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Caveolins in Vascular Smooth Muscle: Form Organizing Function

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Abstract

Caveolae are becoming increasingly recognized as an important organizational structure for a variety of signal and energy transducing systems in vascular smooth muscle (VSM). In this review, we discuss the emerging role of the caveolins in organizing and modulating the basic functions of smooth muscle: contraction, growth/proliferation, and the energetic support systems that support these functions. With clear alterations in cell metabolism and function in VSM with altered caveolin-1 (Cav-1) protein expression and with cardiovascular abnormalities associated with Cav-1 null mice, the caveolin family of proteins may play an important role in the function and dysfunction of VSM.

Introduction

Caveolae are flask-shaped invaginations of 50 –100 nm in size at the plasma membrane that were first described in 1953 by George Palade from electron micrographs of endothelial cells [1]. Then, in 1955, Yamada described similar invaginations in the gall bladder epithelium and created the term caveolae to reflect their appearance as little caves [2]. Biochemically, caveolae represent a subdomain of the plasma membrane enriched in cholesterol, sphingolipids, and a family of integral membrane proteins named caveolins (for review see [3]). Caveolins are a family of 21 kDa to 24 kDa integral membrane proteins with three mammalian isoforms identified as caveolin-1 (Cav-1), caveolin-2 (Cav-2), and caveolin-3 (Cav-3) [3]. Caveolins have the ability to form higher-order oligomeric complexes with themselves that result in a caveolin-rich domain within the plasma membrane, and can bind to additional proteins via its caveolin scaffolding domain (CSD) located in the carboxy- terminus [3]. Cav-1 and Cav-2 are widely expressed whereas expression of Cav-3 is limited to muscle cells [3]. The expression of all three isoforms of caveolin has been found only in smooth muscle cells as tested so far, with all caveolin isoforms assuming a predominantly plasma membrane distribution [4]. Intriguingly, the expression of Cav-3 appears to be less than that of the other caveolins in smooth muscle [5]. Studies in the hamster vasculature demonstrated that Cav-1 was expressed in smooth muscle cells from both the arterial and venous vasculature; whereas Cav-3 was expressed in smooth muscle cells from the arterial but not the venous vasculature [6]. Additionally, studies in Cav-1 null mice demonstrated suppression of caveolae in vascular smooth muscle cells, while caveolae formation remained present in striated muscle types suggesting that Cav-1 is required for caveolae formation in smooth muscle and that Cav-3 cannot compensate for the physiological function of Cav-1 in smooth muscle cells [3]. Moreover, in Cav-3 null mice, skeletal and heart muscle lack caveolae, whereas smooth muscle still demonstrated formation of caveolae invaginations [3]. A recent study by Woodman et al. (2004) in mice urogenital smooth muscle demonstrated that loss of bladder Cav-1 results in a marked decrease in Cav-2 but not Cav-3 expression; whereas ablation of Cav-3 fails to alter

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Cav-1 or Cav-2 expression. Also, deletion of Cav-1 resulted in the almost complete loss of caveolae, while Cav-2 null and Cav-3 null mouse smooth muscle showed a normal number of caveolae [7]. Therefore, it is reasonable to predict that Cav-1 is the major structural caveolin isoform in smooth muscle and that Cav-2 and Cav-3 may play roles other than simply formation of caveolae, such as modulation of Cav-1 expression, modulation of cell signaling and modulation of metabolism. Several recent reviews have described the physiological and pathophysiological roles of caveolae in the cardiovascular system [8,9]. Therefore, the main focus of this review is to outline the role of caveolae and Cav-1 in the functional physiology of vascular smooth muscle cells.

In the simplest analysis, the functions of vascular smooth muscle can be viewed as two-fold: to appropriately alter contractile activity and to alter phenotype to grow or remain contractile. This review will focus on the role caveolae may play in these two functions in smooth muscle and on the energy supply needed to sustain these functions.

