A New Role for Caveolin-1

Regulation of Guanosine Triphosphate Cyclohydrolase I and Tetrahydrobiopterin in Endothelial Cells

Yan-Hua Du, Alex F. Chen

Caveolae was initially described as plasmalemmal vesicles in the endothelium of the heart in the early 1950s. Since then, caveolae have been identified as the 50- to 100-nm flask-shaped invaginations of the plasma membrane in a wide variety of tissues and cell types. In the past decade, a great deal of effort has been expended to determine the biological role of caveolae. Although detailed morphological examinations have provided some insights into their function, it was not until the discovery of the caveolar coat proteins in the early 1990s that the true nature and importance of these organelles were realized. To date, 3 caveolin proteins have been identified and termed as caveolin-1, -2, and -3, possessing molecular masses between 18 and 24 kDa. These proteins serve as markers for and are the major components of caveolae, consisting of a hairpin-like structure with both the N and C terminus exposed to the cytoplasm. The N-terminal domain contains a caveolin scaffolding domain (residues 82 to 101), which is essential for the formation of caveolin oligomers and interaction with other proteins. Given their widespread tissue distributions, a number of in vitro and in vivo studies have implicated caveolae and caveolins in the pathogenesis of diabetes mellitus, atherosclerosis, cardiac hypertrophy, heart failure, pulmonary fibrosis, degenerative muscular dystrophies, and cancer. The recent generation of caveolin null mice has made it possible to evaluate the significance of each caveolin protein in the context of whole animal physiology and pathology (see Table).

As the major coat protein for caveolae assembly, caveolin-1 is particularly abundant in endothelial cells, which are critically involved in the regulation of vascular tone and cardiovascular homeostasis through NO produced by endothelial NO synthase (eNOS). The identification of eNOS targets to caveolae of the plasma membrane and Golgi apparatus, where it is inhibited by binding to caveolin-1. It is only on the release of eNOS from caveolin-1 that the enzyme can be fully activated. Calcium/calmodulin and binding of heat shock protein 90 have been shown to increase eNOS activity in caveolae through a variety of mechanisms involving the prevention of caveolin binding to eNOS. A cell-permeable peptide containing the caveolin-1 scaffolding domain that binds to eNOS was shown to inhibit acetylcholine-induced NO production and vasodilation in mouse aorta. Conversely, relaxation response to acetylcholine was markedly enhanced in aortas derived from caveolin-1 knockout mice. Although the aforementioned evidence validates the negative regulatory influence of caveolin-1 on eNOS function, a more mechanistic understanding is necessary to fully appreciate how caveolin-1 influences eNOS function given the complex regulation of eNOS activity by various factors.

In the present issue of Hypertension, the study by Peterson et al\(^5\) has made an important contribution to this field. It provides novel insights into the role of caveolae/caveolin-1 and how they regulate GTP cyclohydrolase (GTPCH) I, the rate-limiting enzyme for de novo synthesis of the essential eNOS cofactor tetrahydrobiopterin (BH\(_4\)) in endothelial cells. BH\(_4\) is necessary for the synthesis of NO by eNOS through mechanisms including facilitating electron transfer in the eNOS catalytic domain, stabilizing eNOS in its active dimeric form, and promoting eNOS phosphorylation.\(^6,7\) BH\(_4\) deficiency seems to be a common feature in diverse vascular diseases states that characterizes endothelial dysfunction. The critical role of GTPCH/BH\(_4\) in eNOS activity and endothelial function has been described recently in a variety of animal models and human diseases.

Although recent studies have focused on GTPCH I as a target to define the role of BH\(_4\) in eNOS function, little is known about the subcellular localization of this enzyme. Peterson et al\(^5\) filled a significant gap in such knowledge in the current study. Using human umbilical vein endothelial cells as an in vitro cell model, the authors found that a considerable amount of GTPCH I was localized in the caveolae-rich cell membrane along with caveolin-1, with the remaining parts of the enzyme located in cytosol and noncaveolar-associated membrane fractions. Similar results were also obtained in vivo, with the findings that GTPCH I protein and activity were concentrated in the caveolae-rich membrane fraction from the mouse lung. In addition, morphological studies also revealed that caveolin-1 and GTPCH I reside in close proximity to each other in the section of the mouse aorta. Most importantly, the concept that GTPCH I

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localizes in caveolae was further validated by the observation that GTPCH I activity is significantly increased in caveolae-rich membrane fractions of the lung from transgenic mice with endothelial-specific overexpression of GTPCH I as compared with their wild-type littermates. These findings provide strong evidence that a significant portion of GTPCH I was colocalized with caveolin-1 in the caveolae of endothelial cells.

Given the colocalization of GTPCH I and caveolin-1 in endothelial caveolae, the question was raised regarding its functional significance. To this end, the authors determined that disruption of caveolin-1 (ie, in Cav1<sup>−/−</sup> mice) resulted in a 2-fold increase in GTPCH I activity of both the aortas and lung, accompanied by an increase in BH<sub>4</sub> levels when compared with the wild-type controls, suggesting that caveolin-1 may exert an inhibitory effect on basal GTPCH I activity in vivo. The inhibitory effect of caveolin-1 on GTPCH I activity was further demonstrated by the fact that there was a significant reduction of GTPCH I activity in human umbilical vein endothelial cells transfected with adenoviral vector encoding caveolin-1 (Ad-Cav1). Because caveolae are surface microdomains that are particularly rich in free cholesterol to which caveolin-1 binds to obtain the characteristic shape, cholesterol-binding agents, such as filipin and methyl-β-cyclodextrin, have been used to disrupt the integrity of caveolae to study its role in various cellular processes.<sup>8</sup> In the current study, the authors further demonstrated that treatment with methyl-β-cyclodextrin resulted in significantly increased BH<sub>4</sub> levels in isolated aortas of normal mice. Taken together, these in vitro and in vivo findings suggest that caveolin-1 may exert the role as a negative regulator on enzymatic activity of GTPCH I in endothelial cells.

