Membrane Microdomains and Vascular Biology

Emerging Role in Atherogenesis

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The classic model of cell plasma membrane organization is that of a uniform, fluid environment in which protein and lipid constituents diffuse rapidly in an unrestricted fashion. Recent experimental evidence, however, reveals a far more complex membrane organization consisting of microdomains assembled from lipid constituents that have distinct biophysical characteristics. Such microdomains or “lipid rafts” are typically detergent-resistant and highly enriched with cholesterol and sphingolipids compared with the overall membrane lipid bilayer environment. These defined regions of lipid domains sequester proteins that mediate signal transduction in a variety of cell types, including endothelial cells and myocytes. Lipid rafts move or “float” as a coherent structural unit within the liquid-disordered lipid bilayer and can also cluster with other rafts to form larger platforms. The basis for differences in fluidity between rafts and the surrounding membrane is the degree of hydrocarbon chain saturation; domains are characterized by sphingolipids containing saturated fatty acids that attract unesterified cholesterol. The planar sterol nucleus of cholesterol further restricts the mobility and rotation of sphingolipid acyl chains, resulting in limited random diffusion and an expanded molecular width as compared with the rest of the membrane.

The plasma membrane caveola is a lipid raft subtype that is the subject of intensive investigation. Caveolae typically appear as microscopic, flask-shaped invaginations along the membrane surface and are commonly found in endothelial cells, adipocytes, and smooth muscle cells (Figure). The principal protein component of caveolae is caveolin, a scaffolding protein that binds cholesterol efficiently and interacts with various signaling macromolecules, including G proteins and calcium regulating proteins. Caveolin is also a potent inhibitor of endothelial nitric oxide synthase (eNOS) as it binds directly to the enzyme, blocking access of the co-factor, calcium/calmodulin. Caveolin may also regulate intracellular surface cholesterol levels. Genetically engineered animals that lack caveolin-1 protein, and thus caveolae, demonstrate marked defects in arterial relaxation, myogenic tone, and exercise tolerance as a result of abnormalities in cell signaling and NO metabolism. Conversely, the expression of caveolin is markedly elevated under conditions of hypercholesterolemia because of enrichment of plasma membrane cholesterol levels. In addition to atherosclerosis, lipid rafts have been shown to play an important role in other disease processes, including hypertension, Alzheimer’s disease, prion disease, and viral infection.

Pharmacological inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase leads to potent stimulation of eNOS, independently of changes in extracellular low-density lipoprotein (LDL) concentrations. By lowering the levels of plasma membrane cholesterol and potentially interacting with specific lipids, the HMG-CoA reductase inhibitor atorvastatin was shown to attenuate the expression of caveolin-1 and the abundance of caveolae in endothelial cells. These effects were observed in the absence of changes in cytosolic eNOS levels and reversed with mevalonate. When incubated with increasing amounts of extracellular LDL, atorvastatin also promoted the agonist-induced association of eNOS and the chaperone Hsp90, resulting in potent eNOS activation. This finding provides insight into the complex biochemical relationships between membrane cholesterol levels, microdomain abundance, and endothelial function (Figure). Indeed, restoration of normal endothelium-derived NO production with a statin has important benefits for treating the clinical manifestations of atherosclerosis. The release of NO promotes vasodilatation while interfering with various atherogenic pathways, such as platelet adhesion, superoxide formation, expression of adhesion molecules, and smooth muscle cell proliferation.

The existence of a unique membrane microdomain in vascular smooth muscle cells and macrophages that is causally related to cholesterol enrichment during atherosclerosis has been recently characterized using biophysical approaches. This microdomain consists exclusively of unesterified cholesterol and is found prominently in atherosclerotic tissues. Small-angle x-ray diffraction analyses have been used to directly characterize cholesterol monohydrate domains, which are identified as highly ordered lipid structures that have a consistent width of 34 Å and are contained within the surrounding plasma membrane (Figure). These distinct membrane regions consist of cholesterol in a tail-to-tail orientation, because a single cholesterol monohydrate crystal has a long-axis dimension of 17 Å. Cholesterol microdomains have a remarkably smaller intra-bilayer dimension as compared with the overall smooth muscle cell plasma membrane, which has a typical molecular width of 54 to 60 Å.
have also observed that certain oxidized derivatives of cholesterol form microdomains in a manner dependent on their 3-dimensional structure and intermolecular packing constraints. The stability of membrane cholesterol domains is dependent on numerous factors, including temperature, membrane cholesterol-to-phospholipid mole ratio, composition of the surrounding phospholipid acyl chains (eg, degree of saturation), and the extent of lipid peroxidation.

