that for NO release to occur, the inhibitory clamp by caveolin must be overcome. CaM has been proposed to be solely responsible for the dissociation of eNOS from caveolin (Michel et al., 1997a). However, the relationship between caveolin as an inhibitor of eNOS and CaM as its allosteric modulator has not been examined in light of new findings demonstrating a role for other positive and negative regulators of eNOS activation. Another important issue is that there are no direct data showing more NO release from cells that do not express caveolins or that disruption of the eNOS-caveolin complex can lead to increased or prolonged NO release from cells, fundamental experiments if caveolin-1 truly negatively regulates eNOS and NO release.

In Vivo Evidence Supporting the eNOS-Caveolin Interaction. To date, caveolin knockout mice are not available; therefore, examining endothelial function in these mice is not yet feasible. However, recent work using the caveolin scaffolding domain as a surrogate for caveolin has demonstrated that eNOS can be regulated in situ. Exposure of permeabilized cardiac myocytes to the caveolin-3 scaffolding domain peptide (amino acids 55–74), but not a scrambled version, antagonized the negative chronotropic actions of carbachol (Feron et al., 1998). Our group recently used a membrane-permeable form of the caveolin-1 scaffolding domain (amino acids 82–101) by fusing it to a cell-permeable leader sequence (Bucci et al., 2000a). Exposure of the peptide to blood vessels resulted in uptake into the endothelium and adventitia and blockade of ACh-induced relaxations, with no effect on relaxant responses to sodium nitroprusside or the release of prostacyclin, showing that in an intact blood vessel, the caveolin peptide is a potent inhibitor of eNOS. In addition, the peptide also blocked inflammation in two different models by influencing vascular permeability, suggesting that peptidomimetics may be useful therapeutically. With respect to disease mechanisms that may influence the caveolin/eNOS interaction, there is evidence that in a rat model of cirrhosis, caveolin-1 is over-expressed, more caveolin-1 interacts with eNOS, and the basal and stimulated production of NO is depressed (Shah et al., 1999a), suggesting that this interaction may increase portal pressures and contribute to the disease state.

Intracellular Domains of G-Protein-Coupled Receptors. Work by Venema et al. (1996) has shown that the intracellular domain 4 (ID4) of the bradykinin 2 (B2) and the angiotensin II R1 receptors can negatively regulate eNOS activity in vitro (Ju et al., 1998). Indeed, eNOS coprecipitated with the B2 receptor and in vitro interacted with a GST fusion of ID4, and synthetic peptides from ID4 inhibited eNOS activity in a dose-dependent manner in vitro. Mechanistically, the ID4 peptide has been shown to affect NO catalysis by interference with flavin to heme electron transfer (Golser et al., 2000). The concept that a receptor can directly interact with eNOS is extremely novel, suggesting that signaling, albeit negative signaling to eNOS, can occur in the absence of a G-protein intermediate. However, direct evidence supporting the physiological relevance of this interaction is presently unavailable.

NOSIP. NOSIP is the newest protein to interact with eNOS (Dedio et al., 2001). NOSIP is a 34-kDa protein that was initially identified as an eNOS binding partner. The interaction between NOSIP and eNOS has been shown both in vitro and in vivo, and through deletion analysis, NOSIP was shown to bind eNOS between amino acids 366 and 486. Stimulation of cells with calcium ionophore does not change the association of NOSIP and eNOS; however, a peptide derived from the scaffolding domain of caveolin (82–101) is able to displace eNOS from NOSIP. NOSIP does not affect eNOS activity assays in vitro but, when coexpressed in cells, does reduce ionomycin-stimulated NO release. The ability of NOSIP to reduce NO release from intact cells is due to the redistribution of eNOS from the plasma membrane to intracellular compartments. The specificity of NOSIP to eNOS and the true function of NOSIP are not known.

