# ***β***-Adrenergic Stimulation and the Progression of Cardiac Pathology: How Ca$^{2+}$n Calcium Help Explain Heart Failure

Liam Gannon
Temple University, Philadelphia, Pennsylvania 19122, United States

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**ABSTRACT:** As the average life expectancy around the globe increases with access to medicine, diseases which once plagued humanity have largely been eradicated. However, one disease that has seen a large uptick in incidence is heart failure (HF), which afflicts about 6.7 million Americans over the age of 20, meaning that 1 in 4 people are projected to develop HF in the United States during the course of their life. [1] Certain co-morbidities make the likelihood of developing HF greater, such as in the case of myocardial infarctions (MI) and the ensuing ischemic death of cardiac muscle tissue.[2] The specific role of compensatory measures that the heart enacts to counteract the symptoms of HF, along with the downstream signaling pathways that these initiates have yet to be fully understood. In order to understand the link between cardiac muscle death, calcium dysregulation, and cardiac remodeling, this review aims to investigate the altered intracellular signaling pathways in pathology, and how this translates to the physiological effects that are seen in the symptoms of HF. It aims to cover both calcium signaling in the healthy and diseased heart, as well as cardiac remodeling and physiology, and the inter-relatedness of these two processes. G-protein coupled receptors (GPCRs) represent a large number of the therapeutic targets which are studied in HF. [3] It is evident that their signaling pathways play a large role in cardiomyocyte contractility and pathology seen in HF. The development of HF results from aberrant calcium signaling from chronic stimulation of β-adrenergic (βAR) signaling, causing the development of the hypertrophic phenotype and disruption of the cardiac dyad observed in HF. This signaling can largely be attributed to the action of PKA.[22] Understanding this pathway is critical to progressing patient care and outcomes for those who are suffering from HF.

## INTRODUCTION:

G- protein coupled receptors (GPCRs) represent nearly 40% of the therapeutic targets which are approved by the FDA.[5] GPCRs compose a very large class of transmembrane receptors which are coupled to a G-protein, which allows for the initiation of large secondary messenger cascades and signal amplification via the action of extracellular ligand activation.[6] The G- protein receptor is coupled to the heterotrimeric G-protein, and involves three subunits (α, β, and γ). These transmembrane portions are alpha helices, which anchor the receptor inside the plasma membrane. The G-protein exhibits translocase activity, where in the event of ligand binding to the receptor, causes a conformational change to the transmembrane domains and the replacement of GDP with GTP in the α subunit. While over 33 classes of GPCRs exist and carry out various functions, there are three overarching classes of GPCRs: Gαs (stimulatory of cAMP production), Gαi (inhibitory of cAMP production), and Gαq, the latter of which involves the effector phospholipase C and down-stream activation of calcium signaling. There are several amplification mechanisms to GPCRs, including GTPase-activating proteins, which work to accelerate the activity and downstream signaling of the Gα monomer.[6] The activation of second messengers is critical to the transduction cascade of phosphorylation. A major class of second messenger include cAMP, which is activated by the enzyme bound protein adenylyl cyclase. These signaling cascades lead to the activation of protein kinases such as PKA and PKC, which catalyze phosphorylation and further downstream signaling, which is depicted in **Figure 1**.

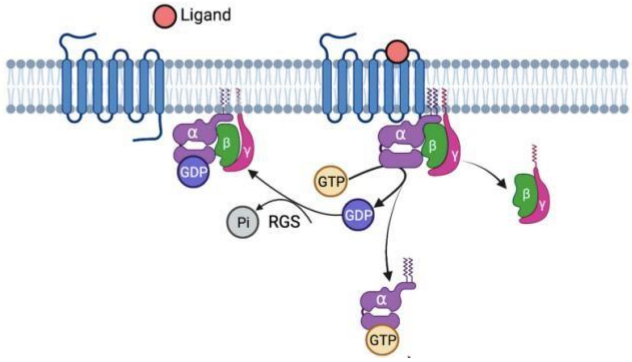

**Figure 1***:* Depiction of the canonical GPCR signaling pathway, with second messengers and phosphorylation mechanism shown. *Kaur et al (2023).*

The exact mechanistic pathways through which GPCRs contribute to the progression of disease (such as HF) are not fully understood due to the numerous changes that take place in the heart during this time. However, the impacts of β-adrenergic (βAR) signaling have been linked to HF progression post-myocardial infarction. A myocardial infarction (MI) is the process by which blood flow is stopped to a specific location on the heart, usually the left ventricle. This is often a threat posed with atherosclerosis and the formation of fatty deposits within the coronary artery leading to these blockages. The occurrence of an MI during one's life increases the risk for HF development. Many of the symptoms associated with HF can be attributed to the actions of βAR. βARs are a specific type of GPCR that respond to catecholamines such as epinephrine, and signal using canonical GPCR pathways like PKA. During MI, βARs are impacted by the lack of oxygenated blood to cardiac tissue due to their high amount tissue specific expression here. In this review, I aim to explain the correlation of βAR signaling and HF and show how the progression of these symptoms is critically interlinked with βAR signaling due to its involvement with calcium signaling and initiating contractility.

