Caveolin-Dependent Angiotensin II Type 1 Receptor Signaling in Vascular Smooth Muscle

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Angiotensin II (Ang II) is a pluripotent hormone in vascular smooth muscle cells (VSMCs) and stimulates arterial hypertrophy, a hallmark of remodeling in hypertension. These effects are mediated primarily through the G protein–coupled receptor Ang II type 1 receptor (AT\(_1\)R). In VSMCs, AT\(_1\)R-mediated signaling is biphasic, and internalization of the agonist/receptor complex into what we called a “signaling domain” is required for the tonic phase of signaling (initially characterized by phospholipase D activation) but not for the initial phospholipase C stimulation, which occurs at the cell surface. This evidence was consistent with the existence of spatially discrete Ang II signaling domains. We showed that sequential Ang II–induced phospholipase activation is mediated through G\(\alpha\)q subunits (G\(\alpha_q\) and G\(\alpha_{12/13}\)), as well as their associated G\(\beta\)\(\gamma\) components. In addition, various nonreceptor tyrosine kinases, including cAbl and some from the Src family, as well as mitogen-activated protein kinases and Akt, are activated by Ang II and mediate VSMC hypertrophy and growth. Activation of these pathways is in part dependent on tyrosine phosphorylation (transmodulation) of the epidermal growth factor receptor (EGF-R), which serves as a “scaffold” for the assembly of cSrc and Pyk2, leading to downstream activation of extracellular-regulated kinase (ERK)1/2 and Akt. These results are consistent with a model that requires temporal dispersion and organization of the AT\(_1\)R signaling repertoire in VSMCs.

Accumulating evidence suggests that receptors and the signaling molecules with which they associate are not randomly distributed in the cell membrane but are localized in specialized signaling domains. Functionally distinct microdomains, formed by the lateral packing of glycosphingolipids and cholesterol within the membrane bilayer, have been identified in plasma membranes. These domains, called “lipid rafts,” have been implicated in membrane trafficking and cellular signaling mechanisms and serve as “scaffolds” to facilitate the association of signaling complexes, thereby increasing the rate of interactions of receptors, coupling and adaptor molecules, and signaling proteins. Caveolae are cell membrane invaginations that contain the major structural protein caveolin-1 (Cav1) and are thought to be subsets of rafts. They are postulated to be platforms for the coordination of certain signaling pathways. In VSMCs, Ang II stimulation promotes AT\(_1\)R association with Cav1 and trafficking of the receptor from heavy density membrane fractions into relatively buoyant Cav1-enriched lipid rafts, which is required for transactivation of EGF-R at focal adhesions, another membrane signaling domains associated with growth factor and integrin signaling. The full expression of the AT\(_1\)R signaling repertoire depends on caveolae/lipid rafts and reactive oxygen species (ROS) production by reduced nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase. Several recent reviews have described the physiological and pathophysiological roles of caveolae and caveolins in the cardiovascular system generally. The main focus of this review is to summarize the recent progress on the emerging understanding of the role of Cav1 as a central organizer for the spatially and temporally regulated, ROS-dependent, growth-related AT\(_1\)R signaling in VSMCs.

Lipid Rafts/Caveolae and Caveolins

The morphological identification of “lipid rafts” is still unclear. Lipid rafts are resistant to low-temperature solubilization by nonionic detergents, such as Triton X-100, a property that allows their separation by means of differential flotation after density-gradient centrifugation. Caveolae are 50- to 100-nm flask-shaped invaginations of plasma membranes that were originally identified by electron microscopy in endothelial cells in capillaries (Figure 1a). Caveolae exist, putatively, as a subset of lipid rafts and also are enriched in cholesterol and sphingolipids. The shape and structural organization of caveolae result from the presence of their signature protein, caveolin, that binds cholesterol and self-assembles into high-mass oligomers to form caveolae (Figure 1a and 1c). Caveolae/lipid rafts function as signaling organizing centers and platforms by exploiting multiple protein–lipid and protein–protein interactions to link the cytoplasmic tail of transmembrane receptors with other protein scaffolds to assemble kinases, phosphatases, and other catalytically active molecules to generate specific signals that are temporally and spatially controlled. Caveolins are a family of 21- to 24-kDa integral membrane proteins.
with 3 mammalian isoforms identified as Cav1, caveolin-2 (Cav2), and caveolin-3 (Cav3) Figure 1b).22 Cav1 and Cav2 are widely expressed, whereas expression of Cav3 is limited to skeletal and cardiac myocytes. Caveolin serves as a scaffold protein involved in the recruitment/association of specific signaling complexes into caveolae.25 Caveolin interacts with a variety of signaling molecules through the caveolin scaffolding domain (CSD) Figure 1b and 1c), corresponding with amino acids 82 to 101, that interacts with consensus motifs (ΦXΨXXXΦXXΨ, Φ=aromatic residue) present in several proteins, including AT1Rs, EGF-Rs, G proteins, and the Src family kinases.25,26 Recent evidence suggests that Cav1 is also found at many intracellular locations, as well as in the plasma membrane. Variations in subcellular localization are paralleled by a plethora of ascribed functions for the caveolins.27

