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Review

Involvement of lipid rafts and caveolae in cardiac ion channel function

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Abstract

A variety of lipid microdomains, including caveolae, have been shown to play an important role in both protein targetting and in controlling protein–protein interactions. There is increasing evidence for significant ion channel localization in lipid rafts. Cardiac channel subunits known to localize in lipid rafts include Kv1.4, Kv1.5, Kv2.1, Kv4, Kir2, Kir3, K_{ATP}, Nav and Cav subunits. This article reviews what is known about the occurrence and functional significance of cardiac ion channel/lipid raft interactions. Much remains to be learned about this area of potentially enormous importance to cardiac function in health and disease.

Keywords: Potassium channel; Caveolae; Connexins; Sodium channel; Calcium channel

1. Introduction

Ion channels play a critical role in shaping the cardiac action potential (AP), which governs regional electrical activity [1]. The physiological function of ion channels is affected by interactions with proteins that modulate their activity and/or localization.

The fluid-mosaic model of Singer and Nicholson (1972) considered the membrane as a fluid bilayer with homogeneous lipid distribution [2]. The cell membrane contains >2000 species of lipids [3], some of which associate, whereas others are exclusionary [4]. Such behavior leads to formation of distinct lipid structures resulting in "phase separation" [5]. Phospholipids with relatively long saturated acyl chains and cholesterol pack together to create a liquid-ordered phase (Lo), and bulk phospholipids composed of phosphoglycerolipids with polyunsaturated fatty acids make up the more fluid liquid-disordered phase (Ld) [5]. Lo microdomains, called "lipid rafts", float in the Ld phase, like icebergs on the ocean, and have revolutionized our notions of membrane-protein targeting and organization [4,6]. Post-translational protein-modifications like palmitoylation and myristoylation favor protein-localization in lipid rafts [7]. Glycosyl phosphatidyl inositol moieties, known as "GPI anchors" also promote lipid raft localization [8]. Specific transmembrane domain residues govern localization in cholesterol enriched domains and membranes [9]. Protein targetting toward lipid rafts may result from lipid raft specific targeting signals as well as via interactions with other proteins.

The presence of proteins in lipid rafts depends on both lipid and protein content. Current membrane models incorporate clusters of proteins and lipids and consider dynamic remodeling of individual microdomains with protein and lipid inclusion or exclusion followed by microdomain fusion (Fig. 1). Lipid rafts participate actively in signal transduction and cellular adaptation to changing environments.

Small invaginated membrane structures called "caveolae" are the best characterized lipid rafts (Fig. 1). First discovered in the early 1950s using electron microscopy [10], these invaginated membrane structures are enriched in cholesterol

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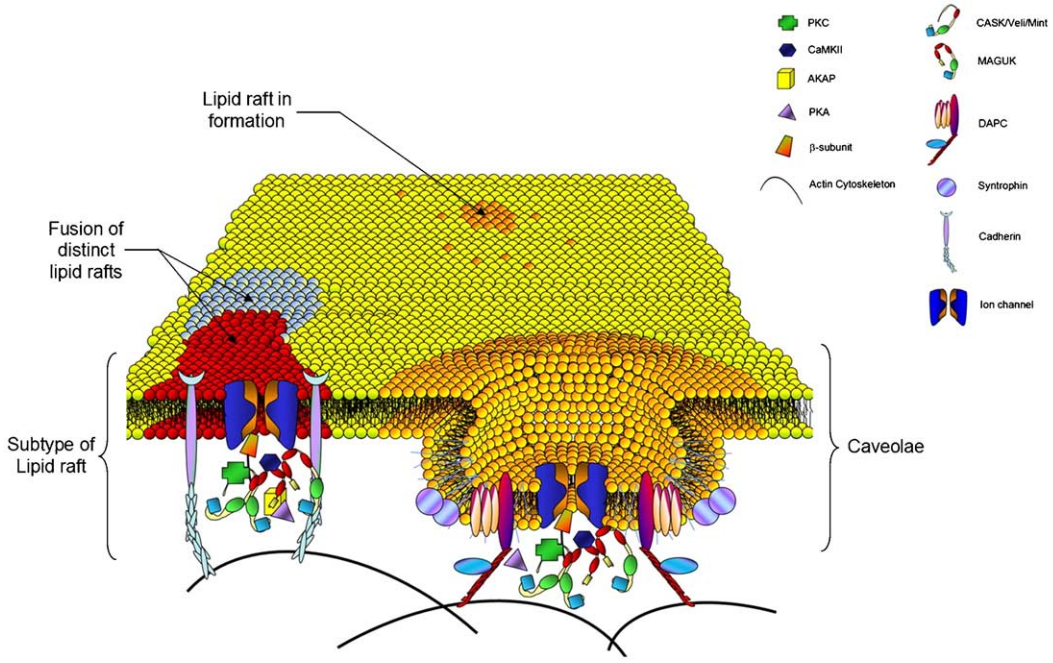

