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Review

Adrenergic regulation of cardiac ionic channels ☆
Role of membrane microdomains in the regulation of kv4 channels

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ABSTRACT

The heart must constantly adapt its activity to the needs of the body. In any potentially dangerous or physically demanding situation the activated sympathetic nervous system leads a very fast cardiac response. Under these circumstances, α1-adrenergic receptors activate intracellular signaling pathways that finally phosphorylate the caveolae-located subpopulation of Kv4 channels and reduce the transient outward K^+ current (I_{to}) amplitude. This reduction changes the shape of the cardiac action potential and makes the plateau phase to start at higher voltages. This means that there are more calcium ions entering the myocyte and the result is an increase in the strength of the contraction. However, an excessive reduction of I_{to} could dangerously prolong action potential duration and this could cause arrhythmias when the heart rate is high. This excessive current reduction does not occur because there is a second population of I_{to} channels located in non-caveolar membrane rafts that are not accessible for α1-AR mediated regulation. Thus, the location of the components of a given transduction signaling pathway in membrane domains determines the correct and safe behavior of the heart. This article is part of a Special Issue entitled: Reciprocal influences between cell cytoskeleton and membrane channels, receptors and transporters. This article is part of a Special Issue entitled: Reciprocal influences between cell cytoskeleton and membrane channels, receptors and transporters. Guest Editor: Jean Claude Hervé.

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1. Introduction

When working, exercising or simply sleeping, the heart must adapt its activity in order to fulfill the needs of the body. The sympathetic nervous system plays a key role in regulating the heart rate and the ventricular contraction force. The sympathetic neurotransmitter norepinephrine activates intracellular signaling pathways that affect the cardiac ionic channels and thereby changes the electrical behavior of the heart. However, as some components of the transduction systems can be preassembled, the signaling mechanism depends on the integrity of the cytoskeleton. Moreover, it also depends on the precise location of the channels within the cell membrane. Thus, for Kv4 channels activation of α_1-adrenoceptors only regulates the caveolae located population.

In this review we will describe some of the effects of sympathetic innervation on the electrical activity of the heart, which are determined by changes in the electrical activity of every single cardiac cell, which in turn are due to changes in the behavior of each individual ionic channel. We will focus on the regulation of the transient outward potassium current, I_{to}, by norepinephrine discussing how cytoskeleton-mediated compartmentalization provides signal specificity.

1.1. Adrenergic regulation of cardiac ionic channels

In cardiac myocytes, the action potential is divided in phases 0 to 4. The inward Na^+ current (I_{Na}) is responsible for the rapid upstroke or phase 0 of the action potential. The transient outward potassium current, I_{to}, is responsible for the initial rapid repolarization or phase 1. The inward L-type calcium current ($I_{Ca\text{-}L}$), counterbalanced by the rapid and slow delayed rectifiers I_{Kr} and I_{Ks} are responsible for the plateau or phase 2. The rapid final repolarization or phase 3 is due to the closing of Ca^{2+} channels and the increase in K^+ currents. Finally, the inward rectifier I_{K1} is responsible for the maintenance of the resting membrane potential or phase 4.

The sympathetic innervation of the heart affects the shape and duration of the cardiac action potential as it regulates the ionic currents involved in the repolarization. Thus, norepinephrine reduces the amplitude of the transient outward potassium current through α1-adrenoceptors (α1-AR) [1] and therefore reduces the phase 1 of the cardiac action potential. As a consequence, there is a larger calcium influx into the cell during the subsequent plateau that increases the force of the contraction [2]. On the other hand, stimulation of β-adrenoceptors (β-AR) is necessary to maintain I_{to} current at physiological levels [1].

The sympathetic nervous system also regulates the Phase 2 of the cardiac action potential, affecting the flow of both potassium and calcium ions. In fact, perhaps the best known effect of β-AR stimulation in the heart is the activation of the cascade of Gαs protein and adenylyl cyclase that leads to the phosphorylation of the Cav1.2 channel by protein kinase A (PKA), which increases the inward L-type calcium current and therefore the force of contraction [3]. The A kinase Anchoring Proteins or AKAPs might help these components to differently associate in separated membrane microdomains of the cardiomyocyte [4]. The increase in the magnitude of ICa-L would prolong the duration of phase 2. However, this effect is counterbalanced by the effect on K^+ currents.