One caveat should be made at the outset. Although we have made reasonable efforts to limit the review to studies actually done in smooth muscle, the reader should bear in mind that the great majority of the work, including that from our own laboratory, has been done on cultured smooth muscle cells that likely are phenotypically altered. Therefore extrapolation of results in cultured smooth muscle cells to results expected in “real” smooth muscle cells should be done with considerable caution.

Role of caveolae in vascular smooth muscle contractile regulation

Several laboratories have generated mice with a disrupted caveolin gene for all independent caveolin isoforms and also a double knockout for Cav-1 and Cav-3. As previously reviewed [3], all caveolin null mice are viable and fertile, but have cardiovascular problems. Cav-1 null mice developed cardiomyopathy, severe pulmonary hypertension, and exaggerated eNOS activity [10-12]. Studies in isolated rings from Cav-1 null mice demonstrated that Cav-1 is essential in the regulation of vascular tone via the modulation of eNOS activity. In the absence of Cav-1, eNOS activity does not respond to regulatory signals and consequently NO levels remain constitutively elevated [10,11]. Isolated aortic rings from Cav-1 null mice exhibited higher basal release of NO and three-fold higher cGMP levels as compared to wild-type mice [10]. Also, Cav-1 null aortic rings were unable to maintain a constant contractile tone [10,11] and had a marked relaxation in response to acetylcholine as compared to aortic rings from wild-type mice [11]. In addition, the vasodilatory response of aortic rings isolated from Cav-1 null mice is completely restored by the addition of the eNOS inhibitor, L-NAME [11]. All together, these data confirm the well-established inhibitory effect of caveolin on basal eNOS activity and its consequent role in the regulation of vascular tone. Interestingly, there is recent evidence that supports the concept of the “caveolar paradox” [13] which proposes that caveolin has the ability to both inhibit eNOS activity on basal conditions and to promote its stimulation upon agonist stimulation (for a review see [14]). For example, recently [15] used CAV-1 null mice to examine the impact of caveolae supression in a model of post-ischemic revascularization (induced by resection of a femoral artery segment) demonstrating how the lack of Cav-1 results in a dramatic defect in post-ischemic neovessel formation instead of promoting NO-mediated angionesis. Furthermore, studies in isolated endothelial cells from Cav-1 null mice aorta validated these results [15]. Therefore, both the enrichment of eNOS in caveolae (compartmentation) and the paradoxical interaction of eNOS with caveolin-1 (caveolar paradox) add complexity to the understanding of the role of caveolae in the regulation of the NO pathway and its consequent effects on smooth muscle contractility. Although the above mentioned studies in isolated rings from Cav-1 null mice validate the negative regulatory influence of Cav-1 on eNOS function, a more mechanistic understanding is necessary to fully appreciate how caveolae and Cav-1 influences eNOS and consequently vascular smooth

