The Caveolar Paradox
Suppressing, Inducing, and Terminating eNOS Signaling

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It is now established that specialized plasmalemmal lipid microenvironments, termed lipid rafts by Simons and Toomre, take part in various signal transduction processes. One subset of lipid rafts (which contain mostly cholesterol and sphingolipids) is found in plasmalemmal vesicles termed caveolae. The term caveolae (“little caves”) was introduced more than 40 years ago to describe plasma membrane invaginations identified by electron microscopy in a wide variety of cell types. Originally, these 50- to 100-nm plasmalemmal vesicles were shown to participate in the transcellular transport of macromolecules (transcytosis) and in the uptake of small molecules (potocytosis). However, it is only recently, with the identification of caveolins as the structural coat component of caveolae, that it has been recognized that caveolae are involved in signal transduction by ensuring the compartmentation of signaling molecules, such as G protein and tyrosine kinase–associated receptors, as well as endothelial nitric oxide synthase (eNOS). The identification of such distinct roles raises the question of how the same organelle can participate in these apparently quite different functions simultaneously. However, in the case of eNOS, recent data suggest that both of these functions (ie, as signaling platforms and intracellular trafficking modules) are, in fact, intimately related and complementary.

Repressing Basal Activity of eNOS

Although both eNOS and caveolins have several consensus sequences that have been proposed to participate in protein-protein interactions, evidence for a functional association between eNOS and caveolins exists only for the caveolin scaffolding domain (CSD), a juxtamembrane region of 20 amino acids in the C-terminal moiety of caveolin. Like other modular protein domains, the scaffolding domain of caveolin facilitates the generation of preassembled oligomeric proteins and, in addition, maintains these various signaling proteins in their off state. Note, however, that the presence of a CSD consensus–binding sequence does not necessarily imply that a given protein will interact with one or more caveolins; for example, several of the toll-like receptors contain CSD-binding domains, but there is no evidence to date of either caveolar targeting or functional regulation of toll-like receptor signaling by caveolins (S. Franz, T. Bourcier, R.A. Kelly, unpublished observations).

The physiological relevance of the inhibitory interaction of caveolar targeting on basal NO production was recently provided in a study on intact endothelial cells exposed to high levels of LDL cholesterol. As originally identified by Fielding and Fielding, caveolae also participate in reverse cholesterol transport by increasing caveolin abundance to promote cholesterol trafficking and efflux. The consequence for eNOS function of this cholesterol-induced increase in caveolin abundance is a marked decline in basal NO release, suggesting that the equilibrium between eNOS bound to caveolin and caveolin-free eNOS determines the basal component of eNOS-dependent NO release in endothelial cells. This interaction may be required to protect the cell from undesired, potentially cytotoxic, or nonphysiological bursts of NO in response to small fluctuations in intracellular calcium ([Ca2+]).

Promoting Stimulation of eNOS

In the presence of increased [Ca2+], calmodulin binding to caveolin antagonizes the blockade (or slowing) of electron transfer attributable to the binding of the scaffold protein to eNOS. In addition, transient increases in [Ca2+], attributable to agonist activation or increases in vascular flow have been shown to promote the dissociation of eNOS from caveolin in endothelial cells. Therefore, conversely, an increase in caveolin abundance in endothelial cells could account for alterations in agonist or flow-mediated stimulation of NO production. In addition to hypercholesterolemia, mentioned above, data from 2 other studies provide support for this hypothesis. Shah et al reported that a decrease in perfusion pressure in cirrhotic liver correlated with a marked increase in caveolin abundance and stabilization of the caveolin/eNOS inhibitory complex. In addition, Pelligrino et al documented that in ovariectomized rats, a decrease in acetylcholine-induced pial arteriolar vasodilation was associated with both a decrease in eNOS abundance and an increase in caveolin-1 abundance.

Importantly, persuasive evidence has also been provided that eNOS localization in caveolae per se (versus other intracellular locales of the enzyme) is key for agonist-stimulated NO release. Blair et al, for example, reported that endothelial cell exposure to oxidized LDL, which results in caveolar cholesterol depletion, rapidly caused the translocation of both eNOS and caveolin from caveolae, thereby leading to a marked decline in acetylcholine-induced eNOS activation. Together with a previous study documenting the
obligatory caveolar location of eNOS for the muscarinic cholinergic stimulation of eNOS in cardiac myocytes,12 these data point to the key role of eNOS localization within plasmalemmal caveole for its functional coupling to specific agonists. This paradigm must be modified in part, however, because of the known direct inhibitory interactions between caveolin and receptors such as the sphingosine 1-phosphate receptor EDG-1.13 Igarashi and Michel,13 for example, reported that caveolin overexpression markedly attenuates sphingosine 1-phosphate–mediated eNOS activation but not basal rates of NO production, suggesting a more proximal inhibitory effect of caveolins on EDG-1 receptor activation or coupling than on eNOS activity per se.

