Cardiac voltage gated calcium channels and their regulation by β-adrenergic signaling

Neema Kumari, Himanshu Gaur, Anamika Bhargava

PII: S0024-3205(17)30683-5
DOI: https://doi.org/10.1016/j.lfs.2017.12.033
Reference: LFS 15495

To appear in: *Life Sciences*

Received date: 23 October 2017
Revised date: 17 December 2017
Accepted date: 24 December 2017

Please cite this article as: Neema Kumari, Himanshu Gaur, Anamika Bhargava , Cardiac voltage gated calcium channels and their regulation by β-adrenergic signaling. The address for the corresponding author was captured as affiliation for all authors. Please check if appropriate. Lfs(2017), https://doi.org/10.1016/j.lfs.2017.12.033

Cardiac voltage gated calcium channels and their regulation by β-adrenergic signalling

Neema Kumari[a] , Himanshu Gaur[a] and Anamika Bhargava[a,b]

[a]Ion channel Biology lab, Department of Biotechnology, Indian Institute of Technology Hyderabad, Telangana-502285, India.

[b]Correspondence at abhargava@iith.ac.in, Ion Channel Biology Lab, Department of Biotechnology, Indian Institute of Technology Hyderabad (IITH), Kandi, Telangana-502285, India. Tel: +91-8331008833.

HIGHLIGHTS

- Voltage-gated calcium channels (VGCCs) are implicated in heart failure being the major source of calcium influx in cardiomyocytes.
- So far, data regarding the role of VGCCs in heart failure is divergent.
- VGCCs are modulated by β-adrenergic receptor (βAR) stimulation during fight and flight response.
- Over the last decade, it is shown that heart failure is also a structural disease where T-tubule microdomains are lost leading to functional and structural de-coupling of VGCCs and βARs.
- Recently, using the combination of state of the art "scanning ion conductance microscopy" and FRET/patch-clamp, it is shown that both βARs and VGCCs are dislocated from their native locations, which may partly explain the pathology of the disease.

ABSTRACT

Voltage-gated calcium channels (VGCCs) are the predominant source of calcium influx in the heart leading to calcium-induced calcium release and ultimately excitation-contraction coupling. In the heart, VGCCs are modulated by the β-adrenergic signaling. Signaling through β-adrenergic receptors (βARs) and modulation of VGCCs by β-adrenergic signaling in the heart are critical signaling and changes to these have been significantly implicated in heart failure. However, data related to calcium channel dysfunction in heart failure is divergent and contradictory ranging from reduced function to no change in the calcium current. Many recent studies have highlighted the importance of functional and spatial microdomains in the heart and that may be the key to answer several puzzling questions. In this review, we have briefly discussed the types of VGCCs found in heart tissues, their structure, and significance in the normal and pathological condition of the heart. More importantly, we have reviewed the modulation of VGCCs by βARs in normal and pathological conditions incorporating functional and structural aspects. There are different types of βARs, each having their own significance in the functioning of the heart. Finally, we emphasize the importance of location of proteins as it relates to their function and modulation by co-signaling molecules. Its implication on the studies of heart failure is speculated.

Key words: Heart failure, voltage gated calcium channels, β-adrenergic receptors, microdomains, T-tubules, Crest

INTRODUCTION

Cardiomyocyte contraction depends upon synchronous movements of calcium into and out of the cell and fast cycling of calcium between the cytosol and sarcoplasmic reticulum (SR). The calcium influx is predominantly carried out by cardiac L-type calcium channels (LTCCs), a class of voltage-gated calcium channels (VGCCs). In the embryonic heart, contraction depends on the calcium ions that enter the cytoplasm through extracellular space whereas in the adult heart it also depends on the calcium ions released from intracellular stores such as SR. Initially, in response to depolarizing stimulus, calcium ions enter cardiac myocytes through the activated LTCCs causing a localized increase in the calcium concentration (Bers, 2008). Calcium influx through LTCCs continue till they are inactivated in a negative feedback manner due to resulting high calcium in dyadic cleft. These calcium ions then bind to ryanodine-sensitive calcium receptors, RyR2- calcium channels, located on SR and activate them to release calcium ions from the SR termed as "calcium-induced-calcium-release" (CICR) (Bers, 2008, Mukherjee and Spinale, 1998, Richard et al. , 2006). The calcium released from the SR rapidly increases the calcium concentration in the dyadic cleft, which within tens of milliseconds diffuses into the rest of the cytosol and leads to an increase in the cytosolic calcium. The calcium ions then bind to heart troponin C subunit, moving the troponin complex away from actin. It unmasks the myosin binding site on actin so that myosin head can now bind to actin utilizing ATP hydrolysis, forming a cross-bridge which pulls the actin filament towards the centre of the sarcomere, contracting the muscle. The whole process is termed as excitation-contraction coupling (EC-coupling) (Bers, 2002). Following influx and release from SR, calcium from the cytosol must be removed and cytosolic calcium concentration must return to its resting level to allow cardiomyocyte relaxation. Calcium removal from the cytosol is controlled by calcium transport by (1) the SR calcium ATPase (SERCA) 2) the sodium-calcium (Na-Ca) exchanger, and (3) slower systems

(including calcium transport by mitochondria and the sarcolemmal calcium-pump) (Aronsen et al. , 2016). As the increase in cytosolic calcium is predominantly due to calcium release from SR, recycling of calcium into the SR by SERCA is the principal determinant of cytosolic calcium removal. Its activity is also controlled by binding of the small inhibitory protein phospholamban. Phosphorylation of phospholamban relieves the inhibition causing an increase in SERCA activity (Kranias and Hajjar, 2012). To maintain steady state contraction and relaxation, the calcium removal from the sarcolemma must be equal to the calcium influx and also SR reuptake must be equal to the calcium released from the SR during CICR. There are two feedback mechanisms in place that lead to the maintenance of calcium concentrations during contraction and relaxation. First mechanism is calcium-dependent inactivation (CDI) of LTCCs. Due to the proximity of LTCCs to RyRs in the dyadic cleft, the release of calcium from RyRs during CICR rapidly increases the dyadic calcium concentration, triggering the inactivation of LTCCs. This works like a localized negative feedback loop whereby a large calcium release into the cytosol restricts further calcium influx through the LTCCs. Second is the balance between the activity of SERCA and Na-Ca exchanger. SERCA and Na-Ca exchanger mediated calcium removal from the cytosol together determines the amount of calcium uptake by the SR or extruded from the cell, respectively. High SERCA activity and low Na-Ca exchanger activity will increase SR calcium uptake and thus increase the calcium transient magnitude in the next heartbeat (Aronsen et al., 2016). However, Na-Ca exchanger activity is regulated by the cytosolic calcium, promoting high calcium extrusion when cytosolic calcium concentration is high. This tends to reduce SR calcium load. This mechanism also works like a negative feedback loop controlling steady state excitation-contraction coupling (for reviews see (Aronsen et al., 2016, Bers, 2008, Eisner et al. , 2013, Shannon and Bers, 2004). Both CICR and CDI are involved in the control of the duration of

an action potential and have a significant function in the remodelling of calcium signalling during the action potential.

Contraction and relaxation of the heart is tightly regulated by autonomic and hormonal control, which is caused by catecholamine binding to β-adrenergic receptors (βARs), which are heteromeric GTP binding protein (G-protein) coupled receptors (GPCRs) that trigger GDP-GTP exchange at the stimulatory G-protein subunit $G\alpha_s$. All these processes of calcium release and contraction can be either enhanced or reduced by calcium channels and βAR agonists or calcium channel blockers respectively (Mukherjee and Spinale, 1998).

Given the importance of calcium signaling in the functioning of heart, several cardiovascular disorders have been characterized with accompanying changes in calcium homeostasis or changes in calcium handling proteins. Alterations in calcium handling proteins and/or calcium cycling processes have been extensively reviewed before (for reviews see (Aronsen et al., 2016, Davlouros et al. , 2016, Kho et al. , 2012, Lindner et al. , 2002, Yano et al. , 2005). As an example, congestive heart failure (CHF) and hypertrophy, which are characterized by the structural and functional impairment of ventricle to get filled with or eject blood, involves a reduction in the calcium entry, EC-coupling and myocyte sensitivity to calcium concomitant with myocyte hypertrophy, fibrosis, and increased cardiac cell death. This is accompanied by the activation of the sympathetic nervous system, renin-angiotensin system and chronic hyper-activation of the βARs (Baker, 2014, Woo and Xiao, 2012). The importance of involvement of both VGCCs and βARs is highlighted by the fact that blockers of both are used as therapeutic agents (Aronow, 2010, Pascual et al. , 2016). Modulation of cardiac LTCCs by βARs has also been described using cells isolated from animal models. However, the extent of dysfunction of LTCCs in heart failure and up to what extent their modulation is modified in heart failure is debatable.

We have focussed this review on 1) Brief knowledge of cardiac VGCCs, βAR and their spatial location 2) Modulation of VGCCs by βARs 3) Changes in the modulation of VGCCs by βARs during pathological conditions 4) Modifiers of functional and structural coupling between VGCCs and βARs. Of note, our review highlights the importance of spatial location of VGCCs and βARs on the cardiomyocyte sarcolemma in the context of structural remodelling that occurs during heart failure.

CARDIAC VOLTAGE-GATED CALCIUM CHANNELS

Two types of VGCCs are expressed on the sarcolemma of cardiomyocytes- LTCCs and T-type voltage-gated calcium channels (TTCCs). They are distinguished based on their gating properties and their differential sensitivity to pharmacological agents (Hess et al. , 1986). LTCCs express throughout the lifespan and have a main role in maintaining the plateau phase of the action potential by allowing calcium influx for EC-coupling in the heart (Bers, 2002). On the other hand, functional roles of TTCCs are diverse depending on the mammalian species, heart region, age and various cardio pathology (Ono and Iijima, 2010).

L-type voltage-gated calcium channels

LTCC derives its name from the slow kinetics of current decay (L for long-lasting, current: ICaL) and it belongs to the category of high voltage-activated channels. It is also called by the name dihydropyridine receptor in cardiac biology. The LTCC activates at potentials positive to −40 mV, peaks at 0 to +10 mV, and tends to reverse at +60 to +70 mV, following a bell-shaped current–voltage relationship (Reuter, 1979, Reuter et al. , 1988, Wang et al. , 2004). LTCCs are located in the T-tubules where they are in close proximity to RyR2 channels on SR (Jaleel et al. , 2008, Treinys and Jurevicius, 2008, Weiss et al. , 2013). There are several isoforms of LTCCs out of which, isoform $Ca_v1.2$ conducts the primary calcium current in cardiac myocytes (Dolphin, 2006, Fu et al. , 2014, Hulme et al. , 2006). Upon

activation, calcium enters through these channels and stimulates the release of calcium by RyR2 channels by the process of CICR (Bers, 2002).

The sympathetic nervous system releases catecholamines such as epinephrine and norepinephrine during the fight-or-flight response and increases ICaL through $Ca_v1.2$ isoform leading to enhanced cardiac performance (Baker, 2014, Hulme et al., 2006). However excess calcium influx through $Ca_v1.2$ has been linked to necrotic myocyte death, cardiac dysfunction and premature death (Jaleel et al., 2008).

Structurally, LTCCs are heteromeric proteins with four subunits- α_1, $\alpha_2\delta$, β and γ (Dolphin, 2006, Treinys and Jurevicius, 2008, Van Petegem and Minor, 2006). But, cardiac $Ca_v1.2$ consists only of three subunits- α_1, $\alpha_2\delta$, and β (Hofmann et al. , 2014, Mukherjee and Spinale, 1998, Treinys and Jurevicius, 2008). α_1 is the pore-forming subunit which also determines the important characteristics such as channel selectivity for calcium, voltage-dependent activation and the sensitivity to agonists and antagonists (Hofmann et al., 2014). It consists of four covalently linked homologous domains (I-IV) and cytoplasmic N-terminus and C-terminus. Each domain consists of six transmembrane helices (S1 to S6) linked by variable cytoplasmic loops. S4 transmembrane helix in each domain consists of positively charged amino acids lysine and arginine which collectively form voltage sensor. Voltage sensor induces voltage dependent conformational changes in the channel for activation. The P loops between S5 and S6 helices line the pore of the channel (Dolphin, 2006, Hofmann et al., 2014). α_1 is 242 kDa in its full-length, but *in vivo*, it is 210 kDa due to the proteolytic processing of the distal C-terminus (dCT) at Ala1800. This processed dCT remains non-covalently associated with the truncated α_1 subunit at the proximal C-terminus (pCT) and exhibits an autoinhibitory role on channel function. Auxiliary subunits i.e $\alpha_2\delta$, and β are associated with the subunit and are involved in anchorage, trafficking, and regulatory functions. β-subunit can modulate the expression of $Ca_v1.2$ at the cell surface (Fu et al. ,

2011, Hofmann et al., 2014, Hulme et al., 2006, Oz et al. , 2017, Stolting et al. , 2015). Using co-sedimentation assays and FRET experiments Stölting et al. have shown a direct interaction of β-subunit and actin in mouse cardiomyocyte HL-1 cell line. Actin interacts with Src homology 3 domain on β-subunit. This association facilitates the forward trafficking of the $Ca_v1.2$, hence leading to increased calcium current density on the plasma membrane (Stolting et al., 2015). Further, upregulation of β-subunit is reported to be associated with heart failure (Hullin et al. , 2007). Detailed reports on the expression and function of LTCCs in the heart and cardiovascular disorders have been published before (for reviews see (Benitah et al. , 2010, Benitah et al. , 2002, Bodi et al. , 2005, Hess et al., 1986, Mukherjee and Spinale, 1998, Treinys and Jurevicius, 2008). Overall, most of the studies have shown that while the amplitude of ICaL is increased in hypertrophied and failing myocytes, its density on the cell surface (normalized to cell capacitance, as an indirect measure of cell surface) is similar to the control myocytes (Benitah et al., 2002, Loyer et al. , 2008, Song et al. , 2005).