muscle cell contractility given the complex regulation of eNOS activity in light of the hypothesis proposed by the caveolar paradox. On the other hand, a substantial body of data suggests a role for smooth muscle Cav-1 in the coordination of the signaling events leading to the regulation of smooth muscle contractility (for review see [8,9]). Myogenic tone in smooth muscle is regulated by a feedback mechanism tightly linking spontaneous transient outward currents (STOCs) to local Ca^{2+} increases in the adjacent sarcoplasmic reticulum (Ca^{2+} sparks) (for review see [16] and [17]). Ca^{2+} sparks are a result of the spontaneous activation of ryanodine receptors (RyRs) from the sarcoplasmic reticulum (SR). These sparks normally remain solitary and confined to an area of ~ 2.0 μm in diameter. However, a Ca^{2+} spark can activate release from neighboring RyRs to form a compound spark that can evolve into propagating Ca^{2+} waves ([16] and [17]). It has been suggested that these effects are due to the alignment of channels in the plasma membrane with RyRs in the SR. Recent studies assume that the presence of caveolae membrane invaginations provides an increased probability of proximity between the channels in the plasma membrane and those in the SR by providing spatial precision for the feedback mechanism between sparks and STOCS. Therefore, these two events have been suggested to be dependent on the presence of caveolae [18]. For example, studies in Cav-1 null mice exhibited reduced STOCs indicating that caveolae is necessary for efficient Ca^{2+} signaling in vascular smooth muscle [10]. The close proximity between the SR and the surface membrane has long been known. However the specific study of these plasma membrane-SR microdomains and their role in Ca^{2+} signaling is only now emerging. Research data indicate that Ca^{2+} sparks that occur in close proximity to the plasma membrane can stimulate both L-Type Ca2+ channels and Ca^{2+}-activated K^+ channels (BK channels) at the plasma membrane (for review see [17]). Moreover, data indicates that the coupling between L-type Ca^{2+} channels and RyRs in smooth muscle is relatively loose given the low number of sparks discharge sites generated by L-type Ca^{2+} channels. On the other hand, coupling between RyRs and BK channels is considered tighter given the high coincidence of sparks and STOCs [19]. Therefore, the emerging view is that aggregation of BK channels into caveolae is the only hypothesis that will allow the close proximity between the membrane and the SR to explain the high coincidence of sparks and STOCs between RyRs and BK channels. Furthermore, other biophysical properties of BK channels are taken into consideration under this hypothesis, like the low Ca^{2+} sensitivity of BK channels as compared to L-type Ca^{2+} channels, the spark volume which only covers a limited membrane area, and the constraint of BK channel distribution based on channel density. In fact, BK channels clustering have been observed in ~200 nm membrane patches [20] consistent with the hypothesis that caveolae constitute the membrane domain were RyRs and BK channels communicate. Moreover, a study by Ohi et al. [21] calculated the average BK channels density at the plasma membrane domains that are adjacent to the Ca^{2+} spark to be 3-4 channels μm^2 in smooth muscle cells from both guinea-pig vas deferens and from urinary bladder. Additionally, immunofluorescence microscopy demonstrated a punctuate staining of BK channels and RyRs in discrete junctional areas of the plasma membrane with peripheral SR fragments. Another research group demonstrated that vasoconstrictors can inhibit BK channels activity by direct Src-dependent phosphorylation [22] consistent with the hypothesis regarding localization of BK channels to caveolae, given that Src-family tyrosine kinases have long been known to copurify with Cav-1 [23].

Ca^{2+} influx across the plasma membrane following depletion of the intracellular Ca^{2+} stores is known as store-operated Ca^{2+} entry (SOCE) [24]. A relation between SOCE and the members of the transient receptor potential canonical (TRPC) family of proteins has recently emerged. TRP1, TRP3 and TRP4 have been suggested to associate with caveolae via interaction with Cav-1 [25-27]. Bergdahl et al. [25] have demonstrated that TRPC1 is functionally coupled to ET-1 type receptors in intact arteries and that it is localized to caveolae. Additionally, disruption of caveolae and lipid rafts by cholesterol depletion resulted in increased sensitivity of TRPC1 to SOCE and ET-1 responses [25]. A recent report by the same group demonstrated that the plasticity of TRPC expression is enhanced by vascular injury in arterial smooth muscle, that

TRPC expression correlates with cellular Ca2+ handling, and that TRPC1 is a subunit that is highly up-regulated by SOCE channels [28].