Fig. 1. Schematic representation of lipid raft structures in a plasma membrane. Lipid rafts float in the membrane, constituting distinct signalling platforms depending on lipid raft subtype and composition. While some are invaginated structures (caveolae), others constitute planar plaques. Lipid rafts can fuse, favouring interactions between constituent proteins. Ion channel regulating complexes in cardiomyocytes are also illustrated. DAPCs (Dystrophin Associated Protein Complexes) are anchored in caveolae and stabilize structures interacting with the actin cytoskeleton, whereas other lipid rafts localized at intercalated disks preferentially interact with cadherin complexes for anchorage to the actin cytoskeleton. In both cases, MAGUK proteins favour the clustering of distinct proteins that regulate channel function. For discussion of specific proteins in complexes, see text.

and sphingolipids, along with the small (21–25 kD) cholesterol-binding protein "caveolin" [11]. Caveolin has several isoforms [12] but caveolin-1 and caveolin-3 are the best-characterized. Caveolin-3 is expressed exclusively in myocytes [13]. Caveolins contain a highly hydrophobic 33-amino acid membrane-spanning core. The invaginated caveolar structure results from a core hairpin loop in caveolin [11]. Myocardial caveolin-3 is found in cardiomyocyte t-tubules and sarcolemma but is absent from intercalated disks [14]. Although research on cardiomyocyte microdomains has focused on caveolae, increasing evidence points to heterogeneity of cardiac lipid raft structures [4,15].

2. Technical considerations

2.1. Biochemical approaches

Lipid rafts are insoluble in non-ionic detergents [4]. Cholesterol and sphingolipids are so tightly packed together in lipid rafts that detergents are generally not strong enough to destabilize them or solubilize proteins anchored in them. Lipid raft microdomains can be separated according to their buoyant density with sucrose or Optiprep gradients. Different detergents possess selectivity for specific lipid raft extraction. Triton X-100 and CHAPS are more selective than Tween-20 and Brij58 (Table 1) [16]. Some detergents solubilize lipids and proteins only partially, yielding an incomplete picture of raft composition [17]. Some detergents can provoke lipid raft fusion, favouring non-physiological protein interactions [17]. To avoid these artifacts, detergent-free isolation techniques have been developed [18], involving mechanical disruption followed by buoyant-density gradient separation. Recent modifications avoid artifacts due to associated non-lipid raft proteins [18].

2.2. Imaging approaches

The localization of ion channels and regulatory partners can be observed with immunofluorescence techniques [19]. One limitation of using constitutively localized proteins as lipid raft markers is partial lipid raft population labeling. New tools allow labeling of the whole lipid raft population [19–22]. Fluorescence Recovery After Photobleaching

Table 1
Detergent selectivity profile in MDCK cells

Detergent	Selectivity	DRM cholesterol/GPL ratio	DRM sphingolipid/GPL ratio
Triton X-100	+++	4.6	3.2
CHAPS	+++	4	3.5
Brij 98	++	2.3	2.3
Brij 96	++	1.7	1.7
Brij 58	++	1.3	1.3
Lubrol	++	1.3	1.4
Tween 20	+	0.8	0.8

DRM=detergent-resistant membrane; GPL=glycophospholipid.

