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# Man Overboard!

## *Rescuing Myocardium with Membrane Rafts*

VOLATILE anesthetics produce important cardioprotective effects by stimulating a series of intracellular signaling events that ultimately render the myocardium resistant to infarction. Anesthetics are known to protect the heart in a temporal manner. An initial early window of myocardial protection lasts hours after exposure to isoflurane or other volatile agents, and myocardial protection reappears again 24–48 h later. The mechanisms of early and delayed anesthetic preconditioning differ. Anesthetics activate various intracellular kinases, which phosphorylate and subsequently modify the activity of downstream proteins (*e.g.*, endothelial nitric oxide synthase [eNOS] and adenosine triphosphate-regulated potassium channels), that are important in mediating cardioprotection. During the early preconditioning phase, modification of preexisting proteins leads to protection, whereas after 24 h, cardioprotection relies on the synthesis of new proteins. The complexity of these signal transduction events requires both functional and spatial organization and coordination of the activity of a large number of intracellular proteins. In this issue of ANESTHESIOLOGY, Tsutsumi *et al.*[1] demonstrate that isoflurane produces delayed protection against myocardial infarction by modulating a key protein, caveolin-3, found in membrane (lipid) rafts (fig. 1).

An extension of the classic fluid lipid bilayer model of the plasma membrane, lipid or membrane rafts are small (10–200 nm) microdomains enriched in sterols, sphingolipids, and cholesterol "floating" in a sea of phospholipids.[2] These lipid domains form docking platforms that control the location of intracellular signal transduction events. Rafts are located in the plasma membrane and are found in the endoplasmic reticulum and mitochondria as well. Membrane rafts function to regulate cellular processes by concentrating proteins to highly specific intracellular locations. The formation of lipid rafts is highly dynamic, and this property allows for temporal regulation of protein signaling and trafficking. A subclass of membrane rafts is the caveolae, which are flask-like invaginations of the cellular membrane (60–80 nm), distinguished by the presence of scaffolding proteins caveolin-1, -2, and -3.[3] Caveolins-1 and -2 are highly expressed in adipocytes, endothelial cells, and fibroblasts, whereas caveolin-3 is expressed predominantly in skeletal, cardiac, and smooth muscle cells. Caveolae are disrupted in caveolin-1 and caveolin-3 knockout mice but are preserved in caveolin-2 mutants.[4] Caveolins bind proteins through a specific domain that enables conformational changes to occur, and this action regulates the activity of signal transduction molecules. Caveolins are required for caveolae formation, and their expression indirectly regulates the number of caveolae available for functional signal transduction. Caveolins can alter the fluidity of membrane rafts through the binding of cholesterol, which in turn alters membrane composition and signaling effects.[4] Tsutsumi *et al.*[5] have previously shown that cardiac-specific overexpression of caveolin-3 decreases myocardial infarction and mimics ischemic preconditioning. The current results extend these previous findings and demonstrate that isoflurane produces delayed preconditioning by up-regulating caveolin-3 and by increasing the colocalization of caveolin-3 with glucose transporter (GLUT)-4 in membrane rafts (caveolar fraction).

GLUT-4 is the major transporter responsible for glucose uptake into cells. During ischemia, cardiac myocyte metabolism is altered to favor anaerobic glycolysis, and increased GLUT-4 translocation from intracellular compartments to the plasma membrane facilitates substrate availability. GLUT-4 has previously been implicated in the cardioprotective effect of both early and delayed forms of ischemic preconditioning, a phenomenon in which brief periods of myocardial ischemia up to 2 (early) or 24–48 h (delayed) before a prolonged period of coronary artery occlusion and reperfusion decreases the extent of subsequent infarction. Myocardial ischemia seems to increase GLUT-4 expression and translocation. GLUT-4 protein is up-regulated after ischemic preconditioning along with increased expression of caveolin-3, phosphorylated eNOS, and phosphorylated Akt. Preconditioning stimuli not only increase the expression of these cardioprotective proteins but also stimulate transloca-

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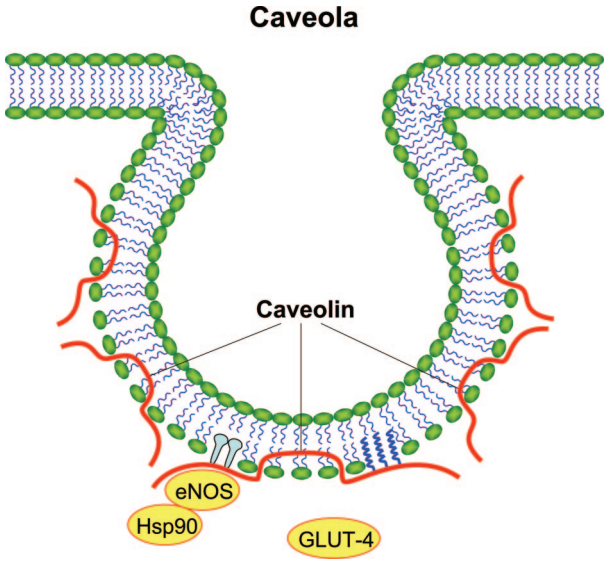