Ca^{2+} sensitization is a mechanism in which an increase in the contractile output of the smooth muscle cell in response to receptor agonists can be observed independently of any changes in intracellular Ca^{2+} concentration. Several studies indicate that the activity Rho-A associated kinase (ROK) acts as the primary Ca^{2+} sensitization mechanism in smooth muscle (for review see [29]). In arterial smooth muscle KCl causes contraction by elevation of the free intracellular Ca^{2+} store, whereas receptor stimulation activates an additional mechanism termed Ca^{2+} sensitization that can involve activation of the GTPase RhoA which results in downstream activation of ROK and PKC. To induce Ca^{2+} sensitization, ROK must interact with the plasma membrane [29]. The role of caveolae as a loci for receptor agonist-induced Ca^{2+} sensitization was proposed by Taggart [4], who demonstrated that receptor agonist stimulation of smooth muscle causes a redistribution of RhoA, ROK, and PKC-α from the cytoplasm to the cell periphery [30] and that this redistribution could be inhibited by a peptide corresponding to the caveolin scaffolding domain of Cav-1 [31]. Moreover, a study by Urban et al. [32] extended this model to include the effects of K^+ depolarization in rabbit arterial smooth muscle cells. The authors demonstrated how stimulation with KCl can induce Ca^{2+} sensitization by causing a Ca^{2+} -dependent ROK translocation to caveolae [32]. These data supported the hypothesis that any stimulus that increases intracellular Ca^{2+} in the arterial smooth muscle will cause increased ROK translocation to caveolae at the cell periphery were additional signaling events that lead to sustained myosin light chain phosphorylation and force may be coordinated, especially in the face of a temporary falling of intracellular Ca^{2+} levels.

A recent study took an alternative approach in which a synthetic Cav-1 scaffolding domain peptide was loaded into contractile smooth muscle cells to test the hypothesis that the scaffolding domain of Cav-1 participates in the coordination of signal transduction that regulates contractility [33]. Chemical loading of the synthetic peptide into smooth muscle cells inhibited PKC-dependent increases in contractility, resulted in activation of ERK1/2 and stimulated phosphorylation of the actin binding protein caldesmon [33]. These results are consistent with a role for Cav-1 in the coordination of the signaling mechanisms leading to the regulation of smooth muscle contractility. However, to date, there is no evidence of altered hemodynamics in Cav-1 null mice to support these *in vitro* findings.

Additional research suggests that caveolae and caveolins may help modulate smooth muscle cell contraction via interaction with several ion channels and receptors. Caveolae have been implicated in the signal transduction of 5-HT_{2A} receptor, which is the primary mediator of smooth muscle contraction in response to 5-HT. A study by Dreja et al. [34] in rat caudal arteries reported that 5-HT_{2A} receptors were enriched in caveolae fractions isolated by sucrose density fractionation and that disruption of caveolae by cholesterol depletion impairs 5-HT-induced contraction. Moreover, 5-HT_{2A} receptors had been found to reside in smooth muscle caveolae using immunogold labeling and electron microscopy [35]. Additionally, siRNA downregulation of Cav-1 in C6 glioma cells resulted in complete abolishment of 5-HT_{2A} receptor signaling while keeping protein expression levels unaffected [36].

Voltage-gated L-type Ca^{2+} channels are key route for Ca^{2+} entry into smooth muscle, making them important regulators of smooth muscle cell contraction. However, research data provide divergent results regarding the role of caveolae in the regulation of voltage-gated L-type Ca^{2+} channels. Darby et al. [37] found that detergent resistant membranes from canine tracheal smooth muscle were enriched in Cav-1, L-type Ca^{2+} channels, and Ca^{2+} binding proteins like calsequestrin and calreticulin, supporting caveolae involvement in smooth muscle Ca^{2+} handling. On the other hand, Löhn et al. [18] found that depletion of caveolae and lipid rafts by cyclodextrin resulted in a dose-dependent decreased of the Ca^{2+} spark frequency, amplitude

and spatial size in arterial smooth muscle cells. However, L-type Ca^{2+} channels currents were unaffected after cyclodextrin treatment suggesting that the function of L-type Ca^{2+} channels is independent of caveolae or lipid rafts.

Role of caveolae in the organization of metabolism in VSM

Smooth muscle has traditionally been viewed as the disorganized (and hence less "differentiated" for function) sibling to the striated muscles. Coincident with the development of our understanding of vascular smooth muscle as a dynamic cell type (capable of responding to growth stimuli for phenotypic transformation and cell migration as well as responding with changes in vascular tone in response to endothelial-derived factors, stretch, metabolites and ions, etc.), there has been an increased understanding of the complexity of VSM cell energetics. Indeed, a framework is emerging of multiple energy producing compartments localized to various energy consuming processes (contraction, ion pumping, signal transduction cascades, etc.) in smooth muscle. Perhaps the best elucidated compartment for energetic support of ATP-requiring processes is that created by the plasma membrane.