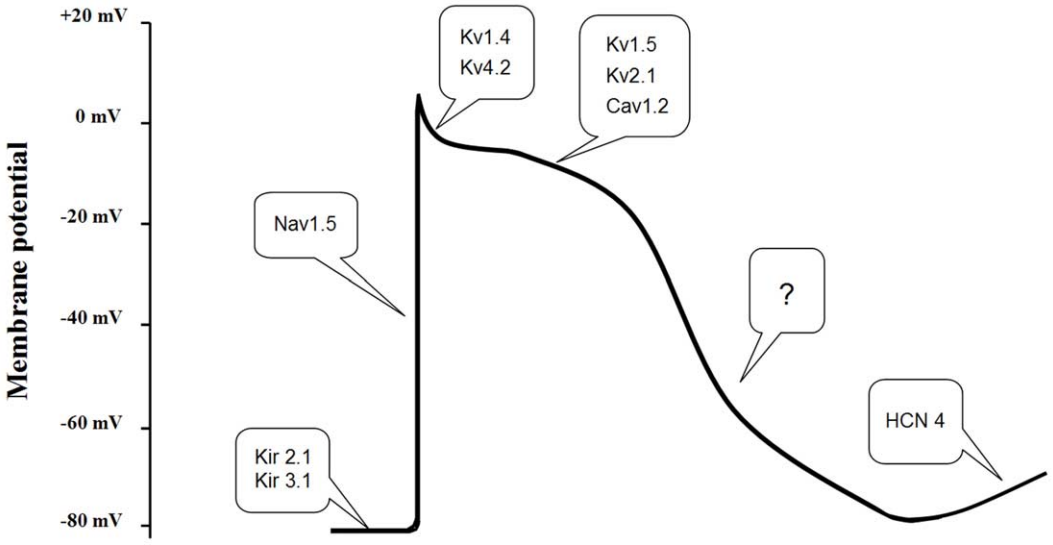

Fig. 2. Illustration of cardiac ion channels known to localize into lipid rafts and their involvement in distinct phases of cardiomyocyte APs. There is still limited knowledge about the lipid raft localization of the subunits (like HERG, KvLQT1 and minK) that control phase-3 repolarization (represented by ?).

(FRAP) is widely used to determine ion channel translational mobility. An early study showed that the membrane-associated guanylate kinase (MAGUK) protein PSD-95 immobilizes Kv1.4 channels at the plasma membrane [23]. Increasing evidence suggests that mobility depends on ion channel localization [24,25]. Caveolae are highly immobile microdomains [26]. Although lipid rafts exhibit distinct rates of lateral diffusion, diffusional mobility is more correlated with membrane anchorage than with raft association [27].

2.3. Functional approaches

Another way to study lipid raft ion channel targeting is to analyze ion channel function after disruption of raft structure. Several reagents have been used (Table 2). A frequently used technique is membrane-cholesterol depletion with methyl-β-cyclodextrin (MCD) [28]. MCD possesses high affinity for cholesterol, removing it from membranes and causing lipid raft disruption [29].

3. Evidence for ion channel localization in lipid rafts/caveolae

A wide range of cardiac ion channel subunits have been localized to lipid rafts, as detailed below. Fig. 2 illustrates the role of these subunits in cardiac APs.

3.1. K^+ channels

K^+ channels are key regulators of the resting membrane potential, which governs excitability, of diastolic conductance, which affects pacemaking function, and of AP repolarization, which determines AP duration and susceptibility to a variety of arrhythmia mechanisms.

3.1.1. Shab-family (Kv2)

The first reported cardiac lipid-microdomain ion channel compartmentalization was for Kv2.1 channels. Kv2.1 localized preferentially into the low buoyant-density sarcolemma fraction after cold-extraction with 1% Triton X-100, indicating anchoring in cholesterol-enriched microdomains [30]. Distinct localization patterns for caveolin-1 and Kv2.1 suggest anchoring in non-caveolar lipid rafts [30]. Colcemide, which disrupts microtubules and causes caveolin internalization, did not affect Kv2.1 localization but dramatically altered caveolin-1 localization [30]. In pancreatic β-cells, lipid raft disruption causes a hyperpolarizing shift of Kv2.1 inactivation [31].

