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Editorial

Review Focus Series

Segregation and integration: Roles played by caveolae and caveolins in the cardiovascular system

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See reviews in the series by Feron and Balligand [15] (pages 788–797), Maguy et al. [21] (pages 798–807), and Hardin and Vallejo [24] (pages 808–815) in this issue and by Mineo and Shaul [26] and Schwencke et al. [30] in next month's issue.

Original articles in the series are by Calaghan and White [16] (pages 816–824) and Shaw et al. [25] (pages 825–835) in this issue.

Over the last two decades our view of the cell membrane has progressed from that of a relatively homogenous protein-containing lipid bilayer that simply separates the cytosol from the extracellular milieu. We now know that the cell membrane is composed of many and varied types of lipids and that their properties largely determine protein localisation and thus protein–protein interactions and cellular signalling. There has also been a veritable explosion of knowledge regarding lipid membrane compartmentalisation, and this Review Focus Series on "Caveolae in Cardiovascular Signalling" provides a timely reminder of the importance of caveolae in the regulation of cardiac myocytes, vascular smooth muscle, and endothelial cell function.

Caveolae were first described over 50 years ago using electron microscopy as flask-like invaginated structures of the plasma membrane [1]. These cellular micro-domains are enriched in cholesterol and sphingolipids as well as in specific proteins such as the scaffolding protein caveolin (for recent comprehensive reviews see Refs. [2–4]). Endothelial cells are one of the most abundant sources of caveolae, and the plasma membrane surfaces of the endothelial cells that face the blood contain numerous caveolae. These "little caves" have been attributed several functions, and a series of elegant electron microscopic studies provided the first hint that caveolae act as trans-endothelial carriers by demonstrating the sequential movement of probes from one side of the endothelial cell layer to the other in a process that seems to involve the budding of caveolae from one side of the endothelial cells, transfer through the intracellular milieu, and fusion with the opposite membrane [5,6]. While the latter observations remain controversial [7–9], our knowledge regarding the role played by caveolae and the caveolins in the regulation of cell signalling has increased markedly (see for example Refs. [10,11]). The list of proteins that can be found in caveolae is still increasing as are the roles for the marker protein caveolin. For example, caveolins (caveolin-1, -2 and -3) play a role in maintaining the lipid composition of caveolae, as well as their morphology, and the signals that originate from these sites [4].

1. Caveolar signalling in the heart

2. Regulation of the endothelial nitric oxide synthase (eNOS) in the heart by caveolins

In the mid-1990s, our concept regarding the regulation of eNOS was revolutionised following reports that the enzyme associates with caveolin-1 and caveolin-3 in endothelial

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cells and cardiac myocytes, respectively [12–14]. The subsequent dissection of these interactions led to the identification of multiple binding sites within both proteins and the competitive displacement of caveolin and calmodulin from eNOS by each other. Not only does the association of eNOS with caveolin regulate enzymatic activity, the localisation of the eNOS signalosome and its individual constituents are also largely determined by caveolin. The review by Feron and Balligand [15] in this issue is a highly informative summary of the regulation of eNOS by caveolin in the heart and the constituents of the signalosome complex. Some of the most interesting work on the regulation of eNOS by caveolins in the heart is linked to signalling by the β_2 adrenoceptor. While Feron and Balligand provide an overview of the state of the art, an original communication by Calaghan and White [16] reports that caveolae modulate excitation–contraction coupling and β-adrenergic stimulation in adult rat cardiac myocytes. The reason for studying adult cardiomyocytes lies in the fact that these cells possess caveolae- and caveolin-3-containing t-tubules [17], while neonatal cells, which are more frequently studied, do not [18,19]. Moreover, t-tubule anomalies have been described in caveolin-3-deficient mice [20]. Using methyl-β-cyclodextrin to deplete cells of membrane cholesterol, Calaghan and White [16] were able to demonstrate a reduction in the shortening of myocytes that correlated with an attenuation of the Ca^{2+}-induced Ca^{2+} release. β-Adrenoceptor agonist stimulation, on the other hand, was enhanced by the detergent treatment, an effect that could be mimicked by dialysing cells with an antibody to caveolin-3. Although it is tempting to speculate that changes in caveolin expression in the adult heart with aging and disease will have consequences for baseline cardiac function as well as responsiveness to β-adrenergic stimulation, additional experimental work is required to clarify these points.