VSM has long been known to be a highly glycolytic cell type with a robust metabolism enabling resistance to metabolic challenges [38,39]. Some of the first insights into the organization of metabolism in VSM came from Paul and others who demonstrated a compartmentalization of glycolysis and glycogenolysis in VSM [40-42]. Further work established that a glycolytic compartment is somehow localized to the plasma membrane of smooth muscle and may be couple to the plasmalemmal calcium pump [43,44] and the sodium pump [45]. However, the mechanism of the localization of glycolytic enzymes to the plasma membrane has remained unknown until recently.

The concept of multiple membrane subdomains in the organization of metabolism in VSM became necessary with the discovery that VSM could carry out a portion of gluconeogenesis by use of exogenously provided fructose-1,6-bisphosphate [46] and that disruption of lipid rafts/caveolae by use of β-cycodextrin resulted in increased gluconeogenesis and decreased glycolysis suggesting that lipid raft/caveolae domains organize and localize glycolysis but not gluconeogenesis [47]. Early work suggested a role for caveolae in calcium transport that was metabolically sensitive. In pulmonary artery VSM cells from hypertensive rats, hypoxia induced a shift in calcium accumulation to the caveolae [48]. Therefore, the caveolae were a reasonable structure in the membrane that might be responsible for a localized glycolytic pathway. The hypothesis that a glycolytic compartment was localized to the caveolae was supported by a localization of the glycolysis specific enzyme phosphofructokinase (PFK) to caveolae and the ability of Cav-1 to immunoprecipitate PFK [49]. Furthermore, siRNA silencing of Cav-1 expression resulted in elimination of caveolar ultrastructure in VSM cells and in a redistribution of PFK away from the VSM cell periphery and towards the center of the [49]. These examples are consistent with Cav-1 acting as a scaffolding to localize glycolysis to the caveolae. Indeed, recent results in our lab indicate similar results for other glycolytic enzymes (e.g. aldolase, unpublished observations). Recent evidence also suggests that the K_{ATP} channel in smooth muscle may be localized near protein kinase A in smooth muscle [50] indicating possible co-localization of one key ATP-requiring process with the relevant signaling molecules and ATP synthesizing pathways in caveolae. In cardiac myocytes glycolysis was found to specifically fuel K_{ATP} channels [51] and whether a specific reliance on caveolae associated K_{ATP} channels and caveolae-associated glycolysis exists in VSM has yet to be explored. Also, whether all caveolae have a glycolytic compartment and whether individual caveolae act independently of other caveolae in the plasma membrane are important unanswered questions in understanding the role of the caveolae in the organization of cell signaling and in the organization of metabolism to support the that cell signaling. That caveolins act as a scaffold for localization of glycolytic enzymes does not appear to be unique to smooth

muscle. Recent work indicates that Cav-3 is important for the localization of PFK in skeletal muscle [52] In addition, GLUT-4 has been reported to be localized to the caveolae in other tissues [53] as has the active insulin receptor [54]. Therefore, the organization of metabolism with signaling kinases in caveolae may be a more general feature of the co-organization of these cascades in a wide variety of cell types.

Finally, recent work with Cav-1 null mice suggest an important range of whole body metabolic changes including high fatty acids and triglycerides, decreased leptin, reduced glucose uptake and reduced insulin receptor protein levels [11]. Whether these changes occur specifically in the VSM is not clear. However, considering the parallels between striated muscle and smooth muscle in the localization of glycolytic machinery to the caveolae, it is likely that some metabolic changes occur due directly to the ablation of caveolin production. It is well known that VSM cells of the proliferative phenotype have decreased caveolae content [55,56] as well as increased overall rates of glycolysis [38,57]. Whether the changes in metabolism are directly consequent or simply coincident of decreased caveolae is not clear. However, marked changes in glycolytic enzyme localization are observed with modulation of Cav-1 and hence caveolae formation [49,58]. Therefore it may be expected that the number of caveolae would indeed be an important modulator of overall metabolism in VSM.

