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Membrane rafts and caveolae in cardiovascular signaling

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Abstract

Purpose of review—Substantial evidence documents the key role of lipid (membrane) rafts and caveolae as microdomains that concentrate a wide variety of receptors and post-receptor components regulated by hormones, neurotransmitters and growth factors.

Recent findings—Recent data document that those microdomains are important in regulating vascular endothelial and smooth muscle cells and renal epithelial cells, and in particular in signal transduction across the plasma membrane.

Summary—Raft/caveolae domains are cellular regions, including in cardiovascular and renal epithelial cells, that organize a large number of signal transduction components, thereby providing spatially and temporally efficient regulation of cell function.

Keywords

Caveolin; vascular endothelium; vascular smooth muscle; eNOS; myocardial ischemia

INTRODUCTION

Signal transduction promoted by neurotransmitters, circulating hormones and growth factors is critically important in the regulation of the kidney and vasculature. Many such regulators produce their effects by interaction with plasma membrane receptors and subsequent perturbation of pathways that modulate metabolic activity, growth, death and differentiated functions of the target cells. The application of various experimental approaches and the elucidation of the crystal structure of G-protein-coupled receptors (GPCR) and certain tyrosine kinase receptors (RTK) has provided new insights regarding their activation and regulation [1–12].

Studies of receptor structure have occurred in parallel with those that have defined the expression and organization in the plasma membrane of receptors and post-receptor components. The concept that membrane lipids and proteins are found in microdomains is supported by a vast literature [13–17]. Data obtained from the application of various biophysical and biochemical approaches have furthered the concept that certain lipids, e.g., cholesterol and sphingolipids, selectively localize in membrane (lipid) raft domains that have decreased fluidity (increased “liquid order”) and greater buoyancy (following fractionation by density gradient centrifugation) compared to other portions of the plasma membrane [18,19]. Inclusion of the protein caveolin (there are 3 isoforms: caveolins-1, -2 and -3) in raft domains

promotes the formation and detection (by electron microscopy) of caveolae ("little caves"), 50–100 nm diameter membrane invaginations. Recent data indicate that the protein cavin, also known as polymerase I and transcript release factor, is important for the formation and maintenance of caveolae [20,21].

Membrane rafts and caveolae concentrate a subset of membrane constituents, including proteins (Figure 1) and other components involved in transport and signal transduction [13, 15–17,22,23]. The ability to detect morphologic caveolae and the use of anti-caveolin antibodies for immunoprecipitation and co-localization of caveolin-interacting proteins has helped identify partners that bind to caveolins and localize in caveolae. Proteomic and lipidomic methods should aid in assessment of raft/caveolae constituents and their changes in physiologic states and disease settings [24–27].

Rafts/caveolae have a non-homogeneous organization of signaling entities that facilitate temporally and spatially efficient cellular regulation by extracellular hormones and growth factors. The interiors of cells have gradients of second messengers and effectors (e.g., cAMP, Ca^{2+} , protein kinases/phosphatases, etc.), implying that local enrichment of messengers and effectors create "signaling factories" surrounded by moats that delimit signals [23,28–35]. Other such components include phosphodiesterases that hydrolyze cyclic nucleotides, thereby curtailing activation of effectors (e.g., protein kinase A, Epac, cyclic nucleotide-gated channels in the case of cAMP), protein phosphatases that limit the extent and duration of protein phosphorylation, and A-Kinase Anchoring Proteins (AKAPs), which bind and organize proteins involved in cAMP-regulated signal transduction, including by vasopressin in the kidney [36–38].

In addition to proteins that regulate cAMP generation and action, lipids and proteins involved in other types of signal transduction localize to rafts and caveolae, especially in cardiovascular cells [31,39]. The precise determinants for such localization are not fully understood but one contributor is an amino acid region on caveolins, the caveolin scaffolding domain (CSD), to which partners bind [16,17,39]. Peptides that mimic the CSD, including cavtratin, a synthetic cell-permeable caveolin-1 CSD peptide, can block caveolin-mediated signaling and responses and may have therapeutic potential for inflammation, tissue fibrosis, pulmonary hypertension and unstable atherosclerotic plaques [40–44]. Membrane rafts that lack caveolins also concentrate signaling molecules, implying that other factors (e.g., binding to lipids) contribute to interaction of signaling entities with rafts and caveolins. Use of multiple techniques to assess rafts/caveolae and their partners is preferred, since no one approach is ideal and complementary results from the use of multiple techniques strengthen conclusions regarding localization of components, functional roles of rafts/caveolae and their contribution in physiologic and pathophysiologic settings. Recent publications review ways to study rafts and caveolae [16, 17,45].