3.1.2. Shaker-family (Kv1)

Kv1.5-subunits underlie an important repolarizing current (I_{Kur}) in atrial myocytes [32]. Kv1.5 function is regulated by various associated proteins, including Kvβ−subunits [33], MAGUK proteins [34,35] and protein

Table 2
Chemicals used for disruption of lipid raft structure

Molecule	Effect	Comments	References
5-methyl-β-cyclodextrin	Cholesterol depletion	Lipid raft disruption	[24]
2-hydroxypropyl-β-cyclodextrin	Cholesterol depletion	Lipid raft disruption	[24]
Fumonisin B	Shingolipids biosynthesis inhibitor	Lipid raft disruption	[24]
Sphingomyelinase	Sphingolipid disruption	Lipid raft disruption	
Cytochalasin D	Cytoskeleton disruption	Caveolae endocytosis	[26]
Colcemide	Cytoskeleton disruption	Caveolae endocytosis	[35]

kinases [36]. Like Kv2.1, Kv1.5 channels are found in low-density Triton X-100 insoluble fractions [24]. In contrast to Kv2.1, Kv1.5 localization follows caveolin-1 after microtubule disruption-induced caveolar internalization [24]. Treatment with MCD slightly shifts Kv1.5 voltage-dependent activation and inactivation [24]. Association of the MAGUK protein SAP97 with Kv1.5 channels in lipid rafts accelerates C-type inactivation. Acceleration of inactivation is abolished by lipid raft disruption with MCD and recovers with cholesterol replacement [37]. SAP97 acts as a scaffolding protein, favoring Kv1.5-interaction with lipid-microdomain regulatory proteins. A SAP97-caveolin-3 complex in COS-7 cells recruits Kv1.5-subunits via multiple protein–protein interactions [38]. In atrial myocardium, Kv1.5 channels localize in intercalated disks, which lack caveolin-3. Kv1.4 channels have also been found in cholesterol-enriched Triton X-100 insoluble fractions in neurons and HEK cells [39], but not in pancreatic β-cells [31]. This may be due to a requirement for another MAGUK protein, PSD-95, for Kv1.4 micro-localization [39].

3.1.3. Shal-family (Kv4)

Kv4.2/4.3 is the molecular basis for the Ca^{2+}-independent transient outward K^+ current (I_{to}), which is activated immediately after the cardiac AP upstroke. There is evidence for lipid raft Kv4.2-localization in rat brain and transfected HEK 293 cells [39]. Kv4.2 requires PSD-95 for targetting to the Triton X-100 insoluble fraction. Removal of the putative PDZ-interacting sequence, which permits channel-binding to MAGUK proteins, diminishes Kv4.2 channel lipid raft localization [39]. Thus, other PDZ-domain proteins may be needed for Kv4.2 lipid raft localization. KChIP is a good candidate, since KChIP profoundly affects Kv4.2 intracellular trafficking and function [40]. Palmitoylation, required for efficient Kv4.3–KChIP interaction [41], also favors lipid raft localization [4].

3.1.4. Inwardly rectifying K^+ channels

3.1.4.1. Kir2. Kir2-subunits are essential for the primary inward-rectifier conductance controlling the resting membrane potential in working myocardium. There is presently no biochemical evidence for Kir2-localization into Triton X-100 insoluble membrane fractions. However, membrane cholesterol content modulates Kir2.1 current in aortic endothelial cells [42]. Increased plasma membrane cholesterol decreases Kir2.1 current density, whereas cholesterol-depletion increases current density [42]. Single channel properties of Kir2.1 are not modified, suggesting that cholesterol modulates the number of active membrane Kir2.1 channels [42].

3.1.4.2. Kir3. Kir3 subunits underlie the G-protein-coupled acetylcholine-dependent current that is a key modulator of heart rate. Kir3.1 subunits co-precipitate with Gβγ, PKAc, PP1c and PP2A [43]. Most of these localize into lipid rafts [44]. In neurons and CHO cells, Kir3.1 channels localize in low buoyant-density fractions following extraction with 1% Triton X-100 and sucrose-gradient separation [45].

3.1.4.3. ATP-sensitive K^+ current (K_{ATP}). K_{ATP} channels serve as endogenous homeostatic transducers, balancing cellular resources and metabolic demands. Cardiac K_{ATP} channels protect against the metabolic insult of ischemia and contribute to adaptive responses to metabolic stress [46]. K_{ATP} channels regulate vascular delivery of metabolic resources [47]. While ATP-sensitive K^+ channels in the adult heart classically consist of Kir6.2 and SUR2A-subunits, Kir6.1, SUR1 and SUR2B-subunits are also expressed in adult mouse hearts [48].

