Published in final edited form as:

Curr Pharm Des. 2014 ; 20(36): 5681–5689.

Signaling epicenters: The role of caveolae and caveolins in volatile anesthetic induced cardiac protection

Yousuke T. Horikawa, M.D., Ph.D.^{1,*}, Yasuo M. Tsutsumi, M.D., Ph.D.^{2,*}, Hemal H. Patel, Ph.D.^{3,4}, and David M. Roth, Ph.D., M.D.^{3,4}

¹Department of Pediatrics, University of California, San Diego, La Jolla, California, USA

²Department of Anesthesiology, The University of Tokushima, Tokushima, JAPAN

³Department of Anesthesiology, University of California, San Diego, La Jolla, California, USA

⁴Department of Anesthesiology, Veterans Affairs San Diego Healthcare System, San Diego, California, USA

Abstract

Caveolae are flask-like invaginations of the cell surface that have been identified as signaling epicenters. Within these microdomains, caveolins are structural proteins of caveolae, which are able to interact with numerous signaling molecules affecting temporal and spatial dimensions required in cardiac protection. This complex moiety is essential to the mechanisms involved in volatile anesthetics. In this review, we will outline a general overview of caveolae and caveolins and their role in protective signaling, with a focus on the effects of volatile anesthetics. These recent developments have allowed us to better understand the mechanistic effect of volatile anesthetics and their potential in cardiac protection.

Keywords

caveolae; caveolin; lipid raft; volatile anesthetics; cardiac protection

Introduction

Cardiac disease remains the largest cause of mortality within the USA.[1] As a result, many have investigated the role of cardioprotective agents and interventions including opioids,[2] sub-lethal ischemia,[3] and volatile anesthetics.[4] Exploring the mechanisms of action of these agents has produced possible pharmaceutical targets to help prevent cardiac disease, yet none have been clinically translated. In an effort to further understand cardiac protective signaling, caveolae, invaginations within the cell membrane and their structural components caveolins, have been shown to be essential to these mechanisms. This review will highlight a series of investigations that elucidate the multifaceted role of caveolae and caveolins

Address correspondence to Dr. Roth: VA Medical Center, San Diego (125) 3350 La Jolla Village Drive, San Diego, CA 92161-5085; Telephone: (858) 552-8585, x1091; FAX (858) 534-0104; droth@ucsd.edu.

*These authors contributed equally to this work

within cardiac protection by altering temporal and spatial recruitment and organization of molecular signaling.

Caveolae and Caveolins

The “fluid-mosaic model” suggested by Singer and Nicolson [5] described the membrane as a fluid bilayer with a homogeneous lipid distribution. In recent years this model has been refined as the plasma membrane has been shown to consist of defined lipid rich regions interspersed with more fluidic membrane regions resulting in a much more complex view of the membrane than originally hypothesized. Caveolae are subcellular structures that were first described using electron microscopy in 1953 by George Palade.[6] Initially, these invaginations were identified as *plasmalemmal vesicles*. Two years later, similar structures were reported in the gall bladder epithelium,[7] and were described as *caveolae intracellulares* due to their cave-like, invaginated appearance. Since their initial discovery, caveolae have been found in almost all cell types,[8] with certain exceptions (e.g. erythrocytes, lymphocytes, and neurons[9–11]). Recent studies have shown that caveolar microdomains are more than lipid enriched invaginations of the plasma membrane.[6, 7] Caveolae play an important role in physiological functions such as cell surface signaling, [12–16] endocytosis,[17] calcium homeostasis,[18–20] adrenergic receptor regulation,[21] and intracellular cholesterol transport (Figure 1).[22, 23] The lipid composition of caveolae includes cholesterol,[22, 24] sphingolipids (such as sphingomyelin, ceramide and gangliosides),[25–27] glycosphingolipids[28] and fatty acids.[29]

Caveolins, structural proteins essential for caveolae formation, are present in three isoforms (Cav-1, -2, and -3). Cav-1 was the first member of the caveolin family to be identified as a phosphorylated protein in *v-Src* transformed cells.[30] Cloning of the Cav-1 complementary DNA (cDNA) revealed that it was identical to another protein, VIP21 which was a component of trans-Golgi-derived vesicles.[31, 32] Cav-1 is a 22-kDa phosphoprotein and has two isoforms.[33, 34] Cav-1 is phosphorylated on Tyr14 by the tyrosine kinase Src[35] and contains three residues (Cys133, Cys143, and Cys156)[36] that are palmitoylated, stabilizing the protein at the membrane.. Cav-2 and Cav-3 were identified in 1996. Cav-2 was identified by microsequencing of a 20-kDa protein co-purified with adipocyte-derived caveolar membranes,[37] and Cav-3 was discovered through cDNA library screening in an attempt to find Cav-1 homologs.[38] Cav-2 has three known isoforms (α , β , and γ) and is phosphorylated on Tyr19 by Src and Ser23 and Ser36 by casein kinase II,[39, 40] whereas Cav-3 is not known to be phosphorylated.

Cav-1 and Cav-2 have similar tissue distribution being expressed in most cell types, while Cav-3 exists primarily in muscle cells.[41–43] Ablation of Cav-1 (i.e., Cav-1 KO mice) results in complete loss of caveolar invaginations in endothelial cells, adipocytes, fibroblasts, and pneumocytes, while caveolae were still present in muscle and cardiac cells.[44–46] Similarly, Cav-3 KO mice do not have any invaginated formations resembling caveolae (Figure 2) in muscle cells; however, caveolar structures are present in other cell types.[47, 48] Interestingly, overexpression of Cav-3 in cardiac myocytes dramatically increases the number of caveolae (Figure 1).[49] Moreover, in Cav-2 KO mice, caveolae remained unchanged.[50] These data suggest when both Cav-1 and Cav-3 are expressed,

such as in cardiac muscle, Cav-3 is the dominant protein necessary for caveolae formation; [51] however, there may be a significant but as of yet not clearly understood role for Cav-1 in cardiac physiology.

All three caveolins have an invariant structural motif.[36, 38, 42, 52, 53] Additionally, Cav-1 and Cav-2 can hetero-oligomerize in most cell types, whereas Cav-3 forms homo-oligomeric complexes in striated myocytes.[38, 54] Caveolins can form hetero- or homo-oligomer complexes composed of 14–16 monomers.[53, 55] Human Cav-3 recently has been shown to form a disc-shaped nonamer.[56]

Cholesterol is essential for caveolae formation through its ability to bind caveolins and regulate caveolin transcription.[22, 57] Caveolin binds to phospholipid liposomes only upon cholesterol incorporation, and this caveolin-cholesterol interaction promotes caveolin oligomerization,[22, 24] suggesting a dependence on cholesterol for protein oligomerization and insertion into membranes. Cells treated with agents that remove cholesterol (e.g. filipin, methyl- β -cyclodextrin, or nystatin) lose caveolin and caveolae, resulting in flattened plasma membranes as visualized by electron microscopy.[58–60]

Molecular trafficking via caveolae and caveolins

Caveolin contains a scaffolding domain (CSD) that is largely responsible for many of the functions of caveolins.[12, 61] Components involved in G-protein-coupled receptor (GPCR) signaling, (*e.g.* G-proteins and G-protein regulated effectors) have been shown to localize in association with the CSD while GPCRs are found in caveolae.[13, 62–65] Numerous signaling components, including tyrosine kinases,[35, 66] phosphatidylinositol-3-kinase/protein kinase B (PI3K/Akt),[67, 68] protein kinase C (PKC),[69, 70] mitogen activated protein kinases (MAPK) including extracellular-signal regulated kinase (ERK) and p38 MAPK,[71, 72] nitric oxide synthase (NOS),[73, 74] and adenylyl cyclase[14, 15, 75, 76] interact with the CSD of caveolins (Figure 1). Recently, it has also been suggested that caveolins directly interact with organelles such as the sarcoplasmic reticulum[56] and mitochondria[77] facilitating and regulating cellular processes and metabolism. These data suggest that caveolins function as scaffolds to support compartmentalization of signaling molecules in caveolae providing a mechanism for temporal and spatial regulation of signal transduction and cross-talk among signaling molecules.[8]

GPCRs are seven-transmembrane-domain receptors that comprise a large superfamily of signaling receptors involved in numerous cellular processes. Compartmentalization of these receptors and other proteins in microdomains located within the plasma membrane, such as in caveolae, may lead to optimal signaling and intracellular regulation.[78] Beta 2-adrenergic receptors have recently been identified to be negatively regulated via caveolae within cardiac myocytes.[21] Not only are GPCRs (*e.g.*, endothelin, bradykinin, serotonin, angiotensin-1, opioid, adenosine, adrenergic) found in caveolae, but G proteins can also interact directly with caveolins via their CSD.[79, 80] Furthermore, inactive G α subunits have been observed to concentrate in caveolae and activation of the subunit causes G α to move out of caveolae.[65, 81] Additionally, caveolae play a role in endocytosis of ligand bound GPCRs, which likely initiates, terminates, or maintains a signal.[82, 83] However, the

molecular mechanisms of ligand-induced movement out of caveolae, receptor-caveolae endocytosis, or the importance of caveolin in these processes are currently not well understood.