Publications on rafts and caveolae are occurring at a very rapid rate (>500 in the past year). Here, we review a sampling of such data with a focus on expression and functions of vascular endothelial and smooth muscle, cardiac and renal caveolae and the contribution of rafts and caveolae to renal and cardiovascular pathophysiology.

VASCULAR ENDOTHELIAL AND RENAL EPITHELIAL CAVEOLAE

Substantial data, including electron microscopic, immunochemical and biochemical analyses, indicate that vascular endothelial cells are highly enriched in caveolae. Pulmonary vascular endothelial cells have been used for many types of studies, including proteomic identification of caveolin-associated proteins (e.g., [25,46]). Proteomic methods reveal that vascular endothelial cell caveolae contain >100 proteins [25] and suggest that caveolae interact with intracellular organelles, including the endoplasmic reticulum and mitochondria [47]. Further

use of proteomic and lipidomic approaches should aid in identifying endothelial raft/caveolae-associated constituents (e.g., [48]).

A large number of signaling molecules that regulate vascular endothelial cells localize in rafts/caveolae. These include receptors (e.g., RTK, GPCR, certain steroid receptors), low molecular weight and heterotrimeric G-proteins, and “downstream” enzymes and components that serve as effectors of extracellular signals and in transport (e.g., endocytotic) machinery. Such downstream components can be regulated by RTK, GPCR and G-proteins (reviewed in: [16, 49–52]). Caveolin-1 has been shown to interact with activin receptor-like kinase (ALK)1, a transforming growth factor (TGF)- β type I membrane receptor expressed primarily on endothelial cells and involved in vascular remodeling and angiogenesis; other data indicate that TGF- β type I receptor, ALK5, and the TGF- β type II receptor localize in caveolae [53]. Increased cholesterol can inhibit TGF- β responsiveness by increasing accumulation of TGF- β receptors in rafts/caveolae, thereby facilitating degradation of TGF- β and suppressing TGF- β -induced signaling; conversely, treatments that deplete cholesterol (e.g., statins, methyl- β -cyclodextrin [M β CD]) can enhance response to TGF- β by increasing non-raft accumulation of TGF- β receptors and facilitating TGF- β -induced signaling. Such results suggest that alteration in TGF- β responsiveness contributes to the promotion of atherogenesis in vascular cells by increased cholesterol levels [54]. Caveolae and caveolin-1 also participate in endothelium-derived hyperpolarizing factor-mediated relaxation by regulating the location and activity of TRPV4 channels and connexins [55]. Other data indicate that important signaling events, including redox signaling and response to shear stress, occur in endothelial caveolae and may contribute to cardiovascular health and disease [56–58].

Endothelial nitric oxide synthase (eNOS, NOS3) is the most extensively studied caveolae-localized signaling molecule that is important for vascular function [58–63]. Recent studies in endothelial cells emphasize internalization of caveolae in regulating activation of eNOS [64], a role for protein nitration by eNOS and NADPH oxidase co-localized in rafts/caveolae [65], the regulation of localization and cellular trafficking of eNOS by S-nitrosylation [66], and ability of the heat shock protein HSP90 to dissociate eNOS from caveolae and protect eNOS from proteolysis by calpain, a calcium-activated protease [67]. Caveolins and caveolae have been implicated in the dietary sodium-induced changes in vascular tone regulated by eNOS [68], in the inhibition of eNOS by AMP-activated protein kinase that acts via Rac1 and Akt [69] and in the regulation by NO of MMP-13 protein-tyrosine nitration that leads to its release from endothelial cells, angiogenesis and wound repair [70].

Transcytosis is an important property of the vascular endothelium, whereby molecules are exchanged bidirectionally between the vascular lumen and the cells and across the cells into the subendothelial environment. Such bidirectional movement occurs across endothelial caveolae via one or more mechanisms that include fluid phase, adsorptive and receptor-mediated endocytosis. Transcytosis via caveolae regulates endothelial barrier function, participates in angiogenesis and contributes, along with changes in signal transduction, to vascular disease (for recent reviews, see [58,71–76]).

Caveolae and rafts regulate renal function in vascular endothelial cells and tubular epithelial cells along the nephron. Lipid rafts play important roles in intestinal and renal epithelial brush border membranes [77]. Recent proteomic data define proteins enriched in detergent-resistant membranes, i.e., rafts, in rat renal inner medullary collecting duct cells, in particular in response to vasopressin [78]. Renal epithelial cells provide model systems to explore the expression and functional roles of caveolins in membrane structure and transport [79–82] and cellular invasion by pathogens [83,84]. Expression of caveolin-1 occurs at various locations in the kidney, for example, trafficking of epithelial sodium channel in mouse cortical collecting duct cells [85]. In humans, caveolin-1 localizes in parietal epithelial cells within Bowman’s capsule; decreased