Pancreatic β-cell Kir6.2 and SUR1-subunits localize to the bulk plasma membrane following detergent-free extraction and separation on sucrose step-gradients [31]. In rat aortic smooth muscle cells, Kir 6.1 colocalizes with an upstream signaling partner, adenylyl cyclase, in caveolin-enriched low buoyant-density fractions after detergent-free extraction and sucrose-gradient separation [49]. Caveolin co-immunoprecipitates with Kir6.1 from arterial homogenates, supporting Kir6.1-caveolin co-localization. Lipid raft structure disruption reduces the cAMP-dependant, protein-kinase A (PKA)-sensitive component of K_{ATP} current, indicating that lipid raft integrity is important for adenylyl cyclase-mediated channel modulation [49].

3.1.5. Ca^{2+}-activated K^+ channels

Recently, high-conductance Ca^{2+}-activated K^+ channels (K_{Ca}) have been shown to contribute to ischemic preconditioning [50,51], primarily in the early trigger-phase [51]. There is no direct evidence for K_{Ca} channel localization in cardiomyocyte-membrane lipid rafts. PKC inhibits [52], whereas PKA activates [53], K_{Ca} channels. Both PKA and PKC appear to localize in caveolae [54,55]. Membrane cholesterol content modulates high-conductance K_{Ca} activation in colonic epithelia [56]. The h*Slo* channel, the molecular equivalent of K_{Ca}, is found in Triton X-100 insoluble fractions of transfected MDCK cells [57].

3.1.6. Summary

Compartmentalization of cardiac K^+ channels illustrates lipid raft heterogeneity. While some channels localize in caveolae, others associate with other lipid raft subtypes (Table 3). These results also point to the differential targetting of channel function and regulation. Finally, some channels require interaction with other proteins, such as MAGUK, for localization in membrane microdomains.

3.2. Na^+ channels

Phase-0 Na^+-flow through voltage-gated Na^+ channels produces activation and electrical-impulse conduction. Nav1.5 sodium channel mutations lead to arrhythmic channelopathies like congenital long QT syndrome, Brugada syndrome, conduction disorders and sudden death [58].

Table 3
Summary of presently recognized cardiac ion channel localization in lipid raft structures

Channel-subunit	Membrane localization	Cell type studied	References
Kv1.4	Non-caveolar lipid raft	HEK cells	[39]
Kv1.4	Bulk plasma membrane	Pancreatic beta-cells	[31]
Kv1.5	Caveolae	L-cells, Ventricular cardiomyocytes	[24,38]
Kv1.5	Non-caveolar lipid raft	Atrial cardiomyocytes	[37]
Kv2.1	Non-caveolar lipid raft	L-cells	[30]
Kv4.2	Subtype of lipid rafts	HEK cells	[39]
Kir2.1	Cholesterol enriched lipid raft	Aortic endothelial cells	[42]
Kir3.1	Subtype of lipid rafts	CHO cells, neurons	[45]
Kir6.1	Cholesterol enriched lipid rafts	Rat aortic SMC	[49]
Kir6.2	Bulk plasma membrane	Pancreatic beta-cells	[31]
K_{Ca} channel	Subtype of lipid rafts	MDCK cells	[57]
Nav1.5	Caveolae	Ventricular myocytes	[14]
Cav1.2	Caveolae	Cardiomyocytes, SMC, Pancreatic beta-cells	[25,31,68,69]
IP3-receptor	Caveolae	Various	[77]
HCN4	Caveolae	Sinus-node cardiomyocytes	[80]
Connexin-43	Caveolae	Alveolar epithelial cells, Various transfected cell types	[25,81,82]

HEK=Human Embryonic Kidney; CHO=Chinese Hamster Ovary; SMC=Smooth muscle cells.