A myriad of protein kinases have been identified that can interact with caveolins via the CSD and alter cellular processes. Tyrosine kinases (Src, Lck, Fyn, and Lyn) are thought to be enriched in caveolae and interact with Cav-1, *via* the CSD. [35] Phosphorylation of caveolins via Src kinase can influence shape changes, muscle degeneration, inflammatory gene expression, transcytosis of albumin, the development of cancer, cardiac, and neurological protection from ischemia-reperfusion injury and cardiac failure.[84–88] MAPKs are also found within caveolae. Regulation of MAPK via caveolins has been identified in numerous cellular interactions including: lung fibrosis,[89] cellular proliferation,[90] cardiac hypertrophy,[91] and angiogenesis.[92] Sheer, mechanical, and osmotic stress have been shown to induce tyrosine phosphorylation, MAPK activation, and caveolae formation.[84, 93, 94] Furthermore, increased ERK activity downregulates Cav-1 mRNA and protein and overexpression of Cav-1 inhibits the MAPK/ERK signaling pathways, an inhibition that is dependent on the CSD.[71] Consistent with these results, both Cav-1 knockout (KO) mice and Cav-3 KO mice show increased activation of p42/44 MAPK.[95, 96] PI3K/Akt are essential cellular survival and proliferation kinases. PI3K and Akt have both been shown to interact with caveolins and localize to caveolae. [97, 98] Additionally, Akt and caveolins are vital to insulin signaling, which can be altered by insulin resistance.[99–102] Furthermore, isoforms of protein kinase C (PKC) can target to caveolae enhancing the regulation of caveolar-localized proteins.[69] This is observed with ceramides, which can recruit and activate PKC and Akt into caveolae where activation of PKC not only stabilizes the kinase complex but also enhances its ability to negatively regulate Akt.[70, 103]

Subcellular compartmentalization of ion channels facilitates and regulates signaling. Ca, K, Na, and Cl channels are concentrated and targeted to caveolae and can associate with caveolins.[104, 105] Among them, voltage-dependent potassium (Kv) channels were reported first within cardiac lipid-microdomains.[106, 107] In pancreatic β -cells, lipid raft disruption inactivates Kv2.1.[108] Adenosine triphosphate-sensitive potassium (K_{ATP}) channels, which are important for the regulation of vascular tone and cardiac protection from ischemia,[109–111] forms a complex with adenylyl cyclase in caveolae, allowing for sustained activation by protein kinase A.[112] Additionally, angiotensin II-activated PKC targets to caveolae to inhibit aortic K_{ATP} channels.[113] Recent investigations have also suggested that hyperpolarization-activated cyclic nucleotide-gated channel (HCN) 4 is closely regulated by Cav-3 during development[114] and for daily pacemaking.[115] Furthermore, Cav-3 can also negatively regulate Ca-activated potassium channels in transfected cells, facilitating channel trafficking.[116]

Cardiac Effects of Caveolae/Caveolin

Several investigators have generated Cav-1 and Cav-3 KO mice where either one or both genes are deleted and Cav-3 transgenic mice.[44, 45, 95, 117, 118] The regulation of cardiac physiology by caveolins may depend on cell specific expression and compartmentalization

of signaling complexes. As previously studied, all caveolin null mice have cardiovascular dysfunction. Cav-3 is mainly expressed within cardiac and skeletal muscle and is regulated in cardiac hypertrophy.[119] Conversely, Cav-3 KO mice, which lack morphologically caveolae, develop cardiomyopathy characterized by cardiac hypertrophy, dilation and reduced contractility as well.[95] Additionally, Cav-3 gene ablation leads to increased activity of the MAPK cascade in cardiac myocytes.[95] Park *et al.*[117] reported that Cav-1/3 double KO mice, which are deficient in all three caveolin proteins, have cardiac hypertrophy with cardiomyopathy and decreased contractility. Supporting these data, adenovirus-mediated overexpression of Cav-3 in neonatal cardiac myocytes decreases the ability of the adrenergic agonist phenylephrine or endothelin-1 to increase cell size, suggesting Cav-3 behaves as a negative regulator of hypertrophic responses.[91] However, global overexpression of Cav-3 results in Duchenne-like muscular dystrophy phenotype resulting in cardiac degeneration, fibrosis and reduced cardiac NOS activity and cardiac function.[118] By utilizing the alpha myosin heavy chain promoter our laboratory generated a cardiac-specific Cav-3 overexpressing mouse (Cav-3 OE) that was strikingly different.[49] These mice did not resemble a Duchenne's phenotype nor did they develop cardiomyopathy as they aged, in fact these mice were protected against cardiac failure.[88]

Myocardial ischemia/reperfusion injury and subsequent myocardial infarction remains a leading cause of morbidity and mortality. However, there are many beneficial experimental interventions that produce cardiac protection. Interestingly, perhaps the most effective is produced following brief (as short as 5 mins) ischemic exposure to the myocardium. This cardiac protection is termed ischemic preconditioning (IPC).[3] IPC has been described as a biphasic event where there is an acute phase, which occurs immediately after the IPC stimulus and lasts approximately 1–3 h and is transient.[120] The delayed phase of protection is observed 12–24 h after the initial stimulus and lasts up to 72 h.[121] Clinically relevant cardiac protection can be induced at the time of reperfusion and has been termed postconditioning.[122] Preconditioning and postconditioning are mediated via three parallel signaling pathways including GPCR and natriuretic peptide induced nitric oxide signaling, the reperfusion injury salvage kinase (RISK) pathway, and the survival activating factor enhancement (SAFE) pathway.[123, 124] Many of the molecules involved in these signaling pathways interact with caveolae and caveolins, including (GPCRs), receptor tyrosine kinases, TNF receptors, Src kinases, G-proteins, H-Ras, nitric oxide synthases, protein kinase C (PKC), phosphatidylinositol 3-kinase (PI3K), and MEK/ERK kinases.[97, 125] Interestingly, infusion of the caveolin scaffolding domain peptide of Cav-1 into ischemic/reperfused hearts increased the recovery of cardiac function where the effects were largely attributed to regulation of endothelial cell function.[126] Subsequently, it was shown that cardiac injury activated mitogen-activated protein kinases, and regulated Cav-3, and Cav-1, limiting the eNOS production suggesting a potential role of NO in IPC.[72, 127] Others have shown that IPC can modulate the microenvironment within caveolae to enrich cardioprotective proteins, including eNOS and the glucose transporter (GLUT-4).[128] Recently, an integral role for caveolae in IPC induced nitrosylation of mitochondrial proteins has been proposed.[129]

Intralipid, a commercially available lipid emulsification that has traditionally been used in parenteral nutrition, has been shown to reduce myocardial damage after a lethal hypoxic insult by inhibiting mitochondrial permeability transition pore and activating the cardio-protective GSK-3 β pathway.[130, 131] This suggests a possible clinical treatment to decrease myocardial damage that may relate to lipid loading as a therapeutic endpoint. Further work needs to be undertaken to define the caveolin versus lipid enrichment component of this protective mechanism. It is possible that introduction of lipids to the membrane protects this vital structure from injury and maintains cellular integrity during stress, whether this effect is caveolin dependent or independent is not clear.

Caveolae/Caveolin in Anesthetic Effects

Volatile anesthetics were first used over 150 years ago, but their exact mechanism of action remains unknown. Volatile anesthetics are short chain halogenated alkanes and ethers that interact with cell membrane lipids and directly or indirectly interact with membrane bound proteins to produce a number of cellular effects. Thus, volatile anesthetics likely affect lipid microdomains, such as caveolae. Interestingly, emulsified volatile anesthetics were successfully used for anesthesia and enhanced preconditioning-induced cardiac protection, [132–135] suggesting that halogenated ethers dissolved in the lipid phase of micelles interact directly with caveolae.[136]

Many studies have shown that volatile anesthetics[4, 137] can exert cardiac protection similar to IPC and that anesthetic induced preconditioning (APC) is mediated by many of the same signal transduction elements involved in IPC.[138–153] APC is a biphasic event as well.[120, 121, 154–157] Within the first 1–2 hours, an acute phase is observed immediately after the preconditioning stimulus that involves pre-existing proteins, ion channel modifications, post-translational changes, and reactive oxygen species.[4, 150, 158] Approximately, 12–24 hours after the preconditioning stimulus a delayed phase is observed that involves *de novo* synthesis of proteins and translocation of molecules.[51, 155, 159] Additionally, subsequent work has shown the cardiac protective mechanisms include putative triggers (GPCRs, RTKs, G-proteins), mediators (protein kinases), and end-effectors (K_{ATP} channel and Glut4).[160–163]

APC-induced cardiac protection involves many different signaling mechanisms that have been shown to localize and interact with caveolin and caveolae including: GPCRs (opioid and adenosine receptors)[62, 64, 164, 165] and signaling mediators such as the G α subunit of heterotrimeric G-proteins, Src kinases, PI3K, eNOS, PKC isoforms and ERK kinase.[35, 66–74] Furthermore, K_{ATP} channels, a possible end-effector involved in APC, are thought to be concentrated within caveolae.[112, 146]

Utilizing *in vitro* and *in vivo* experimental mouse models, we have shown that isoflurane increases the total number of caveolae at the sarcolemmal surface (Figure 2). We identified the importance of temporal signaling in Cav-1 activation involved in APC. Although, Src kinase is essential to phosphorylation of Cav-1, just as importantly we observed that inactivation of Src via C-terminal Src kinase (CSK) was necessary in order maintain acute APC (Figure 3).[86] Additionally, our laboratory reported that Cav-3 KO mice lacking

caveolae but expressing Cav-1 were resistant to acute APC. Pharmacological disruption of caveolae also prevented acute APC-induced cardiac protection, indicating that the presence of caveolae and the expression of Cav-3 were essential.[60] To further test the importance of Cav-3 in the heart, adult rat cardiac myocytes were isolated and infected with Cav-3 virus, which increased the number of surface caveolae and upregulated protective signaling pathways.[49] Likewise, cardiac specific overexpression of Cav-3 in mice resulted in innate cardiac protection from ischemia/reperfusion injury via down regulation of pro-apoptotic genes and upregulation of survival kinases.[49] Collectively, these data implicate a role for both Cav-1 and Cav-3 and the presence of caveolae in the acute phase of cardiac protection from ischemia-reperfusion injury.

More recently, we observed Cav-1 deficient mice still exhibited delayed cardiac protection from ischemia-reperfusion injury, whereas, Cav-3 KO mice completely lacked this ability. [51] Unlike acute APC, delayed APC has been suggested to require *de novo* gene and protein expression, which can ultimately lead to increased protein synthesis. Cav-1 KO mice still expressed Cav-3 and had caveolae. Pharmacological disruption of caveolae at the time of the protective stimulus had no effect on delayed APC, although, later disruption inhibited any protective effects. As a result, we hypothesized that Cav-3 was responsible for translocation of Glut4 to caveolae. We observed that both WT and Cav-1 KO mice were able to co-localize and translocate Cav-3 with the glucose transporter 4 (GLUT4) to caveolae following delayed APC stimulation, indicating that delayed cardioprotective effects are dependent on caveolae, Cav-3 and GLUT4 translocation (Figure 3).[51] A recent study looking at human Cav-3 has identified direct interaction sites with ryanodine receptors found on sarcoplasmic reticulum,[56] whereas our laboratory has recently identified caveolae directly interacting with mitochondria[77] suggesting an integral role for caveolins in cellular organelle regulation.