Regarding the slow delayed rectifier I_{Ks}, formed by the Kv7.1 channel together with the accessory protein KCNE1 or mink [5,6], the sympathetic innervation increases current amplitude and therefore shortens the repolarization. Again, this effect is caused by the stimulation of β-AR, Gαs protein, adenylyl cyclase and the phosphorylation of the channel by PKA. In this intracellular pathway receptor, channel, PKA and protein phosphatase 1, that is the phosphatase that reverts the phosphorylation, are associated to form a supramolecular complex brought together through the anchoring protein Yotiao [7,8].

Last, the sympathetic regulation of the rapid delayed rectifier I_{Kr}, driven by the HERG channel, is more elusive. In heterologous expression systems norepinephrine causes opposite effects depending on the concentration and receptor type. Initially, stimulation of β-AR triggers the phosphorylation of HERG channel by PKA, which together with cAMP direct interaction with an intracellular CNBD (Cyclic Nucleotide Binding Domain) of the channel reduces the amplitude of the I_{Kr} current. However, the subsequent binding of the phosphorylated channel to the 14-3-3 protein increases current amplitude [9–11]. Similarly, the regulation of the channel upon α1-AR stimulation is also confusing. Apparently, stimulation of the α1-adrenergic receptor might inhibit the channel by a mechanism involving channel phosphorylation. However, the deletion of most of the phosphorylation consensus sites for Ser/Thr protein kinases does not reduce the effect on the channel [12].

In the global picture, both inward Ca^{2+} and outward K^+ currents are increased after sympathetic innervation but the effect on K^+ repolarizing currents is stronger. That means that in the sympathetic stimulated heart the repolarization is faster and the action potential duration is shorter. This is required for the heart to beat at a higher frequency.

2. The transient outward K^+ current, I_{to}

2.1. Molecular correlates of the transient outward K^+ current, I_{to}

The transient outward is a fast activating and rapidly inactivating potassium current (Fig. 1), responsible for the phase 1 of the cardiac action potential, the initial rapid repolarization from the peak of the action potential until the plateau level [13].

There are at least two different phenotypes of I_{to} in the mammalian hearts that differ in the velocity of recovery from inactivation. I_{to} fast ($I_{to,f}$) has a recovery of inactivation rate of 70 ms approximately, whereas I_{to} slow ($I_{to,s}$) recovers in 1 s. These two phenotypes indicate that there are at least two types of I_{to} channel forming proteins or "α subunits". Several genes of the Kv (Voltage-dependent K^+ Channels) family encode I_{to} channels: *KCNA4*, *KCND2* and *KCND3* genes [14–17]. *KCNA4* gene encodes the Kv1.4 channel responsible for the $I_{to,s}$ with the characteristic slow recovery rate that is found in rabbit ventricle [18] and human endocardium [19]. However, $I_{to,f}$ is the predominant phenotype [20] and is currently accepted that *KCND2* and *KCND3* genes encode the Kv4.2 and Kv4.3 proteins, responsible for the $I_{to,f}$ channels in rat cardiomyocytes [16,21,17].

In rat ventricle, the transient outward K^+ current is generated through two different channels: a homotetramer formed by Kv4.3 channels in endocardium and a mixture of Kv4.2 and Kv4.3 channels in epicardium. In humans, I_{to} is mainly driven by Kv4.3 channels because that is the only isoform expressed in enough amounts [17]. Like in other species, in human ventricle the I_{to} current gradient between endocardium and epicardium is observed. Since the expression of Kv4.3 channel is homogeneous throughout the ventricle, the