Positive Regulatory Proteins

Calmodulin. The first protein shown to be involved in eNOS regulation was calmodulin (CaM). Early studies on neuronal NOS and eNOS (Bredt and Snyder, 1990; Forstermann et al., 1991), demonstrated that purified NOS utilized CaM as an activator of NO synthesis. Mechanistically, CaM binding to a canonical CaM binding motif can displace an
adjacent autoinhibitory loop on eNOS and neuronal NOS, thus facilitating NADPH-dependent electron flux from the reductase domain of the protein through to the oxygenase domain. The terminal electron acceptor in the oxygenase domain is heme, which can bind oxygen for insertion into the NOS substrate, L-arginine. To date, there are no papers documenting that CaM can actually be recruited to eNOS in a stimulus-dependent manner and that the recruitment occurs contemporaneously with NO release. Pharmacological evidence using inhibitors of CaM or calcium-free buffers have indirectly shown the requirement for CaM. Recent work has shown that eNOS immunoprecipitated from human endothelial cells has immunoreactive CaM bound to it (Russell et al., 2000). Moreover, upon challenge of the cells with estrogen, the amount of CaM recovered in the eNOS immunocomplex does not change. This suggests that CaM may serve as a tightly bound prosthetic group, akin to CaM found in inducible NOS (Cho et al., 1992), and that regulation of the affinity of CaM interactions with NOS may occur through subtle changes in free calcium levels.

**Heat Shock Protein 90 (hsp90).** The hsp90 family is a group of highly conserved stress proteins that are expressed in all eukaryotic cells (Pratt, 1997). Two genes encode hsp90, with the human gene products hsp90α and hsp90β having 86% sequence homology. The hsp90 is highly abundant in cells, accounting for 1 to 2% of cytosolic protein, and is localized to the cytoplasm, with a small amount found in the nucleus and cytoskeleton (Pratt, 1997). The main function of hsp90 has been its involvement in a multicomponent chaperone system that is responsible for the proper folding of proteins such as steroid receptors and cell cycle-dependent kinases (Pratt, 1997). However, the abundance of hsp90 associated with newly synthesized proteins suggests that this may not be its only function. There is increasing evidence that hsp90 may be an integral part of signal transduction in all cells. Indeed, hsp90 orthologs are important for tyrosine kinase signaling in *Drosophila* and receptor-G-protein signaling in yeast.

Previously it had been shown that eNOS coprecipitated with a 90-kDa tyrosine-phosphorylated protein, later shown to be hsp90 (Venema et al., 1996; Garcia-Cardena et al., 1998). Indeed, hsp90 was associated with eNOS in resting endothelial cells; and treatment of cells with four distinct stimuli that cause NO release, vascular endothelial growth factor (VEGF), histamine, fluid shear stress, and estrogen, all enhanced the interaction between hsp90 and eNOS in a time frame consistent with NO release (Garcia-Cardena et al., 1998; Russell et al., 2000). The rapid stimulus-dependent formation of the hsp90-eNOS hetero-complexes suggests that it occurs simultaneously with other signaling events such as the mobilization of intracellular calcium and/or protein phosphorylation.

The mechanism of how hsp90 regulates eNOS function is less clear. Previously we have shown that hsp90 can directly activate eNOS in vitro (Garcia-Cardena et al., 1998), and coexpression of eNOS with hsp90 in COS cells increased NO activity in broken cell lysates. These results suggest that hsp90 may act as an allosteric modulator of eNOS by inducing a conformational change in the enzyme that results in increased activity or possibly stabilize the “activation” complex. A recent paper has shown that hsp90 increases CaM affinity for neuronal NOS (Song et al., 2001). Alternatively, hsp90 may act as a scaffold for the recruitment of other regulatory molecules including kinases and phosphatases that may influence eNOS function.