Recent studies using Cav1−/− mice provided evidence supporting the physiological and pathophysiological importance of Cav1 in vivo.15,17,22 Cav1−/− mice show: (1) lack of caveolae in the tissues examined; (2) vascular dysfunction with impaired endothelium-dependent relaxation, contractility, and maintenance of myogenic tone; (3) hyperproliferation of mouse embryo fibroblasts in culture and hypercellularity of lung endothelial cells; (4) dilated cardiomyopathy and pulmonary hypertension, as well as cardiac hypertrophy; and (5) increased smooth muscle cell proliferation in a carotid artery blood flow cessation model.28–32 These studies suggest that Cav-1 normally functions as a negative regulator of cell growth. In contrast, Cav1−/− and apolipoprotein E−/− (apoE−/−) double-knockout mice showed that the lack of Cav1 in apoE−/− mice significantly reduces the development of atherosclerosis in the aorta.33 Although underlying molecular mechanisms are incompletely understood, these findings suggest that Cav1 has multiple functions in cardiovascular homeostasis.

Caveolin and AT1R Trafficking

The phenomenon of agonist-induced AT1R trafficking has been studied extensively and has focused generally on “internalization” in the context of receptor desensitization that leads to degradation or recycling. In this canonical scenario, activated G protein–coupled receptors are inactivated by G

Figure 1. Caveolae and caveolin. (a) Presence of flask shaped caveolae (arrows) in VSMC plasma membrane as visualized by an electron microscopy (scale bar=200 nm). Reproduced, with permission, from Ushio-Fukai et al.13 (b) The caveolin gene family has 3 members: Cav1, Cav2, and Cav3. Both Cav1 and Cav2 exist in different isoforms. The N-terminal region contains the CSD, which is essential for both the formation of caveolin oligomers and the interaction of caveolins with other proteins. MS indicates the membrane-spanning domains. (c) Plasma membrane caveolae are anchored by the actin cytoskeleton, which is mediated through filamin. Two Cav1 monomers form a single homo-oligomer, and the membrane-spanning domain forms a hairpin-like loop within the membrane bilayer. (d) Cav1 seems to have principle functions in lipid transport, membrane trafficking, and cell signaling. Caveolae deliver their contents, including caveolins, to the caveosomes through Cav1-containing vesicles (cavicles), which are distinct and separate mechanisms from those involved in clathrin-coated pits-mediated endocytosis.
protein receptor kinase–mediated phosphorylation of serine residues in the cytoplasmic tail with the subsequent binding of β-arrestin, which guides trafficking to clathrin-coated pits leading to intracellular degradation or recycling pathways. This model has been modified to incorporate observations that the β-arrestin–associated β2-adrenergic receptors and AT,R internalize and activate the ERK1/2.