caveolin-1 expression occurs in the kidneys of patients with focal segmental glomerulosclerosis, lupus glomerulonephritis and unilateral ureteropelvic junction obstruction [86,87]. Increased expression of a caveolin-associated protein PV-1 has been observed in the glomerular capillaries of renal transplant glomerulopathy, the extent of such expression correlating with the grade of glomerulopathy and proteinuria [88]. Epithelial differentiation may, at least in part and perhaps indirectly, depend on caveolin-1 expression in adjacent stromal cells [89]. Caveolin-1/caveolae have been implicated in regulating TGF- β -induced RhoA activation that leads to collagen and fibronectin accumulation in mesangial cells and perhaps in fibrotic renal disease [90]. Other data indicate that renal proximal tubule epithelial cells in spontaneous hypertensive rats (SHR) and Wistar Kyoto (WKY) controls differ in terms of sensitivity to angiotensin II (Ang II) with higher H₂O₂ generation in SHR leading to enhanced expression by rafts of glycosylated and nonglycosylated AT(1) receptor forms [91]. In addition, an interaction has been proposed between cAMP formation and annexin-2 in lipid rafts in the trafficking of aquaporin-2 to the apical plasma membrane [92], although others question whether another aquaporin, aquaporin-1, localizes in lipid rafts [93].

VASCULAR SMOOTH MUSCLE CAVEOLAE

The lungs, in particular the pulmonary vasculature, have the highest expression of caveolae in the body [94]. Vascular caveolae are functionally important in both the endothelium and smooth muscle, providing a location for interaction of receptors, ion channels, signal transduction, kinases and other effector molecules, thereby helping to regulate cellular signaling pathways [17]. Ca²⁺ flux and intracellular Ca²⁺ levels are important regulators of smooth muscle physiology; numerous components in the Ca²⁺-handling machinery (plasma membrane Ca²⁺ pump, sodium-calcium exchanger, voltage dependent Ca²⁺ channels, and transient receptor potential channels [TRPC]) localize to and are regulated in caveolae [95, 96]. The suggestion that caveolae form “nanocontacts” with the sarcoplasmic reticulum [97] provides an additional level of organization and control of Ca²⁺ signaling in smooth muscle. This concept may help explain how Ca²⁺-induced Ca²⁺ release and triggering of store-operated Ca²⁺ channels (a subset of TRPC) occur. Recent data implicate caveolae in the regulation of Ca²⁺ sensitization in smooth muscle contraction and in downstream kinase signaling associated with shear stress. The CSD of caveolin may be sufficient to produce physiological effects: Treatment of human arterial smooth muscle cells with the CSD of caveolin-1 or -3 can inhibit Ang II-promoted increase in intracellular Ca²⁺ [42]. This result raises the intriguing possibility that caveolin, in particular the 20 amino acid region that comprises the CSD, might be a target in the treatment of vascular disease. In addition, caveolar structure modulates responses to physiological agonists: its disruption perturbs responses as a function of the proteins in caveolae [98]. For example, caveolae may be conduits for the regulation of intracellular Ca²⁺ and may enrich Ca²⁺-sensitive signaling [99].

A setting of smooth muscle pathophysiology that is affected by aberrant Ca²⁺ signaling is pulmonary arterial hypertension (PAH), which can be idiopathic (IPAH, with unknown etiology) or secondary to other disorders [100]. The level of caveolin expression may contribute to the PAH phenotype although there is controversy as to whether expression is increased or decreased. Caveolin-1 knockout (KO) mice have PAH, which appears attributable to remodeling of pulmonary precapillary vessels [101]. Caveolin-1 expression is reduced in the lungs of patients with IPAH and in animal models of PAH, such as hypoxic- and monocrotaline-treated rats [102,103]. A reduction in caveolin-1 expression is observed in the plexiform lesions (comprised of proliferating pulmonary arterial endothelial cells [PAEC]) that accompany IPAH and because of the high expression of caveolae in PAEC, likely accounts for the overall reduction in caveolin expression in the lungs of IPAH patients [102]. In contrast, caveolin-1 and caveolae expression are increased in pulmonary arterial smooth muscle cells (PASMC) from IPAH patients in association with increased function, including hyperproliferation and

enhanced Ca^{2+} -induced Ca^{2+} entry [103]. The decrease in caveolin-1 expression in IPAH-PAEC vs. the increase in IPAH-PASMC indicate that cell-specific changes in caveolin-1 expression and function occur in this disease—and perhaps others.