**Interactions Between Caveolin, CaM, and hsp90.** As mentioned previously, CaM has been proposed to be exclusively responsible for the dissociation of eNOS from caveolin. Recently the relationship between caveolin as an inhibitor of eNOS and CaM as its allosteric modulator has been examined in light of hsp90 as an additional regulatory protein. Labeling of endothelial cells with [35S]methionine followed by immunoprecipitation of eNOS resulted in the appearance of several co-associated radiolabeled proteins interacting substoichiometrically with eNOS (Gratton et al., 2000). Western blotting of these immunoprecipitated proteins demonstrated the presence of eNOS, caveolin-1, and hsp90 in the same complex. Moreover, the addition of exogenous CaM weakly displaced caveolin from CaM. Reconstitution of the heterotrimeric complex in vitro showed the eNOS interaction with both hsp90 and caveolin, but the latter proteins did not interact with each other, demonstrating that eNOS was the bridge holding the complex together. Interestingly, the binding of caveolin to eNOS was displaced by the caveolin scaffolding domain peptide, but not by calcium-activated CaM, demonstrating that CaM cannot physically disrupt the eNOS-caveolin complex in vitro. However, hsp90, per se, did not influence the eNOS/caveolin interaction but facilitated the ability of CaM to displace caveolin from eNOS. These data are consistent with two potential models: 1) perhaps the “recruitment or activation” of hsp90 and CaM to eNOS results in weak physical displacement of eNOS from caveolin,

### TABLE 1

<table>
<thead>
<tr>
<th>Protein</th>
<th>Agonist</th>
<th>Association</th>
<th>Effect of Bound Protein</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caveolin</td>
<td>A23187/bradykinin</td>
<td>Decreased/increased</td>
<td>Inhibitory</td>
<td>Ju et al., 1997; Michel et al., 1997b</td>
</tr>
<tr>
<td>B2 receptor</td>
<td>Bradykinin, ionophore</td>
<td>Decreased</td>
<td>Inhibitory</td>
<td>Golser et al., 2000</td>
</tr>
<tr>
<td>NOSIP</td>
<td>Ionomycin</td>
<td>Decreased</td>
<td>Inhibitory</td>
<td>Dedio et al., 2001</td>
</tr>
<tr>
<td>Calmodulin</td>
<td>VEGF, histamine, shear</td>
<td>Increased</td>
<td>Stimulatory</td>
<td>Venema et al., 1996; Garcia-Cardena et al., 1998</td>
</tr>
<tr>
<td>hsp90</td>
<td>VEGF, histamine, shear</td>
<td>Increased</td>
<td>Stimulatory</td>
<td>Venema et al., 1996; Garcia-Cardena et al., 1998</td>
</tr>
<tr>
<td>Dynamin-2</td>
<td>Ionomycin</td>
<td>Increased</td>
<td>Stimulatory</td>
<td>Cao et al., 2000</td>
</tr>
<tr>
<td>Erk 1/2</td>
<td>Bradykinin</td>
<td>Decreased</td>
<td>Inhibitory</td>
<td>Bernier et al., 2000</td>
</tr>
<tr>
<td>Raf-1</td>
<td>Bradykinin</td>
<td>Decreased</td>
<td>Inhibitory</td>
<td>Bernier et al., 2000</td>
</tr>
<tr>
<td>Akt</td>
<td>Bradykinin</td>
<td>Decreased</td>
<td>N.D.</td>
<td>Bernier et al., 2000</td>
</tr>
</tbody>
</table>

N.D., not determined.

* It is presumed that more CaM is bound.
the protein that influences eNOS activity. The relevance of Akt binding is clear based on in vitro and in vivo evidence describing the phosphorylation site (see below), whereas the phosphorylation by Erk has not been characterized. However, the association of these proteins to eNOS following agonist activation is indicative of a dynamic multiprotein signaling complex influencing eNOS function (i.e., NOS-ome).