Alterations of Vascular Cell Membrane Structure in Atherosclerosis

Unesterified or free cholesterol is a major constituent of the vascular cell plasma membrane, where it regulates lipid bilayer dynamics and structure by modulating the packing of neighboring phospholipid molecules. The unesterified cholesterol molecule is oriented in the membrane such that the long axis of the highly planar sterol lies parallel to adjacent phospholipid acyl chains, thereby increasing the order parameter associated with the acyl chain region of the membrane. By restricting the random motion of membrane lipids and the mean cross-sectional area occupied by neighboring phospholipid acyl chains, cholesterol has a pronounced condensing effect on biological membranes. Investigators have proposed that certain proteins preferentially localize to cholesterol-enriched regions of the membrane (eg, caveolae), as the microenvironment created by cholesterol enrichment may be essential for maintaining the tertiary structure and function of restricted proteins.

Abnormal accumulation of cholesterol in vascular cells during atherosclerosis, however, has deleterious effects on membrane function, including ion transport and signal transduction mechanisms. In endothelial cells, excessive membrane cholesterol incorporation during hyperlipidemia may interfere with the active transport of amino acids, such as L-arginine. As a result, activation of eNOS leads to overproduction of superoxide, the alternative product of NO synthase when quantities of L-arginine or essential cofactors are insufficient. By modulating the physicochemical properties of membrane lipids, cholesterol enrichment disrupts the function of other transport proteins, including voltage-sensitive ion channels. In single-channel electrophysiological recordings of calcium-activated K⁺/H⁺ channels, cholesterol enrichment caused the ion channel pore to favor the closed state, as a result of increased intra-bilayer structural stress and lateral elastic stress energy.

In vascular smooth muscle cells obtained from atherosclerotic plaque, calcium transport mechanisms and basal intracellular calcium levels are disrupted by increased membrane cholesterol content. These changes have important consequences for atherosclerosis, because calcium participates directly in signal transduction pathways that promote smooth muscle cell proliferation and migration, among other effects.
Collectively, these observations provide compelling experimental evidence supporting the concept that membrane cholesterol levels are carefully regulated within a certain physiological range to facilitate normal activity of constituent proteins. Normal cholesterol levels are also necessary in the formation of lipid rafts, such as caveolae and detergent-resistant membrane domains. An excess amount of cholesterol results in adverse consequences for vascular biology.

Membrane Cholesterol Domains Precede Formation of Crystals

Unstable atherosclerotic lesions are characterized by large extracellular lipid deposits consisting of cholesterol (both free and esterified), phospholipids, and lesser amounts of triacylglycerol and fatty acids. The lipid core is bounded by its luminal side by a fibrous cap and on its abluminal side by the plaque base. Disruption in the integrity of the collagen-rich fibrous cap exposes elements of the blood to the thrombogenic lipid core, resulting in rapid thrombus formation.

Unesterified cholesterol in the plaque is associated with membrane phospholipid or extracellular crystals, a prominent feature of atherosclerotic lesions in humans and animals. Although noncystalline membrane cholesterol can readily exchange from the plaque with plasma lipoprotein particles, cholesterol in a crystalline state appears to be inert and resistant to reverse transport mechanisms.

In mouse macrophage cells, formation of free cholesterol crystals is enhanced with an acyl-CoA-cholesterol acyl transferase inhibitor, as esterified cholesterol hydrolysis leads to free cholesterol accumulation. Microscopic cholesterol crystals form and extend out from the subcellular compartments with various morphologies that include plates, needles, and helices, as observed by scanning electron microscopy approaches. Interestingly, formation of membrane cholesterol microdomains, as measured by x-ray diffraction approaches, precedes any evidence of extracellular cholesterol crystals. This observation suggests that membrane microdomains may represent a key nucleating site for extracellular cholesterol crystals. Preventing crystal formation is an important goal, as these rigid macromolecules contribute to mechanisms of cell injury and death, including apoptosis. Because cholesterol in this state does not respond well to pharmacological interventions that promote lesion regression, early intervention is essential. Cholesterol crystalline domain formation may be slowed or blocked by modulating the chemical (eg, degree of acyl chain saturation and oxidation) and physical (eg, temperature) properties of the membrane, thereby slowing or even preventing subsequent extracellular crystal development.