2.1. Ion channels

There is increasing evidence for significant ion channel localization in lipid rafts, which are cholesterol- and sphingolipid-rich microdomains in cell membranes, and in caveolae. Potassium ion channel subunits known to govern regional electrical activity and to localize in lipid rafts include K^+ channels of the shab (Kv2), shaker (Kv1) and shal (Kv4) families as well as inwardly-rectifying, ATP-sensitive, and Ca^{2+}-activated K^+ channels. In addition to this heterogeneous group of K^+ channels, caveolae also contain Na^+ channels and the Ca^{2+} channels investigated by Calaghan and White [16], as well as the hyperpolarization-activated, cyclic nucleotide-binding channels. The article by Maguy et al. [21] provides a detailed and concise review of current knowledge of the relationships between cardiac lipid rafts, caveolae and ion channel function, and the molecular mechanisms involved in channel activation. Certain pathological conditions are associated with changes in the cholesterol content of cells and with the structural remodelling of caveolae. For example, in heart failure there is a significant redistribution of caveolin-3 out of caveolae [22], a finding that fits well with the reported gradual development of cardiomyopathy in caveolin-$3^{-/-}$ mice [23].

3. Caveolar signalling in vascular smooth muscle cells

Caveolae are also found in vascular smooth muscle cells, and Hardin and Vallejo [24] have done an excellent job of sorting through the literature relating specifically to caveolar signalling in vascular smooth muscle cells. This review highlights the differences in the functions attributed to caveolae and caveolin between smooth muscle and other cell types.

3.1. Caveolae and myogenic tone

In addition to the well-documented effects of caveolin-1 on eNOS function, which affects basal vascular tone as well as the response to agonist stimulation, there is convincing evidence indicating a role for smooth muscle caveolin-1 in coordination of the signalling events leading to the regulation of smooth muscle contractility. While this topic is comprehensively reviewed by Hardin and Vallejo [24], an original communication by Shaw et al. [25] presents data supporting the concept that the structural arrangement of caveolae in the proximity of the peripheral sarcoplasmic reticulum in smooth muscle cells from pressurised resistance arteries serves to regulate Ca^{2+} oscillations and contractile activity.

4. Caveolar signalling in disease

Early morphological studies showed that the membrane associated with surface caveolae has a distinct sterol composition, and later studies showed that the caveolar structure is particularly sensitive to cholesterol perturbation. Such findings indicated that free cholesterol and caveolin are closely linked, but the exact role of caveolin and caveolae in cholesterol homeostasis still remains unclear especially as no clear disruption of cholesterol regulation has been reported in caveolin-deficient cells [3]. The evidence indicating that alterations in cellular cholesterol affect vascular cell signalling is more convincing, and the review by Mineo and Shaul [26] in next month's issue highlights the link between alterations in the lipid composition of caveolae and cardiovascular diseases. These authors have also made a significant contribution to this field by showing that oxidized low density lipoprotein displaces eNOS from plasmalemmal caveolae and impairs its activation [27], while high density lipoprotein maintains the lipid environment in caveolae [28]. Moreover, C-reactive protein (CRP), an acute-phase reactant that is positively correlated with cardiovascular disease risk and

endothelial dysfunction, prevents endothelial NO synthase (eNOS) activation by diverse agonists. The latter effect is dependent on Fcγ receptors [29].

The review by Schwencke et al. [30] in the April issue concentrates on a larger spectrum of diseases, most notably muscular dystrophy, vascular proliferative disease, cardiac hypertrophy, heart failure, and cancer. Indeed, given that caveolin-1 plays a role as an endogenous inhibitor of numerous signalling proteins and the fact that it inhibits cell proliferation [31–33], it is logical to suggest that a decrease in caveolin levels would be associated with enhanced cellular proliferation and eventually tumour formation. However, much of the data indicating that caveolin-1 has tumour suppressor properties obtained to date has been based on in vitro studies and on data obtained using genetically modified mice. The latter findings appear to contrast with the fact that several human tumours have been linked to increased caveolin levels, and a possible tumour-promoting action of caveolin-1 has also been suggested [30].