**Protein Phosphorylation.** Another post-translational modification that can potentially regulate eNOS activity is through protein phosphorylation (Fig. 1; Table 2). eNOS is primarily phosphorylated on serine residues and to a lesser extent on tyrosine and threonine residues (Michel et al., 1993; Corson et al., 1996; García-Cardena et al., 1996). The ability of pharmacological inhibitors of phosphatidylinositol 3-kinase (PI-3K; i.e., wortmannin and LY294002) to reduce insulin- and VEGF-stimulated NO release provided the first evidence that a downstream effector of PI-3K could modulate eNOS activity (Zeng and Quon, 1996; Papapetropoulos et al., 1997). The protein kinase Akt is activated by the 3-phosphorylated inositol lipids generated by PI-3K, and is known to phosphorylate a limited number of cellular substrates according to a distinct substrate motif that is found in eNOS (RRXXRXS/T). Akt can directly phosphorylate recombinant eNOS or eNOS in situ, at serine 1177 (human)/1179 (bovine) (Dimmeler et al., 1999; Fulton et al., 1999; Gallis et al., 1999; Michel et al., 1999). Due to the promiscuous nature of kinase cascades, it is much more difficult to demonstrate that eNOS is a direct Akt substrate in vivo; however, several lines of evidence support this idea. Cotransfection of wild-type Akt and eNOS in COS cells increases eNOS phosphorylation in a wortmannin-sensitive manner and is inhibited by mutation of serine 1177/1179 to alanine (Dimmeler et al., 1999; Fulton et al., 1999). Stimulation of endothelial cells with VEGF and shear stress phosphorylates and activates Akt, and within a similar time frame, eNOS is phosphorylated on serine 1179 in a PI-3K-dependent manner (Dimmeler et al., 1999; Fulton et al., 1999; Gallis et al., 1999; Michel et al., 1999). Akt-phosphorylated eNOS is 15- to 20-fold more active than unphosphorylated eNOS (Gallis et al., 1999) or more active at lower levels of calcium or calmodulin (Fulton et al., 1999; Michel et al., 1999). Mutation of serine 1177 to aspartate (S1177D), which mimics the negative charge afforded by phosphorylation, results in an enzyme that is constitutively active at low levels (10 nM) of calcium (Dimmeler et al., 1999), whereas mutation of serine 1177/1179 to alanine prevents Akt-dependent NO release (Fulton et al., 1999). Adenoviral-mediated gene transfer of constitutively active Akt to endothelial cells markedly increases basal NO release. Activated Akt potentiated and the activation-deficient Akt inhibited VEGF-stimulated NO release by approximately 50 to 70%, similar to levels after wortmannin treatment (Papapetropoulos et al., 1997; Fulton et al., 1999). As stated previously, the subcellular localization of eNOS is crucial for agonist-induced NO release; therefore, we tested whether Akt-dependent activation of eNOS was influenced by its distribution (Fulton et al., 1999). Transfection of cells with a mutant form of eNOS that cannot be acylated prevents the ability of Akt to stimulate NO release, suggesting that eNOS must be membrane-associated for this interaction to occur, and may explain in part the reduction in agonist-induced NO.
release from cells where eNOS is mislocalized (Sessa et al., 1995; Liu et al., 1996).

Wortmannin does not completely block agonist-induced phosphorylation of eNOS on serine 1177/1179, suggesting that there are other kinases capable of phosphorylating serine 1177/1179. Moreover, there appears to be a discrepancy in the ability of VEGF and IGF-1 to phosphorylate Akt and eNOS. IGF-1 induces greater phosphorylation of this residue by Akt, protein kinase A (PKA), or AMP-dependent kinase (AMPK) is associated with increased enzyme activity. Other proteins have been shown to be associated with increased eNOS activity or NO release such as dynamin (Dyn). The role of nitric oxide synthase-interacting protein (NOSIP) is less clear, but over-expression of this protein mislocalizes eNOS.

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Serine 1177/1179 is not the only eNOS phosphorylation site. For example, shear stress induces the phosphorylation of another site on eNOS, serine 116 (Gallis et al., 1999). The region surrounding this site does not conform to the accepted Akt motif, thus the kinase and the functional significance are
the resting diameter was significantly larger than control (2000). In femoral arteries infected with the myr-Akt virus, modified by adenoviruses encoding constitutively active Akt, Akt activity in the endothelium of blood vessels was identified in blood vessels (McCabe et al., 2000). eNOS Phosphorylation.

It is unlikely that phosphorylation-induced changes in eNOS activity can be entirely attributed to changes in eNOS-associated proteins because recombinant-phosphorylated eNOS activity can be entirely attributed to changes in eNOS-associated proteins because recombinant-phosphorylated eNOS and eNOS S1179D are more active than unphosphorylated eNOS (McCabe et al., 2000).