Role of caveolae in VSM cell differentiation and proliferation and atherosclerosis

It is well established that VSM is capable of exhibiting a range of phenotypes within the vascular wall. Phenotypic transformation to a proliferative phenotype, or selection for survival of a proliferative phenotype, underlies neointimal hyperplasia and is a hallmark of early atherosclerosis and of restenosis. The involvement of caveolae in VSM phenotype transformation and proliferation is suggested by the finding that there is a greater prevalence of caveolae in VSM of the contractile phenotype compare to the proliferative phenotype [56]. Cav-1 knock-out experiments showed inhibition of caveolae formation in VSM [11,52,59]. However, it is becoming clear that the role of caveolins in VSM is not only to act as the structural meshwork for formation of caveolae. VSM is capable of expressing all three forms of caveolin [4] and since caveolins may have important signaling roles, the roles of Cav-2 and Cav-3 in VSM proliferation are unclear. In bladder smooth muscle, Cav-1 expression inhibits Cav-2 expression [7]. If similar results are found in VSM, the possibility is raised that Cav-2 may play a role in initiation or maintenance of the proliferative phenotype.

The regulatory role of the localization of signaling molecules in the caveolae remains unclear. Some cascades seem to be active when localized in caveolae including glycolysis [47,49,59, 60] and insulin signaling [54] while other signaling pathways, notably those for growth, seem inhibited by localization in caveolae (see [61] for a review). Ironically, most studies examining the role of caveolins in the regulation of proliferation are done in cultured cells which already have many characteristics of the proliferative phenotype. It has been long recognized that VSM response to platelet-derived growth factor (PDGF) was a key event in switching to the proliferative phenotype early in the process of atherogenesis. Recent work showed that when cultured human coronary VSM cells were treated with PDGF, Cav-1 protein expression was decreased; the loss of Cav-1 immunostaining was also observed in the neointima after balloon injury in ileac artery [62]. However, Cav-1 mRNA levels increased with the increased lysosomal degradation of Cav-1, likely accounting for the mismatch in mRNA and protein levels. In VSM cells from wild type mice, provision of serum stimulated proliferation and decreased caveolin-1 expression [63]. In the same study, when Cav-1 null mice were transfected to express Cav-1, proliferation was inhibited. Taken together with their findings that VSM cells of human atheroma had decreased Cav-1 levels, the findings of Schwencke et al. clearly imply a role of Cav-1 in checking VSM cellular proliferation. When Cav-1 was

overexpressed in human coronary VSM cells, PDGF administration shifted the VSM cells towards apoptosis and inhibited cyclin D expression. In a different study by Carlin et al. [64] caveolae and lipid raft disruption by cyclodextrin potentiated PDGF-induced chemotaxis suggesting a role for caveolae in cell migration associated with atherogenesis. Therefore, Cav-1 may be responsive to PDGF and play a key role in VSM proliferation and apoptosis in vascular disease. One study utilized a Cav-1 null mouse model combined with arterial ligature to develop a vascular lesion model [65]. With the lack of Cav-1, mice having their carotids ligated exhibited significantly more intimal hyperplasia compared with ligated arteries from control mice. Concomitant with the VSM hyperplasia was an increase in the MAP kinase and cyclin D immunostaining. However, it remains unclear whether the changes observed were due to simply the lack of Cav-1 in VSM cells and downstream sequellae or whether global changes in proatherscleratic factors (such as the reported elevated plasma cholesterol, elevated plasma triglycerides) in the Cav-1 null mice mediated these changes. Indeed, the same group interestingly noted that the offspring of Cav-1 null mice bred with ApoE null mice appeared to be protected from atherosclerosis compared to ApoE null mice [66]. More mechanistic studies are needed to address this important issue, especially using in vivo models where VSM cell phenotype isn't altered by culture conditions.