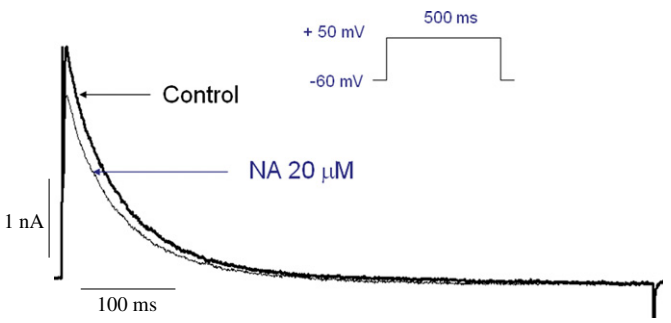

Fig. 1. I_{to} current recording from a ventricular cardiomyocyte. The cell was maintained at a holding potential of −60 mV and depolarized during 500 ms to +50 mV. The current activates very fast, in few milliseconds, and inactivates rapidly. Noradrenaline (NA) reduces the current amplitude in a concentration dependent manner.

responsible for the transmural variability of the current might be the regulatory subunit KChIP2 [22].

2.2. Adrenergic regulation of the transient outward K^+ current, I_{to}

Norepinephrine has different effects on I_{to} current. Thus, the basal β-AR stimulation has a trophic effect that ensures I_{to} expression at physiological levels, whereas the acute α1-adrenergic receptors (α1-AR) stimulation decreases the current amplitude [1].

2.2.1. β_2-adrenoceptor mediated trophic effect on I_{to}

β-adrenoceptors are major regulators of cardiac physiology as they mediate catecholamine-induced inotropic, chronotropic and lusitropic responses. Therefore, β-ARs transduction pathways have been extensively studied.

Although they differ in physiological roles and pharmacological properties, both β1 and β2-ARs activate the classic Gαs-AC-cAMP-PKA pathway, whereas only β2 activate also Gαi protein [23,24]. Our group has found that this dual coupling of β-ARs to Gαs and Gαi proteins described in cultured cells [23,25–27] is in fact active in rat isolated adult cardiomyocytes [28]. Thus, in cardiac myocytes β2-AR stimulation activates the Gαs protein, adenylyl cyclase and PKA. The PKA-phosphorylated receptor then switches to a Gαi protein whose βγ complex activates β-ARK1. Once dually phosphorylated by PKA and β-ARK1, the β2-AR internalizes in a process that requires Arrestin [26,27]. The internalized β2-Adrenoceptor-Arrestin complex recruits cSrc and activates Ras, which thereby activates MEK1/2 and finally ERK1/2. We recently found a physiological function for this intracellular pathway [28], where the MAPK cascade modulates the Kv4.2 and Kv4.3 protein levels in the cell membrane, and therefore I_{to} current amplitude, in response to tonic β-adrenergic stimulation (Fig. 2).

It is noteworthy that the increase in I_{to} current amplitude induced by the activation of the MAP kinase cascade is due to an increased channel protein synthesis [29], and this effect depends on the integrity of the cytoskeleton, probably in order to allow the translocation of newly formed channels to the plasma membrane [30].

2.2.2. α_1-adrenoceptor mediated reduction on I_{to} current

Several studies have reported that acute noradrenergic stimulation reduces the transient outward potassium current amplitude in different models (Fig. 1). The current reduction is due to α1-receptor activation, since α1-adrenergic blocker prazosin, but not the β-blocker propranolol completely prevents the effect [31,32].

α1-adrenergic receptors classically couple to the Gαq protein-PLC pathway. However, in heterologous expression systems they can alternatively couple to Gαs proteins [33,34]. Moreover, in rat cardiomyocytes, the mechanism responsible for the α1-adrenoceptor induced I_{to} reduction requires the activation of Gαs protein. This coupling activates the cAMP/PKA pathway, which finally phosphorylates the I_{to} channel (Fig. 3) and reduces the amplitude of the transient outward potassium current up to a 35% [32,35].

The reduction of the amplitude of the I_{to} current induced by noradrenaline is concentration dependent and saturates with 30 μM norepinephrine [32]. This result is surprising and arises some questions like why does noradrenaline reduce I_{to} current only a third; why do not higher noradrenaline concentrations fully abolish I_{to} current; or how does α1-AR stimulation activate PKA without affecting i.e. L-type calcium channels. In addition, if cAMP/PKA intracellular pathway phosphorylates the I_{to} channel, why does β-adrenoceptors stimulation have no effect on I_{to}? These questions will be discussed below.