Structural location of LTCCs in cardiomyocytes

The questions of the fate of LTCCs in heart failure, their regulation by βARs in heart failure and inconsistent data across researchers have puzzled the scientific community for a long time. This field has upto a certain extent advanced through the use of scanning ion conductance microscopy (SICM) and patch clamp to address this question. SICM is a non-contact and non–optical microscopy method which uses a glass pipette as a scanning probe (Hansma et al. , 1989) for visualization of the three-dimensional topography of live cells (Korchev et al. , 2000, Novak et al. , 2009). It is based on measuring the changes in the ion flow through the pipette (pipette similar to glass pipette used in patch-clamp) positioned at the cell surface and allows resolution of the structural features of cardiomyocytes, such as Z-grooves, cell crests located between them and T-tubules. The SICM pattern of T-tubule distribution co-localizes well with the T-tubular marker di-8-ANEPPS, a voltage sensitive

dye (Nikolaev et al. , 2010). The technique of combined SICM and patch clamp called as "super-resolution scanning patch-clamp" developed and validated by Gorelik and colleagues can provide a map of functional ion channels on the cell surface with nanoscale resolution (Bhargava et al. , 2013). With this technique, it was possible to locate ion channels within T-tubule and crest microdomains. Using "super-resolution scanning patch-clamp" it was recently shown that functional LTCCs (through which currents could be recorded) predominantly reside in T-tubules. In this method, first the topography of cardiomyocyte is obtained and then the patch-pipette is lowered at a precise location on the sarcolemma, in this case, either T-tubule or crest. Though the location of LTCCs on the sarcolemma was described before as membrane localization, super-resolution scanning patch-clamp for the first time gave direct evidence of physical location of clusters of functional LTCCs in T-tubules and very little functional LTCCs on the crest sarcolemma (Bhargava et al., 2013) (Fig. 1). Deduction of information of spatial location of functional LTCCs was not possible with conventional microscopy or convention patch clamp techniques. The T-tubular structural location of LTCCs is of prime importance in light of the fact that, in heart failure, structural abnormalities accompany a number of functional changes in cardiomyocytes. Overall, there is a clear evidence of T-tubule loss and disorganization during pathological alterations in the heart ((Nikolaev et al., 2010, Swift et al. , 2012), for reviews see (Crossman et al. , 2017, Crossman et al. , 2011, Gorelik et al. , 2013, Guo et al. , 2013, Ibrahim et al. , 2011)) (Fig. 2). This T-tubule loss and disorganization is consistently observed in isolated cardiomyocytes from different animal models of heart failure of different species including human cardiomyocytes (Guo et al., 2013). In the beginning, the T-tubule remodelling was probed using microscopy in fixed and stained cells. Gorelik group from Imperial College London, for the first time, obtained topography of live, unfixed and unstained cardiomyocytes using SICM. They also confirmed the loss of T-tubule openings on the

surface of human cardiomyocytes (Lyon et al. , 2009) which was consistent with the disorganization seen in a rat model of heart failure (Lyon et al., 2009).

Most interestingly, using the same rat model of heart failure, it is now shown that, not only the function of LTCCs, but their spatial location is also altered in heart failure. Normally LTCCs are found deep inside the T-tubules, but during heart failure, LTCCs are not only present in T-tubules but also at crest locations where they are rarely present in control cells (Sanchez-Alonso et al. , 2016) (Fig. 2). Moreover, open probability of only those LTCCs was increased which were moved from their native locations (Sanchez-Alonso et al., 2016). Using computational models and calcium imaging it was demonstrated that this relocated LTCC current could contribute to early-after depolarizations and initiate re-entrant arrhythmias (Sanchez-Alonso et al., 2016). This observation is of prime importance in light of the fact that early studies on failing human cells also suggested that open probability of LTCC increases in heart failure but in the absence of spatial location nothing could be said about the changing location of those dysfunctional channels. This also provides answer to various studies where no change was detected in the LTCC channel activity during heart failure. It could be due to the masking of microdomain readout in the whole cell recording conditions.

T-type voltage-gated calcium channels

TTCCs (T for transient, current: ICaT) are distinguished from LTCCs on the basis of their kinetics of activation and inactivation. They are low voltage activated with activation threshold for ICaT being −70 to −60 mV. ICaT is fully activated at −30 to −10 mV at physiological calcium concentration. ICaT is completely inactivated at membrane potentials more positive than −40 mV (Ono and Iijima, 2010). Earlier, TTCC was considered to play a minor functional role in cardiomyocytes. However, after the discovery of TTCC antagonists and cloning of its isoforms- $Ca_v3.1$, $Ca_v3.2$, and $Ca_v3.3$ in the early 90s, its importance was acknowledged. TTCCs show significant contribution in cardiac automaticity, development

and EC-coupling in normal cardiac myocytes (Ono and Iijima, 2010, Zhou and January, 1998). TTCCs also play a major role in the pacemaker activity of adult sinus node. This property is attributed to their low threshold of voltage activation which allows them to get activated with even a slight change in the membrane potential from the resting potential (Katz, 1996, Yasui et al. , 2005). This was supported by a number of studies that reported high expression of TTCCs in SA node (Bohn et al. , 2000, Hagiwara et al. , 1988, Ono and Iijima, 2005). Knock out mouse models of TTCCs further emphasized the role of T-type channel isoforms in cardiac pacemaking and impulse conduction (Chen et al. , 2003, Mangoni et al. , 2006, Thuesen et al. , 2014). Along the same lines, isoform specific $Ca_v3.1$ knockout mice shows bradycardia and slowing of the atrioventricular conduction (Mangoni et al., 2006).

Electrophysiological and immunostaining techniques have shown that TTCCs are highly concentrated on cardiomyocytes surface than in T-tubules (Jaleel et al., 2008). Hence, calcium entering through TTCCs is not taken up by the SR to the same extent as the calcium entering through LTCCs. This explains why calcium influx from TTCCs induces only little calcium release from SR (Jaleel et al., 2008). It is also reported that calcium influx through TTCCs doesn't cause increased myocyte contractility, calcium mediated pathologies and death (Jaleel et al., 2008, Katz, 1996). $Ca_v3.1$ and $Ca_v3.2$ are the major isoforms expressed in cardiomyocytes of an embryo, although $Ca_v3.3$ mRNA was also reported to be expressed in Purkinje fibers. During fetal period, current density and expression of TTCCs changes and they contribute to electrical activity in the early embryonic stages. $Ca_v3.1$ has been reported to be functional in mouse hearts at the middle embryonic stage and both $Ca_v3.1$ and $Ca_v3.2$ in rat hearts at the middle embryonic to perinatal stages (Ono and Iijima, 2010, Yasui et al., 2005). With age, their expression is lost, almost undetectable in ventricular myocytes of the adult heart. But they are prevalent in the sinoatrial node, facilitating pacemaker

depolarization. In pathological conditions such as cardiac hypertrophy and heart failure, TTCCs have been found to be re-expressed in atrial and ventricular myocytes, contributing abnormal electrical activity and excitation-contraction coupling through a small contribution of calcium release from SR (Ono and Iijima, 2010, Vassort et al. , 2006, Wang et al. , 2013, Yasui et al., 2005). This increased ICaT has relatively high sensitivity towards Ni^{2+} suggesting that $Ca_v3.2$ is the re-expressed TTCC under pathological conditions (Vassort et al., 2006, Yasui et al., 2005). It has been shown that increased calcium influx through $Ca_v3.2$ induces calcineurin/NFAT (nuclear factor of activated T-cell) hypertrophic signaling (Houser, 2009, Houser and Molkentin, 2008, Ono and Iijima, 2010, Yasui et al., 2005). Various studies have investigated the role of TTCCs in the pathology of heart disorders. For details on TTCCs in the heart and pathological disorders, please refer to the detailed reviews published before (Ono and Iijima, 2010, Yasui et al., 2005). Spatial location of functional TTCCs on the cardiomyocyte sarcolemma has not been investigated so far. This is limited by the fact that there is no/little expression of TTCCs in healthy adult ventricular cardiomyocytes and that the TTCCs have a tiny conductance. However, it would be interesting to investigate the spatial location of TTCCs in heart failure where they are re-/overexpressed.

Unlike LTCCs, TTCCs are found to consist only of α_1 subunit (Ono and Iijima, 2010). Electrophysiological studies have shown that recombinant LTCC requires auxiliary subunits for normal gating behaviour, but in the case of recombinant $Ca_v3.1$ and $Ca_v3.2$, currents similar to native ICaT was seen without auxiliary subunits (Zhang et al. , 2013). It's not yet clear whether auxiliary subunits for native TTCCs exist or not.

β-ADRENERGIC RECEPTOR IN CARDIAC MYOCYTE

The βARs are G-protein-coupled receptors (GPCRs) found on the membrane of cardiac myocytes. The sympathetic nervous system modulates cardiac function (such as heart rate

and contractility) by activating βARs during fight or flight response. Acute βAR stimulation regulates cardiac output in the fight-or-flight response, whereas chronic βAR stimulation plays an important role in physiological and pathological cardiac remodelling (Woo and Xiao, 2012). Due to insufficient cardiac function in pathological conditions, the sympathetic system releases norepinephrine which initiates the chronic activation of βAR (Baker, 2014, Davidson and Koch, 2001, Engelhardt et al. , 1999, Nikolaev et al., 2010, Woo and Xiao, 2012). When catecholamines bind to βARs, conformational changes occur in the receptor enabling its coupling to heterotrimeric G-proteins. This results in the substitution of the GDP on the Gα subunit of G-proteins by GTP and subsequent dissociation of the heterotrimer into active Gα-GTP and Gβγ subunits to mediate downstream signalling (Wess, 1997). Gα-GTP then stimulates adenylyl cyclase (AC) which results in the formation of cyclic AMP (cAMP). Increased cAMP levels regulate a wide variety of cellular processes activating a number of downstream signaling molecules. One such pathway involves the activation of cAMP dependent protein kinase A (PKA) by the dissociation of regulatory subunits (PKA-RS) from the catalytic subunits (PKA-CS). This activated PKA then phosphorylates further targets including cardiac calcium channels (An et al. , 1996, Baker, 2014, Bernstein et al. , 2011, Davidson and Koch, 2001, Fujita and Ishikawa, 2011, Kamp and Hell, 2000, Lohse et al. , 2003, Madamanchi, 2007, Marsh, 1989, Oz et al., 2017, Weiss et al., 2013, Woo and Xiao, 2012). Calcium channel modulation by PKA is described in detail in the next section. There are extensive changes in the downstream cAMP signaling in heart failure which include decreased cellular levels of cAMP due to increased activity of protein phosphatase 1 (PP1) (Movsesian, 2004) and due to desensitization of βARs (Perera and Nikolaev, 2013). Rat models of heart failure showed a dramatic decrease in the expression and activity of phosphodiesterases (PDEs) in the heart (Perera and Nikolaev, 2013). Therefore, it is proposed

that this decrease in cAMP-hydrolyzing PDE activity could be a short-term adaptive mechanism to counterbalance the reduction in cAMP production by desensitized βARs.