In models of atherosclerosis, systematic changes in the cholesterol content of vascular cell membranes have been measured and shown to correlate with the development of cholesterol microdomains. Cholesterol enrichment had remarkably consistent effects on the molecular dimensions and lipid organization of plasma membranes derived from an intact animal model and from those obtained from smooth muscle cells grown in vitro. Under atherosclerotic-like conditions, prominent cholesterol domains were observed in smooth muscle cell plasma membranes as free cholesterol levels in the membrane increased in parallel with serum LDL levels. In both model and biological membranes, oxidized cholesterol derivatives also formed domains within the membrane lipid bilayer. The development of such cholesterol domains is associated with extracellular crystal formation and cellular apoptosis and appears to be highly dependent on sterol 3-dimensional structure.

Cholesterol Influences Drug Availability to Cellular Receptor Sites

Numerous cardiovascular drugs bind to protein receptors (eg, ion channels) embedded in vascular cell plasma membranes after intercalation and diffusion of the drug through the surrounding lipid bilayer. Calcium channel blockers (CCBs), in particular, modulate excitation-contraction mechanisms in smooth muscle cells by regulating the transmembrane influx of calcium ions through voltage-sensitive ion channels. CCB receptor binding affinity and kinetics can be precisely calculated from their membrane concentration in equilibrium with the L-type calcium channel protein receptor.

The available volume in the plasma membrane is an important determinant of the concentration of drug available for receptor binding, a parameter highly influenced by membrane cholesterol content in an inverse fashion. This relationship between membrane cholesterol content and drug interactions is dependent, in turn, on the lipophilicity and other physico-chemical properties of the compound. Reduction in membrane available free volume, and hence drug-membrane partitioning, can also be accomplished by changes in temperature and degree of acyl chain saturation of the phospholipid constituents.

Dihydropyridine-type CCBs with high affinity for the membrane lipid bilayer under normal and even cholesterol-enriched conditions are characterized by favorable pharmacokinetics, including a slow onset and long duration of activity. These agents, referred to as third-generation CCBs, have effects on membrane function that may help to elucidate differences in their clinical benefit among patients with coronary artery disease as compared with older, less lipophilic members of this class. In prospective, randomized trials, highly lipophilic CCBs have been shown to reduce cardiovascular events in patients with documented coronary artery disease as compared with placebo or less lipophilic CCBs. These pharmacological and clinical observations suggest that the activity of cardiovascular drugs that target receptor sites in vascular membranes may be highly influenced by the concentration and organization of cholesterol in the lipid bilayer as a function of hyperlipidemia.

Conclusion

The use of high-resolution imaging techniques, such as x-ray diffraction and electron microscopy, has provided direct physical evidence for structurally distinct microdomains in the cell plasma membrane. These lipid rafts, which include detergent-resistant membranes and caveolae, consist of lipids with unique lateral diffusion properties and molecular dimensions. Among other functions, lipid domains provide sites for sequestering proteins that mediate signal transduction and NO metabolism in vascular cells. Using small-angle x-ray scattering techniques, we have characterized a novel microdo-
main that is causally linked to atherogenesis and consists of unesterified cholesterol molecules organized into crystalline-like structures. These cholesterol domains disrupt cellular function, influence drug access to membrane receptors, and serve as potential nucleating sites for the formation of extracellular crystals, a hallmark feature of the advanced plaque. Pharmacological agents that interfere with the stability of membrane cholesterol microdomains may have therapeutic benefit by facilitating normal removal of cholesterol by reverse transport mechanisms. Collectively, these findings support an emerging model of the cell plasma membrane that is remarkably complex with respect to function, structure, and molecular organization.

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References

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