Fig. 1. A schematic representation of a caveola, a subtype of a membrane (lipid) raft. Caveolae contain the protein caveolin that interacts with glucose transporter (GLUT)-4, heat shock protein (Hsp) 90, and endothelial nitric oxide synthase (eNOS). These proteins have all been shown to play a role during anesthetic preconditioning.

tion of GLUT-4 to the caveolar-rich membrane fraction, thereby sustaining activation of signaling molecules.[6] Interestingly, eNOS, which is known to play an important role during both early and delayed phases of anesthetic preconditioning, is reciprocally regulated by interactions with caveolins-1 and -3 and GLUT-4. The activity of eNOS is decreased when this enzyme is associated with caveolin-1. Conversely, disassociation of caveolin-1/eNOS interaction and increased translocation of GLUT-4 and its enhanced association with caveolin-3 activate eNOS.[7] Although caveolin-1 is an important mediator of the early phase of isoflurane preconditioning, there seems to be almost no role for this protein in delayed protection. These findings indicate a differential role of caveolins during anesthetic cardioprotection that may be both temporal (early *vs.* delayed) and cell lineage (endothelial cell *vs.* cardiomyocyte) specific. Caveolin-1, while negatively regulating eNOS under basal conditions, also facilitates eNOS signaling during stimulation by compartmentalizing proteins in the appropriate intracellular locations. This condition is referred to as "caveolar paradox" and illustrates the complex nature of protein–protein interactions that ultimately impact cell survival after ischemia and reperfusion.

Other binding proteins, such as heat shock protein-90, have also been implicated in the regulation of eNOS by caveolins. For example, caveolin-1, eNOS, and heat shock protein 90 can be coimmunoprecipitated from endothelial cells, and the presence of heat shock protein 90 decreases the inhibitory effect of caveolin-1 on eNOS activity.[8] Tsutsumi *et al.*[1] did not investigate eNOS regulation by GLUT-4 or caveolin-3 interactions during delayed anesthetic preconditioning; however, it is interesting to speculate that interactions between these molecules and other binding partners, such as heat shock protein 90, may be spatially regulated in caveolae by lipophilic volatile anesthetics.

GLUT-4 is the major insulin-responsive GLUT. Insulin-induced translocation of GLUT-4 to the membrane from intracellular vesicles stimulates glucose uptake in muscle and adipose tissues. However, the exact mechanism whereby translocation of GLUT-4 to the plasma membrane induces cardioprotection is unknown. The current findings that GLUT-4 translocation is enhanced by delayed preconditioning with isoflurane in caveolin-1 but not caveolin-3 knockout mice also implicate caveolins as potential targets of disease processes, such as diabetes, that modulate the efficacy of preconditioning. For example, diabetes and acute hyperglycemia attenuate reduction of myocardial infarct size elicited by diverse preconditioning stimuli, and this occurs through impaired eNOS regulation by heat shock protein 90.[9] The caveolins are also modulated by diabetes. Caveolin-3 content is reduced in lipid rafts from diabetic myocardium,[7] and disruption of caveolae in adipocytes renders these cells insulin resistant.[10] Caveolin-3 knockout mice exhibit insulin resistance *in vivo*, and acute hyperglycemia alone disturbs lipid raft stability by interfering with cholesterol synthesis.[11] Thus, defects in membrane raft composition and caveolin expression induced by disease states could underlie the lack of cardioprotection observed in some clinical studies using volatile anesthetics, although this hypothesis remains to be specifically tested.

The work by Tsutsumi *et al.*[1] highlighted in this issue of ANESTHESIOLOGY emphasizes that anesthetic cardioprotection requires functional lipid domains, such as caveolae, and their associated proteins, the caveolins. Membrane rafts regulate the intracellular location of signaling molecules activated by volatile anesthetics, and these microdomains may be the interface through which anesthetics directly mediate protection. Moreover, lipid rafts may represent a new target for the rescue of ischemic myocardium and provide a new therapeutic approach in the treatment of patients with cardiovascular disease.

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## ANESTHESIOLOGY REFLECTIONS

# Rolland Whitacre's Wandering Testament

Less than 6 yr after serving as president of the American Society of Anesthesiologists (ASA) and 5 yr after coinventing his namesake spinal needle, Dr. Rolland Whitacre (*above right*) suffered a fatal heart attack in 1956. He received the ASA's Distinguished Service Award later that year. More than half a century later, a New Testament (*above left*) bearing the inscription "Rolland John Whitacre" appeared in an online auction. Deemed outside the Wood Library-Museum's collecting parameters, the book was purchased with personal funds and mailed as a gift in 2008 from the WLM curator to Whitacre's family. When the wandering book returned to the WLM curator as a "lost shipment" in early 2010, he shipped it, finally successfully, by registered mail to Whitacre's daughter. (Copyright © the American Society of Anesthesiologists, Inc. This image appears in color in the *Anesthesiology Reflections* online collection available at www.anesthesiology.org.)

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