The importance of refocusing studies on caveolae and caveolar proteins in native tissues cannot be stressed enough as there are marked differences in the makeup of these micro-domains in vivo and in vitro. This point was recently highlighted by the report that more than 40% of the proteins expressed in luminal endothelial cell plasma membranes in vivo (rat lung) could not be detected in the same cells in culture [34]. This heterogeneity is most probably also regulated by the environment of a given tissue, which means that caveolar signalling in one tissue is likely to differ from that of another. It will be interesting to follow developments in this field to determine the differential role of caveolae in the pathogenesis of cardiovascular disease and cancer.

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References

[1] Palade GE. An electron microscope study of the mitochondrial structure. J Histochem Cytochem 1953;1:188–211.
[2] Cohen AW, Hnasko R, Schubert W, Lisanti MP. Role of caveolae and caveolins in health and disease. Physiol Rev 2004;84:1341–79.
[3] Martin S, Parton RG. Caveolin, cholesterol, and lipid bodies. Semin Cell Dev Biol 2005;16:163–74.
[4] Li XA, Everson WV, Smart EJ. Caveolae, lipid rafts, and vascular disease. Trends Cardiovasc Med 2005;15:92–6.
[5] Bruns RR, Palade GE. Studies on blood capillaries. II. Transport of ferritin molecules across the wall of muscle capillaries. J Cell Biol 1968;37:277–99.
[6] Simionescu N, Simionescu M, Palade GE. Permeability of muscle capillaries to small heme-peptides. Evidence for the existence of patent transendothelial channels. J Cell Biol 1975;64:586–607.
[7] Parton RG. Caveolae-from ultrastructure to molecular mechanisms. Nat Rev Mol Cell Biol 2003;4:162–7.
[8] Rippe B, Rosengren BI, Carlsson O, Venturoli D. Transendothelial transport: the vesicle controversy. J Vasc Res 2002;39:375–90.
[9] Hommelgaard AM, Roepstorff K, Vilhardt F, Torgersen ML, Sandvig B, van Deurs B. Caveolae: stable membrane domains with a potential for internalization. Traffic 2005;6:720–4.
[10] Rizzo V, Sung A, Oh P, Schnitzer JE. Rapid mechanotransduction in situ at the luminal cell surface of vascular endothelium and its caveolae. J Biol Chem 1998;273:26323–9.
[11] Rizzo V, McIntosh DP, Oh P, Schnitzer JE. In situ flow activates endothelial nitric oxide synthase in luminal caveolae of endothelium with rapid caveolin dissociation and calmodulin association. J Biol Chem 1999;273:34724–9.
[12] García-Cardena G, Fan G, Stern DF, Liu J, Sessa WC. Endothelial nitric oxide synthase is regulated by tyrosine phosphorylation and interacts with caveolin-1. J Biol Chem 1996;271:27237–40.
[13] García-Cardena G, Oh P, Liu J, Schnitzer JE, Sessa WC. Targeting of nitric oxide synthase to endothelial cell caveolae via palmitoylation: implications for nitric oxide signaling. Proc Natl Acad Sci U S A 1996;93:6448–53.
[14] Feron O, Belhassen L, Kobzik L, Smith TW, Kelly RA, Michel T. Endothelial nitric oxide synthase targeting to caveolae-specific interactions with caveolin isoforms in cardiac myocytes and endothelial cells. J Biol Chem 1996;271:22810–4.
[15] Feron O, Balligand J-L. Caveolin and the regulation of endothelial nitric oxide synthase in the heart. Cardiovasc Res 2006;69:788–97.
[16] Calaghan S, White E. Caveolae modulate excitation–contraction coupling and b2-adrenergic signalling in adult rat ventricular myocytes. Cardiovasc Res 2006;69:816–24.
[17] Scriven DR, Klimek A, Asghari P, Bellve K, Moore ED. Caveolin-3 is adjacent to a group of extra-dyadic ryanodine receptors. Biophys J 2005;89:1893–901.
[18] Lohn M, Furstenau M, Sagach V, Elger M, Schulze W, Luft FC, et al. Ignition of calcium sparks in arterial and cardiac muscle through caveolae. Circ Res 2000;87:1034–9.
[19] Xiang Y, Rybin VO, Steinberg SF, Kobilka B. Caveolar localization dictates physiologic signaling of b2-adrenoceptors in neonatal cardiac myocytes. J Biol Chem 2002;277:34280–6.
[20] Galbiati F, Engelman JA, Volonte D, Zhang XL, Minetti M, Li M, et al. Caveolin-3 null mice show a loss of caveolae, changes in the microdomain distribution of the dystrophin–glycoprotein complex, and t-tubule abnormalities. J Biol Chem 2001;276:21425–33.
[21] Maguy A, Hebert T, Nattel S. Involvement of lipid rafts and caveolae in cardiac ion channel function. Cardiovasc Res 2006;69:798–807.
[22] Ratajczak P, Damy T, Heymes C, Oliviero P, Marotte F, Robidel E, et al. Caveolin-1 and -3 dissociations from caveolae to cytosol in the heart during aging and after myocardial infarction in rat. Cardiovasc Res 2003;57:358–69.
[23] Woodman SE, Park DS, Cohen AW, Cheung MW, Chandra M, Shirani J, et al. Caveolin-3 knockout mice develop a progressive cardiomyopathy and show hyperactivation of the p42/44 MAPK cascade. J Biol Chem 2002;277:38988–97.
[24] Hardin CD, Vallejo J. Caveolins in vascular smooth muscle: form organizing function. Cardiovasc Res 2006;69:808–15.
[25] Shaw L, Sweeney M, O'Neill SC, Jones CJP, Austin C, Taggart MJ. Caveolae and sarcoplasmic reticular coupling in smooth muscle cells of pressurised arteries: the relevance for Ca^{2+} oscillations and tone. Cardiovasc Res 2006;69:825–35.
[26] Mineo C, Shaul PW. Circulating cardiovascular disease risk factors and signaling in endothelial cell caveolae. Cardiovasc Res 2006;70: in press.
[27] Blair A, Shaul PW, Yuhanna IS, Conrad PA, Smart EJ. Oxidized low density lipoprotein displaces endothelial nitric-oxide synthase (eNOS) from plasmalemmal caveolae and impairs eNOS activation. J Biol Chem 1999;274:32512–9.