Three subtypes of βARs have been cloned (β_1AR, β_2AR, β_3AR). β_1AR is found primarily in the heart and comprises 75-80% of the βARs found in the heart. The β_2AR is expressed in various tissues including heart and comprises 20-25% of cardiac βARs. β_1ARs and β_2ARs are expressed in the ratio of 70-80%:30-20% in the human ventricle and 60-70%:40-30% in human atrium (Baker, 2014, Biolo et al. , 2006, Gorelik et al., 2013, Lohse et al., 2003, Madamanchi, 2007, Wallukat, 2002, Woo and Xiao, 2012). The β_3AR is found in the adipose tissue, and only minimally in the heart. Another subtype, β_4AR is also known which is considered as a low affinity state of β_1AR, which awaits genetic and pharmacologic characterization (Madamanchi, 2007). There are significant differences in the signaling pathways and cellular responses of the different βAR subtypes as described below (Baker, 2014, Bernstein et al., 2011, Biolo et al., 2006, Gorelik et al., 2013, Woo and Xiao, 2012). The different signaling pathways used by the β_1ARs and the β_2ARs also suggest that these proteins may be physically separated in the cardiomyocyte membrane providing evidence for spatial and functional signaling microdomains. Signaling through β_1ARs and β_2ARs is described below in brief highlighting the differences in the signaling.

Signaling through β_1ARs

When stimulated, cardiomyocyte β_1ARs primarily binds to the G stimulatory (Gs) protein. The Gα subunit of the Gs protein (Gαs) activates AC and PKA. Through β_1AR signaling, PKA phosphorylates a number of key proteins such as LTCCs, RyR2 receptors, phospholamban and cardiac contractile proteins, generating a positive inotropic and lusitropic effect. During heart failure β_1AR levels reduce up to 50% reflecting severely diminished β_1AR signaling likely due to sustained elevated catecholamine levels (Freedman and Lefkowitz, 2004). β_1AR knockout mice showed no response to catecholamine stimulation

despite the presence of β_2ARs (Rohrer et al. , 1996). On the other hand, cardiomyocyte-targeted β_1AR overexpressing mice developed dilated cardiomyopathy and heart failure at young age. This is similar to the pathology caused by chronic catecholamine overstimulation (Engelhardt et al., 1999) which reinforces the view that increased βAR signaling that may happen in chronic stimulation plays an important role in pathological cardiac remodelling.

Signaling through β_2ARs

β_2ARs can activate PKA in the same manner as described above for β_1ARs. However, through β_2AR signaling, only LTCCs are phosphorylated with smaller inotropic and no lusitropic effects (Gorelik et al., 2013). In addition to Gs, the β_2ARs can couple to pertussis toxin - sensitive G inhibitory (Gi) protein upon sustained activation opposing the positive inotropic effect (Baker, 2014, Bernstein et al., 2011, Lohse et al., 2003, Madamanchi, 2007, Steinberg, 1999). Gi coupling releases the activated Giα subunit, which inhibits AC activity, which causes downstream activation of the mitogen-activated protein kinases (MAPK). Of note, Giα coupling also activates the cytosolic effector molecule phospholipase A2 (cPLA2), which causes cAMP-independent enhancement of calcium signaling and cardiac contraction. This β_2AR-cPLA2 pathway is reported to operate during inefficient activation of β_1AR-Gs-cAMP pathway thus supplementing defective β_1AR signalling (Pavoine and Defer, 2005). Due to the dual capability of β_2ARs to couple either to Gs or Gi, the effect of their activation varies depending on the species, and the developmental or pathophysiological state of the heart. Of note, β_2AR levels are reported to remain constant during heart failure (Madamanchi, 2007). The β_2AR knockout mice did not suffer from developmental defects and showed no significant differences in the cardiovascular phenotype which is in sharp contrast to β_1AR knockouts. The β_2AR knockout mice also showed typical response to catecholamine stimulation which indicates that β_1AR is the signaling pathway for the catecholamine-induced changes in cardiac physiology. Interestingly, mice with cardiac-specific overexpression of

β_2ARs displayed enhanced basal contractility and cardiac function with minimal pathology (Milano et al. , 1994). This led to many studies probing the potential of β_2AR overexpression as a therapeutic approach (Akhter et al. , 1997, Dorn et al. , 1999), however this line of research is hampered by many conflicting studies (Du et al. , 2000, Freeman et al. , 2001).

Termination of βAR signaling following stimulation

To prevent the overstimulation of βAR receptors, which can be detrimental, there are several regulatory mechanisms in place (Ferguson, 2001). These processes involve receptor desensitization which acts as a negative feedback to the adrenergic signal, under conditions of prolonged agonist stimulation. The first of these processes is the deactivation of G-proteins. Soon after the dissociation of the coupled G-protein into active Gα and Gβγ subunits, conformational changes occur that again causes formation of inactive G-protein. This process is rapid and ensures dose dependent nature of βAR signal propagation. Prolonged catecholamine stimulation overthrows the short-term active/inactive cycling of the G-protein and triggers further negative feedback regulation of βAR activity. Second, upon activation βARs undergo homologous desensitization which occurs within minutes of agonist stimulation (Barak et al. , 1999a, Barak et al. , 1999b). Homologous desensitization begins when the dissociated Gβγ subunit binds with the active form of a βAR kinase 1 (βARK1). βARK1 is translocated to the activated receptor after binding with the activated Gβγ subunit, where it phosphorylates the agonist-occupied βAR (Freedman and Lefkowitz, 2004). βARK1 is the most prominent cardiac G-protein coupled receptor kinase. Phosphorylation of the receptor occurs at the C-terminus and targets it for arrestin-induced uncoupling from the G-protein. βARs can also undergo heterologous desensitization which occurs when the βARs are phosphorylated through PKA (Freedman et al. , 1995). Decreased density of βARs on the cell surface was also demonstrated in cultured neonatal cardiomyocytes upon agonist exposure leading to the attenuation of βAR signal (Sibley and Lefkowitz, 1985). In both

desensitization cases, βAR phosphorylation allows for β-arrestin to bind and interfere with future association with G-proteins, functionally uncoupling the receptor (Perry et al. , 2002). Binding to β-arrestin increases βAR's affinity for adaptor protein (AP)-2 and clathrin (Claing et al. , 2002). The receptors are then internalized via clathrin-coated vesicles, within minutes of stimulation (Zhang et al. , 1996). Following internalization, receptors are transported to endosomes where they can be recycled back to the plasma membrane for the next cycle of activation (Claing et al., 2002). In response to prolonged receptor stimulation, the internalized receptor can instead be degraded in the liposomes. This down-regulation process lowers the total number of receptors in the cell to relieve chronic overstimulation. Down-regulation is initiated within hours to days after consistent receptor internalization. Of note, down-regulation is reported to occur in the early development of the heart disease, but slowly than uncoupling, taking hours to days (Biolo et al., 2006, El-Armouche et al. , 2007, Engelhardt et al., 1999, Ungerer et al. , 1993, Ungerer et al. , 1994, Wallukat, 2002).

Spatial location of βARs

After successful application of SICM to image normal and failing cardiomyocyte topography, Gorelik group for the first time combined SICM with fluorescence resonance energy transfer (FRET) imaging to probe the structural location of βARs. Non-specific agonist isoproterenol was applied together with the specific inhibitor of either β_1ARs (CGP) or β_2ARs (ICI) to record specific signaling through either β_2ARs or β_1ARs respectively (Nikolaev et al., 2010). Interesting results were obtained when the drugs were applied either to T-tubules or crest. The results suggested that functional β_1ARs are located across the entire surface of cardiomyocytes whereas β_2ARs are located exclusively in the deep T-tubules (Nikolaev et al., 2010) (Fig. 1). And hence, cAMP signaling via β_2ARs gives a fine control on LTCC modulation which also resides deep within T-tubules. Using a rat model of chronic heart failure and using the same combination of SICM-FRET technique they further showed that

during heart failure, β_2ARs were redistributed from the T-tubules to the cell crests (Fig. 2.). This redistribution lead to the diffused receptor-mediated cAMP-signaling. cAMP generated by activation of β_2ARs, usually confined to smaller microdomains, was generated globally in heart failure (Nikolaev et al., 2010). It is possible that this redistribution of β_2ARs in heart failure changes compartmentation of cAMP and contributes to the failing myocardial phenotype.

MODULATION OF LTCC ACTIVITY IN THE HEART BY β-ADRENERGIC RECEPTOR SIGNALING

$Ca_v1.2$ channels are modulated by βARs/cAMP signaling pathway in the heart. In the fight-or-flight response, sympathetic system activates βARs transiently. This transient stimulation of βARs has different functional consequences as against sustained βAR activation which occurs in pathological states as described in the previous section. Both β_1 and β_2ARs couple to AC via Gs proteins, thus ensuring the cascade: activation of AC, increase in cAMP, activation of protein kinase A (PKA) and calcium channel phosphorylation. Many different receptors can stimulate cAMP synthesis in the heart but only βARs can produce cAMP-dependent stimulation of the LTCCs in cardiomyocytes (Warrier et al. , 2007). This difference is due to the compartmentation of generated cAMP. βARs can stimulate cAMP production in both cytosolic and caveolar compartments as opposed to other receptors which only stimulate cAMP production in the cytosolic compartment. Caveolar compartments are associated with βAR regulation of LTCC function and this is the reason why receptors which can stimulate cAMP in caveolar compartments can modulate LTCCs (Warrier et al., 2007). At the molecular level, dCT of LTCCs contains a leucine zipper motif that interacts with A-kinase anchoring protein (AKAP15). PKA anchors dCT through AKAP15, this complex interacts directly with the β subunit of LTCCs through pCT and phosphorylates LTCC $Ca_v1.2$. Phosphorylation leads to 3- to 4-fold increase in ICaL in the cell and increased

cardiac contractile force (Catterall, 2015, Fu et al. , 2013, Fu et al., 2014, Fuller et al. , 2010, Hofmann et al., 2014, Hulme et al. , 2003, Hulme et al., 2006, Mukherjee and Spinale, 1998, Oz et al., 2017, Treinys and Jurevicius, 2008, Yang et al. , 2016). Voltage dependence of ICaL also shifts to more hyperpolarized potentials (Benitah et al., 2010, Chen et al. , 2002, Collis et al. , 2007) resulting in a gain of function. This shift has only been reported by few groups and has not been reproduced in many studies (Reuter, 1987). Also upon βAR stimulation, inactivation of LTCC is mainly due to calcium (calcium dependent inactivation, CDI), due to a slow-down of VDI (Findlay et al. , 2008) . The fast inactivation time constant of ICaL of rat ventricular cardiomyocytes was "unexpectedly" slowed down after ICaL was increased by βAR stimulation (Alvarez et al. , 2004, Haase et al. , 2005) which would also result in increased calcium influx. At the level of single channel activity, βAR stimulation leads to an increase in the channel's open probability shifting to Mode 2 (Reuter, 1987, Reuter et al. , 1986, Tsien et al. , 1986, Yue et al. , 1990). It is important to note that cardiac LTCCs have multiple gating modes: mode 0 in which channels do not open or open very rarely, mode 1 in which the open probability is low, and mode 2 in which the open probability is higher and the openings are long-lasting (Reuter et al., 1986, van der Heyden et al. , 2005). Early studies by Maki et al. have shown that in cultured rat neonatal ventricular myocytes, exposure to norepinephrine, results in increased mRNA levels of α_1 subunit and number of functional VGCCs on the membrane (Maki et al. , 1996). However, there is no further evidence in literature supporting this.

Multiple LTCC sites for phosphorylation by PKA have been identified in α_1 subunit *in vitro*, but most of them are not required for βAR regulation *in vivo* (Fu et al., 2014, Hulme et al., 2006, Yang et al., 2016). Hulme et al. showed in dissociated rat ventricular myocytes that on isoproterenol treatment, a non-selective activator of βAR, there was an increased phosphorylation of S1928 in dCT. A similar result was seen with forskolin, a direct activator

of AC, indicating the role of cAMP and PKA in the phosphorylation (Hulme et al., 2006). In embryonic heart, deletion of dCT resulted in the loss of βAR regulation of ICaL thereby causing the death of embryo due to heart failure (which model which system). This result showed that autoinhibitory signaling complex formed with $Ca_v1.2$ and dCT anchored with PKA via AKAP15 is a modulatory substrate for βAR regulation and its disruption leads to heart failure (Fu et al., 2014, Fu et al., 2011, Yang et al., 2016). On pre-treatment with selective β_1AR and β_2AR antagonists, there was a reduction in phosphorylation of S1928 by 79% and 42% respectively. When both the antagonists were used together, there was a complete blockage of phosphorylation. In presence of 1,2-bis(2-aminophenoxy) ethane-*N*_, *N*_- tetraacetic acid (BAPTA)–acetoxymethyl ester to buffer intracellular calcium, only phosphorylation by β_1AR stimulation was seen. Whole-cell patch clamp in presence of BAPTA showed 98% increase in current through β_1AR stimulation. This indicated that the phosphorylation of S1928 occurs via both β_1AR and β_2AR stimulation at an elevated level of calcium, but at basal calcium levels only β_1AR was responsible for phosphorylation (Hulme et al., 2006).