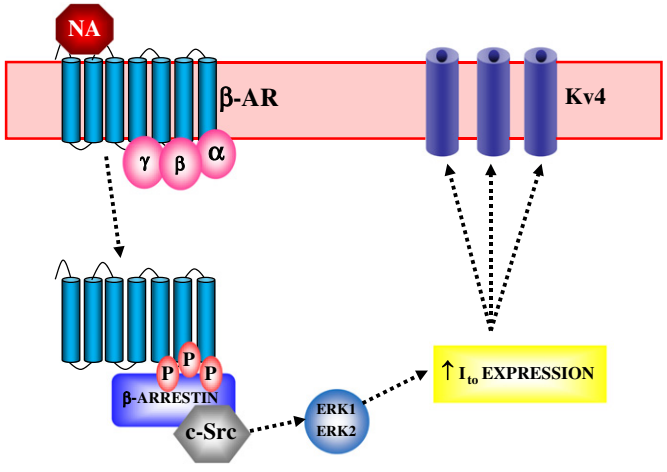

Fig. 2. Intracellular pathway connecting the β-Adrenoceptor activation with the I_{to} channel expression through the activation of the MAP kinase cascade by the internalized receptor. In cardiac myocytes β_2-AR sustained stimulation results in receptor desensitization and internalization in a process that requires Arrestin. The β_2-Adrenoceptor-Arrestin complex recruits cSrc and activates Ras, which thereby activates MEK1/2 and ERK1/2. Finally, the MAPK cascade modulates the Kv4.2 and Kv4.3 protein levels in the cell membrane, and therefore I_{to} current amplitude, in response to sympathetic stimulation.

3. Compartmentalization mechanisms

Traditionally, the interaction Receptor-G Protein-Efector was believed to be based on random collision of its components in the Fluid Mosaic of the membrane. But this model does not fit with what today is known as the non-randomness of the membranes. A clear example of that is the regulation of I_{to} current by adrenergic receptors [35].

α1- and β-adrenergic receptors activate the cAMP/PKA intracellular pathway, but only α1-adrenoceptor activation phosphorylates the I_{to} channel. This pathway requires a large number of proteins interacting together in milliseconds. One of the proteins involved is adenylate cyclase, which accounts for 0.001% of the membrane proteins, making its random collision with other involved molecules unlikely in the milliseconds frame. It is therefore necessary the preassembly of the components of the signaling as this would accelerate the response at the same time that separates responses carried by same effectors, but triggered by various agonists [36,37]. Some of the mechanisms involved in the compartmentalization of these signals include AKAPs and membrane rafts [38,39].

3.1. AKAPs

In cardiac muscle cells, the cAMP-dependent PKA has multiple substrates like L-type Ca^{2+} channels in the sarcolemma [40,41], ryanodine receptors, phospholamban and Cl^- channels in the sarcoplasmic reticulum [42,43] and troponin I in the thick filaments [42]. The need for compartmentalization of PKA in the heart was proposed more than 30 years ago [36,37]. It is currently known that a way of orchestrating cAMP signaling is to maintain local populations of PKA in an inactive form through its association with A-kinase-anchoring proteins. These proteins bind to PKA and direct the enzyme to specific targets by binding also to the cytoskeleton, membranes or organelles [44]. In fact, AKAPs form multifunctional complexes in a way that might ensure the proximity of the various components of a signal transmission system. Moreover, AKAPs may integrate different signaling pathways thereby allowing the reversibility of the signal, for instance, directing the phosphorylation and then the desphosphorylation of a given substrate [45]. Thus, compartmentalization of PKA by AKAP is an important mechanism of regulation of adrenergic signaling in the heart.