[28] Shaul PW. Endothelial nitric oxide synthase, caveolae and the development of atherosclerosis. J Physiol 2003;547:21–33.
[29] Mineo C, Gormley AK, Yuhanna IS, Osborne-Lawrence S, Gibson L, Hahner L, et al. FcgRIIB mediates C-reactive protein inhibition of endothelial NO synthase. Circ Res 2005;97:1124–31.
[30] Schwencke C, Braun-Dullaeus RC, Wunderlich C, Strasser RH. Caveolae and caveolin in transmembrane signaling: implications for human disease. Cardiovasc Res 2006;70:in press. doi:10.1016/j.cardiores.2005.11.029.
[31] Lee SW, Reimer CL, Oh P, Campbell DB, Schnitzer JE. Tumor cell growth inhibition by caveolin re-expression in human breast cancer cells. Oncogene 1998;16:1391–7.
[32] Razani B, Engelman JA, Wang XB, Schubert W, Zhang XL, Marks CB, et al. Caveolin-1 null mice are viable but show evidence of hyperproliferative and vascular abnormalities. J Biol Chem 2001; 276:38121–38.
[33] Liu J, Razani B, Tang S, Terman BI, Ware JA, Lisanti MP. Angiogenesis activators and inhibitors differentially regulate caveolin-1 expression and caveolae formation in vascular endothelial cells. Angiogenesis inhibitors block vascular endothelial growth factor-induced down-regulation of caveolin-1. J Biol Chem 1999;274: 15781–5.
[34] Durr E, Yu J, Krasinska KM, Carver LA, Yates JR, Testa JE, et al. Direct proteomic mapping of the lung microvascular endothelial cell surface in vivo and in cell culture. Nat Biotechnol 2004; 22:985–92.