The sympathetic nervous system modulates cardiac Na^+ channel function via β-adrenergic (βAR) pathways [59]. Both *indirect* PKA-dependant modulation and *direct* G-protein modulation occur [60,61]. I_{Na} enhancement occurs without changes in single channel characteristics or voltage-dependent activation [59,62], suggesting that increased current density may be due to increased numbers of functional channels in the sarcolemma [59]. The short time-frame of I_{Na}-increase implies rapid membrane recruitment of Na^+ channels, suggesting a sub-sarcolemmal reservoir. The localization of βARs in cardiac caveolae [63] and the role of lipid rafts in protein-trafficking make them candidates for the location of "recruitable" Na^+ channels [14]. Dialysis of caveolin-3 antibodies into atrial myocytes abolishes "direct" Gαs-induced I_{Na} increases, Na^+ channels co-localize with Gαs in the caveolin-3 enriched fraction, and caveolin-3 co-precipitates and co-localizes with Na^+ channels and Gαs-subunits [14]. These results suggest that tripartite caveolar complexes of caveolin-3, Na^+ channels, and Gαs constitute the reservoir of functional Na^+ channels recruited by βAR activation, and the site of Na^+ channel phosphorylation by activated PKA.

3.3. Ca^{2+} channels

3.3.1. L-type Ca^{2+} channels and Ca^{2+}-release channels

Depolarization-induced Ca^{2+} entry through L-type Ca^{2+} channels (LTCCs) maintains the AP plateau and activates nearby Ca^{2+}-release channels or "ryanodine-receptors" (RyRs) in the sarcoplasmic reticulum (SR), releasing Ca^{2+} via "Ca^{2+}-induced Ca^{2+} release" (CICR) [64] and producing excitation-contraction coupling. In cardiomyocytes, LTCCs are mainly localized within the t-tubular network. This localization is profoundly altered in caveolin-3 null-mice [65]. Several studies suggest that caveolae are responsible for the LTCC/RyR co-localization that underlies CICR. This function may be particularly important in atrial cardiomyocytes, whose t-tubular system is less developed than in ventricular cells. The most efficacious Ca^{2+}-signal for SR Ca^{2+}-release may be a transient microdomain of high $[Ca^{2+}]_i$ beneath individual, open LTCCs [66]. High-$[Ca^{2+}]_i$ domains may be more easily obtained with invaginated structures like caveolae [67,68], although the precise role of microdomain-structure in Ca^{2+}-dynamics awaits clarification. Cardiomyocyte cholesterol depletion decreases the frequency, amplitude and width of Ca^{2+}-sparks [68]. In pancreatic β-cells, Cav1.2, the pore-forming subunit of LTCCs, is found in low buoyant-density membrane-fractions enriched in caveolin [31]. Moreover, the α1-chain is preferentially found in caveolin-rich detergent-insoluble fractions of smooth muscle cells and cardiomyocytes [25,69].

3.3.2. IP_3 receptors

Inositol-1,4,5-trisphosphate receptors (IP_3-receptors) are intracellular Ca^{2+} channels releasing Ca^{2+} from the sarcoplasmic reticulum. Type 1 and 2 IP_3-receptors are found in the heart, predominantly in atria [70]. Their role is controversial [71]. IP_3-dependant Ca^{2+}-release likely enhances atrial Ca^{2+}-signaling, exerting positive inotropic effects, and may contribute to Ca^{2+}-dependent atrial arrhythmias [72].

IP_3 receptors interact with ankyrin-B [73]. Ankyrins are membrane-associated adapter proteins [74] that bind other proteins via membrane-binding domains. Ankyrin-B knockout mice show mislocalization of cardiac IP_3-receptors, suggesting an essential role for ankyrin-B in their targeting [75]. Ankyrin-B mutations produce long-QT syndrome arrhythmias and sudden death, to which IP3-receptor dysfunction could contribute [76].

IP_3-receptors localize to Triton X-100 insoluble caveolin-enriched fractions and electronic-microscopy localizes IP3-receptors to caveolae-like invaginated structures [77]. A link between ankyrin-B and lipid-microdomain localization of IP_3-receptors is suggested by the following evidence [78]: In aortic endothelial cells, hyaluronan increases the number of

CD44 receptors in the Triton X-100 insoluble caveolin-rich fraction. Immunoprecipitation of the caveolin-rich fraction results in co-precipitation of ankyrin-B, IP_3-receptors and CD44, but in the absence of hyaluronan neither ankyrin-B nor IP_3-receptors co-precipitate with CD44. Lipid raft disruption by cholesterol-depletion inhibits IP_3-receptor association with caveolae and suppresses Ca^{2+}-signaling. Thus, physical association between CD44-containing lipid rafts and IP_3-receptors may be important for triggering Ca^{2+}-release from internal stores.