Anesthetic Pulmonary Effects

Plasma albumin is transported across the endothelium by transcytosis, a process regulated by the trafficking of vesicles.[166–168] The binding of albumin to the 60-kDa albumin-binding protein, gp60, in endothelial cell surface activates albumin transport *via* caveolae.[169–171] Additionally, M β CD, a cholesterol-binding agent that disrupts caveolae, inhibited transcellular albumin transport, suggesting that caveolae are responsible for transcytosis. Studies in Cav-1 KO mice suggest that the absence of caveolae can inhibit albumin uptake. [172]

Hu *et al.*[173] reported in an *ex vivo* rat lung that isoflurane, not sevoflurane, increased pulmonary endothelial albumin permeability. Furthermore, cultured endothelial cells, when exposed to high dose isoflurane resulted in a fourfold increase in fluorescent ¹²⁵I-albumin uptake in rat lung microvascular endothelial cells *via* Src activation as well as phosphorylation of Cav-1. These data suggest that higher doses of isoflurane may cause pulmonary edema as a result of increased albumin permeability secondary to increased caveolin levels.

Questions for the Future

Caveolae and caveolins serve complex roles in cell structure and biology. Caveolae and caveolins are vital to cellular signaling and function via modulation of temporal and spatial signaling components. Several studies have now identified a critical role for caveolae and caveolins in cardiac protection against I/R injury and heart failure. Both caveolae and caveolins serve integral roles in IPC, APC, and cardiac remodeling. However, the question remains whether it is the lipid rich caveolar environment or caveolins themselves that are critical for protection. Unfortunately, isolating caveolins from caveolae or other lipid rich microenvironments is difficult, though more focused work in knockout mice with lipid directed therapies may be a means to address such questions. Furthermore, it is not clear how caveolins are regulated. Recent reports have shown that caveolins can enhance mitochondrial function and that cellular caveolae interact directly with mitochondria.[77, 174] This suggests that caveolins may not only be structural proteins but active regulators of cellular signaling between the cell and its resident organelles. Future investigations will be necessary to identify how caveolins migrate from the cell membrane to the mitochondria and also whether similar mechanisms are involved in APC.

Caveolins are relatively small proteins yet seemingly can target organelles, coordinate cellular function and modulate signaling, suggesting a much more complex cellular distribution pattern than originally defined. Exactly how caveolins can perform these varied functions that are not classically explained by their plasma membrane enrichment in caveolae is unknown. Perhaps, caveolins undergo post-translational modifications such as phosphorylation,[175, 176] glycosylation,[177] or sumoylation,[178] or perhaps certain interactions with other resident proteins alter protein-protein interactions and targeting.

Recent studies have shown that neurons express all three caveolins[179, 180] but lack structural caveolae.[181, 182] These studies suggest that caveolins can still be functional regardless of the caveolar structure, perhaps by serving as scaffolds for signaling complexes involved in neuronal transmission and plasticity. Recent work revealed a key relationship between N-Methyl-D-aspartate (NMDA) receptor signaling and Cav-1 in primary neurons. [87] Interestingly, NMDA receptors have been known targets for anesthesia. Furthermore, Cav-1 has been implicated in enhancing arborization[183] or primary neurons and loss of caveolin-1 presents with an Alzheimer's like condition[184]. These data together, suggest that caveolins are vital to neuronal proliferation and signaling. Defining a role for caveolin in terminally differentiated cells such as cardiac myocytes and neurons may have larger implication to adaptation from stressors such as diabetes, aging, neurodegeneration, and other cardiovascular disorders.

Although caveolin expression in cardiac myocytes is protective, it is important to note that oncogenic cells also express caveolins. During the early phase of tumor development, Cav-1 can act as a tumor suppressor gene; however, Cav-1 has been shown to be up-regulated in numerous multi-drug resistant and metastatic cell lines as well as in human cancer cell types. [185–187] Recently, we have shown that caveolin-1 expression can prevent chemotherapy-induced apoptosis in the presence of volatile anesthetics.[188] These data suggest that the same mechanisms which protect the heart from a lethal ischemic stress, may also protect

oncologic cells against traditional chemotherapeutic strategies, which increase cell death. Furthermore, these data suggest that the use of volatile anesthetics prior to chemotherapies (such as during tumor resection) may actually be detrimental to overall outcome. In the future, perhaps targeting caveolins will be a possible therapy or adjuvant for decreasing the morbidity and mortality of many of these cancers.

Within the past decade, many new scientific discoveries have been made regarding caveolae and caveolins especially within cardiac protection. We now know that caveolins are not merely structural proteins but key components in pro-survival and many other cellular regulatory pathways. Given that caveolins have been shown to greatly enhance resistance to ischemic injury and cardiac remodeling, caveolins maybe an ideal target for future therapeutic agents though validation studies in large animal models and humans need to be performed.

Acknowledgments

This work was supported by the National Institutes of Health Grants HL091071 (awarded to Hemal H. Patel), HL10107200 (awarded to Hemal H. Patel and David M. Roth) and by Veterans Administration Merit Awards BX000783 (awarded to David M. Roth)

References

1. Hoyert DL. 75 years of mortality in the United States, 1935–2010. NCHS data brief. 2012;1–8. [PubMed: 22617094]
2. Schultz JE, Hsu AK, Gross GJ. Morphine mimics the cardioprotective effect of ischemic preconditioning *via* a glibenclamide-sensitive mechanism in the rat heart. *Circ Res*. 1996; 78:1100–1104. [PubMed: 8635241]
3. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation*. 1986; 74:1124–1136. [PubMed: 3769170]
4. Kersten JR, Schmeling TJ, Pagel PS, Gross GJ, Warltier DC. Isoflurane mimics ischemic preconditioning *via* activation of K_{ATP} channels: Reduction of myocardial infarct size with an acute memory phase. *Anesthesiology*. 1997; 87:361–370. [PubMed: 9286901]
5. Singer SJ, Nicolson GL. The fluid mosaic model of the structure of cell membranes. *Science*. 1972; 175:720–731. [PubMed: 4333397]
6. Palade G. Fine structure of blood capillaries. *J of Appl Phys*. 1953; 24:1424–1436.
7. Yamada E. The fine structure of the gall bladder epithelium of the mouse. *J Biophys Biochem Cytol*. 1955; 1:445–458. [PubMed: 13263332]
8. Patel HH, Murray F, Insel PA. Caveolae as organizers of pharmacologically relevant signal transduction molecules. *Annu Rev Pharmacol Toxicol*. 2008; 48:359–391. [PubMed: 17914930]
9. Fra AM, Williamson E, Simons K, Parton RG. *De novo* formation of caveolae in lymphocytes by expression of VIP21-caveolin. *Proc Natl Acad Sci U S A*. 1995; 92:8655–8659. [PubMed: 7567992]
10. Gorodinsky A, Harris DA. Glycolipid-anchored proteins in neuroblastoma cells form detergent-resistant complexes without caveolin. *J Cell Biol*. 1995; 129:619–627. [PubMed: 7537273]
11. Mirre C, Monlauzeur L, Garcia M, Delgrossi MH, Le Bivic A. Detergent-resistant membrane microdomains from Caco-2 cells do not contain caveolin. *Am J Physiol*. 1996; 271:C887–C894. [PubMed: 8843719]
12. Lisanti MP, Scherer PE, Tang Z, Sargiacomo M. Caveolae, caveolin and caveolin-rich membrane domains: A signalling hypothesis. *Trends Cell Biol*. 1994; 4:231–235. [PubMed: 14731661]
13. Steinberg SF, Brunton LL. Compartmentation of G protein-coupled signaling pathways in cardiac myocytes. *Annu Rev Pharmacol Toxicol*. 2001; 41:751–773. [PubMed: 11264475]