Interestingly, calcium current through $Ca_v1.2$ channels with S1928 mutated to Ala (S1928A) could still be regulated by βAR stimulation but the level of stimulation was 70-80% compared to that of wild-type $Ca_v1.2$, indicating that S1928 is responsible only for 20-30% of total response and not required for significant βAR stimulation (Benitah et al., 2010, Ganesan et al. , 2006, Hulme et al., 2003, Hulme et al., 2006). By reconstitution studies and *in vivo* proteomics analysis Fu et al. had revealed S1700 to be the key amino acid for phosphorylation, located at the interface between the pCT and dCT, mediating the fight-or-flight response, cardiac homeostasis and normal physiological regulation (Fu et al., 2014). Mutation of S1700 to Ala (S1700A/SA) in mice eliminated the phosphorylation by PKA. This reduced the basal ICaL in neonatal as well as adult cardiomyocytes by ~37% of WT

ICaL value. It also reduced the up-regulation of $Ca_v1.2$ channel current via βAR/PKA pathway. Still, SA mice have shown to be regulated by PKA phosphorylation (Fu et al., 2014). This regulation might be due to the additional phosphorylation sites, residing either in α_1 or β subunits. Phosphorylation at another conserved phosphorylation site Thr-1704 (T1704) has been shown to be involved in normal βAR regulation of LTCCs. Mutation of T1704 to Ala (T1704A) had shown similar results as SA (Fu et al., 2013, 2014). When both the mutations were present together i.e. S1700A and T1704A (STAA) there was also a reduction in basal ICaL, cellular contractility, impaired exercise capacity and sensitivity to βAR agonists, but complete elimination of βAR stimulation was not seen (Fu et al., 2013). Effect of this double mutation was greater at low levels of βAR stimulation, which shows that effective phosphorylation of S1700 is enhanced by the phosphorylation of T1704, diminishing autoinhibition with an increase in channel activity (Fu et al., 2013). Notably, Thr1704 is phosphorylated by casein kinase II, implicating this constitutively active protein kinase in the control of basal $Ca_v1.2$ channel activity as well (Emrick et al. , 2010, Fuller et al., 2010). Casein kinase II conserved phosphorylation site was originally found in skeletal muscle $Ca_v1.1$ channels (Emrick et al., 2010). However, Fuller et al. have shown that stimulation of $Ca_v1.2$ activity through the phosphorylation by PKA requires only S1700 (Fuller et al., 2010). All these studies indicate that multiple phosphorylation sites are responsible for the increase in LTCC current upon phosphorylation by PKA and oppose the long-standing belief that S1928 is the key phosphorylation site.

Phosphorylation of the regulatory β2 subunit also plays a crucial role in the PKA-mediated up-regulation of ICaL. In dog cardiomyocytes, after phosphorylation of calcium channels by PKA, followed by immunoprecipitation using a polyclonal antibody against C-terminal of β_2 subunit, Haase et al. found that 62kDa β_2 subunit was the major PKA-substrate in the immunoprecipitate and hence suggested that the phosphorylation of β_2 subunit has a major

role in the βAR upregulation of cardiac LTCCs (Haase et al. , 1993). In 1999, using phosphopeptide mapping and micro sequencing, Gerhardstein et al. identified the phosphorylation sites of PKA in β2 subunit as serine478 (S478) and serine479 (S479) (Gerhardstein et al. , 1999). To confirm the significance of β2 subunit phosphorylation by PKA, Bunemann et al. used a truncated α_1 subunit lacking S1928 ($\alpha_{1C}\Delta1905$) which is a PKA-substrate and WT β2 subunit. Using the truncated channel in HEK cells, they found some barium currents on depolarization of cells. To confirm that this current was due to the phosphorylation of β2 subunit, they mutated S478 and S479 to alanine (S478A, S479A) and added PKA in the cell system. They observed no current augmentation with $\alpha_{1C}\Delta1905$ and S478A/S479A β2 subunit. These results showed that β2 subunit has a major role in the upregulation of LTCC current upon phosphorylation by PKA (Bunemann et al. , 1999).

The underlying molecular mechanisms of how phosphorylation of sites located either on $Ca_v1.2$ or β2 results in increased channel opening probability are not completely understood. This could be due to the fact that several other players of calcium handling are also involved.

Modifiers of βAR modulation of LTCCs (functional coupling)

So far, a few other proteins have been identified that modify β-adrenergic regulation of LTCCs (Fig. 1).

A) Sphingosine-1-phosphate (S1P): S1P is a circulating bioactive sphingolipid and has been identified as an important signaling mediator implicated in the regulation of several cellular processes including cardiac calcium handling (Means and Brown, 2009). Early evidence suggested an important regulatory role of S1P in regulating electrophysiological and contractile properties of cardiac myocytes under both basal and βAR stimulation conditions (Landeen et al. , 2008, Means and Brown, 2009). Only recently, Egom and colleagues reported that SIP partially reverses the effect of the βAR activation on LTCC current through signalling pathway that involves Pak1 (P21-activated kinase 1) - PP2A (protein phosphatase

2A) interaction. The basal LTCC current, however, was unaffected by S1P (Egom et al. , 2016). Which subtype of βAR signalling was involved was not investigated. More studies are needed to elucidate the role of S1P in cardiovascular disorders although currently available data indicates that that S1P can produce beneficial effects in disease models of cardiac hypertrophy, although it may induce cardiac hypertrophy in normal condition (Li and Zhang, 2016).

B) Ahnak: Another modifier of LTCC-βAR coupling is an important protein "ahnak" that is tightly bound to the LTCC β2 subunit in cardiomyocytes. Ahnak was originally identified in 1992 as tumour-related gene by Shtivelman and co-workers (Shtivelman et al. , 1992). Ahank derives its name due to its large size which is approximately 700kDa. β2 Subunit interaction with ahnak was identified in several cardiac preparations derived from rodent to human heart (Haase, 2007). In humans, polymorphism is identified in the gene loci encoding ahnak. One such ahnak polymorphism, I5236T, interferes with adrenergic stimulation of ICaL (Haase, 2007). Ahnak requires the conserved β subunit modules, SH3 and/or GK, for efficient ahnak/$Ca_v1.2$ channel interaction. Ahnak's role as a modifier of LTCC-βAR coupling can be summarised as: ahnak acts as a repressor of ICaL by β2 subunit sequestration. PKA phosphorylation relieves the inhibition imposed by ahnak. PKA phosphorylation can be mimicked by ahnak-derived fragments carrying a naturally occurring missense mutation Ile5236Thr. Therefore, ahnak-derived peptides are emerging as potent modulators of LTCC current in the adult mammalian heart (Haase, 2007). Ahnak's interaction with LTCC β2 subunit during heart failure remains to be determined. Also which subtype of βARs is involved is not known, though ahnak is expressed throughout the sarcolemma (Hohaus et al. , 2002).

C) Rem GTPase: Rem GTPase is a member of Rad/Rem/Rem2/Gem/Kir (RGK) GTPases that belong to the Ras superfamily of monomeric G-proteins (Colicelli, 2004). Like Ras,

RGK proteins contain a nucleotide binding domain (NBD) that can cycle between GTP- and GDP-bound states. However, these proteins also have long N- and C-terminus extensions, and non-conservative substitutions of residues important for nucleotide binding and hydrolysis compared to Ras (Colicelli, 2004, Finlin and Andres, 1997, Finlin et al. , 2000). Rem GTPase inhibits LTCCs in the heart cells by arresting LTCCs in a low open probability state, without changing the number of channels at the cell surface (Xu et al. , 2010) by binding to the LTCC β2 subunit (Finlin et al. , 2003). More importantly, the Rem-inhibited calcium channels can be rescued by LTCC activators such as BayK8644, but not by the βAR stimulation (Xu et al., 2010). This indicates that Rem completely eliminated βAR stimulation of LTCCs when overexpressed. Rem proteins are found in heart, and their levels are up/down regulated in cardiac diseases (Chang et al. , 2007, Finlin and Andres, 1997, Tan et al. , 2002). Therefore, they present as important drug targets modulating LTCCs.

Potential modifiers of βAR modulation of LTCCs (structural coupling)

A) **Bridging integrator 1(BIN1):** BIN1 is essential for localization of LTCCs to T-tubules. This was demonstrated using human and mouse adult cardiomyocytes and using complementary immunocytochemistry, electron microscopy with dual immunogold labeling, and co-immunoprecipitation techniques. BIN1 is a member of the BAR domain protein superfamily which is involved in membrane invagination and endocytosis (Hong et al. , 2010). BIN1 is also known to initiate T-tubule genesis in skeletal muscle cells (Hong et al., 2010). The delivery of LTCCs to BIN1 structures is not a specific property of cardiomyocytes as it also occurs in non-myocyte cell lines. This indicates that other myocyte-specific structures are not essential for this trafficking and that there is an intrinsic relationship between LTCC delivery and its BIN1 scaffold. Therefore up/down regulation of BIN1 may significantly affect LTCC localization to T-tubules and therefore its regulation by βARs.

B) **Caveolin-3**: In ventricular cardiomyocytes a subpopulation of LTCCs localize to caveolae. Caveolae are specialized microdomains of the plasmamembrane rich in signaling molecules and supported by the structural protein caveolin-3. Of note, caveolin protein expression decreases with age and studies on caveolin knock out (KO) mice support the hypothesis that the loss of caveolin protein causes an aged phenotype (Kawabe et al. , 2001, Ratajczak et al. , 2003). Immunofluorescence studies have shown that LTCCs colocalize with caveolin-3 (the abundant isoform of caveolin in heart) in caveolae microdomains present in the cardiomyocyte sarcolemma as a part of a macromolecular signaling complex necessary for β_2AR regulation of LTCC current (Balijepalli et al. , 2006, Makarewich et al. , 2012). This is evidenced by the fact that the stimulation of LTCC current by β_2AR stimulation was eliminated by disruption of caveolae or by small interfering RNA directed against caveolin-3, whereas β_1AR stimulation of LTCC current remained unaltered (Balijepalli et al., 2006). This means that there is a specific coupling of LTCCs with β_2ARs within caveolae and also suggests that LTCCs coupled to β_1AR signaling may be located outside of caveolar microdomains.

MODULATION OF T-TYPE VOLTAGE-GATED CALCIUM CHANNEL ACTIVITY IN THE HEART BY β-ADRENERGIC RECEPTOR SIGNALING

Various neurotransmitters and hormones such as endothelin, bradykinin, ATP, β-adrenergic agonists etc, are known to affect the activity of TTCCs (Vassort et al., 2006). In the literature, βAR modulation of ICaT is less reported. It could be due to the fact that TTCCs are rarely expressed in adult cardiomyocytes. There is some evidence of an increase of ICaT by βAR stimulation and G-proteins as early as 1992, under more physiological conditions (Vassort et al., 2006). In guinea pig ventricular myocytes, isoproterenol increased ICaT (25–100% increase) only at high concentrations (1M) while ICaL was increased at much lower concentration (10 nM) (Mitra and Morad, 1986). Modulation of ICaT is also reported in atrial

myocytes of frog where almost 100% increase was seen in 50% of the cells (Alvarez et al. , 1996, Alvarez and Vassort, 1992). However, the general consensus is that TTCCs are resistant to stimulation by βAR stimulation (Vassort et al., 2006). Presence of TTCCs in adult human cardiomyocytes is never reported. Because TTCCs reappear during cardiovascular disorders, it would be interesting to investigate the modulation of TTCCs by βAR signaling in those pathological conditions, if any?