In VSMCs, Ang II induces rapid (<2 minutes) translocation of a portion of the AT,R from heavy membrane fractions to Cav1-enriched lipid rafts, and AT,R and Cav1 become associated. We explored the mechanisms involved in AT,R trafficking to the Cav1-enriched/lipid raft signaling locus and found that it depends on Cav1. Moreover, Cav3 has been shown to act as a chaperone for the AT,R, allowing the receptor to traffic through the exocytic pathway and to localize at the cell membrane in transfection systems. This interaction seems to be mediated by the CSD and is required to prevent the mislocalization of AT,R to lipid bodies or Golgi, which results in aberrant maturation and surface expression of AT,R. Thus, Ang II–promoted interaction of AT,R with Cav1 may serve as a fundamental mechanism by which Cav1 may function as a scaffold protein and/or a chaperone for proper AT,R targeting into caveolae/lipid rafts. This mechanism is required for activation of downstream signaling events, such as transactivation of EGF-R and Akt phosphorylation in VSMCs (Figure 2). In a recent commentary, the large signaling complex in hepatocyte C9 cells that was Ang II stimulated and Cav1 dependent and that contained not only Cav1 but also AT,R and EGF-R was referred to as a “signalplex.”

Moreover, intactness of the microtubule and of the actin cytoskeletal systems and the presence of the actin-binding, nonreceptor tyrosine kinase cAbl, which is a substrate of cSrc and binds to Cav1, are essential for proper AT,R targeting into Cav1-enriched lipid rafts in VSMCs. Mundy et al reported that microtubules and the actin cytoskeleton are involved in trafficking, bidirectionally, of Cav1 containing vesicles (“cavicles”) between the cell membrane and intracellular compartments, called caveosomes (Figure 1d). The dense cortical actin network beneath the plasma membrane likely inhibits, basally, the clavicle/caveosome trafficking, as well as lateral movement within the cell membrane. The stable cortical actin may limit, in the absence of agonist stimulation, access to caveolae of proteins, such as AT,R, that bind to Cav1 after Ang II stimulation and migrate into Cav1-enriched lipid rafts. An important function of Cav1 may be to tether caveolae to the actin cytoskeleton through binding to the actin-binding protein filamin, thereby maintaining caveolae at the cell surface until stimulants trigger detachment from the cytoskeleton (Figure 1c).

Cortical actin remodeling is modulated by Ang II–induced ROS- and cSrc-dependent tyrosine phosphorylation of cortactin. Cortactin interacts with the actin nucleation factors actin-related protein 2/3 and N-Wiskott-Aldrich syndrome protein to facilitate actin branching and binds to cSrc and the GTPase dynamin, which is involved in membrane remodeling. In VSMCs, Ang II stimulates cSrc-dependent tyrosine phosphorylation of cortactin. Ang II promotes recruitment of cSrc, Cav1, and cAbl to the AT,R, which is required for actin cytoskeleton reorganization, as well as AT,R trafficking into caveolae/lipid rafts. Recently, we
found that cAbl is a major upstream mediator for tyrosine phosphorylation of cortactin (L. Zuo, M. Ushio-Fukai, and R.W. Alexander, unpublished observations, 2006). Thus, activation of cSrc/cAbl/cortactin pathways is important for promoting actin remodeling, thereby increasing the motility of AT,R from actin-enriched heavy membrane fractions into Cav1-enriched lipid rafts fractions (Figure 1).

Caveolin and ROS-Dependent AT,R Signaling

Major outputs of the AT,R signaling repertoire are dependent on transactivation of the EGF-R, which is required for activation of downstream target Akt, leading to vascular hypertrophy in VSMCs (Figure 2).6,9 Intact caveolae/lipid rafts are essential for transactivation of EGF-R, as inferred from experiments in which cholesterol was extracted from VSMCs by β-cyclodextrin, and Ang II–induced tyrosine phosphorylation of EGF-R was inhibited.13 Unlike the AT,R in VSMC plasma membranes, the EGF-R basally resides primarily in Cav1-enriched light membrane fractions.7,10 Ang II stimulation promotes AT,R binding to Cav1, as well as migration of AT,R into, and the simultaneous egress of EGF-R out of, Cav1-enriched/lipid rafts, events that are cotemporaneous with the appearance of phosphorylated EGF-R and Cav1 in focal adhesions.7 Because AT,R has a CSD consensus binding sequence (Y302GF304LGKKF309GGY312), this site may mediate binding for Cav1. Of note, knockdown of Cav1 using small interfering RNA (siRNA) interferes with the trafficking of both receptors, EGF-R transactivation, and Akt phosphorylation.10 These findings suggest that Cav1 is essential for the spatial-temporal organization of AT,R signaling in VSMCs (Figure 2) and provide additional support for the importance of Cav1 in cardiovascular regulation.