14. Ostrom RS, Gregorian C, Drenan RM, Xiang Y, Regan JW, Insel PA. Receptor number and caveolar co-localization determine receptor coupling efficiency to adenylyl cyclase. *J Biol Chem.* 2001; 276:42063–42069. [PubMed: 11533056]
15. Ostrom RS, Liu X, Head BP, Gregorian C, Seasholtz TM, Insel PA. Localization of adenylyl cyclase isoforms and G protein-coupled receptors in vascular smooth muscle cells: Expression in caveolin-rich and noncaveolin domains. *Mol Pharmacol.* 2002; 62:983–992. [PubMed: 12391260]
16. Williams TM, Lisanti MP. The caveolin proteins. *Genome Biol.* 2004; 5:214. [PubMed: 15003112]
17. Anderson RG. Potocytosis of small molecules and ions by caveolae. *Trends Cell Biol.* 1993; 3:69–72. [PubMed: 14731772]
18. Fujimoto T, Nakade S, Miyawaki A, Mikoshiba K, Ogawa K. Localization of inositol 1,4,5-trisphosphate receptor-like protein in plasmalemmal caveolae. *J Cell Biol.* 1992; 119:1507–1513. [PubMed: 1334960]
19. Fujimoto T. Calcium pump of the plasma membrane is localized in caveolae. *J Cell Biol.* 1993; 120:1147–1157. [PubMed: 8382206]
20. Scriven DRL, Klimek A, Lee KL, Moore EDW. The molecular architecture of calcium microdomains in rat cardiomyocytes. *Ann N Y Acad Sci.* 2002; 976:488–499. [PubMed: 12502603]
21. Macdougall DA, Agarwal SR, Stopford EA, Chu H, Collins JA, Longster AL, Colyer J, Harvey RD, Calaghan S. Caveolae compartmentalise beta2-adrenoceptor signals by curtailing cAMP production and maintaining phosphatase activity in the sarcoplasmic reticulum of the adult ventricular myocyte. *Journal of molecular and cellular cardiology.* 2012; 52:388–400. [PubMed: 21740911]
22. Murata M, Peranen J, Schreiner R, Wieland F, Kurzchalia TV, Simons K. VIP21/caveolin is a cholesterol-binding protein. *Proc Natl Acad Sci U S A.* 1995; 92:10339–10343. [PubMed: 7479780]
23. Smart EJ, Ying YS, Donzell WC, Anderson RGW. A role for caveolin in transport of cholesterol from endoplasmic reticulum to plasma membrane. *J Biol Chem.* 1996; 271:29427–29435. [PubMed: 8910609]
24. Li S, Song KS, Lisanti MP. Expression and characterization of recombinant caveolin. Purification by polyhistidine tagging and cholesterol-dependent incorporation into defined lipid membranes. *J Biol Chem.* 1996; 271:568–573. [PubMed: 8550621]
25. Parton RG. Ultrastructural localization of gangliosides; GM1 is concentrated in caveolae. *J Histochem Cytochem.* 1994; 42:155–166. [PubMed: 8288861]
26. Liu P, Anderson RG. Compartmentalized production of ceramide at the cell surface. *J Biol Chem.* 1995; 270:27179–27185. [PubMed: 7592974]
27. Fujimoto T. GPI-anchored proteins, glycosphingolipids, and sphingomyelin are sequestered to caveolae only after crosslinking. *J Histochem Cytochem.* 1996; 44:929–941. [PubMed: 8756764]
28. Fra AM, Masserini M, Palestini P, Sonnino S, Simons K. A photo-reactive derivative of ganglioside GM1 specifically cross-links VIP21-caveolin on the cell surface. *FEBS Lett.* 1995; 375:11–14. [PubMed: 7498456]
29. Trigatti BL, Anderson RG, Gerber GE. Identification of caveolin-1 as a fatty acid binding protein. *Biochem Biophys Res Commun.* 1999; 255:34–39. [PubMed: 10082651]
30. Glenney JR Jr, Zokas L. Novel tyrosine kinase substrates from Rous sarcoma virus-transformed cells are present in the membrane skeleton. *J Cell Biol.* 1989; 108:2401–2408. [PubMed: 2472406]
31. Kurzchalia TV, Dupree P, Parton RG, Kellner R, Virta H, Lehnert M, Simons K. VIP21, a 21-kD membrane protein is an integral component of trans-Golgi-network-derived transport vesicles. *J Cell Biol.* 1992; 118:1003–1014. [PubMed: 1512286]
32. Glenney JR Jr. The sequence of human caveolin reveals identity with VIP21, a component of transport vesicles. *FEBS Lett.* 1992; 314:45–48. [PubMed: 1360410]
33. Kogo H, Fujimoto T. Caveolin-1 isoforms are encoded by distinct mRNAs. Identification Of mouse caveolin-1 mRNA variants caused by alternative transcription initiation and splicing. *FEBS Lett.* 2000; 465:119–123. [PubMed: 10631317]

34. Kogo H, Aiba T, Fujimoto T. Cell type-specific occurrence of caveolin-1 α and -1 β in the lung caused by expression of distinct mRNAs. *J Biol Chem*. 2004; 279:25574–25581. [PubMed: 15067006]
35. Li S, Couet J, Lisanti MP. Src tyrosine kinases, Galpha (Rep) subunits, and H-Ras share a common membrane-anchored scaffolding protein, caveolin. Caveolin binding negatively regulates the auto-activation of Src tyrosine kinases. *J Biol Chem*. 1996; 271:29182–29190. [PubMed: 8910575]
36. Dietzen DJ, Hastings WR, Lublin DM. Caveolin is palmitoylated on multiple cysteine residues. Palmitoylation is not necessary for localization of caveolin to caveolae. *J Biol Chem*. 1995; 270:6838–6842. [PubMed: 7896831]
37. Scherer PE, Okamoto T, Chun M, Nishimoto I, Lodish HF, Lisanti MP. Identification, sequence, and expression of caveolin-2 defines a caveolin gene family. *Proc Natl Acad Sci U S A*. 1996; 93:131–135. [PubMed: 8552590]
38. Tang Z, Scherer PE, Okamoto T, Song K, Chu C, Kohtz DS, Nishimoto I, Lodish HF, Lisanti MP. Molecular cloning of caveolin-3, a novel member of the caveolin gene family expressed predominantly in muscle. *J Biol Chem*. 1996; 271:2255–2261. [PubMed: 8567687]
39. Lee H, Park DS, Wang XB, Scherer PE, Schwartz PE, Lisanti MP. Src-induced phosphorylation of caveolin-2 on tyrosine 19. Phospho-caveolin-2 (TyRP)19 is localized near focal adhesions, remains associated with lipid rafts/caveolae, but no longer forms a high molecular mass hetero-oligomer with caveolin-1. *J Biol Chem*. 2002; 277:34556–34567. [PubMed: 12091389]
40. Sowa G, Pypaert M, Fulton D, Sessa WC. The phosphorylation of caveolin-2 on serines 23 and 36 modulates caveolin-1-dependent caveolae formation. *Proc Natl Acad Sci U S A*. 2003; 100:6511–6516. [PubMed: 12743374]
41. Song KS, Scherer PE, Tang Z, Okamoto T, Li S, Chafel M, Chu C, Kohtz DS, Lisanti MP. Expression of caveolin-3 in skeletal, cardiac, and smooth muscle cells. Caveolin-3 is a component of the sarcolemma and co-fractionates with dystrophin and dystrophin-associated glycoproteins. *J Biol Chem*. 1996; 271:15160–15165. [PubMed: 8663016]
42. Razani B, Woodman SE, Lisanti MP. Caveolae: from cell biology to animal physiology. *Pharmacol Rev*. 2002; 54:431–467. [PubMed: 12223531]
43. Cohen AW, Hnasko R, Schubert W, Lisanti MP. Role of caveolae and caveolins in health and disease. *Physiol Rev*. 2004; 84:1341–1379. [PubMed: 15383654]
44. Drab M, Verkade P, Elger M, Kasper M, Lohn M, Lauterbach B, Menne J, Lindschau C, Mende F, Luft FC, Schedl A, Haller H, Kurzchalia TV. Loss of caveolae, vascular dysfunction, and pulmonary defects in caveolin-1 gene-disrupted mice. *Science*. 2001; 293:2449–2452. [PubMed: 11498544]
45. Razani B, Engelman JA, Wang XB, Schubert W, Zhang XL, Marks CB, Macaluso F, Russell RG, Li M, Pestell RG, Di Vizio D, Hou H Jr, Kneitz B, Lagaud G, Christ GJ, Edelmann W, Lisanti MP. Caveolin-1 null mice are viable but show evidence of hyperproliferative and vascular abnormalities. *J Biol Chem*. 2001; 276:38121–38138. [PubMed: 11457855]
46. Zhao YY, Liu Y, Stan RV, Fan L, Gu Y, Dalton N, Chu PH, Peterson K, Ross J Jr, Chien KR. Defects in caveolin-1 cause dilated cardiomyopathy and pulmonary hypertension in knockout mice. *Proc Natl Acad Sci U S A*. 2002; 99:11375–11380. [PubMed: 12177436]
47. Hagiwara Y, Sasaoka T, Araishi K, Imamura M, Yorifuji H, Nonaka I, Ozawa E, Kikuchi T. Caveolin-3 deficiency causes muscle degeneration in mice. *Hum Mol Genet*. 2000; 9:3047–3054. [PubMed: 11115849]
48. Galbiati F, Engelman JA, Volonte D, Zhang XL, Minetti C, Li M, Hou H Jr, Kneitz B, Edelmann W, Lisanti MP. 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; 276:21425–21433. [PubMed: 11259414]
49. Tsutsumi YM, Horikawa YT, Jennings MM, Kidd MW, Niesman IR, Yokoyama U, Head BP, Hagiwara Y, Ishikawa Y, Miyanoara A, Patel PM, Insel PA, Patel HH, Roth DM. Cardiac-specific overexpression of caveolin-3 induces endogenous cardiac protection by mimicking ischemic preconditioning. *Circulation*. 2008; 118:1979–1988. [PubMed: 18936328]
50. Razani B, Wang XB, Engelman JA, Battista M, Lagaud G, Zhang XL, Kneitz B, Hou H Jr, Christ GJ, Edelmann W, Lisanti MP. Caveolin-2-deficient mice show evidence of severe pulmonary

dysfunction without disruption of caveolae. *Mol Cell Biol.* 2002; 22:2329–2344. [PubMed: 11884617]