3.2. Membrane rafts and caveolae

On the other hand, there are membrane microdomains, the membrane rafts, where different receptors co-localize with components of intracellular signaling cascades. Rafts host, in a very selective

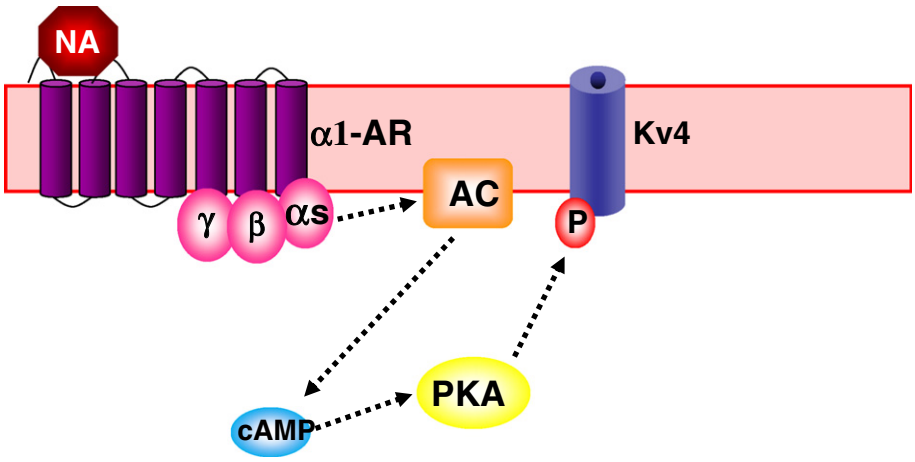

Fig. 3. Intracellular pathway connecting the α_1-Adrenoceptor activation with the I_{to} channel phosphorylation and subsequent reduction of current amplitude. Activated α_1-AR couples to a $G\alpha_s$ protein and activates the cAMP/PKA signaling cascade, which leads to Kv4 channels phosphorylation and reduction of I_{to}.

way, a large number of molecules including enzymes involved in the regulation of ionic channels [46]. Basically, membrane rafts are rich in cholesterol and sphingolipids, which give them a stiffer consistency than the rest of the membrane [47]. However, based on its structure and the presence of specific markers there are different types of rafts and, among them, caveolae are the best studied.

Unlike other membrane rafts, caveolae form invaginations in the plasma membrane of approximately 50–100 nm size (Fig. 4). They are especially abundant in the cells of the cardiovascular system including endothelial and smooth muscle cells, cardiac myocytes and fibroblasts [48]. The hallmark of caveolar microdomains is the presence of caveolin [49], the protein responsible for the characteristic invaginated morphology.

Caveolae concentrates a wide variety of molecules involved in cell signaling, such as G protein coupled receptor, protein kinases, ionic channels [50] and even the endothelial nitric oxide synthase, an enzyme that is not found in other areas of the plasma membrane [51]. Some of these proteins are bound to caveolin through the caveolin scaffolding domain (CSD). This binding is important because keeps the enzyme in an inactive state until the right activator appears and releases the inhibition [52,53]. Caveolin-3 is the isoform expressed in cardiac myocytes and in skeletal muscle fibers [54,55] and different studies have demonstrated that both the deficit of caveolin-3 as its overexpression triggers cardiac pathologies [56–58].

One of the first molecules identified in caveolae was the α subunit of the G protein [52], A distribution analysis of G protein subunits in cardiomyocytes shows that whereas proteins Gαi/0 and Gαq are highly concentrated in caveolar regions, Gαs proteins and βγ subunit locate equally in and out caveolae [59,60]. Similarly, adenylyl cyclase and PKA are also found in caveolae in cardiomyocytes [59,61–63]. As mentioned above, when in caveolae G proteins, adenylyl cyclase or PKA display an inactive conformation due to its interaction with caveolin. [59,62–64]. Thus, the particular localization of these enzymes provides a mechanism to inhibit their activity in the absence of activators. In addition, palmitoylation processes can help the location of some proteins in membrane rafts and coating of this palmitoylation can help the movement of molecules in and out of the membrane rafts in response to stimulation by agonists [55,65,66].

4. Intracellular transduction components and ionic channels locate in membrane rafts

4.1. Ionic channels in membrane rafts

Ionic channels do not locate randomly along the cell membrane, but rather follow a particular distribution that allows for the correct electrical behavior of the cell. For instance, in cardiac myocytes Kv1.5 channels concentrate in regions of cell-to-cell contact [67]. Moreover, brain ion channels not only show different distribution between axon and dendrites, but also along the dendrites [68].