CHANGES IN β-ADRENERGIC REGULATION OF CARDIAC VOLTAGE-GATED CALCIUM CHANNELS IN HEART FAILURE

During heart failure pathology, there is an inconsistent change in the basal ICaL and $Ca_v1.2$ becomes consistently less responsive to βAR stimulation and PKA activation, resulting in diminished contractile force and fight-or-flight response (Mukherjee and Spinale, 1998, Nikolaev et al., 2010, Saraiva et al. , 2003, Wallukat, 2002, Zheng et al. , 2013). Reduced responsiveness of $Ca_v1.2$ to βAR stimulation can be attributed to the greater basal $Ca_v1.2$ availability and open probability as compared to the normal heart, studied in ventricular myocytes from patients with heart failure. This increased basal $Ca_v1.2$ availability and open probability is likely because of the hyper-phosphorylation or persistent phosphorylation of the channel by PKA which limits the channel stimulation by βAR agonists (Chen et al., 2002, Zheng et al., 2013) and increases calcium influx causing cardiac hypertrophy and pathological remodelling of the ventricles as seen in both animal models and human patients. In hypertrophied rat myocytes (Scamps et al. , 1990), dogs (Kaab et al. , 1996) and rabbits (Rozanski et al. , 1997) with pacing-induced left-ventricular dysfunction, a significant lowering of βAR augmentation of ICaL was reported. In hypertensive rat myocytes, ICaL was augmented with βAR stimulation as compared to controls (Xiao and McArdle, 1994). In human patients with CHF, reduction in the number of βARs was found in association with increased plasma catecholamine concentrations and hence ICaL augmentation was reduced

by βAR stimulation with the development of CHF (Brodde et al. , 1995). It is also reported that prolonged βAR stimulation also internalizes LTCC in a macromolecular complex together with β-arrestin into clathrin-coated vesicles (Lipsky et al. , 2008). However, there are also studies which suggest that there are no changes in the expression and function of LTCCs per se in heart failure. Studies by Yang et al. have shown that in SA mice (described in the previous section) there is a reduced βAR regulation, causing reduced contractile function, cardiac hypertrophy and heart failure without changing expression, location or function of $Ca_v1.2$ (Yang et al., 2016). The immunocytochemical technique was used by them to identify the location of Cav1.2 (Yang et al., 2016). This indicates the higher sensitivity of the heart to even partial disruption of the normal upregulation of $Ca_v1.2$ by the mutation at S1700. STAA mice showed accelerated hypertrophy, heart failure, and death (Yang et al., 2016). Cardiac hypertrophy increased with voluntary wheel-running exercise and βAR stimulation in SA mice. Exercised SA mice showed an increase in heart weight (HW)/ body weight (BW) ratio compared to non-exercised SA mice of the same age. While exercised wild-type (WT) mice had similar HW/BW ratio as non-exercised WT mice. It shows that SA mice are more sensitive to exercise-induced cardiac hypertrophy, than WT mice (Yang et al., 2016).

Structurally, in heart failure (Fig. 2), LTCCs are dislocated from their localization in T-tubules and so are β_2ARs. LTCCs and β_2ARs reside in T-tubule signaling microdomain together with a number of other proteins, a few of which maintain the structure of T-tubules. Since T-tubule structure is lost in heart failure, these proteins were investigated. Any change in the expression of these structural proteins will not only affect the structure of T-tubules, but also the coupling between β_2ARs and LTCCs. In this context, BIN1 transcription is reported to be reduced in the heart failure which leads to intracellular localization of LTCCs (Hong et al. , 2012). These intracellular channels can neither participate in calcium influx nor

βAR regulation. Therefore one can speculate that in failing myocytes where BIN1 transcription is reduced, LTCC current upon βAR stimulation may not show an increase due to channels not being targeted to T-tubule microdomains. Similarly, caveolin-3 expression is reduced in human heart failure and rodent models of heart failure (Feiner et al. , 2011) and overexpression of caveolin-3 is cardioprotective (Horikawa et al. , 2011, Tsutsumi et al. , 2008). Mice lacking caveolin-3 showed no presence of caveolae (Galbiati et al. , 2001) and developed hypertrophy (Woodman et al. , 2002), indicating that absence of caveolar microdomains is detrimental. This knock out mice possibly lacks of β_2AR modulation of LTCCs and thus contributing to cardiac pathological phenotype. Microdomain (T-tubule or crest) specific changes in the βAR modulation of LTCCs is yet to be investigated. Also βAR modulation of over/re-expressed TTCCs remains to be determined.

CONCLUSIONS AND FUTURE PROSPECTS: LESSONS FROM MICRODOMAINS

Contradictory data obtained from different cellular and animal models regarding functioning and regulation of cardiac VGCCs in cardiovascular pathology suggests that the information is still not complete. However, the amelioration of pathological symptoms by calcium channel blockers suggest an unequivocal causal role of cardiac calcium channels. The recently developed technique of super-resolution scanning-patch clamp (Bhargava et al., 2013) provides some missing answers. This technique developed and validated by Gorelik and colleagues provided a map of functional ion channels on the cardiomyocyte surface with nanoscale resolution (Bhargava et al., 2013). Their investigation concluded that not only function, but location of LTCCs is disturbed in heart failure (Sanchez-Alonso et al., 2016). Since heart failure is a structural disease where T-tubules are reduced and disorganized, using the combination of SICM and FRET, Nikolaev et al. showed that β_2ARs also change locations (Nikolaev et al., 2010). Similar to LTCC, β_2ARs are usually restricted to T-tubules but redistribute to crest locations in heart failure (Nikolaev et al., 2010). How this change

affects the modulation of LTCCs by βARs in T-tubule and crest microdomains is yet to be investigated. To investigate this, perhaps a triple combination of SICM-FRET-patch clamp would be required. It is certainly challenging but worth exploiting. One could include the βAR agonist in the glass pipette used for SICM and patch clamp or an additional pipette may be used to deliver agonist locally. This would give critical insights on the outcome of β-blockers and calcium channels blocker (CCBs) therapy. The spatial concept underscores the fact that not all LTCCs are dysfunctional and by using CCBs, the therapy is targeted towards blocking all LTCCs which may not be beneficial in the long term.

FIGURE LEGENDS

Figure 1. Healthy Cardiomyocte. In healthy cardiomyocytes, LTCCs, β2ARs, AHNAK, REM and Bin1 colocalize in T-tubule scaffolds. REM inhibits LTCCs and eliminates its modulation by βARs (2). AHNAK inhibits LTCCs but this inhibition can be relieved by βAR modulation of LTCCs (3). SIP modulates the βAR regulation of LTCCs via Pak-PP2A pathway (4). A subset of LTCCs also colocalize with β2ARs in caveolae. TTCCs are present in sarcolemma only.

Figure 2. Failing cardiomyocyte. In failing cardiomyocytes, T-tubule scaffolds are disorganised with reduced expression of BIN1 and caveolin-3 (Cav3). The proximity between LTCCs and RyRs is reduced. LTCCs and β_2ARs redistribute to sarcolemma. TTCCs are overexpressed on the sarcolemma. Regulation of these overexpressed channels by βARs is not defined. Also, regulation of coupling between LTCCs and βARs by REM, AHNAK and SIP is not defined in heart failure.

ACKNOWLEDGMENT

This work was partly supported by grants to AB: BioCARe, DBT, India, (BT/BioCARe/01/9701/2013-14), seed grant, IITH (SG/IITH/F145/2016-17/SG-27), UGC-JRF fellowship (NK) and MHRD, India fellowship (HG). The authors would like to thank Dr Yogesh Bhargava (Dr Hari Singh Gour Central University, sagar) for his comments on the manuscript and help with illustrations.

DISCLOSURE STATEMENT

The authors declare having no financial competing interest.

REFERENCES

1. Akhter SA, Skaer CA, Kypson AP, McDonald PH, Peppel KC, Glower DD, et al. Restoration of beta-adrenergic signaling in failing cardiac ventricular myocytes via adenoviral-mediated gene transfer. Proc Natl Acad Sci U S A. 1997;94:12100-5.
2. Alvarez J, Hamplova J, Hohaus A, Morano I, Haase H, Vassort G. Calcium current in rat cardiomyocytes is modulated by the carboxyl-terminal ahnak domain. J Biol Chem. 2004;279:12456-61.
3. Alvarez JL, Rubio LS, Vassort G. Facilitation of T-type calcium current in bullfrog atrial cells: voltage-dependent relief of a G protein inhibitory tone. J Physiol. 1996;491 (Pt 2):321-34.
4. Alvarez JL, Vassort G. Properties of the low threshold Ca current in single frog atrial cardiomyocytes. A comparison with the high threshold Ca current. J Gen Physiol. 1992;100:519-45.
5. An RH, Davies MP, Doevendans PA, Kubalak SW, Bangalore R, Chien KR, et al. Developmental changes in beta-adrenergic modulation of L-type Ca2+ channels in embryonic mouse heart. Circ Res. 1996;78:371-8.
6. Aronow WS. Current role of beta-blockers in the treatment of hypertension. Expert Opin Pharmacother. 2010;11:2599-607.
7. Aronsen JM, Louch WE, Sjaastad I. Cardiomyocyte Ca2+ dynamics: clinical perspectives. Scand Cardiovasc J. 2016;50:65-77.
8. Baker AJ. Adrenergic signaling in heart failure: a balance of toxic and protective effects. Pflugers Arch. 2014;466:1139-50.
9. 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. Proc Natl Acad Sci U S A. 2006;103:7500-5.

10. Barak LS, Warabi K, Feng X, Caron MG, Kwatra MM. Real-time visualization of the cellular redistribution of G protein-coupled receptor kinase 2 and beta-arrestin 2 during homologous desensitization of the substance P receptor. J Biol Chem. 1999a;274:7565-9.

11. Barak LS, Zhang J, Ferguson SS, Laporte SA, Caron MG. Signaling, desensitization, and trafficking of G protein-coupled receptors revealed by green fluorescent protein conjugates. Methods Enzymol. 1999b;302:153-71.

12. Benitah JP, Alvarez JL, Gomez AM. L-type Ca(2+) current in ventricular cardiomyocytes. J Mol Cell Cardiol. 2010;48:26-36.

13. Benitah JP, Gomez AM, Fauconnier J, Kerfant BG, Perrier E, Vassort G, et al. Voltage-gated Ca2+ currents in the human pathophysiologic heart: a review. Basic Res Cardiol. 2002;97 Suppl 1:I11-8.

14. Bernstein D, Fajardo G, Zhao M. THE ROLE OF beta-ADRENERGIC RECEPTORS IN HEART FAILURE: DIFFERENTIAL REGULATION OF CARDIOTOXICITY AND CARDIOPROTECTION. Prog Pediatr Cardiol. 2011;31:35-8.

15. Bers DM. Cardiac excitation-contraction coupling. Nature. 2002;415:198-205.

16. Bers DM. Calcium cycling and signaling in cardiac myocytes. Annu Rev Physiol. 2008;70:23-49.

17. Bhargava A, Lin X, Novak P, Mehta K, Korchev Y, Delmar M, et al. Super-resolution scanning patch clamp reveals clustering of functional ion channels in adult ventricular myocyte. Circ Res. 2013;112:1112-20.

18. Biolo A, Rosa AS, Mazzotti NG, Martins S, Bello-Klein A, Rohde LE, et al. The role of adrenergic receptor polymorphisms in heart failure. Braz J Med Biol Res. 2006;39:1281-90.

19. Bodi I, Mikala G, Koch SE, Akhter SA, Schwartz A. The L-type calcium channel in the heart: the beat goes on. J Clin Invest. 2005;115:3306-17.

20. Bohn G, Moosmang S, Conrad H, Ludwig A, Hofmann F, Klugbauer N. Expression of T- and L-type calcium channel mRNA in murine sinoatrial node. FEBS Lett. 2000;481:73-6.

21. Brodde OE, Michel MC, Zerkowski HR. Signal transduction mechanisms controlling cardiac contractility and their alterations in chronic heart failure. Cardiovasc Res. 1995;30:570-84.

22. Bunemann M, Gerhardstein BL, Gao T, Hosey MM. Functional regulation of L-type calcium channels via protein kinase A-mediated phosphorylation of the beta(2) subunit. J Biol Chem. 1999;274:33851-4.

23. Catterall WA. Regulation of Cardiac Calcium Channels in the Fight-or-Flight Response. Curr Mol Pharmacol. 2015;8:12-21.

24. Chang L, Zhang J, Tseng YH, Xie CQ, Ilany J, Bruning JC, et al. Rad GTPase deficiency leads to cardiac hypertrophy. Circulation. 2007;116:2976-83.

25. Chen CC, Lamping KG, Nuno DW, Barresi R, Prouty SJ, Lavoie JL, et al. Abnormal coronary function in mice deficient in alpha1H T-type Ca2+ channels. Science. 2003;302:1416-8.

26. Chen X, Piacentino V, 3rd, Furukawa S, Goldman B, Margulies KB, Houser SR. L-type Ca2+ channel density and regulation are altered in failing human ventricular myocytes and recover after support with mechanical assist devices. Circ Res. 2002;91:517-24.

27. Claing A, Laporte SA, Caron MG, Lefkowitz RJ. Endocytosis of G protein-coupled receptors: roles of G protein-coupled receptor kinases and beta-arrestin proteins. Prog Neurobiol. 2002;66:61-79.

28. Colicelli J. Human RAS superfamily proteins and related GTPases. Sci STKE. 2004;2004:RE13.

29. Collis LP, Srivastava S, Coetzee WA, Artman M. beta2-Adrenergic receptor agonists stimulate L-type calcium current independent of PKA in newborn rabbit ventricular myocytes. Am J Physiol Heart Circ Physiol. 2007;293:H2826-35.