51. Tsutsumi YM, Kawaraguchi Y, Horikawa YT, Niesman IR, Kidd MW, Chin-Lee B, Head BP, Patel PM, Roth DM, Patel HH. Role of caveolin-3 and glucose transporter-4 in isoflurane-induced delayed cardiac protection. *Anesthesiology.* 2010; 112:1136–1145. [PubMed: 20418694]
52. Dupree P, Parton RG, Raposo G, Kurzchalia TV, Simons K. Caveolae and sorting in the trans-Golgi network of epithelial cells. *EMBO J.* 1993; 12:1597–1605. [PubMed: 8385608]
53. Monier S, Parton RG, Vogel F, Behlke J, Henske A, Kurzchalia TV. VIP21-caveolin, a membrane protein constituent of the caveolar coat, oligomerizes in vivo and in vitro. *Mol Biol Cell.* 1995; 6:911–927. [PubMed: 7579702]
54. Das DK, Maulik N, Sato M, Ray PS. Reactive oxygen species function as second messenger during ischemic preconditioning of heart. *Mol Cell Biochem.* 1999; 196:159–167.
55. Sargiacomo M, Scherer PE, Tang Z, Kubler E, Song KS, Sanders MC, Lisanti MP. Oligomeric structure of caveolin: Implications for caveolae membrane organization. *Proc Natl Acad Sci U S A.* 1995; 92:9407–9411. [PubMed: 7568142]
56. Whiteley G, Collins RF, Kitmitto A. Characterization of the molecular architecture of human caveolin-3 and interaction with the skeletal muscle ryanodine receptor. *The Journal of biological chemistry.* 2012; 287:40302–40316. [PubMed: 23071107]
57. Fielding CJ, Bist A, Fielding PE. Caveolin mRNA levels are up-regulated by free cholesterol and down-regulated by oxysterols in fibroblast monolayers. *Proc Natl Acad Sci U S A.* 1997; 94:3753–3758. [PubMed: 9108050]
58. Rothberg KG, Heuser JE, Donzell WC, Ying YS, Glenney JR, Anderson RG. Caveolin, a protein component of caveolae membrane coats. *Cell.* 1992; 68:673–682. [PubMed: 1739974]
59. Christian AE, Haynes MP, Phillips MC, Rothblat GH. Use of cyclodextrins for manipulating cellular cholesterol content. *J Lipid Res.* 1997; 38:2264–2272. [PubMed: 9392424]
60. Horikawa YT, Patel HH, Tsutsumi YM, Jennings MM, Kidd MW, Hagiwara Y, Ishikawa Y, Insel PA, Roth DM. Caveolin-3 expression and caveolae are required for isoflurane-induced cardiac protection from hypoxia and ischemia/reperfusion injury. *J Mol Cell Cardiol.* 2008; 44:123–130. [PubMed: 18054955]
61. Okamoto T, Schlegel A, Scherer PE, Lisanti MP. Caveolins, a family of scaffolding proteins for organizing "preassembled signaling complexes" at the plasma membrane. *J Biol Chem.* 1998; 273:5419–5422. [PubMed: 9488658]
62. Patel HH, Head BP, Petersen HN, Niesman IR, Huang D, Gross GJ, Insel PA, Roth DM. Protection of adult rat cardiac myocytes from ischemic cell death: role of caveolar microdomains and delta-opioid receptors. *Am J Physiol Heart Circ Physiol.* 2006; 291:H344–H350. [PubMed: 16501018]
63. Head BP, Patel HH, Roth DM, Lai NC, Niesman IR, Farquhar MG, Insel PA. G-protein-coupled receptor signaling components localize in both sarcolemmal and intracellular caveolin-3-associated microdomains in adult cardiac myocytes. *J Biol Chem.* 2005; 280:31036–31044. [PubMed: 15961389]
64. Lasley RD, Narayan P, Uittenbogaard A, Smart EJ. Activated cardiac adenosine A(1) receptors translocate out of caveolae. *J Biol Chem.* 2000; 275:4417–4421. [PubMed: 10660613]
65. Oh P, Schnitzer JE. Segregation of heterotrimeric G proteins in cell surface microdomains. G_q binds caveolin to concentrate in caveolae, whereas G_i and G_s target lipid rafts by default. *Mol Biol Cell.* 2001; 12:685–698. [PubMed: 11251080]
66. Labrecque L, Nyalendo C, Langlois S, Durocher Y, Roghi C, Murphy G, Gingras D, Beliveau R. Src-mediated tyrosine phosphorylation of caveolin-1 induces its association with membrane type 1 matrix metalloproteinase. *J Biol Chem.* 2004; 279:52132–52140. [PubMed: 15466865]
67. Shack S, Wang XT, Kokkonen GC, Gorospe M, Longo DL, Holbrook NJ. Caveolin-induced activation of the phosphatidylinositol 3-kinase/Akt pathway increases arsenite cytotoxicity. *Mol Cell Biol.* 2003; 23:2407–2414. [PubMed: 12640124]
68. Fecchi K, Volonte D, Hezel MP, Schmeck K, Galbiati F. Spatial and temporal regulation of GLUT4 translocation by flotillin-1 and caveolin-3 in skeletal muscle cells. *Faseb J.* 2006; 20:705–707. [PubMed: 16455755]

69. Rybin VO, Xu X, Steinberg SF. Activated protein kinase C isoforms target to cardiomyocyte caveolae : stimulation of local protein phosphorylation. *Circ Res.* 1999; 84:980–988. [PubMed: 10325235]
70. Fox TE, Houck KL, O'Neill SM, Nagarajan M, Stover TC, Pomianowski PT, Unal O, Yun JK, Naides SJ, Kester M. Ceramide recruits and activates protein kinase C zeta (PKC zeta) within structured membrane microdomains. *J Biol Chem.* 2007; 282:12450–12457. [PubMed: 17308302]
71. Engelman JA, Chu C, Lin A, Jo H, Ikezu T, Okamoto T, Kohtz DS, Lisanti MP. Caveolin-mediated regulation of signaling along the p42/44 MAP kinase cascade in vivo. A role for the caveolin-scaffolding domain. *FEBS Lett.* 1998; 428:205–211. [PubMed: 9654135]
72. Ballard-Croft C, Locklar AC, Kristo G, Lasley RD. Regional myocardial ischemia-induced activation of MAPKs is associated with subcellular redistribution of caveolin and cholesterol. *Am J Physiol Heart Circ Physiol.* 2006; 291:H658–H667. [PubMed: 16565301]
73. Feron O, Dessy C, Opel DJ, Arstall MA, Kelly RA, Michel T. Modulation of the endothelial nitric-oxide synthase-caveolin interaction in cardiac myocytes. Implications for the autonomic regulation of heart rate. *J Biol Chem.* 1998; 273:30249–30254. [PubMed: 9804784]
74. Kamoun WS, Karaa A, Kresge N, Merkel SM, Korneszczyk K, Clemens MG. LPS inhibits endothelin-1-induced endothelial NOS activation in hepatic sinusoidal cells through a negative feedback involving caveolin-1. *Hepatology.* 2006; 43:182–190. [PubMed: 16374854]
75. Huang C, Hepler JR, Chen LT, Gilman AG, Anderson RG, Mumby SM. Organization of G proteins and adenylyl cyclase at the plasma membrane. *Mol Biol Cell.* 1997; 8:2365–2378. [PubMed: 9398661]
76. Ostrom RS, Violin JD, Coleman S, Insel PA. Selective enhancement of beta(Rep)-adrenergic receptor signaling by overexpression of adenylyl cyclase type 6: Colocalization of receptor and adenylyl cyclase in caveolae of cardiac myocytes. *Mol Pharmacol.* 2000; 57:1075–1079. [PubMed: 10779394]
77. Fridolfsson HN, Kawaraguchi Y, Ali SS, Panneerselvam M, Niesman IR, Finley JC, Kellerhals SE, Migita MY, Okada H, Moreno AL, Jennings M, Kidd MW, Bonds JA, Balijepalli RC, Ross RS, Patel PM, Miyanojara A, Chen Q, Lesnefsky EJ, Head BP, Roth DM, Insel PA, Patel HH. Mitochondria-localized caveolin in adaptation to cellular stress and injury. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology.* 2012; 26:4637–4649. [PubMed: 22859372]
78. Ostrom RS. New determinants of receptor-effector coupling: trafficking and compartmentation in membrane microdomains. *Mol Pharmacol.* 2002; 61:473–476. [PubMed: 11854426]
79. Ostrom RS, Insel PA. The evolving role of lipid rafts and caveolae in G protein-coupled receptor signaling: implications for molecular pharmacology. *Br J Pharmacol.* 2004; 143:235–245. [PubMed: 15289291]
80. Insel PA, Head BP, Patel HH, Roth DM, Bunday RA, Swaney JS. Compartmentation of G-protein-coupled receptors and their signalling components in lipid rafts and caveolae. *Biochem Soc Trans.* 2005; 33:1131–1134. [PubMed: 16246064]
81. Li S, Okamoto T, Chun M, Sargiacomo M, Casanova JE, Hansen SH, Nishimoto I, Lisanti MP. Evidence for a regulated interaction between heterotrimeric G proteins and caveolin. *J Biol Chem.* 1995; 270:15693–15701. [PubMed: 7797570]
82. Escriche M, Burgueno J, Ciruela F, Canela EI, Mallol J, Enrich C, Lluís C, Franco R. Ligand-induced caveolae-mediated internalization of A1 adenosine receptors: morphological evidence of endosomal sorting and receptor recycling. *Exp Cell Res.* 2003; 285:72–90. [PubMed: 12681288]
83. Rapacciuolo A, Suvarna S, Barki-Harrington L, Luttrell LM, Cong M, Lefkowitz RJ, Rockman HA. Protein kinase A and G protein-coupled receptor kinase phosphorylation mediates beta-1 adrenergic receptor endocytosis through different pathways. *J Biol Chem.* 2003; 278:35403–35411. [PubMed: 12821660]
84. Volonte D, Galbiati F, Pestell RG, Lisanti MP. Cellular stress induces the tyrosine phosphorylation of caveolin-1 (TyR14) via activation of p38 mitogen-activated protein kinase and c-Src kinase. Evidence for caveolae, the actin cytoskeleton, and focal adhesions as mechanical sensors of osmotic stress. *J Biol Chem.* 2001; 276:8094–8103. [PubMed: 11094059]