Most of ionic channels, such as: Kv1.4, Kv1.5, Kv2.1, Kv4, or Cav are located on membrane rafts [69,70,59,71,72] sometimes in a tissue specific manner. Thus, the voltage dependent potassium channels Kv1.5 and Kv4.3 locate in caveolae in mouse L cells and in cardiomyocytes respectively, whereas Kv2.1 channels are found in non caveolar membrane rafts of L cells and neurons [70–72]. The location in rafts facilitates the interaction with different proteins, and directly modulates the activity of these channels. In this sense, the rupture of membrane rafts alters the biophysical behavior of Kv1.5, Kv2.1 or Kv11.1 channels in different cell types [70,71,73].

In some cases, posttranslational modifications such as palmitoylation and myristoylation [74], the fractions of glycosyl phosphatidyl inositol known as “GPI anchors” [75] or transmembrane domains residues [76] favor its localization in membrane rafts. In other cases, the channels are

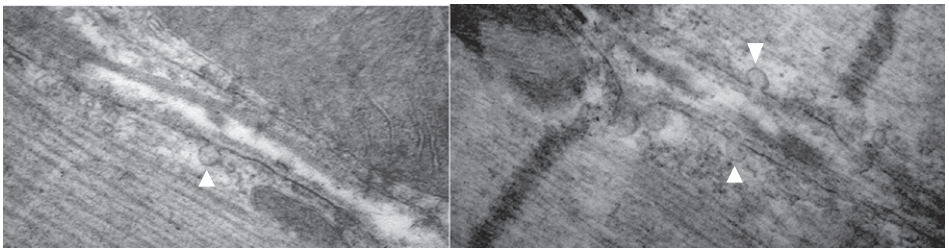

Fig. 4. Caveolae (white arrowheads) in the membrane of cardiac myocytes. Membrane rafts are microdomains enriched in sphingolipids and cholesterol which can concentrate proteins involved in cellular signaling, including receptors, protein kinases and phosphstases and ionic channels. Caveolae are membrane rafts with a characteristic flask shape due to the presence of the protein caveolin.

not able to place themselves in membrane rafts and they must be directed to their final localization by the interaction with other proteins such as other ionic channels or MAGUK proteins [77–79].

4.2. There are two distinct populations of I_{to} channels, one caveolin-associated and another one caveolin-independent

Regarding the localization of I_{to} channel forming proteins Kv4.2 and Kv4.3, both membrane fractionation and coimmunoprecipitation experiments showed that they localize in membrane rafts in rat cardiomyocytes. When membrane microdomains are isolated by centrifugation in density gradients, Kv4 channels are found in low density fractions or membrane rafts and, as expected, raft disruption with cholesterol extracting agents displaces Kv4 channels to high density fractions [72].

However, this method does not distinguish whether or not these membrane rafts are caveolae. Since cytoskeleton disruption leads to caveolae internalization, disrupting agents such as colchicine or cytochalasin are typically used to discriminate between caveolar and non-caveolar membrane rafts [80–82]. When the isolation of membrane microdomains in density gradients is performed in the presence of colchicine, only half of the Kv4 channels migrate to high density fractions, suggesting the existence of two different channel populations, one in caveolae and another one in planar membrane rafts. Coimmunoprecipitation experiments between caveolin and Kv4 channels, as well as electron microscopy immunostaining [72] confirmed the existence of a caveolin-associated and a caveolin-independent population of Kv4 channels (Fig. 5).

In rat ventricular cardiomyocytes, heterotetramers of Kv4.2 and Kv4.3 proteins form the channel responsible for the I_{to} current [83]. However, this raises an important issue in relation to the expression of these channels in caveolae, as Kv4.3 channels are localized in membrane rafts in cardiac myocytes [72], but the localization of Kv4.2 is controversial. Thus, whereas Kv4.2 channels are not found in any type of membrane rafts in mouse L cells or hyppocampal neurons [69], they locate in caveolae of TS201 cells, cardiac myocytes and surprisingly hyppocampal neurons [84,72]. Furthermore, the association of Kv1.4 channel with PSD95 protein, which also associates to the channel Kv4.2, translocates these channels to caveolae [71,84]. This could mean that the Kv4.2 channel is unable to locate in rafts by itself, but can be transferred to rafts by the proteins it associates with. In this sense, is interesting to point out that the Kv.3 protein has a consensus sequence for caveolin binding (ΦxxxxΦxxΦ, aminoacids 165–173) and Kv4.2 does not. Thus, the Kv4.3 could be the scaffolding protein that locates the Kv4.2 channel in membrane rafts in cardiac myocytes.