Terminating eNOS Signaling

In this issue of Circulation Research, Li et al14 propose that eNOS-dependent signaling can be terminated by a NO/cGMP-dependent process that leads to the disruption of caveolin oligomers. The hypothesis put forward by these authors is that NO disrupts caveolar signaling by distancing elements of the cascade. In their study, they document that exposing endothelial cells or smooth muscle cells to NO donors alters the coupling of caveolar resident proteins, such as bradykinin or endothelin receptors, with downstream signal-transduction cascades that result in calcium release from intracellular stores. This interpretation is potentially confounded by the relatively high and noncompartmentalized release of concentrations of NO, by NO donors, which could, for example, affect [Ca2+]i transients induced by receptors or ion channels not located in caveolae. The authors also propose that NO signaling itself can be terminated by a decrease in plasmalemmal L-arginine transport through NO-mediated disruption of caveolae. However, the authors provide no evidence that L-arginine–induced NO production, which presumably results in a physiologically relevant increase in NO production, leads to the degree of caveolin redistribution that was observed in their NO donor experiments. Other caveats should also be noted. First, none of the data provided causally associates the change in the oligomeric status of caveolin with any of the functional alterations in intracellular signaling tested. Second, the proportion of disrupted caveolin complexes on NO donor exposure remains limited when compared with the total cellular pool of caveolins. Third, the data documenting caveolin deoligomerization may not be relevant to the activity of physiological, compartmentalized release of NO by eNOS.

Nevertheless, the NO/cGMP-evoked decrease in the relative abundance of high molecular weight complexes of caveolins has been well documented by independent methodological approaches and certainly emphasizes the plasticity of function of lipid rafts and caveolar microdomains. Accordingly, the biophysical changes observed by Li et al14 may be related to alterations in detergent solubility of the caveolin heterocomplex on agonist stimulation, as recently reviewed by Fleming and Busse.15 Intracellular trafficking of caveolin and lipid microdomains may correspond to plasmalemmal caveolae after budding from the plasmalemmal membrane. Indeed, the GTPase dynamin, which is known to play an essential regulatory role in the process of endocytosis through clathrin-coated pits, was recently found to promote cholera toxin B chain internalization within caveolae after budding from the plasmalemmal membrane.16 Therefore, both caveolar fission and apparent caveolin deoligomerization could be the same phenomenon. Li et al,14 for example, also used the cholera toxin B subunit as a marker for caveolae and demonstrated its reversible translocation from the plasmalemmal membrane after exposure to sodium nitroprusside. This is consistent with the observation that the M1 muscarinic acetylcholine receptor also follows this mode of sequestration and internalization through budded caveolae,17 thereby leading to a desensitization of downstream NO signaling after exposure to muscarinic agonists. Although dynamin was documented to be necessary for caveolar-plasmalemmal fission, it was not sufficient. Additional, presently unidentified mechanisms dependent on agonist-receptor binding apparently are also required for caveolar budding.

Caveolae as eNOS Organelles

Although controversy remains regarding the subcellular location of all eNOS moieties within cells, the caveolar location of eNOS can be considered a major locale, at least for the activatable pool of the enzyme. Indeed, 2 studies have reported that increasing caveolin abundance resulted in a marked decline in agonist-stimulated NO generation, mimicking the phenotype observed in cells isolated from eNOS knockout animals. CSD peptides, for example, when introduced into cardiac myocytes by reversible permeabilization, mimic the inhibitory effects of full-length caveolin (caveolin-3 in the case of cardiac myocytes) and lead to a decrease in cGMP production on agonist stimulation.12 Moreover, muscarinic agonist regulation of myocyte beating rate could be inhibited by suppressing eNOS activation with 20-residue CSD caveolin surrogates. Recently, Bucci et al18 confirmed in mice that the activity of eNOS is highly sensitive to inhibition by CSD peptides. They showed that systemic administration of CSD peptides (fused to the homeodomain of antennapedia) suppressed acute inflammation and vascular leak to the same extent as that observed with an NOS inhibitor. Together, these studies demonstrate that expression of CSD peptides in either cardiac myocytes or endothelial cells clearly targets eNOS-dependent signaling.

Considering the many potential targets with which caveolin has been proposed to interact, plasmalemmal caveolae seem to be a well-suited intracellular locale for eNOS and its complex regulation. Tonic repression of basal activity, facilitation of agonist-evoked stimulation, and termination of the signal are 3 apparently paradoxical tasks that seem to be effectively managed in the context of the caveolar/eNOS interaction.

References

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