3.4. Hyperpolarization-activated, cyclic-nucleotide binding channels (HCNs)

The hyperpolarization-activated non-selective cation current, first called "funny current" or I_f, is believed to be important in cardiac automaticity. I_f is carried by HCN-subunits, with HCN4 particularly important in the heart [1]. HCN4-subunit function is finely controlled by βAR and muscarinic-cholinergic regulation. Most proteins in the βAR-pathway are localized in lipid rafts and sinus-node cardiomyocytes are rich in caveolae [79]. HCN4-subunits are found in low-density fractions of sinus-node cardiomyocyte preparations containing flotilin and caveolin [80]. Lipid raft disruption alters HCN4-subunit localization, shifts I_f-activation in the depolarizing direction and reduces deactivation, accelerating diastolic depolarization and heart rate [80].

3.5. Connexins

Myocytes are linked via gap junctions to form an electrically continuous syncitium that permits AP propagation. Gap junctions contain hemi channel connexin proteins which subserve their electrical function. Cardiomyocytes actively adjust their coupling by changes in connexin expression, regulation of connexin trafficking and turnover, and modulation of connexin channel properties [81].

Connexin-43, the major cardiac isoform, co-localizes with caveolin-1 in low buoyant-density Triton X-100 insoluble fractions and co-immunoprecipitates with caveolin-1 [25,81,82]. Caveolin is not found in myocardial intercalated disks, so other lipid raft structures may produce connexin-43 localization to in vivo intercalated-disk microdomains [83,84]. The MAGUK protein ZO-1, a major connexin-interacting protein, mediates delivery of connexin-43 from lipid raft domains to gap-junctional plates [85]. There is increased association of ZO-1 with connexin-43 during cardiac gap-junction remodeling [86].

4. Lipid rafts as a scaffolding structure for ion channel regulation

Ion channel biophysical properties are determined by their 3-dimensional structure and related interactions with regulatory proteins. Structures that include channel-subunits and regulatory proteins have been termed "channelosomes". Channelosome structure depends on the microdomains that incorporate protein constituents. MAGUK proteins appear crucial in forming channelosomes. While PSD-95 is required for Kv1.4 localization in lipid rafts, SAP-97 serves a similar role for Kv1.5 [37–39]. SAP-97 and PSD-95 are also part of Kir2-containing complexes. [87]. These proteins can bind other scaffolding proteins, such as CASK, Veli and Mint, forming the skeleton of the channelosome architecture [88–90]. CASK is present in lipid rafts and the multiprotein complex SAP97/CASK/Veli/Mint1 is essential for trafficking and plasma membrane localization of Kir2 channels [87,91]. CASK interacts with the N-type Ca^{2+} channel [92]. Such proteins bind directly to ion channels, offering protein-binding surfaces such as PDZ-domains and recruiting regulatory proteins to intracellular sites [93]. For instance, SAP-97 favors Kvβ–Kv1.5 association in lipid rafts, increasing C-type inactivation [37]. Kvβ may also recruit PKC to the channel complex by interacting with the PKCζ-interacting (ZIP) protein [94]. SAP-97 and PSD95 also bind type-II Ca^{2+}-activated calmodulin-kinase (CAMKII) [95,96], which regulates numerous ion channels as a function of intracellular Ca^{2+} concentration. Most channels regulated by CAMKII localize in lipid rafts, including Kv1.5, Na^+ channels, LTCCs and IP_3-receptors [97–102], and CAMKII localizes to lipid rafts [103,104].