4.3. Adrenergic receptor location in membrane rafts

Although PKA is a broad-specific kinase activated by cAMP and a number of agonists increase cAMP and activate PKA, they have very different cellular effects [85,86]. This suggests that the α1-AR/PKA/I_{to} pathway can be compartmentalized and limited to specific membrane regions. In fact, the cAMP increase and the PKA dependent phosphorylation of the I_{to} channels after α1-AR stimulation only occur when both the sarcoplasmic membrane and the cytoskeleton integrity are maintained [35].

In this sense, caveolae can host complexes formed by G protein coupled receptors, heterotrimeric G proteins, regulators and effectors. A large number of GPCRs can localize in membrane rafts and in some cases a pre-assembly of signaling complexes with the receptor already exists in membrane rafts. In other cases, the interaction of the agonist with the receptor makes G protein migrate to membrane rafts and take part in receptor desensitization and internalization processes [87,88].

Raft isolation by sucrose density gradients and immunoprecipitation experiments demonstrate that the α1-adrenergic receptor and Gαq protein are located physically together in caveolae [60]. Similar results have been obtained for the components of the β-AR signaling-Gαs protein pathway, which are also localized in membrane rafts and in caveolae in rat ventricular myocytes and in heterologous expression systems [59].

One work demonstrated that when the α1-adrenergic receptor is stimulated in isolated membranes of cardiac myocytes cAMP is not increased. However, when the stimulation is produced in intact cells cAMP levels increase [35]. This suggests that when the cells are lysed the components of the α1-adrenergic receptor pathway separate and as they are not close there is no activation of adenylate cyclase, so that cAMP concentration is not increased.

On the other hand, the increase of cAMP caused by α1-adrenergic receptor represents about 70% of basal cAMP levels, whereas the stimulation of adenylate cyclase with forskolin increases cAMP in 300–400% [35]. This indicates that the effect of each receptor must be compartmentalized, i.e. Gαs protein, adenylate cyclase and PKA must be preassembled and in close proximity to α1-adrenergic receptor. Thus, as the α1-AR, Gαs protein, AC, PKA and I_{to} are preassembled, the 70% increase in cAMP levels reduces I_{to} current amplitude because the increase is restricted to the area surrounding the supramolecular complex. However, broad increases in cAMP such as those caused by stimulation of β-adrenergic receptor have no effect on I_{to} because the second messenger is not increased in the "correct" place.

Moreover, when α1-AR activates adenylate cyclase, cAMP levels increases only in specific subcellular domains and activates only a discrete subpopulation of PKAs. Thus, after α1-adrenergic stimulation

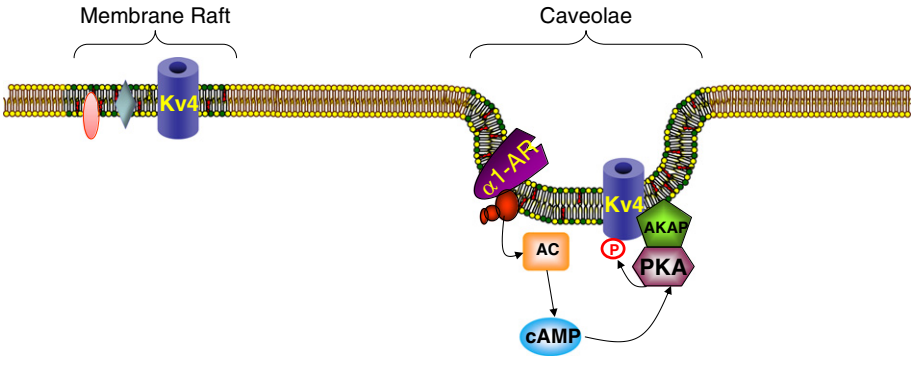

Fig. 5. All Kv4 channels are located in membrane rafts in cardiac myocytes. However, two different Kv4 channel populations exist, one population is located in caveolae and the other is located in planar membrane rafts, leading to the existence of two different populations of Kv4 channels in the membrane of cardiac myocytes also in terms of regulation. Only the caveolae located population of Kv4 channels can be regulated by α1-AR stimulation whereas the channel population located in planar membrane rafts is not accessible to the caveolae-located effectors of α1-ARs.