85. Smythe GM, Eby JC, Disatnik MH, Rando TA. A caveolin-3 mutant that causes limb girdle muscular dystrophy type 1C disrupts Src localization and activity and induces apoptosis in skeletal myotubes. *J Cell Sci.* 2003; 116:4739–4749. [PubMed: 14600260]
86. Patel HH, Tsutsumi YM, Head BP, Niesman IR, Jennings M, Horikawa Y, Huang D, Moreno AL, Patel PM, Insel PA, Roth DM. Mechanisms of cardiac protection from ischemia/reperfusion injury: a role for caveolae and caveolin-1. *FASEB J.* 2007; 21:1565–1574. [PubMed: 17272740]
87. Head BP, Patel HH, Tsutsumi YM, Hu Y, Mejia T, Mora RC, Insel PA, Roth DM, Drummond JC, Patel PM. Caveolin-1 expression is essential for N-methyl-D-aspartate receptor-mediated Src and extracellular signal-regulated kinase 1/2 activation and protection of primary neurons from ischemic cell death. *FASEB J.* 2008; 22:828–840. [PubMed: 17905724]
88. Horikawa YT, Panneerselvam M, Kawaraguchi Y, Tsutsumi YM, Ali SS, Balijepalli RC, Murray F, Head BP, Niesman IR, Rieg T, Vallon V, Insel PA, Patel HH, Roth DM. Cardiac-specific overexpression of caveolin-3 attenuates cardiac hypertrophy and increases natriuretic peptide expression and signaling. *Journal of the American College of Cardiology.* 2011; 57:2273–2283. [PubMed: 21616289]
89. Wang XM, Zhang Y, Kim HP, Zhou Z, Feghali-Bostwick CA, Liu F, Ifedigbo E, Xu X, Oury TD, Kaminski N, Choi AM. Caveolin-1: a critical regulator of lung fibrosis in idiopathic pulmonary fibrosis. *J Exp Med.* 2006; 203:2895–2906. [PubMed: 17178917]
90. Gosens R, Stelmack GL, Dueck G, McNeill KD, Yamasaki A, Gerthoffer WT, Unruh H, Gounni AS, Zaagsma J, Halayko AJ. Role of caveolin-1 in p42/p44 MAP kinase activation and proliferation of human airway smooth muscle. *Am J Physiol Lung Cell Mol Physiol.* 2006; 291:L523–L534. [PubMed: 16617096]
91. Koga A, Oka N, Kikuchi T, Miyazaki H, Kato S, Imaizumi T. Adenovirus-mediated overexpression of caveolin-3 inhibits rat cardiomyocyte hypertrophy. *Hypertension.* 2003; 42:213–219. [PubMed: 12847114]
92. Sonveaux P, Martinive P, DeWever J, Batova Z, Daneau G, Pelat M, Ghisdal P, Gregoire V, Dessy C, Balligand JL, Feron O. Caveolin-1 expression is critical for vascular endothelial growth factor-induced ischemic hindlimb collateralization and nitric oxide-mediated angiogenesis. *Circ Res.* 2004; 95:154–161. [PubMed: 15205364]
93. Boyd NL, Park H, Yi H, Boo YC, Sorescu GP, Sykes M, Jo H. Chronic shear induces caveolae formation and alters ERK and Akt responses in endothelial cells. *Am J Physiol Heart Circ Physiol.* 2003; 285:H1113–H1122. [PubMed: 12763750]
94. Shin J, Jo H, Park H. Caveolin-1 is transiently dephosphorylated by shear stress-activated protein tyrosine phosphatase μ . *Biochem Biophys Res Commun.* 2006; 339:737–741. [PubMed: 16325778]
95. Woodman SE, Park DS, Cohen AW, Cheung MW, Chandra M, Shirani J, Tang B, Jelicks LA, Kitsis RN, Christ GJ, Factor SM, Tanowitz HB, Lisanti MP. Caveolin-3 knock-out mice develop a progressive cardiomyopathy and show hyperactivation of the p42/44 MAPK cascade. *J Biol Chem.* 2002; 277:38988–38997. [PubMed: 12138167]
96. Cohen AW, Park DS, Woodman SE, Williams TM, Chandra M, Shirani J, Pereira de Souza A, Kitsis RN, Russell RG, Weiss LM, Tang B, Jelicks LA, Factor SM, Shtutin V, Tanowitz HB, Lisanti MP. Caveolin-1 null mice develop cardiac hypertrophy with hyperactivation of p42/44 MAP kinase in cardiac fibroblasts. *Am J Physiol Cell Physiol.* 2003; 284:C457–C474. [PubMed: 12388077]
97. Krajewska WM, Maslowska I. Caveolins: structure and function in signal transduction. *Cell Mol Biol Lett.* 2004; 9:195–220. [PubMed: 15213803]
98. Smythe GM, Rando TA. Altered caveolin-3 expression disrupts PI(3) kinase signaling leading to death of cultured muscle cells. *Exp Cell Res.* 2006; 312:2816–2825. [PubMed: 16814768]
99. Cohen AW, Razani B, Wang XB, Combs TP, Williams TM, Scherer PE, Lisanti MP. Caveolin-1-deficient mice show insulin resistance and defective insulin receptor protein expression in adipose tissue. *Am J Physiol Cell Physiol.* 2003; 285:C222–C235. [PubMed: 12660144]
100. Oshikawa J, Otsu K, Toya Y, Tsunematsu T, Hankins R, Kawabe J, Minamisawa S, Umemura S, Hagiwara Y, Ishikawa Y. Insulin resistance in skeletal muscles of caveolin-3-null mice. *Proc Natl Acad Sci U S A.* 2004; 101:12670–12675. [PubMed: 15314230]

101. Ha H, Pak Y. Modulation of the caveolin-3 and Akt status in caveolae by insulin resistance in H9c2 cardiomyoblasts. *Exp Mol Med*. 2005; 37:169–178. [PubMed: 16000870]
102. Hahn-Obercyger M, Graeve L, Madar Z. A high-cholesterol diet increases the association between caveolae and insulin receptors in rat liver. *J Lipid Res*. 2009; 50:98–107. [PubMed: 18757837]
103. Hajduch E, Turban S, Le Liepvre X, Le Lay S, Lipina C, Dimopoulos N, Dugail I, Hundal HS. Targeting of PKC ζ and PKB to caveolin-enriched microdomains represents a crucial step underpinning the disruption in PKB-directed signalling by ceramide. *Biochem J*. 2008; 410:369–379. [PubMed: 17983354]
104. Balijepalli RC, Foell JD, Hall DD, Hell JW, Kamp TJ. Localization of cardiac L-type Ca(2+) channels to a caveolar macromolecular signaling complex is required for beta(2)-adrenergic regulation. *Proceedings of the National Academy of Sciences of the United States of America*. 2006; 103:7500–7505. [PubMed: 16648270]
105. Balijepalli RC, Kamp TJ. Caveolae, ion channels and cardiac arrhythmias. *Progress in biophysics and molecular biology*. 2008; 98:149–160. [PubMed: 19351512]
106. Martens JR, Navarro-Polanco R, Coppock EA, Nishiyama A, Parshley L, Grobaski TD, Tamkun MM. Differential targeting of Shaker-like potassium channels to lipid rafts. *J Biol Chem*. 2000; 275:7443–7446. [PubMed: 10713042]
107. Martinez-Marmol R, Villalonga N, Sole L, Vicente R, Tamkun MM, Soler C, Felipe A. Multiple Kv1.5 targeting to membrane surface microdomains. *J Cell Physiol*. 2008; 217:667–673. [PubMed: 18668522]
108. Xia F, Gao X, Kwan E, Lam PP, Chan L, Sy K, Sheu L, Wheeler MB, Gaisano HY, Tsushima RG. Disruption of pancreatic beta-cell lipid rafts modifies Kv2.1 channel gating and insulin exocytosis. *J Biol Chem*. 2004; 279:24685–24691. [PubMed: 15073181]
109. Nichols CG, Lederer WJ. Adenosine triphosphate-sensitive potassium channels in the cardiovascular system. *Am J Physiol*. 1991; 261:H1675–H1686. [PubMed: 1750525]
110. Yokoshiki H, Sunagawa M, Seki T, Sperelakis N. ATP-sensitive K⁺ channels in pancreatic, cardiac, and vascular smooth muscle cells. *Am J Physiol*. 1998; 274:C25–C37. [PubMed: 9458709]
111. Gross GJ, Peart JN. KATP channels and myocardial preconditioning: an update. *Am J Physiol Heart Circ Physiol*. 2003; 285:H921–H930. [PubMed: 12915383]
112. Sampson LJ, Hayabuchi Y, Standen NB, Dart C. Caveolae localize protein kinase A signaling to arterial ATP-sensitive potassium channels. *Circ Res*. 2004; 95:1012–1018. [PubMed: 15499025]
113. Sampson LJ, Davies LM, Barrett-Jolley R, Standen NB, Dart C. Angiotensin II-activated protein kinase C targets caveolae to inhibit aortic ATP-sensitive potassium channels. *Cardiovasc Res*. 2007; 76:61–70. [PubMed: 17582389]
114. Bosman A, Sartiani L, Spinelli V, Del Lungo M, Stillitano F, Nosi D, Mugelli A, Cerbai E, Jaconi M. Molecular and functional evidence of HCN4 and caveolin-3 interaction during cardiomyocyte differentiation from human embryonic stem cells. *Stem cells and development*. 2013
115. Ye B, Balijepalli RC, Foell JD, Kroboth S, Ye Q, Luo YH, Shi NQ. Caveolin-3 associates with and affects the function of hyperpolarization-activated cyclic nucleotide-gated channel 4. *Biochemistry*. 2008; 47:12312–12318. [PubMed: 19238754]
116. Suzuki Y, Yamamura H, Ohya S, Imaizumi Y. Direct molecular interaction of caveolin-3 with KCa1.1 channel in living HEK293 cell expression system. *Biochemical and biophysical research communications*. 2013; 430:1169–1174. [PubMed: 23237801]
117. Park DS, Woodman SE, Schubert W, Cohen AW, Frank PG, Chandra M, Shirani J, Razani B, Tang B, Jelicks LA, Factor SM, Weiss LM, Tanowitz HB, Lisanti MP. Caveolin-1/3 double-knockout mice are viable, but lack both muscle and non-muscle caveolae, and develop a severe cardiomyopathic phenotype. *Am J Pathol*. 2002; 160:2207–2217. [PubMed: 12057923]
118. Galbiati F, Volonte D, Chu JB, Li M, Fine SW, Fu M, Bermudez J, Pedemonte M, Weidenheim KM, Pestell RG, Minetti C, Lisanti MP. Transgenic overexpression of caveolin-3 in skeletal muscle fibers induces a Duchenne-like muscular dystrophy phenotype. *Proc Natl Acad Sci U S A*. 2000; 97:9689–9694. [PubMed: 10931944]