PSD-95 and SAP-97 also bind A-kinase anchoring proteins (AKAPs), which localize PKA in close proximity to ion channels. PKA is found in lipid rafts [105,106]. KCNQ1, the pore-forming subunit of the cardiac repolarizing current I_{Ks}, associates with both PKA and protein-phosphatase 1 (PP1) through the protein yotiao, promoting sympathetic regulation [107]. The interaction of AKAP with PKA is required for cAMP-dependent regulation of LTCCs [108]. AKAPs interact with the β_2AR, favoring localization of the β_2-adrenergic pathway within channelosomes [109]. Stimulation of β_2ARs increases cardiac contractility without globally increasing cAMP [110], suggesting that LTCCs and the β_2AR transduction pathway share a privileged signaling microenvironment. LTCCs co-assemble with β_2ARs, $G_{\alpha s}$, adenylyl-cyclase, PKA and phosphatase–PP2A [111]. In adult cardiomyocytes, β_2ARs are found exclusively in low buoyant-density fractions enriched in caveolin-3, together with Gαs and adenylyl-cyclase [112].

5. Interactions with cytoskeletal proteins

Kir2 channels are associated with the dystrophin-associated protein complex (DAPC) [87]. DAPC forms a structural link between the actin cytoskeleton and the extracellular matrix and is especially prevalent in muscle cells [113]. Caveolin-3 null mice show important changes in DAPC distribution and t-tubule abnormalities [65].

Caveolin-3 associates with dystrophin and interacts directly with the C-terminal tail of β-dystroglycan, part of the DAPC [114]. Dystroglycan also regulates caveolin-3 distribution [115]. In *mdx* mice deficient in dystrophin [116], mini-dystrophin restores LTCC current in skeletal-muscle myocytes, suggesting that dystrophin influences LTCCs [117]. Dystrophin and α-actinin regulate caveolae by anchoring them to the actin cytoskeleton. Na^+ channels, which localize in cardiomyocyte t-tubular caveolae, interact specifically with syntrophin, another constitutive DAPC protein [118].

N-cadherin and catenins are principally found in the low buoyant-density fractions of myoblast plasma membranes [119]. SAP97, which co-localizes with Kv1.5 channels at the intercalated disk, also binds cadherin [34,37]. Connexin-43, which is found in intercalated-disk lipid rafts, is also associated with the N-cadherin multiprotein complex. Cell-surface expression of connexin-43 requires N-cadherin and vice versa [120].

6. Pathophysiological implications

Structural remodeling of caveolae occurs in cardiac pathologies. The cardiac distribution of caveolin-3 is dramatically altered by heart failure, with an increased proportion of caveolin-3 in the detergent-soluble fraction [121]. Caveolin-3 knock-out mice exhibit progressive cardiomyopathy with hypertrophy, dilation, and reduced fractional shortening [122]. Caveolin-3 over-expression induces cardiac degeneration, fibrosis and cardiac-function impairment, along with DAPC downregulation [123]. ANP is found in rat-cardiomyocyte caveolae [124]. ANP modulates cardiac hypertrophy and remodeling; ANP deficiency exaggerates hypertrophy and remodeling after pressure overload [125]. ANP binds to caveolar B-type ANP receptors, which possess guanylyl cyclase activity. cGMP formation leads to cGMP-dependent kinase activation, which can inhibit LTCC activity and thereby reduce contractility [124]. Furthermore ANP modulates I_f in human atrial cardiomyocytes [126]. Thus, caveolar integrity may be important in myocardial adaptation to mechanical overload. Lipid rafts and caveolae are acutely affected by mechanical events. Mechanical overload can also induce membrane fusion of caveolae [127].

Statins, which directly act on the cholesterol synthesis pathway, are widely used for the prevention of cardiovascular disease. Recent studies showed that statin therapy affects caveolar turnover by limiting their endocytosis [128]. Atorvastatin has also been shown to modulate the cholesterol enrichment of the lipid rafts [129]. Thus, many effects of these molecules may be due to lipid raft modification.

Given the evidence for ion channel localization in lipid rafts, pathological modifications in lipid raft structures and distribution may underlie ion channel dysfunction and associated arrhythmias in conditions like hypertension, diabetes, ischemic heart disease and heart failure.

7. Conclusions

Lipid rafts constitute dynamic platforms that float in cardiomyocyte membranes. Enriched in signaling molecules and ion channel regulatory proteins, the various forms of lipid rafts exhibit distinct protein populations and membrane-anchoring mechanisms. Thus, they can actively participate in differential cardiomyocyte ion channel targeting and regulation. At present, little is known about the role of lipid rafts in cardiac dysfunction and arrhythmias. Recent technical advances will help to further our understanding of these important membrane structures and their role in cardiac health and disease.

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