PKA phosphorylates I_{to} but not I_{Ca}-L or the ryanodyne receptor, because these targets are regulated by a different pool of PKAs.

The existence of distinct populations of PKAs localized near their specific targets answers the previously raised questions about the ability of α1-AR stimulation to activate PKA without affecting i.e. L-type calcium channels; and why β-adrenoceptors have no effect on I_{to} despite they activate cAMP/PKA pathway.

4.4. α1-adrenoceptors regulate only the caveolae-located subpopulation of cardiac Kv4 channels

It is one question remaining about the α1-AR-Gαs protein-AC-PKA regulation of I_{to}: why does noradrenaline reduce I_{to} current only 35% and why do not higher noradrenaline concentrations reduce I_{to} current 100%?

As explained in the previous section, in ventricular myocytes all the components of the α1-AR/I_{to} signal transduction pathway that includes the receptor, Gαs protein, AC, AKAP100, PKA, Kv4.2 and Kv4.3 are raft-associated [72]. This preassembly within membrane domains allows the quick regulation of the channel in response to α1-adrenergic stimulation. Colchicine disrupts cytoskeleton structure and causes caveolae removal from the membrane. When α1-adrenergic receptor is stimulated in the presence of colchicine the amplitude of I_{to} is not reduced because colchicine causes the molecules involved in the α1-adrenergic receptor transduction pathway to separate, and as they are not preassembled there is no effect on I_{to} [35].

Moreover, coimmunoprecipitation experiments and electron micrographs [72] answer the question regarding α1-Adrenergic regulation of I_{to}. As mentioned, maximum α1-AR stimulation reduces I_{to} current amplitude up to a 35%. The presence of Kv4.3 channels outside caveolae demonstrates that two different I_{to} channel populations exist. One, that is caveolae associated and sensitive to α1-adrenergic regulation and a second channel population localized in non-caveolar membrane rafts and α1-adrenergic independent (Fig. 5).

4.5. Genetic alterations in Caveolin and cardiac ionic channels

Considerable information exists in the literature about functional cardiac abnormalities from caveolin-null mice or from caveolin mutations identified in human patients. Caveolin KO or caveolin mutations results in a phenotype of cardiac hypertrophy, characterized by an increase in cardiomyocyte size and by interstitial fibrosis, which induces contractile dysfunction progressing to diastolic dysfunction [89,90].

In human patients, mutations in caveolin-3 have been associated with long QT syndrome (LQTS) and sudden infant dead syndrome (SIDS). Most of the mutations reported to date affect the cardiac excitability through the alteration in the function of cardiac Na channels, which causes an increase in late sodium current [90–92].

As explained in the previous sections, caveolae contain numerous signaling molecules such us β2- and α1-adrenoceptors, Gαs, Gαi and Gαq proteins, MAPKs, Src kinases, etc, which modulates the expression and function of many cardiac ionic channels. Thus, KO or mutations in caveolin are also expected to produce alterations in the regulation of cardiac ionic channels and, therefore, in the cardiac electrical activity. The extent and the mechanisms involved is a field waiting to be explored.

5. Summary

Location of particular transduction components inside and outside caveolae is an effective way of separating signaling pathways. In the heart, the preassembly of the α1-AR-Kv4 transducing components in caveolae ensures the fast reduction of I_{to} current triggered by sympathetic stimulation when an increased contraction force is required. However, the presence of another population of Kv4 channels located outside caveolae and therefore not accessible to α1-AR mediated regulation protects against the excessive reduction of the current, a potentially arrhythmogenic situation.

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