In Vivo Evidence Supporting a Functional Role for eNOS Phosphorylation. A physiological role of endogenous Akt in vasomotor function has been identified in blood vessels. Akt activity in the endothelium of blood vessels was modified by adenoviruses encoding constitutively active Akt, myr-Akt, and a dominant negative Akt, Akt- AA (Luo et al., 2000). In femoral arteries infected with the myr-Akt virus, the resting diameter was significantly larger than control arteries, and the increase in diameter was reversed by Nω-nitro-1-arginine methyl ester, an inhibitor of nitric oxide synthesis. Also, myr-Akt-infected blood vessels displayed an increase in baseline blood flow. Although dominant negative Akt did not significantly affect resting diameter or blood flow, it did reduce the ability of ACh, but not nitroglycerin, to increase both diameter and blood flow. These data strongly indicate that the Akt-eNOS axis is important for blood flow control in conduit vessels.

For several years, the hydroxymethylglutaryl-coenzyme A reductase inhibitors have been shown to provide beneficial cardiovascular actions that are not related to the ability of these drugs to reduce cholesterol. Although statins have been shown to influence eNOS mRNA and protein levels (Laufs and Liao, 1998), the improvement in endothelial function may not entirely be attributed to changes in gene expression (Kaesemeyer et al., 1999). Recently, it has been shown that simvastatin can activate endothelial Akt, leading to an increase in eNOS phosphorylation and NO release (Kureishi et al., 2000). Simvastatin treatment resulted in an increase in endothelial cell survival and angiogenesis in normocholesterolemic animals. Collectively, these results suggest that endogenous Akt can participate in the release of NO from the endothelium in response to agonist stimulation and that increasing Akt activity, either using adenosinurises or simvastatin, can stimulate NO release.

Summary

Given the fundamental importance of endothelial-derived NO in cardiovascular homeostasis and physiology, elucidation of the enzymatic control mechanisms by the aforementioned protein regulators will increase our understanding of how NO release is controlled in vivo. In addition, perhaps novel insights into the mechanisms of endothelial dysfunction, a manifestation of many cardiovascular diseases, may be attributable to impairments in upstream protein regulators of eNOS function.

Note Added in Proof. Recently, two papers have demonstrated enhanced endothelium-dependent responses in mice lacking the gene for caveolin-1 consistent with the concept that caveolin-1 negatively regulates eNOS function (Drab et al., 2001; Razani et al., 2001).

References


Table 2
Potential kinases that phosphorylate eNOS

<table>
<thead>
<tr>
<th>Kinase</th>
<th>Site</th>
<th>Agonists</th>
<th>eNOS Activity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Akt</td>
<td>S1177/1179</td>
<td>VEGF, IGF, shear stress, estrogen, simvastatin</td>
<td>↑</td>
<td>Dimmel et al., 1999; Fulton et al., 1999; Gallia et al., 1999; Michell et al., 1999</td>
</tr>
<tr>
<td>AMPK</td>
<td>S1177, T495</td>
<td>[AMP],</td>
<td>↑</td>
<td>Chen et al., 1999</td>
</tr>
<tr>
<td>PKC</td>
<td>T495</td>
<td>PMA</td>
<td>↑</td>
<td>Michell et al., 1999</td>
</tr>
<tr>
<td>PKA</td>
<td>S1177, S633</td>
<td>Iloprost</td>
<td>↑</td>
<td>Butt et al., 2000</td>
</tr>
<tr>
<td>PKG</td>
<td>S1177, S633</td>
<td>N.D.</td>
<td>↑</td>
<td>Butt et al., 2000</td>
</tr>
<tr>
<td>MAP kinase</td>
<td>S116</td>
<td>Shear stress</td>
<td>↑</td>
<td>Gallia et al., 1999</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>↓</td>
<td>Bernier et al., 2000</td>
</tr>
</tbody>
</table>

N.D., not determined.

* In the presence of CaM/Ca2+. 


Address correspondence to: Dr. William C. Sessa, Yale University School of Medicine, Boyer Center for Molecular Medicine, Rm 436 D, New Haven, CT 06536. E-mail: william.sessa@yale.edu

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