Major elements of the AT,R signaling repertoire in VSMCs are dependent on ROS derived from NADPH oxidase.46 NADPH oxidases in phagocytic cells consist of the membrane-bound cytochrome b558 composed of the catalytic gp91phox and the p22phox subunits, as well as cytosolic components, including p47phox, p67phox, and the small Rho GTPase Rac1.47 Recently, novel gp91phox (also termed “Nox2”) homologues have been identified in nonphagocytic cells and include Nox1, Nox3, Nox4, and Nox5.48 Lipid rafts have been proposed to function as platforms for compartmentalization of redox signaling events through activation of NADPH oxidase–derived ROS.49 Indeed, NADPH oxidase has been identified in caveolae/lipid rafts in various cells,50–52 which could be important for the control of cell migration and growth.

In VSMCs, Nox1 and Rac1 are important components for Ang II–stimulated NADPH oxidase activation.52,53 Nox1, but not Rac1, is found in caveolae/lipid rafts basally51 and Ang II stimulation promotes cytoskeleton/microtubule-dependent recruitment of Rac1 into these microdomains, which correlates with NADPH oxidase activation.50 Similar dynamic Rac1 association with caveolae/lipid rafts has been reported in other systems.54,55 Ang II stimulates tyrosine phosphorylation of Sos-1 (a Rac–guanine nucleotide exchange factor), which is localized in Cav1-enriched lipid rafts in VSMCs.10 Cav1 siRNA inhibits Ang II–stimulated tyrosine phosphorylation of Sos-1, Rac1 activation, and membrane translocation, as well as H2O2 production.10 In VSMCs, AT,R-mediated activation of cSrc, cAbl, and p38 mitogen-activated protein kinase and Akt, as well as transactivation of the EGF-R, but not ERK1/2, are mediated through ROS derived from NADPH oxidase.5–7,14 All of these ROS-sensitive signaling pathways are dependent on the expression of Cav1 and/or the presence of Cav1-enriched lipid rafts.10,13 Thus, Cav1 plays an essential role in linking AT,R signaling with NADPH oxidases to promote local production of ROS in caveolae/lipid rafts via regulating Rac1 activity, thereby forming platforms to activate redox signaling events in VSMCs (Figure 2).

Caveolin Tyrosine Phosphorylation and AT,R Signaling

Cav1 was first identified as a major tyrosine-phosphorylated protein in v-Src–transformed embryo fibroblasts.56 Cav1 is phosphorylated on tyrosine 14 in response to growth factors,57–59 cellular stresses including oxidative stress,60–62 and Ang II.7,13 Many lines of evidence indicate that Src family kinases play an essential role in this process. In VSMCs, Ang II–stimulated tyrosine phosphorylation of Cav1 is mediated through cAbl,7 which is a substrate of cSrc and one of the important mediators for ROS-dependent tyrosine phosphorylation of Cav1.63 H2O2–stimulated, cSrc-dependent phosphorylation of Cav1 (which may be mediated by cAbl, as noted) inhibits clathrin-dependent internalization and facilitates caveolar-dependent trafficking of EGF-R and Cav1 to the perinuclear region.64 In VSMCs, Ang II induces the formation of an immunocomplex (signaplex) that includes the AT,R, Cav1, cSrc, and cAbl, and knockdown of any 1 of the latter 3 proteins with siRNA inhibits AT,R migration into and EGF-R egress from the caveolae/lipid rafts and EGF-R and Akt phosphorylation (Figure 2).7,10 Given that Cav1 is tyrosine phosphorylated by Src and cAbl, these results suggest that pY14-Cav1 seems to have important roles in trafficking for both EGF-R and AT,R and its associated signaling in VSMCs.