A number of other mediators of VSM proliferation appear to be influenced by caveolins. One mediator that increases Cav-1 expression is n-3 polunsaturated fats. Incorporation of docosahexanoic acid and eicosapentaenoic acid into freshly isolated rat thoracic aorta VSM cells resulted in increased expression of Cav-1 and Cav-3 coincident with inhibition of VSM proliferation mediated by the MAPK pathway [67]. Another pathologic source of VSM hypertrophy is via angiotensin II mediated VSM cell growth. Knock-down of Cav-1 by siRNA results in impaired targeting of the angiotensin II type 1 receptor thus inhibiting ROS-dependent angiotensin II signaling involved in VSM hypertrophy [68]. The role for Cav-1 in stretch-mediated VSM cell proliferation was elucidated by a recent study by Sedding et al. [69]. When VSM cells were stretched there was an activation of PI3-kinase, Akt and Src kinases and their activation was abolished by b-cyclodextrin of with antisense oligobnucleotides against Cav-1. Furthermore, venous VSM exhibited increase Akt activation when exposed to increased pressure and the Akt activation was almost abolished in Cav-1 null mice. VSM cell stretch has been reported to result in a translocation of Cav-1 from caveolae to non-caveolar membrane sites suggesting that proliferative cascades may be regulated by altered localization of Cav-1 in VSM. Finally, A novel role for the Na^+/K^+-ATPase in the regulation of VSM proliferation has been explored by Allen and colleagues as well as others (see [70,71] for example). Low levels of the Na^+-pump inhibitor ouabain activates VSM cell proliferation by interaction of the α-subunit that resides in the caveolae thus recruiting signal transduction proteins involved in proliferation cascades [72]. Since others have shown a coupling of glycolysis with the sodium pump in VSM [45] and a localization of glycolysis to the caveolae [49,59,60], a localization of the Na^+-pump to caveolae would be expected. Combined with growing evidence for the role of caveolae in localizing insulin receptors and GLUT4 (see [54] for a review), a picture is emerging of caveolae localizing coordinated sets of pathways (insulin receptor, glucose transporters, glycolytic enzymes and pumps specifically fueled by glycolysis) in the same membrane subdomain.

No discussion of smooth muscle proliferation should occur without at least a mention of apoptosis. Although the role of the caveolins in apoptosis has been widely studied in other cells types, little is known about its role in smooth muscle cells. One study [62] showed that overexpression of Cav-1 inhibited PDGF-induced cyclin D1 expression and was proapoptotic. Therefore, Cav-1 in VSM may, at least under over-expression situations, become pro-apoptotic. In our own lab with A7r5 cells we have observed increased apoptosis in Cav-1 over-expressing cells (unpublished observations).

Summary

The dynamic nature of vascular smooth muscle and its ability to alter contractility and phenotype in response to varied stimuli is becoming increasingly appreciated. The organization of signaling and metabolism underlying these functions has been incompletely understood. Caveolae are emerging as an organizational structure of central importance for an increasingly complex system of signal transduction. As we learn more about Cav-1 as a scaffolding protein for localization and regulation of signaling and metabolism, our understanding of the roles of Cav-2 and Cav-3 in VSM is far from complete. The co-regulation of the caveolin family of proteins and the range of signaling pathways that may be affected by these proteins remains a rich area of investigation. The cardiovascular abnormalities seen in the Cav-1 knockout mice suggest a potential role of Cav-1 in modulating VSM function and dysfunction. Future studies will be necessary not only to dissect the molecular pathways through which the caveolins regulate vascular smooth muscle cell function, but also to determine whether the caveolins represent viable targets in therapeutic strategies to combat vascular disease.

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