30. Crossman DJ, Jayasinghe ID, Soeller C. Transverse tubule remodelling: a cellular pathology driven by both sides of the plasmalemma? Biophys Rev. 2017;9:919-29.

31. Crossman DJ, Ruygrok PN, Soeller C, Cannell MB. Changes in the organization of excitation-contraction coupling structures in failing human heart. PLoS One. 2011;6:e17901.

32. Davidson MJ, Koch WJ. Genetic manipulation of beta-adrenergic signalling in heart failure. Acta Physiol Scand. 2001;173:145-50.

33. Davlouros PA, Gkizas V, Vogiatzi C, Giannopoulos G, Alexopoulos D, Deftereos S. Calcium Homeostasis and Kinetics in Heart Failure. Med Chem. 2016;12:151-61.

34. Dolphin AC. A short history of voltage-gated calcium channels. Br J Pharmacol. 2006;147 Suppl 1:S56-62.

35. Dorn GW, 2nd, Tepe NM, Lorenz JN, Koch WJ, Liggett SB. Low- and high-level transgenic expression of beta2-adrenergic receptors differentially affect cardiac hypertrophy and function in Galphaq-overexpressing mice. Proc Natl Acad Sci U S A. 1999;96:6400-5.

36. Du XJ, Autelitano DJ, Dilley RJ, Wang B, Dart AM, Woodcock EA. beta(2)-adrenergic receptor overexpression exacerbates development of heart failure after aortic stenosis. Circulation. 2000;101:71-7.

37. Egom EE, Bae JS, Capel R, Richards M, Ke Y, Pharithi RB, et al. Effect of sphingosine-1-phosphate on L-type calcium current and Ca(2+) transient in rat ventricular myocytes. Mol Cell Biochem. 2016;419:83-92.

38. Eisner D, Bode E, Venetucci L, Trafford A. Calcium flux balance in the heart. J Mol Cell Cardiol. 2013;58:110-7.

39. El-Armouche A, Gocht F, Jaeckel E, Wittkopper K, Peeck M, Eschenhagen T. Long-term beta-adrenergic stimulation leads to downregulation of protein phosphatase inhibitor-1 in the heart. Eur J Heart Fail. 2007;9:1077-80.

40. Emrick MA, Sadilek M, Konoki K, Catterall WA. Beta-adrenergic-regulated phosphorylation of the skeletal muscle Ca(V)1.1 channel in the fight-or-flight response. Proc Natl Acad Sci U S A. 2010;107:18712-7.

41. Engelhardt S, Hein L, Wiesmann F, Lohse MJ. Progressive hypertrophy and heart failure in beta1-adrenergic receptor transgenic mice. Proc Natl Acad Sci U S A. 1999;96:7059-64.

42. Feiner EC, Chung P, Jasmin JF, Zhang J, Whitaker-Menezes D, Myers V, et al. Left ventricular dysfunction in murine models of heart failure and in failing human heart is associated with a selective decrease in the expression of caveolin-3. J Card Fail. 2011;17:253-63.

43. Ferguson SS. Evolving concepts in G protein-coupled receptor endocytosis: the role in receptor desensitization and signaling. Pharmacol Rev. 2001;53:1-24.

44. Findlay I, Suzuki S, Murakami S, Kurachi Y. Physiological modulation of voltage-dependent inactivation in the cardiac muscle L-type calcium channel: a modelling study. Prog Biophys Mol Biol. 2008;96:482-98.

45. Finlin BS, Andres DA. Rem is a new member of the Rad- and Gem/Kir Ras-related GTP-binding protein family repressed by lipopolysaccharide stimulation. J Biol Chem. 1997;272:21982-8.

46. Finlin BS, Crump SM, Satin J, Andres DA. Regulation of voltage-gated calcium channel activity by the Rem and Rad GTPases. Proc Natl Acad Sci U S A. 2003;100:14469-74.

47. Finlin BS, Shao H, Kadono-Okuda K, Guo N, Andres DA. Rem2, a new member of the Rem/Rad/Gem/Kir family of Ras-related GTPases. Biochem J. 2000;347 Pt 1:223-31.

48. Freedman NJ, Lefkowitz RJ. Anti-beta(1)-adrenergic receptor antibodies and heart failure: causation, not just correlation. J Clin Invest. 2004;113:1379-82.

49. Freedman NJ, Liggett SB, Drachman DE, Pei G, Caron MG, Lefkowitz RJ. Phosphorylation and desensitization of the human beta 1-adrenergic receptor. Involvement of G protein-coupled receptor kinases and cAMP-dependent protein kinase. J Biol Chem. 1995;270:17953-61.

50. Freeman K, Lerman I, Kranias EG, Bohlmeyer T, Bristow MR, Lefkowitz RJ, et al. Alterations in cardiac adrenergic signaling and calcium cycling differentially affect the progression of cardiomyopathy. J Clin Invest. 2001;107:967-74.

51. Fu Y, Westenbroek RE, Scheuer T, Catterall WA. Phosphorylation sites required for regulation of cardiac calcium channels in the fight-or-flight response. Proc Natl Acad Sci U S A. 2013;110:19621-6.

52. Fu Y, Westenbroek RE, Scheuer T, Catterall WA. Basal and beta-adrenergic regulation of the cardiac calcium channel CaV1.2 requires phosphorylation of serine 1700. Proc Natl Acad Sci U S A. 2014;111:16598-603.

53. Fu Y, Westenbroek RE, Yu FH, Clark JP, 3rd, Marshall MR, Scheuer T, et al. Deletion of the distal C terminus of CaV1.2 channels leads to loss of beta-adrenergic regulation and heart failure in vivo. J Biol Chem. 2011;286:12617-26.

54. Fujita T, Ishikawa Y. Apoptosis in heart failure. -The role of the beta-adrenergic receptor-mediated signaling pathway and p53-mediated signaling pathway in the apoptosis of cardiomyocytes. Circ J. 2011;75:1811-8.

55. Fuller MD, Emrick MA, Sadilek M, Scheuer T, Catterall WA. Molecular mechanism of calcium channel regulation in the fight-or-flight response. Sci Signal. 2010;3:ra70.

56. Galbiati F, Engelman JA, Volonte D, Zhang XL, Minetti C, Li M, et al. 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;276:21425-33.

57. Ganesan AN, Maack C, Johns DC, Sidor A, O'Rourke B. Beta-adrenergic stimulation of L-type Ca2+ channels in cardiac myocytes requires the distal carboxyl terminus of alpha1C but not serine 1928. Circ Res. 2006;98:e11-8.

58. Gerhardstein BL, Puri TS, Chien AJ, Hosey MM. Identification of the sites phosphorylated by cyclic AMP-dependent protein kinase on the beta 2 subunit of L-type voltage-dependent calcium channels. Biochemistry. 1999;38:10361-70.

59. Gorelik J, Wright PT, Lyon AR, Harding SE. Spatial control of the betaAR system in heart failure: the transverse tubule and beyond. Cardiovasc Res. 2013;98:216-24.

60. Guo A, Zhang C, Wei S, Chen B, Song LS. Emerging mechanisms of T-tubule remodelling in heart failure. Cardiovasc Res. 2013;98:204-15.

61. Haase H. Ahnak, a new player in beta-adrenergic regulation of the cardiac L-type Ca2+ channel. Cardiovasc Res. 2007;73:19-25.

62. Haase H, Alvarez J, Petzhold D, Doller A, Behlke J, Erdmann J, et al. Ahnak is critical for cardiac Ca(V)1.2 calcium channel function and its beta-adrenergic regulation. FASEB J. 2005;19:1969-77.

63. Haase H, Karczewski P, Beckert R, Krause EG. Phosphorylation of the L-type calcium channel beta subunit is involved in beta-adrenergic signal transduction in canine myocardium. FEBS Lett. 1993;335:217-22.

64. Hagiwara N, Irisawa H, Kameyama M. Contribution of two types of calcium currents to the pacemaker potentials of rabbit sino-atrial node cells. J Physiol. 1988;395:233-53.

65. Hansma PK, Drake B, Marti O, Gould SA, Prater CB. The scanning ion-conductance microscope. Science. 1989;243:641-3.

66. Hess P, Lansman JB, Nilius B, Tsien RW. Calcium channel types in cardiac myocytes: modulation by dihydropyridines and beta-adrenergic stimulation. J Cardiovasc Pharmacol. 1986;8 Suppl 9:S11-21.

67. Hofmann F, Flockerzi V, Kahl S, Wegener JW. L-type CaV1.2 calcium channels: from in vitro findings to in vivo function. Physiol Rev. 2014;94:303-26.

68. Hohaus A, Person V, Behlke J, Schaper J, Morano I, Haase H. The carboxyl-terminal region of ahnak provides a link between cardiac L-type Ca2+ channels and the actin-based cytoskeleton. FASEB J. 2002;16:1205-16.

69. Hong TT, Smyth JW, Chu KY, Vogan JM, Fong TS, Jensen BC, et al. BIN1 is reduced and Cav1.2 trafficking is impaired in human failing cardiomyocytes. Heart Rhythm. 2012;9:812-20.

70. Hong TT, Smyth JW, Gao D, Chu KY, Vogan JM, Fong TS, et al. BIN1 localizes the L-type calcium channel to cardiac T-tubules. PLoS Biol. 2010;8:e1000312.

71. Horikawa YT, Panneerselvam M, Kawaraguchi Y, Tsutsumi YM, Ali SS, Balijepalli RC, et al. Cardiac-specific overexpression of caveolin-3 attenuates cardiac hypertrophy and increases natriuretic peptide expression and signaling. J Am Coll Cardiol. 2011;57:2273-83.

72. Houser SR. Ca(2+) signaling domains responsible for cardiac hypertrophy and arrhythmias. Circ Res. 2009;104:413-5.

73. Houser SR, Molkentin JD. Does contractile Ca2+ control calcineurin-NFAT signaling and pathological hypertrophy in cardiac myocytes? Sci Signal. 2008;1:pe31.

74. Hullin R, Matthes J, von Vietinghoff S, Bodi I, Rubio M, D'Souza K, et al. Increased expression of the auxiliary beta(2)-subunit of ventricular L-type Ca(2)+ channels leads to single-channel activity characteristic of heart failure. PLoS One. 2007;2:e292.

75. Hulme JT, Lin TW, Westenbroek RE, Scheuer T, Catterall WA. Beta-adrenergic regulation requires direct anchoring of PKA to cardiac CaV1.2 channels via a leucine zipper interaction with A kinase-anchoring protein 15. Proc Natl Acad Sci U S A. 2003;100:13093-8.

76. Hulme JT, Westenbroek RE, Scheuer T, Catterall WA. Phosphorylation of serine 1928 in the distal C-terminal domain of cardiac CaV1.2 channels during beta1-adrenergic regulation. Proc Natl Acad Sci U S A. 2006;103:16574-9.

77. Ibrahim M, Gorelik J, Yacoub MH, Terracciano CM. The structure and function of cardiac t-tubules in health and disease. Proc Biol Sci. 2011;278:2714-23.

78. Jaleel N, Nakayama H, Chen X, Kubo H, MacDonnell S, Zhang H, et al. Ca2+ influx through T- and L-type Ca2+ channels have different effects on myocyte contractility and induce unique cardiac phenotypes. Circ Res. 2008;103:1109-19.

79. Kaab S, Nuss HB, Chiamvimonvat N, O'Rourke B, Pak PH, Kass DA, et al. Ionic mechanism of action potential prolongation in ventricular myocytes from dogs with pacing-induced heart failure. Circ Res. 1996;78:262-73.

80. Kamp TJ, Hell JW. Regulation of cardiac L-type calcium channels by protein kinase A and protein kinase C. Circ Res. 2000;87:1095-102.

81. Katz AM. Calcium channel diversity in the cardiovascular system. J Am Coll Cardiol. 1996;28:522-9.

82. Kawabe JI, Grant BS, Yamamoto M, Schwencke C, Okumura S, Ishikawa Y. Changes in caveolin subtype protein expression in aging rat organs. Mol Cell Endocrinol. 2001;176:91-5.

83. Kho C, Lee A, Hajjar RJ. Altered sarcoplasmic reticulum calcium cycling--targets for heart failure therapy. Nat Rev Cardiol. 2012;9:717-33.

84. Korchev YE, Gorelik J, Lab MJ, Sviderskaya EV, Johnston CL, Coombes CR, et al. Cell volume measurement using scanning ion conductance microscopy. Biophys J. 2000;78:451-7.

85. Kranias EG, Hajjar RJ. Modulation of cardiac contractility by the phospholamban/SERCA2a regulatome. Circ Res. 2012;110:1646-60.