Tyrosine-phosphorylated Cav1 also associates specifically with Grb7, a scaffolding protein involved in growth factor–induced cell migration.65 This association may serve to link pY14-Cav1 to the focal adhesion machinery involved in cell locomotion.65 In VSMCs, EGF-R egress from the caveolae/lipid rafts induced by Ang II stimulation is required for the appearance of transactivated EGF-R at focal adhesions, where they colocalize with vinculin, phospho-paxillin, and pY14-Cav1 (Figure 2).7,13 Nox1 colocalizes, as noted, with Cav1 and is found in Cav1-enriched fractions, whereas Nox4 is localized at focal adhesions in VSMCs (Figure 2).51 In other systems, cAbl has been shown to be localized at focal adhesions and mediates effects of integrins by phosphorylating paxillin.66 The focal adhesion complex contains integrin receptors and many associated signaling and adaptor molecules, including vinculin and paxillin, as well as caveolin.67 Thus, pY14-Cav1 may serve as a scaffold for the formation of active signaling complexes with transactivated EGF-R at focal adhesions, which may be important in the spatial/temporal organization of AT,R signaling (Figure 2).
Functional Role of Caveolin in Vascular Growth/Hypertrophy

In vascular cells, ROS modulate growth/hypertrophy and migration; endothelial function, including endothelial-dependent relaxation and expression of proinflammatory adhesion molecules, chemokines, and cytokines; and modification of the extracellular matrix. All of these processes play important roles in vascular diseases, such as hypertension and atherosclerosis, which are mediated by Ang II. Ang II is a major mediator of ROS generation in vascular cells, and Cav1 is an important organizer of the associated redox-sensitive signaling pathways. Ang II activates the ROS-dependent kinase Akt and ROS-independent kinase ERK1/2, which contribute to Ang II–induced hypertrophy. Cav1 siRNA inhibits Ang II–stimulated ROS-dependent Akt phosphorylation and [3H]leucine incorporation without affecting ROS-independent ERK1/2 phosphorylation. These results suggest that Cav1 is specifically involved in the ROS-dependent AT1R signaling events regulating VSMC hypertrophy (Figure 2). Studies in other systems show an inhibitory or a stimulatory role of Cav1 in activation of ERK1/2 and Akt pathways. Thus, the characteristics of Cav1-mediated responses seem to be highly dependent on the molecular context and cell type, reflecting varying patterns of expression of Cav1, as well as of the multiple proteins with which it can interact.

Some studies using Cav1−/− mice show that Cav1 functions as a negative regulator for cell growth. In contrast and as noted, Cav1−/− and apoE−/− double-knockout mice are relatively protected from the development of atherosclerosis in the aorta compared with the apoE genotype. Cav1 is proatherogenic, as reflected by the exacerbation of the process by Ang II infusion in apoE−/− mice. Given that Cav1 functions as a signaling scaffold, it is reasonable that removal of compartmentalization of ROS-dependent signaling components by knockdown of Cav1 may inhibit proatherogenic stimuli that normally act through caveolae/lipid rafts. Moreover, arteries from Cav1−/− mice show abnormalities in Ang II–induced contractile responses, further supporting a potential role of Cav1 in Ang II–mediated VSMC function in vivo. As noted, Cav1 is a major tyrosine phosphorylation substrate of cAbl, Imapinib (STI571, Gleevec), a selectively selective inhibitor of the Bcr-Abl tyrosine kinase, inhibits Ang II infusion–induced hypertrophy in rat mesenteric arteries in vivo, as well as diabetes-associated atherosclerosis in aorta of apoE−/− mice. Atherosclerosis in this model is highly dependent on AT1R. It will be intriguing to investigate whether pY14-Cav1 is involved in AT1R-mediated proatherogenic effects in vivo in future studies.

Perspectives

As mentioned above, the information presented here is consistent with the notion that Cav1 plays an important role in Ang II–induced AT1R trafficking into the Cav1-enriched lipid rafts and EGF-R egress out of these microdomains, both of which are required for Rac1-dependent NADPH oxidase activation, ROS-dependent EGF-R transactivation, and downstream signaling linked to VSMC hypertrophy. The cardiovascular abnormalities seen in Cav1 knockout mice impugn a potentially important role of Cav1 in modulating VSMC function and dysfunction. Thus, Cav1 is likely a central nidal for the spatially and temporally organized ROS-dependent growth-related AT1R signaling in VSMCs. These findings provide new insights into an essential role of Cav1 in Ang II–mediated vascular pathophysiology and perhaps provide a guide for the development of new therapeutic strategies. Understanding the functional significance of Cav1 in ROS-dependent AT1R signaling in VSMCs in vivo using tissue-specific conditional Cav1 knockout mice is an important objective for future investigation.

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Disclosures

None.

References