119. Kikuchi T, Oka N, Koga A, Miyazaki H, Ohmura H, Imaizumi T. Behavior of caveolae and caveolin-3 during the development of myocyte hypertrophy. *J Cardiovasc Pharmacol*. 2005; 45:204–210. [PubMed: 15725944]
120. Kuzuya T, Hoshida S, Yamashita N, Fuji H, Oe H, Hori M, Kamada T, Tada M. Delayed effects of sublethal ischemia on the acquisition of tolerance to ischemia. *Circ Res*. 1993; 72:1293–1299. [PubMed: 8495557]
121. Marber MS, Latchman DS, Walker JM, Yellon DM. Cardiac stress protein elevation 24 hours after brief ischemia or heat stress is associated with resistance to myocardial infarction. *Circulation*. 1993; 88:1264–1272. [PubMed: 8353888]
122. Zhao ZQ, Corvera JS, Halkos ME, Kerendi F, Wang NP, Guyton RA, Vinten-Johansen J. Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning. *Am J Physiol Heart Circ Physiol*. 2003; 285:H579–H588. [PubMed: 12860564]
123. Hausenloy DJ, Tsang A, Yellon DM. The reperfusion injury salvage kinase pathway: a common target for both ischemic preconditioning and postconditioning. *Trends Cardiovasc Med*. 2005; 15:69–75. [PubMed: 15885573]
124. Heusch G. Cardioprotection: chances and challenges of its translation to the clinic. *Lancet*. 2013; 381:166–175. [PubMed: 23095318]
125. D'Alessio A, Kluger MS, Li JH, Al-Lamki R, Bradley JR, Pober JS. Targeting of tumor necrosis factor receptor 1 to low density plasma membrane domains in human endothelial cells. *The Journal of biological chemistry*. 2010; 285:23868–23879. [PubMed: 20511226]
126. Young LH, Ikeda Y, Lefer AM. Caveolin-1 peptide exerts cardioprotective effects in myocardial ischemia-reperfusion via nitric oxide mechanism. *Am J Physiol Heart Circ Physiol*. 2001; 280:H2489–H2495. [PubMed: 11356603]
127. Der P, Cui J, Das DK. Role of lipid rafts in ceramide and nitric oxide signaling in the ischemic and preconditioned hearts. *Journal of molecular and cellular cardiology*. 2006; 40:313–320. [PubMed: 16337960]
128. Koneru S, Penumathsa SV, Thirunavukkarasu M, Samuel SM, Zhan L, Han Z, Maulik G, Das DK, Maulik N. Redox regulation of ischemic preconditioning is mediated by the differential activation of caveolins and their association with eNOS and GLUT-4. *Am J Physiol Heart Circ Physiol*. 2007; 292:H2060–H2072. [PubMed: 17277024]
129. Sun J, Kohr MJ, Nguyen T, Aponte AM, Connelly PS, Esfahani SG, Gucek M, Daniels MP, Steenbergen C, Murphy E. Disruption of caveolae blocks ischemic preconditioning-mediated S-nitrosylation of mitochondrial proteins. *Antioxidants & redox signaling*. 2012; 16:45–56. [PubMed: 21834687]
130. Rahman S, Li J, Bopassa JC, Umar S, Iorga A, Partownavid P, Eghbali M. Phosphorylation of GSK-3 β mediates intralipid-induced cardioprotection against ischemia/reperfusion injury. *Anesthesiology*. 2011; 115:242–253. [PubMed: 21691195]
131. Li J, Iorga A, Sharma S, Youn JY, Partow-Navid R, Umar S, Cai H, Rahman S, Eghbali M. Intralipid, a clinically safe compound, protects the heart against ischemia-reperfusion injury more efficiently than cyclosporine-A. *Anesthesiology*. 2012; 117:836–846. [PubMed: 22814384]
132. Musser JB, Fontana JL, Mongan PD. The anesthetic and physiologic effects of an intravenous administration of a halothane lipid emulsion (5% vol/vol). *Anesth Analg*. 1999; 88:671–675. [PubMed: 10072026]
133. Zhou JX, Luo NF, Liang XM, Liu J. The efficacy and safety of intravenous emulsified isoflurane in rats. *Anesth Analg*. 2006; 102:129–134. [PubMed: 16368817]
134. Chiari PC, Pagel PS, Tanaka K, Krolikowski JG, Ludwig LM, Trillo RA Jr, Puri N, Kersten JR, Warltier DC. Intravenous emulsified halogenated anesthetics produce acute and delayed preconditioning against myocardial infarction in rabbits. *Anesthesiology*. 2004; 101:1160–1166. [PubMed: 15505452]
135. Rao Y, Wang YL, Zhang WS, Liu J. Emulsified isoflurane produces cardiac protection after ischemia-reperfusion injury in rabbits. *Anesth Analg*. 2008; 106:1353–1359. table of contents. [PubMed: 18420844]

136. Lucchinetti E, Schaub MC, Zaugg M. Emulsified intravenous versus evaporated inhaled isoflurane for heart protection: old wine in a new bottle or true innovation? *Anesth Analg*. 2008; 106:1346–1349. [PubMed: 18420843]
137. Cason BA, Gamperl AK, Slocum RE, Hickey RF. Anesthetic-induced preconditioning: Previous administration of isoflurane decreases myocardial infarct size in rabbits. *Anesthesiology*. 1997; 87:1182–1190. [PubMed: 9366471]
138. Kersten JR, Orth KG, Pagel PS, Mei DA, Gross GJ, Warltier DC. Role of adenosine in isoflurane-induced cardioprotection. *Anesthesiology*. 1997; 86:1128–1139. [PubMed: 9158363]
139. Cope DK, Impastato WK, Cohen MV, Downey JM. Volatile anesthetics protect the ischemic rabbit myocardium from infarction. *Anesthesiology*. 1997; 86:699–709. [PubMed: 9066337]
140. Piriou V, Ross S, Pigott D, Evans R, Foex P. Beneficial effect of concomitant administration of isoflurane and nicorandil. *Br J Anaesth*. 1997; 79:68–77. [PubMed: 9301392]
141. Ismaeil MS, Tkachenko I, Gamperl AK, Hickey RF, Cason BA. Mechanisms of isoflurane-induced myocardial preconditioning in rabbits. *Anesthesiology*. 1999; 90:812–821. [PubMed: 10078683]
142. Toller WG, Montgomery MW, Pagel PS, Hettrick DA, Warltier DC, Kersten JR. Isoflurane-enhanced recovery of canine stunned myocardium: Role for protein kinase C? *Anesthesiology*. 1999; 91:713–722. [PubMed: 10485783]
143. Toller WG, Gross ER, Kersten JR, Pagel PS, Gross GJ, Warltier DC. Sarcolemmal and mitochondrial adenosine triphosphate-dependent potassium channels: Mechanism of desflurane-induced cardioprotection. *Anesthesiology*. 2000; 92:1731–1739. [PubMed: 10839925]
144. Toller WG, Kersten JR, Gross ER, Pagel PS, Warltier DC. Isoflurane preconditions myocardium against infarction *via* activation of inhibitory guanine nucleotide binding proteins. *Anesthesiology*. 2000; 92:1400–1407. [PubMed: 10781287]
145. Roscoe AK, Christensen JD, Lynch C III. Isoflurane, but not halothane, induces protection of human myocardium *via* adenosine A₁ receptors and adenosine triphosphate-sensitive potassium channels. *Anesthesiology*. 2000; 92:1692–1701. [PubMed: 10839921]
146. Zaugg M, Lucchinetti E, Spahn DR, Pasch T, Schaub MC. Volatile anesthetics mimic cardiac preconditioning by priming the activation of mitochondrial K_{ATP} channels *via* multiple signaling pathways. *Anesthesiology*. 2002; 97:4–14. [PubMed: 12131097]
147. Mullenheim J, Ebel D, Frabdorf J, Preckel B, Thamer V, Schlack W. Isoflurane preconditions myocardium against infarction *via* release of free radicals. *Anesthesiology*. 2002; 96:934–940. [PubMed: 11964602]
148. Tanaka K, Weihrauch D, Kehl F, Ludwig LM, LaDisa JF Jr, Kersten JR, Pagel PS, Warltier DC. Mechanism of preconditioning by isoflurane in rabbits: A direct role for reactive oxygen species. *Anesthesiology*. 2002; 97:1485–1490. [PubMed: 12459675]
149. Fujimoto K, Bosnjak ZJ, Kwok WM. Isoflurane-induced facilitation of the cardiac sarcolemmal K_{ATP} channel. *Anesthesiology*. 2002; 97:57–65. [PubMed: 12131104]
150. Patel HH, Ludwig LM, Fryer RM, Hsu AK, Warltier DC, Gross GJ. Delta opioid agonists and volatile anesthetics facilitate cardioprotection *via* potentiation of K_{ATP} channel opening. *FASEB J*. 2002; 16:1468–1470. [PubMed: 12205051]
151. Novalija E, Varadarajan SG, Camara AKS, An J, Chen Q, Riess ML, Hogg N, Stowe DF. Anesthetic preconditioning: Triggering role of reactive oxygen and nitrogen species in isolated hearts. *Am J Physiol Heart Circ Physiol*. 2002; 283:H44–H52. [PubMed: 12063273]
152. Tsutsumi YM, Patel HH, Lai NC, Takahashi T, Head BP, Roth DM. Isoflurane produces sustained cardiac protection after ischemia-reperfusion injury in mice. *Anesthesiology*. 2006; 104:495–502. [PubMed: 16508397]
153. Raphael J. Physiology and pharmacology of myocardial preconditioning. *Seminars in cardiothoracic and vascular anesthesia*. 2010; 14:54–59. [PubMed: 20472628]
154. Tanaka K, Ludwig LM, Krolikowski JG, Alcindor D, Pratt PF, Kersten JR, Pagel PS, Warltier DC. Isoflurane produces delayed preconditioning against myocardial ischemia and reperfusion injury: role of cyclooxygenase-2. *Anesthesiology*. 2004; 100:525–531. [PubMed: 15108964]