86. Landeen LK, Dederko DA, Kondo CS, Hu BS, Aroonsakool N, Haga JH, et al. Mechanisms of the negative inotropic effects of sphingosine-1-phosphate on adult mouse ventricular myocytes. Am J Physiol Heart Circ Physiol. 2008;294:H736-49.

87. Li N, Zhang F. Implication of sphingosin-1-phosphate in cardiovascular regulation. Front Biosci (Landmark Ed). 2016;21:1296-313.

88. Lindner M, Bohle T, Beuckelmann DJ. Ca2+-handling in heart failure--a review focusing on Ca2+ sparks. Basic Res Cardiol. 2002;97 Suppl 1:I79-82.

89. Lipsky R, Potts EM, Tarzami ST, Puckerin AA, Stocks J, Schecter AD, et al. beta-Adrenergic receptor activation induces internalization of cardiac Cav1.2 channel complexes through a beta-arrestin 1-mediated pathway. J Biol Chem. 2008;283:17221-6.

90. Lohse MJ, Engelhardt S, Eschenhagen T. What is the role of beta-adrenergic signaling in heart failure? Circ Res. 2003;93:896-906.

91. Loyer X, Gomez AM, Milliez P, Fernandez-Velasco M, Vangheluwe P, Vinet L, et al. Cardiomyocyte overexpression of neuronal nitric oxide synthase delays transition toward heart failure in response to pressure overload by preserving calcium cycling. Circulation. 2008;117:3187-98.

92. Lyon AR, MacLeod KT, Zhang Y, Garcia E, Kanda GK, Lab MJ, et al. Loss of T-tubules and other changes to surface topography in ventricular myocytes from failing human and rat heart. Proc Natl Acad Sci U S A. 2009;106:6854-9.

93. Madamanchi A. Beta-adrenergic receptor signaling in cardiac function and heart failure. Mcgill J Med. 2007;10:99-104.

94. Makarewich CA, Correll RN, Gao H, Zhang H, Yang B, Berretta RM, et al. A caveolae-targeted L-type Ca(2)+ channel antagonist inhibits hypertrophic signaling without reducing cardiac contractility. Circ Res. 2012;110:669-74.

95. Maki T, Gruver EJ, Davidoff AJ, Izzo N, Toupin D, Colucci W, et al. Regulation of calcium channel expression in neonatal myocytes by catecholamines. J Clin Invest. 1996;97:656-63.

96. Mangoni ME, Traboulsie A, Leoni AL, Couette B, Marger L, Le Quang K, et al. Bradycardia and slowing of the atrioventricular conduction in mice lacking CaV3.1/alpha1G T-type calcium channels. Circ Res. 2006;98:1422-30.

97. Marsh JD. Coregulation of calcium channels and beta-adrenergic receptors in cultured chick embryo ventricular cells. J Clin Invest. 1989;84:817-23.

98. Means CK, Brown JH. Sphingosine-1-phosphate receptor signalling in the heart. Cardiovasc Res. 2009;82:193-200.

99. Milano CA, Allen LF, Rockman HA, Dolber PC, McMinn TR, Chien KR, et al. Enhanced myocardial function in transgenic mice overexpressing the beta 2-adrenergic receptor. Science. 1994;264:582-6.

100. Mitra R, Morad M. Two types of calcium channels in guinea pig ventricular myocytes. Proc Natl Acad Sci U S A. 1986;83:5340-4.

101. Movsesian MA. Altered cAMP-mediated signalling and its role in the pathogenesis of dilated cardiomyopathy. Cardiovasc Res. 2004;62:450-9.

102. Mukherjee R, Spinale FG. L-type calcium channel abundance and function with cardiac hypertrophy and failure: a review. J Mol Cell Cardiol. 1998;30:1899-916.

103. Nikolaev VO, Moshkov A, Lyon AR, Miragoli M, Novak P, Paur H, et al. Beta2-adrenergic receptor redistribution in heart failure changes cAMP compartmentation. Science. 2010;327:1653-7.

104. Novak P, Li C, Shevchuk AI, Stepanyan R, Caldwell M, Hughes S, et al. Nanoscale live-cell imaging using hopping probe ion conductance microscopy. Nat Methods. 2009;6:279-81.

105. Ono K, Iijima T. Pathophysiological significance of T-type Ca2+ channels: properties and functional roles of T-type Ca2+ channels in cardiac pacemaking. J Pharmacol Sci. 2005;99:197-204.

106. Ono K, Iijima T. Cardiac T-type Ca(2+) channels in the heart. J Mol Cell Cardiol. 2010;48:65-70.

107. Oz S, Pankonien I, Belkacemi A, Flockerzi V, Klussmann E, Haase H, et al. Protein kinase A regulates C-terminally truncated CaV 1.2 in Xenopus oocytes: roles of N- and C-termini of the alpha1C subunit. J Physiol. 2017;595:3181-202.

108. Pascual I, Moris C, Avanzas P. Beta-Blockers and Calcium Channel Blockers: First Line Agents. Cardiovasc Drugs Ther. 2016;30:357-65.

109. Pavoine C, Defer N. The cardiac beta2-adrenergic signalling a new role for the cPLA2. Cell Signal. 2005;17:141-52.

110. Perera RK, Nikolaev VO. Compartmentation of cAMP signalling in cardiomyocytes in health and disease. Acta Physiol (Oxf). 2013;207:650-62.

111. Perry SJ, Baillie GS, Kohout TA, McPhee I, Magiera MM, Ang KL, et al. Targeting of cyclic AMP degradation to beta 2-adrenergic receptors by beta-arrestins. Science. 2002;298:834-6.

112. Ratajczak P, Damy T, Heymes C, Oliviero P, Marotte F, Robidel E, et al. Caveolin-1 and -3 dissociations from caveolae to cytosol in the heart during aging and after myocardial infarction in rat. Cardiovasc Res. 2003;57:358-69.

113. Reuter H. Properties of two inward membrane currents in the heart. Annu Rev Physiol. 1979;41:413-24.

114. Reuter H. Calcium channel modulation by beta-adrenergic neurotransmitters in the heart. Experientia. 1987;43:1173-5.

115. Reuter H, Kokubun S, Prod'hom B. Properties and modulation of cardiac calcium channels. J Exp Biol. 1986;124:191-201.

116. Reuter H, Porzig H, Kokubun S, Prod'hom B. Calcium channels in the heart. Properties and modulation by dihydropyridine enantiomers. Ann N Y Acad Sci. 1988;522:16-24.

117. Richard S, Perrier E, Fauconnier J, Perrier R, Pereira L, Gomez AM, et al. 'Ca(2+)-induced Ca(2+) entry' or how the L-type Ca(2+) channel remodels its own signalling pathway in cardiac cells. Prog Biophys Mol Biol. 2006;90:118-35.

118. Rohrer DK, Desai KH, Jasper JR, Stevens ME, Regula DP, Jr., Barsh GS, et al. Targeted disruption of the mouse beta1-adrenergic receptor gene: developmental and cardiovascular effects. Proc Natl Acad Sci U S A. 1996;93:7375-80.

119. Rozanski GJ, Xu Z, Whitney RT, Murakami H, Zucker IH. Electrophysiology of rabbit ventricular myocytes following sustained rapid ventricular pacing. J Mol Cell Cardiol. 1997;29:721-32.

120. Sanchez-Alonso JL, Bhargava A, O'Hara T, Glukhov AV, Schobesberger S, Bhogal N, et al. Microdomain-Specific Modulation of L-Type Calcium Channels Leads to Triggered Ventricular Arrhythmia in Heart Failure. Circ Res. 2016;119:944-55.

121. Saraiva RM, Chedid NG, Quintero HC, Diaz GL, Masuda MO. Impaired beta-adrenergic response and decreased L-type calcium current of hypertrophied left ventricular myocytes in postinfarction heart failure. Braz J Med Biol Res. 2003;36:635-48.

122. Scamps F, Mayoux E, Charlemagne D, Vassort G. Calcium current in single cells isolated from normal and hypertrophied rat heart. Effects of beta-adrenergic stimulation. Circ Res. 1990;67:199-208.

123. Shannon TR, Bers DM. Integrated Ca2+ management in cardiac myocytes. Ann N Y Acad Sci. 2004;1015:28-38.

124. Shtivelman E, Cohen FE, Bishop JM. A human gene (AHNAK) encoding an unusually large protein with a 1.2-microns polyionic rod structure. Proc Natl Acad Sci U S A. 1992;89:5472-6.

125. Sibley DR, Lefkowitz RJ. Molecular mechanisms of receptor desensitization using the beta-adrenergic receptor-coupled adenylate cyclase system as a model. Nature. 1985;317:124-9.

126. Song LS, Pi Y, Kim SJ, Yatani A, Guatimosim S, Kudej RK, et al. Paradoxical cellular Ca2+ signaling in severe but compensated canine left ventricular hypertrophy. Circ Res. 2005;97:457-64.

127. Steinberg SF. The molecular basis for distinct beta-adrenergic receptor subtype actions in cardiomyocytes. Circ Res. 1999;85:1101-11.

128. Stolting G, de Oliveira RC, Guzman RE, Miranda-Laferte E, Conrad R, Jordan N, et al. Direct interaction of CaVbeta with actin up-regulates L-type calcium currents in HL-1 cardiomyocytes. J Biol Chem. 2015;290:4561-72.

129. Swift F, Franzini-Armstrong C, Oyehaug L, Enger UH, Andersson KB, Christensen G, et al. Extreme sarcoplasmic reticulum volume loss and compensatory T-tubule remodeling after Serca2 knockout. Proc Natl Acad Sci U S A. 2012;109:3997-4001.

130. Tan FL, Moravec CS, Li J, Apperson-Hansen C, McCarthy PM, Young JB, et al. The gene expression fingerprint of human heart failure. Proc Natl Acad Sci U S A. 2002;99:11387-92.

131. Thuesen AD, Andersen H, Cardel M, Toft A, Walter S, Marcussen N, et al. Differential effect of T-type voltage-gated Ca2+ channel disruption on renal plasma flow and glomerular filtration rate in vivo. Am J Physiol Renal Physiol. 2014;307:F445-52.

132. Treinys R, Jurevicius J. L-type Ca2+ channels in the heart: structure and regulation. Medicina (Kaunas). 2008;44:491-9.

133. Tsien RW, Bean BP, Hess P, Lansman JB, Nilius B, Nowycky MC. Mechanisms of calcium channel modulation by beta-adrenergic agents and dihydropyridine calcium agonists. J Mol Cell Cardiol. 1986;18:691-710.

134. Tsutsumi YM, Horikawa YT, Jennings MM, Kidd MW, Niesman IR, Yokoyama U, et al. Cardiac-specific overexpression of caveolin-3 induces endogenous cardiac protection by mimicking ischemic preconditioning. Circulation. 2008;118:1979-88.

135. Ungerer M, Bohm M, Elce JS, Erdmann E, Lohse MJ. Altered expression of beta-adrenergic receptor kinase and beta 1-adrenergic receptors in the failing human heart. Circulation. 1993;87:454-63.

136. Ungerer M, Parruti G, Bohm M, Puzicha M, DeBlasi A, Erdmann E, et al. Expression of beta-arrestins and beta-adrenergic receptor kinases in the failing human heart. Circ Res. 1994;74:206-13.

137. van der Heyden MA, Wijnhoven TJ, Opthof T. Molecular aspects of adrenergic modulation of cardiac L-type Ca2+ channels. Cardiovasc Res. 2005;65:28-39.

138. Van Petegem F, Minor DL, Jr. The structural biology of voltage-gated calcium channel function and regulation. Biochem Soc Trans. 2006;34:887-93.

139. Vassort G, Talavera K, Alvarez JL. Role of T-type Ca2+ channels in the heart. Cell Calcium. 2006;40:205-20.

140. Wallukat G. The beta-adrenergic receptors. Herz. 2002;27:683-90.

141. Wang F, Gao H, Kubo H, Fan X, Zhang H, Berretta R, et al. T-type Ca(2)(+) channels regulate the exit of cardiac myocytes from the cell cycle after birth. J Mol Cell Cardiol. 2013;62:122-30.

142. Wang MC, Dolphin A, Kitmitto A. L-type voltage-gated calcium channels: understanding function through structure. FEBS Lett. 2004;564:245-50.

143. Warrier S, Ramamurthy G, Eckert RL, Nikolaev VO, Lohse MJ, Harvey RD. cAMP microdomains and L-type Ca2+ channel regulation in guinea-pig ventricular myocytes. J Physiol. 2007;580:765-76.