CARDIAC CAVEOLAE

Two important aspects of high fidelity signaling are the ability to regulate the “turn on” and “turn off” of the signal. In addition, though, a third key feature in signal transduction is the need to limit basal or “leaky” signaling. In this regard, cardiac caveolae may help limit unsolicited activity of signaling molecules: caveolin-3 KO mice have a ~40% increase in myocardial cAMP content [104] and cardiac myocytes treated with M β CD have a 60–70% increase in phosphorylation of PKA targets, suggesting that disruption of caveolae results in dysregulation of cAMP generation [105]. This dysregulation may be secondary to enhanced Gs-promoted signaling or loss of Gi-mediated inhibition of adenylyl cyclase activity [106]. Other studies show that basal signaling activity of β_2 -adrenergic receptors is enhanced by cholesterol depletion and can be suppressed by increased expression of caveolin or increasing membrane cholesterol [107]. Understanding how caveolae regulate basal signaling and how this is perturbed by disease could provide novel insights into cardiovascular physiology and pharmacology.

Caveolae in cardiac myocytes are major sites of GPCR expression and ionic flux [16,39,108]. The ability of caveolae to enrich and regulate various channels may help coordinate signaling events while defects in these associations may contribute to cardiac arrhythmias [109]. Caveolin-1 can traffic Kv1.5 channels to lipid-rich (raft) regions that modulate channel function [110]; caveolin-trafficking mutants trap Kv1.5 channel expression within the cell, thereby limiting plasma membrane expression. In addition, lipid-rich microenvironments in myocytes distinct from caveolae are key to Kv1.5 channel localization and function: depletion of membrane cholesterol changes channel current in the absence of co-localization with caveolin-3 [111]. Other data confirm that Kv1.5 is not present in cardiac myocyte caveolae [112]. These studies suggest that myocyte caveolins have a trafficking function distinct from a structural role. Since caveolin-3 is the isoform in myocytes that is necessary for caveolae formation, perhaps caveolin-1 has a different role: transporting components to lipid-rich compartments.

The role of caveolae in regulating cardiac injury after ischemia and ischemia/reperfusion has recently received much attention. Studies with adult cardiac myocytes *in vitro* reveal that disruption of caveolae with cholesterol-depleting agents (e.g., M β CD) eliminates protection (induced by a preconditioning, short ischemic stimulus, or opioids) from damage by hypoxia-reperfusion [113]. These data have been confirmed in studies with intact hearts [114]. Compounds such as M β CD do not differentiate between rafts and caveolae and thus, it is important to confirm conclusions obtained by their use in other ways, such as with caveolin-KO mice. Caveolin-1 and -3 KO mice are both resistant to cardiac protection from ischemia/reperfusion injury [115,116] although morphological caveolae are only absent in cardiac myocytes from caveolin-3 KO mice. Thus, caveolins may have roles other than via caveolae in cardiac protection. Though caveolins-1 and -3 exist in atria [117], the data in ventricles are equivocal. Caveolin-1 KO mice have loss of protection in the ventricles and appear to have altered cardiac matrix metalloproteinase (MMP) activity with co-localization of caveolin-1 with MMP-2 in ventricular myocytes [118], suggesting that caveolin-1 has distinct roles in ventricles. Defining the signaling events regulated by caveolin-1 vs. caveolin-3 in cardiac myocytes and in different cardiac regions will be important future investigations. Of note, localization of components in caveolae in sinoatrial regions appears to be important for automaticity in the heart [119].

CONCLUSIONS and FUTURE DIRECTIONS

Since the discovery of caveolae in the 1950s [120,121], in the subsequent 50+ years scientists have been spelunkers in these “caves”, seeking further understanding of physiology and pathophysiology. Caveolae/rafts, lipid-rich platforms in a sea of fluidity, serve as nexuses for signal transduction in virtually all cells and tissues. Deletion of caveolin genes in KO mice is not lethal but absence of caveolins can alter numerous cellular processes [16] and reduce life span [94]; recent data indicate that restoring endothelial-specific caveolin-1 expression in caveolin-1 KO mice can rescue vascular, cardiac and pulmonary defects observed in global knockouts [122]. Caveolae house a large number of signaling molecules—probably too many in number and concentration to be explained by binding to the CSD. Are there caveolae sub-species that have specific roles in compartmentation of signaling molecules? Do post-translational modifications of caveolins (e.g., phosphorylation, nitrosylation, ubiquitination, etc.) regulate the binding of signaling partners to caveolins? Recent evidence suggests that caveolae may be conduits of signaling to non-plasma membrane regions: Understanding how caveolae link to sarcoplasmic reticulum, mitochondria, nuclei, and other cellular compartments may provide insights into the dynamic control of cellular function. The growing number of cellular and animal models of caveolin-associated diseases argues for the need to generate tissue- and cell-specific [122], as well as age-conditional, expression and knockdown of caveolins so as to achieve more precise assessment of caveolins in pathophysiology. Thus, many unanswered issues remain with respect to rafts/caveolins although their role in regulation in cardiovascular cells seems unquestioned.

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Figure 1.
Schematic depicting caveolae and caveolae-resident proteins.