155. Tsutsumi YM, Patel HH, Huang D, Roth DM. Role of 12-lipoxygenase in volatile anesthetic-induced delayed preconditioning in mice. *Am J Physiol Heart Circ Physiol.* 2006; 291:H979–H983. [PubMed: 16648187]
156. Lutz M, Liu H. Inhaled sevoflurane produces better delayed myocardial protection at 48 versus 24 hours after exposure. *Anesth Analg.* 2006; 102:984–990. [PubMed: 16551886]
157. Baxter GF, Goma FM, Yellon DM. Characterisation of the infarct-limiting effect of delayed preconditioning: Timecourse and dose-dependency studies in rabbit myocardium. *Basic Res Cardiol.* 1997; 92:159–167. [PubMed: 9226101]
158. Tsutsumi YM, Yokoyama T, Horikawa Y, Roth DM, Patel HH. Reactive oxygen species trigger ischemic and pharmacological postconditioning: *In vivo* and *in vitro* characterization. *Life Sci.* 2007; 81:1223–1227. [PubMed: 17915258]
159. Tonkovic-Capin M, Gross GJ, Bosnjak ZJ, Tweddell JS, Fitzpatrick CM, Baker JE. Delayed cardioprotection by isoflurane: role of K(ATP) channels. *Am J Physiol Heart Circ Physiol.* 2002; 283:H61–H68. [PubMed: 12063275]
160. Zaugg M, Lucchinetti E, Uecker M, Pasch T, Schaub MC. Anaesthetics and cardiac preconditioning. Part I. Signalling and cytoprotective mechanisms. *Br J Anaesth.* 2003; 91:551–565. [PubMed: 14504159]
161. Tanaka K, Ludwig LM, Kersten JR, Pagel PS, Warltier DC. Mechanisms of cardioprotection by volatile anesthetics. *Anesthesiology.* 2004; 100:707–721. [PubMed: 15108989]
162. De Hert SG, Turani F, Mathur S, Stowe DF. Cardioprotection with volatile anesthetics: mechanisms and clinical implications. *Anesth Analg.* 2005; 100:1584–1593. [PubMed: 15920178]
163. Stadnicka A, Marinovic J, Ljubkovic M, Bienengraeber MW, Bosnjak ZJ. Volatile anesthetic-induced cardiac preconditioning. *J Anesth.* 2007; 21:212–219. [PubMed: 17458651]
164. Schultz JE, Rose E, Yao Z, Gross GJ. Evidence for involvement of opioid receptors in ischemic preconditioning in rat hearts. *Am J Physiol Heart Circ Physiol.* 1995; 268:H2157–H2161.
165. Lasley RD, Konyn PJ, Hegge JO, Mentzer RM Jr. Effects of ischemic and adenosine preconditioning on interstitial fluid adenosine and myocardial infarct size. *Am J Physiol Heart Circ Physiol.* 1995; 269:H1460–H1466.
166. Ghitescu L, Fixman A, Simionescu M, Simionescu N. Specific binding sites for albumin restricted to plasmalemmal vesicles of continuous capillary endothelium: receptor-mediated transcytosis. *J Cell Biol.* 1986; 102:1304–1311. [PubMed: 3007533]
167. Schnitzer JE, Oh P. Albondin-mediated capillary permeability to albumin. Differential role of receptors in endothelial transcytosis and endocytosis of native and modified albumins. *J Biol Chem.* 1994; 269:6072–6082. [PubMed: 8119952]
168. Minshall RD, Tiruppathi C, Vogel SM, Niles WD, Gilchrist A, Hamm HE, Malik AB. Endothelial cell-surface gp60 activates vesicle formation and trafficking via G(i)-coupled Src kinase signaling pathway. *J Cell Biol.* 2000; 150:1057–1070. [PubMed: 10973995]
169. Vogel SM, Minshall RD, Pilipovic M, Tiruppathi C, Malik AB. Albumin uptake and transcytosis in endothelial cells *in vivo* induced by albumin-binding protein. *Am J Physiol Lung Cell Mol Physiol.* 2001; 281:L1512–L1522. [PubMed: 11704548]
170. Shajahan AN, Timblin BK, Sandoval R, Tiruppathi C, Malik AB, Minshall RD. Role of Src-induced dynamin-2 phosphorylation in caveolae-mediated endocytosis in endothelial cells. *J Biol Chem.* 2004; 279:20392–20400. [PubMed: 15007081]
171. Maniatis NA, Brovkovich V, Allen SE, John TA, Shajahan AN, Tiruppathi C, Vogel SM, Skidgel RA, Malik AB, Minshall RD. Novel mechanism of endothelial nitric oxide synthase activation mediated by caveolae internalization in endothelial cells. *Circ Res.* 2006; 99:870–877. [PubMed: 16973909]
172. Schubert W, Frank PG, Razani B, Park DS, Chow CW, Lisanti MP. Caveolae-deficient endothelial cells show defects in the uptake and transport of albumin *in vivo*. *J Biol Chem.* 2001; 276:48619–48622. [PubMed: 11689550]
173. Hu G, Schwartz DE, Shajahan AN, Visintine DJ, Salem MR, Crystal GJ, Albrecht RF, Vogel SM, Minshall RD. Isoflurane, but not sevoflurane, increases transendothelial albumin permeability in

the isolated rat lung: role for enhanced phosphorylation of caveolin-1. *Anesthesiology*. 2006; 104:777–785. [PubMed: 16571974]

174. Garlid KD, Costa AD, Quinlan CL, Pierre SV, Dos Santos P. Cardioprotective signaling to mitochondria. *Journal of molecular and cellular cardiology*. 2009; 46:858–866. [PubMed: 19118560]
175. Sowa G, Pypaert M, Fulton D, Sessa WC. The phosphorylation of caveolin-2 on serines 23 and 36 modulates caveolin-1-dependent caveolae formation. *Proceedings of the National Academy of Sciences of the United States of America*. 2003; 100:6511–6516. [PubMed: 12743374]
176. Patel HH, Tsutsumi YM, Head BP, Niesman IR, Jennings M, Horikawa Y, Huang D, Moreno AL, Patel PM, Insel PA, Roth DM. Mechanisms of cardiac protection from ischemia/reperfusion injury: a role for caveolae and caveolin-1. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*. 2007; 21:1565–1574. [PubMed: 17272740]
177. Kazui A, Ono M, Handa K, Hakomori S. Glycosylation affects translocation of integrin, Src, and caveolin into or out of GEM. *Biochemical and biophysical research communications*. 2000; 273:159–163. [PubMed: 10873579]
178. Fuhs SR, Insel PA. Caveolin-3 undergoes SUMOylation by the SUMO E3 ligase PIASy: sumoylation affects G-protein-coupled receptor desensitization. *The Journal of biological chemistry*. 2011; 286:14830–14841. [PubMed: 21362625]
179. Masserini M, Palestini P, Pitto M. Glycolipid-enriched caveolae and caveolae-like domains in the nervous system. *J Neurochem*. 1999; 73:1–11. [PubMed: 10386949]
180. Gaudreault SB, Blain JF, Gratton JP, Poirier J. A role for caveolin-1 in post-injury reactive neuronal plasticity. *J Neurochem*. 2005; 92:831–839. [PubMed: 15686485]
181. Shyng SL, Heuser JE, Harris DA. A glycolipid-anchored prion protein is endocytosed via clathrin-coated pits. *J Cell Biol*. 1994; 125:1239–1250. [PubMed: 7911471]
182. Lang DM, Lommel S, Jung M, Ankerhold R, Petrausch B, Laessing U, Wiechers MF, Plattner H, Stuermer CA. Identification of reggie-1 and reggie-2 as plasmamembrane-associated proteins which cocluster with activated GPI-anchored cell adhesion molecules in non-caveolar micropatches in neurons. *J Neurobiol*. 1998; 37:502–523. [PubMed: 9858255]
183. Head BP, Hu Y, Finley JC, Saldana MD, Bonds JA, Miyanojara A, Niesman IR, Ali SS, Murray F, Insel PA, Roth DM, Patel HH, Patel PM. Neuron-targeted caveolin-1 protein enhances signaling and promotes arborization of primary neurons. *The Journal of biological chemistry*. 2011; 286:33310–33321. [PubMed: 21799010]
184. Head BP, Peart JN, Panneerselvam M, Yokoyama T, Pearn ML, Niesman IR, Bonds JA, Schilling JM, Miyanojara A, Headrick J, Ali SS, Roth DM, Patel PM, Patel HH. Loss of caveolin-1 accelerates neurodegeneration and aging. *PloS one*. 2010; 5:e15697. [PubMed: 21203469]
185. Goetz JG, Lajoie P, Wiseman SM, Nabi IR. Caveolin-1 in tumor progression: the good, the bad and the ugly. *Cancer metastasis reviews*. 2008; 27:715–735. [PubMed: 18506396]
186. Burgermeister E, Liscovitch M, Rocken C, Schmid RM, Ebert MP. Caveats of caveolin-1 in cancer progression. *Cancer letters*. 2008; 268:187–201. [PubMed: 18482795]
187. Shatz M, Liscovitch M. Caveolin-1: a tumor-promoting role in human cancer. *International journal of radiation biology*. 2008; 84:177–189. [PubMed: 18300018]
188. Kawaraguchi Y, Horikawa YT, Murphy AN, Murray F, Miyanojara A, Ali SS, Head BP, Patel PM, Roth DM, Patel HH. Volatile anesthetics protect cancer cells against tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis via caveolins. *Anesthesiology*. 2011; 115:499–508. [PubMed: 21862885]

Figure 1. Caveolae are signaling epicenters

Caveolae are known to concentrate various signaling receptors (receptor tyrosine kinases, RTK; G-protein coupled receptors, GPCRs; caveolin scaffolding domains, CSD) as well as caveolins which can interact with many protein kinase.

Figure 2. Electron microscopy of caveolae

A) Untreated cardiac myocyte; **B)** Caveolin-3 overexpressing cardiac myocyte have an abundance of caveolae; **C)** Caveolin-3.knockout cardiac myocyte lack caveolae; **D)** Isoflurane induces caveolae formation in cardiac myocytes; scale bar 200nm

Figure 3. The role of caveolae and caveolins in anesthetic induced cardiac protection
 Isoflurane increases caveolae formation at the cell surface. Acute preconditioning requires both caveolin-1 and -3 and activates both Src kinase and PI3K/AKT/GSK pathway, whereas delayed cardiac protection increases caveolin-3 and increases GLUT 4 expression.