Based on this new evidence by Peterson et al,<sup>5</sup> it is tempting to speculate that the interaction among caveolin-1, GTPCH, and eNOS through their colocalizations in endothelial caveolae may provide machinery for optimal NO formation. It is possible that, although maintained in an inhibitory state by caveolin-1 under basal condition, GTPCH I activity may be activated on either physiological (eg, sheer stress) or pharmacological (eg, certain agonists) stimulations to accommodate eNOS activation, resulting in optimal local concentration of BH<sub>4</sub> required for biosynthesis of endothelial NO (see Figure).

Yet, the exact mechanisms underlying caveolin-1 modulation of GTPCH I are incompletely understood. New questions have been raised from the current study by Peterson et al.<sup>5</sup> For instance, whether caveolin-1 exerts its inhibitory role on GTPCH I through the direct interaction between these 2 proteins by their binding or involving other molecules, such as Akt and AMP-activated protein kinase. GTPCH is also close to and inhibited by caveolin-1, and its presence in caveolae may optimize the local BH<sub>4</sub> level required for NO production and, thus, endothelial function.

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**Table. Cardiovascular Phenotypes Observed in Caveolin Knockout Mice**

<table>
<thead>
<tr>
<th>Classifications</th>
<th>Caveolin-1</th>
<th>Caveolin-2</th>
<th>Caveolin-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoforms</td>
<td>α, β</td>
<td>α, β, γ</td>
<td>...</td>
</tr>
<tr>
<td>Expression</td>
<td>Endothelial cells, epithelial cells, smooth muscle cells, macrophages, fibroblasts, adipocytes</td>
<td>Same as cav-1</td>
<td>Skeletal, cardiac and smooth muscle cells</td>
</tr>
<tr>
<td>Phenotypes</td>
<td>Increased: vasorelaxation, NO production, vascular permeability, neointimal hyperplasia, p42/44 MAPK signaling, hypertrophic cardiomyopathy, pulmonary hypertension/fibrosis</td>
<td>Pulmonary hypertension/fibrosis</td>
<td>Hypertrophic cardiomyopathy, increased Ras-p42/44 MAPK signaling</td>
</tr>
</tbody>
</table>

Decreased: angiogenesis, myogenic tone, life span, insulin signal and glucose uptake

MAPK indicates mitogen-activated protein kinase; ..., no isoform.

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**Figure. Putative mechanisms underlying interactions between caveolin-1, eNOS, and GTPCH in endothelial cells.** Caveolin-1, GTPCH, and eNOS are colocalized in the flask-shaped caveolae microdomain of endothelial cells. By its interaction with the caveolin-1 scaffolding domain, eNOS is maintained in an inactive state in caveolae. On stimulation by shear stress or certain agonists, interaction with caveolin-1 is released; eNOS binds positive regulatory proteins, including calmodulin and heat shock protein 90, and is phosphorylated by Akt and AMP-activated protein kinase. GTPCH is also close to and inhibited by caveolin-1, and its presence in caveolae may optimize the local BH<sub>4</sub> level required for NO production and, thus, endothelial function.
as through a GTPCH feedback regulatory protein that governs GTPCH activity, needs to be determined. Caveolin proteins are known to have the capacity to modulate other cellular signaling pathways, including G proteins, Src-like kinases, Ras-Stat, mitogen-activated protein kinase kinase/extracellular signal–regulated kinases, and tyrosine kinase receptors. Given the role of caveolae as an important signaling platform in the plasma membrane where interactions between signaling molecules are regulated and optimized, it would be of interest to examine whether these signaling molecules are also involved in the regulation of GTPCH I by caveolin-1. In addition, because the GTPCH I protein also resides in the cytoplasm apart from the caveolae membrane, whether a translocation of GTPCH I from cytosol to membrane (or inverse) occurs during the process of eNOS activation remains to be determined.

The authors have provided direct evidence that caveolin-1 is a negative regulator of GTPCH I using caveolin-1 knockout mice, but this is clearly under an unnatural condition with a total lack of caveolin-1. The modulation of GTPCH and/or eNOS enzyme activities may actually depend on altered caveolin-1 expression levels and/or membrane dissociation patterns in diseases. Indeed, decreased NO synthase activity in cardiomyocytes from rats with heart failure has been shown to be related to the dissociation of caveolin from caveolae to the cytosol, without a change in the total amount of caveolin protein. Accumulating evidence suggests that a deficiency of the GTPCH/BH4 pathway is a prerequisite of eNOS uncoupling that results in the formation of superoxide anion instead of NO. Because eNOS uncoupling seems to be a common feature in a number of vascular diseases, including diabetes mellitus and hypertension, it will be of significance to elucidate whether and how caveolae/caveolin alterations affect GTPCH/eNOS activities under these conditions in the future.

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**Disclosures**

None.

**References**

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