144. Weiss S, Oz S, Benmocha A, Dascal N. Regulation of cardiac L-type Ca(2)(+) channel CaV1.2 via the beta-adrenergic-cAMP-protein kinase A pathway: old dogmas, advances, and new uncertainties. Circ Res. 2013;113:617-31.

145. Wess J. G-protein-coupled receptors: molecular mechanisms involved in receptor activation and selectivity of G-protein recognition. FASEB J. 1997;11:346-54.

146. Woo AY, Xiao RP. beta-Adrenergic receptor subtype signaling in heart: from bench to bedside. Acta Pharmacol Sin. 2012;33:335-41.

147. Woodman SE, Park DS, Cohen AW, Cheung MW, Chandra M, Shirani J, et al. Caveolin-3 knock-out mice develop a progressive cardiomyopathy and show hyperactivation of the p42/44 MAPK cascade. J Biol Chem. 2002;277:38988-97.

148. Xiao YF, McArdle JJ. Elevated density and altered pharmacologic properties of myocardial calcium current of the spontaneously hypertensive rat. J Hypertens. 1994;12:783-90.

149. Xu X, Marx SO, Colecraft HM. Molecular mechanisms, and selective pharmacological rescue, of Rem-inhibited CaV1.2 channels in heart. Circ Res. 2010;107:620-30.

150. Yang L, Dai DF, Yuan C, Westenbroek RE, Yu H, West N, et al. Loss of beta-adrenergic-stimulated phosphorylation of CaV1.2 channels on Ser1700 leads to heart failure. Proc Natl Acad Sci U S A. 2016;113:E7976-E85.

151. Yano M, Ikeda Y, Matsuzaki M. Altered intracellular Ca2+ handling in heart failure. J Clin Invest. 2005;115:556-64.

152. Yasui K, Niwa N, Takemura H, Opthof T, Muto T, Horiba M, et al. Pathophysiological significance of T-type Ca2+ channels: expression of T-type Ca2+ channels in fetal and diseased heart. J Pharmacol Sci. 2005;99:205-10.

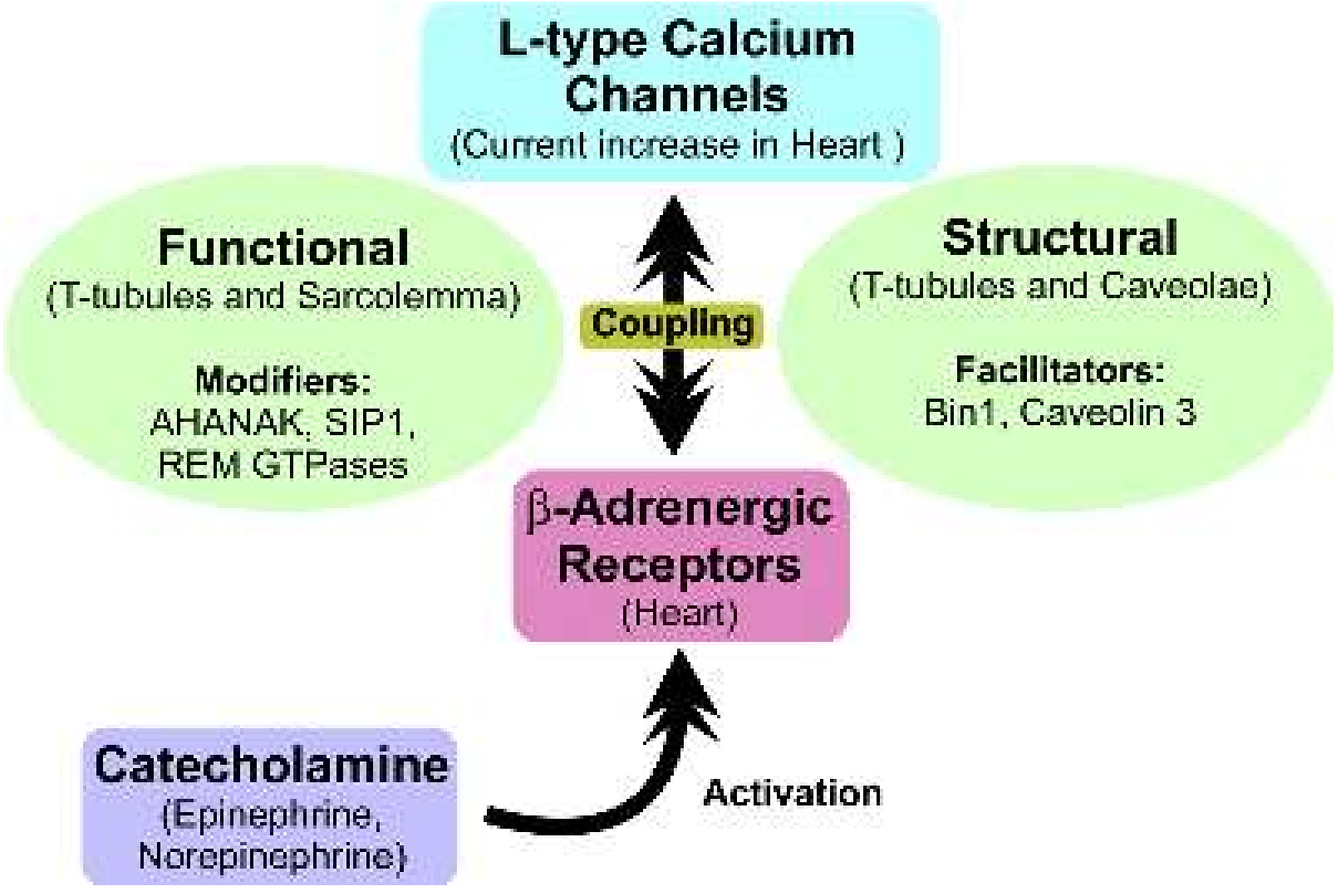
153. Yue DT, Herzig S, Marban E. Beta-adrenergic stimulation of calcium channels occurs by potentiation of high-activity gating modes. Proc Natl Acad Sci U S A. 1990;87:753-7.

154. Zhang J, Ferguson SS, Barak LS, Menard L, Caron MG. Dynamin and beta-arrestin reveal distinct mechanisms for G protein-coupled receptor internalization. J Biol Chem. 1996;271:18302-5.

155. Zhang Y, Jiang X, Snutch TP, Tao J. Modulation of low-voltage-activated T-type Ca(2)(+) channels. Biochim Biophys Acta. 2013;1828:1550-9.

156. Zheng MQ, Li X, Tang K, Sharma NM, Wyatt TA, Patel KP, et al. Pyruvate restores beta-adrenergic sensitivity of L-type Ca(2+) channels in failing rat heart: role of protein phosphatase. Am J Physiol Heart Circ Physiol. 2013;304:H1352-60.

157. Zhou Z, January CT. Both T- and L-type Ca2+ channels can contribute to excitation-contraction coupling in cardiac Purkinje cells. Biophys J. 1998;74:1830-9.



Graphics Abstract

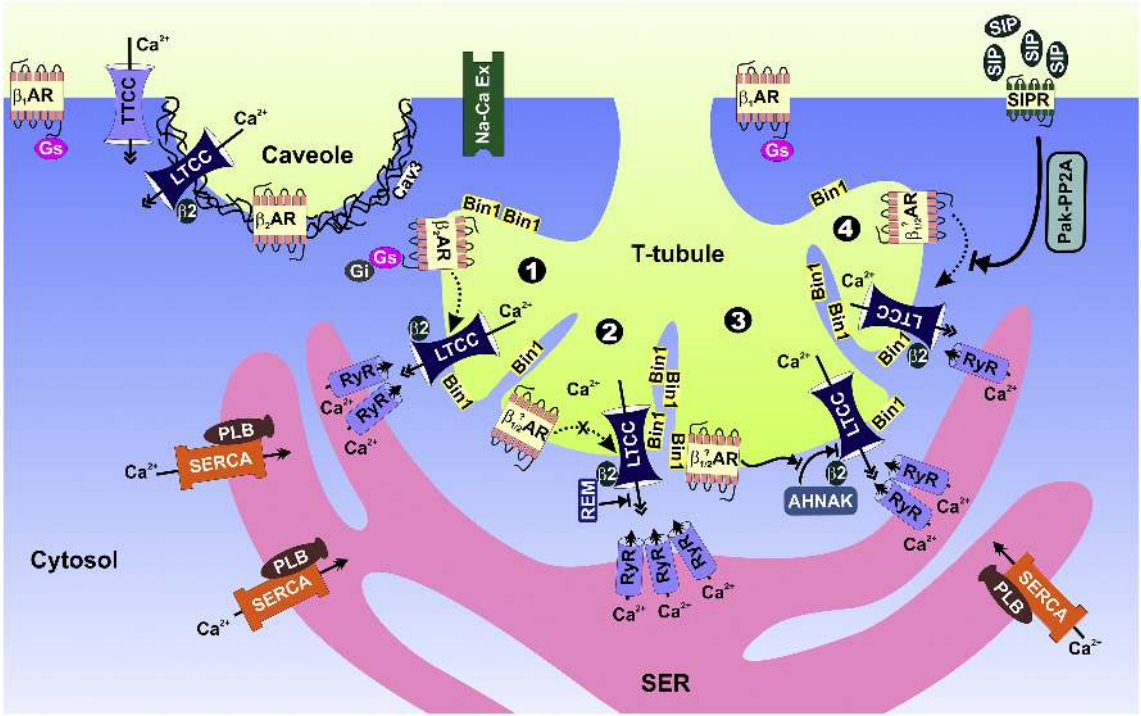

Ca2+
TTCC
β1AR
Gs
LTCC
Caveole
β2
β2AR
Cav3
Na-Ca Ex
Bin1
Gi
T-tubule
1
2
3
4
RyR
PLB
SERCA
Cytosol
REM
AHNAK
SIP
SIPR
Pak-PP2A
SER

Figure 1

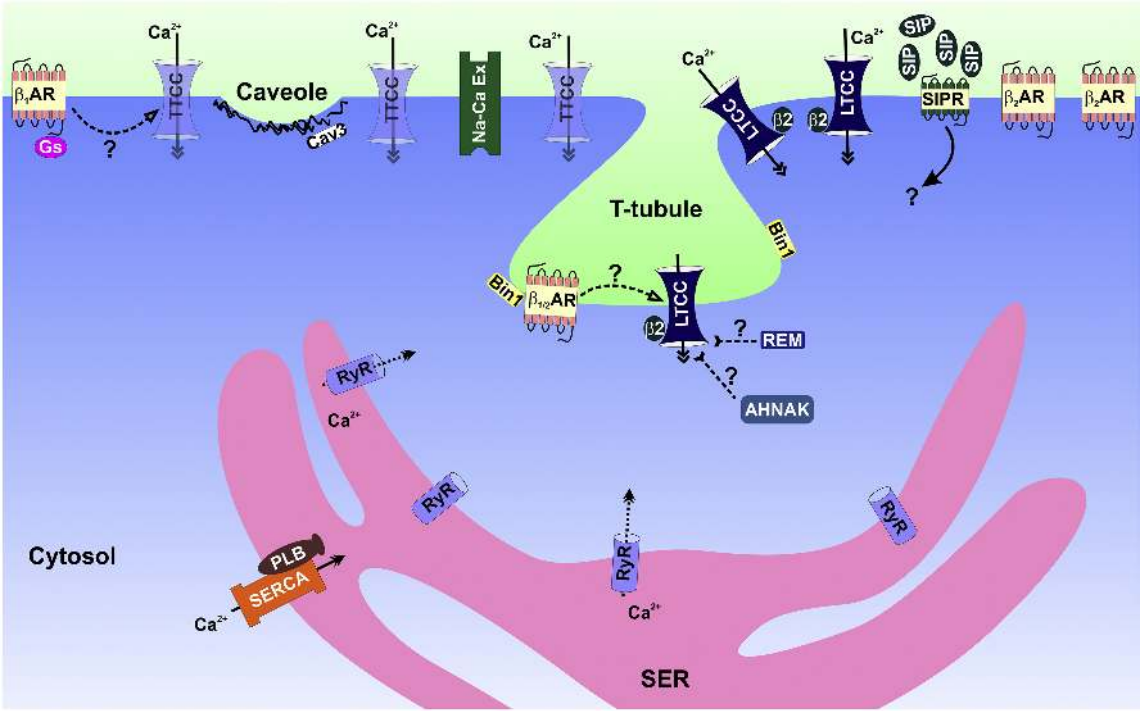

Ca2+
β1AR
Gs
?
TTCC
Caveole
Cav3
Na-Ca Ex
LTCC
β2
SIP
SIPR
β2AR
T-tubule
Bin1
REM
AHNAK
RyR
Cytosol
PLB
SERCA
SER

Figure 2