### *Basic Overview of GPCRs and Calcium Signaling*

Within cardiomyocytes specifically, there are several signaling pathways that contribute to healthy contractility of the heart in relation to GPCRs. The large diversity in the numbers and types of GPCRs is extensive, and accounts for a large number of downstream signaling pathways seen in the heart. For example, heart rate is controlled by acetylcholine-dependent activation of potassium channels within cardiomyocytes. Namely, the G-protein coupled inwardly rectifying potassium channels (GIRKs) become hyperpolarized when interacting with the activated Gα subunit.[4] This allows for normal relaxation kinetics and the uptake/release of calcium from the cell and into the SR. The activation of second messengers by this calcium cascade leads to the stimulation of PKA, which can then activate several contractile elements of the heart via phosphorylation, such as phospholamban, RyRs, and LTCCs, which all work together to carry out calcium induced calcium release (CICR), an essential cardiac process whereby a small amount of calcium released into the cytosol initiates a depolarization event, causing the release of a larger amount of calcium. It also interacts with Troponin I, which is critical to the initiation of the contractile motion of sarcomeres.[25] These points will be touched on more later, as their relation to cardiac remodeling relates to GPCR dysfunction. The desensitization and de-activation of the channels is marked by the dissociation of the Gα subunit and the reformation of the G α, β, and γ heterotrimeric protein complex. The receptor itself can be desensitized in a variety of ways, such as with the recruitment of proteins known as β-arrestins, which initiate receptor internalization and ubiquitination.[3] Importantly, these processes are disrupted in pathology, leading to aberrant signaling of second messengers and the appearance of clinical symptoms seen in HF.

### *Healthy Cardiac Modeling*

A typical, healthy cardiomyocyte in humans is around 100 **µ**M long, and 10-25 **µ**M in diameter.[10] Within the cardiomyocytes are the sarcomeres, which are the contractile unit of the cell. At rest, they are around 1.8-2.4 **µ**M in length, and composed of thick and thin filaments. The thick filament, otherwise known as myosin, has a barbed end and a globular head, the latter of which being the location of actin (thin filament) binding. The cycle begins when tropomyosin binds to the cleft where myosin and actin meet, where the crossbridge can be formed and protected from binding of calcium. A part of the actin filament known as the troponin complex contains three proteins: troponin I, T, and C. Troponin T anchors tropomyosin to the crossbridge, while Troponin I maintains structural integrity of the crossbridge. Importantly, Troponin C initiates a conformational change of the crossbridge, causing the initiation of the contractile cycle (or systolic phase) upon calcium binding.[10] These details can be viewed in **Figure 2**, where the structure of the sarcomere with labeled locations of troponin and tropomyosin are indicated.

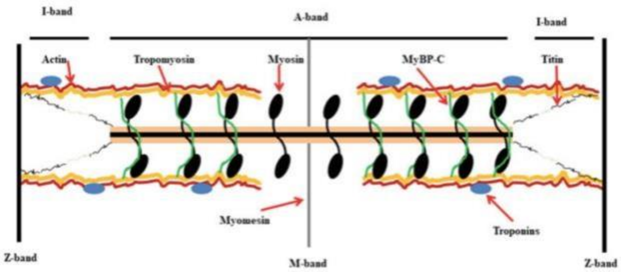

**Figure 2**: Diagram of the sarcomere as seen in the cardiac muscle cells. Calcium binding to troponin initiates the dissociation of ADP and inorganic phosphate to initiate the power stroke of contraction. *Kartha et al. 2021.*

### *Organization of the Cardiac Dyad*

Along the basement membrane and sarcolemma of the cardiomyocytes, there are many ion channels that assist in contraction via electrical potential differences. A rapid influx of sodium into the cell followed by potassium efflux, and subsequent calcium influx mediated by the SR is what initiates the binding of calcium to Troponin C and allows for contraction to begin. This action potential is the driving force of how the heart operates. When looking further into calcium signaling specifically, much calcium is stored within the cell in the sarcoplasmic reticulum (SR). The SRs within the cardiomyocytes are located in close proximity to T-tubules, which are ~200 nm wide invaginations on the surface membranes of the cardiomyocytes. Association is maintained by protein complexes, namely the protein junctophilin.[11] This same association protein is seen to be decreased or degraded in pathology. The space where the SR and the T-tubules associate is known as the dyadic space. Many T-tubules are surrounded almost entirely with SRs, as coordination between the SR and various calcium channels that line the invagination is necessary in controlling ion gradients and voltage differences. Important to note is that the number of T-tubules observed in cardiomyocytes decreases in heart failure, thereby increasing the dissociation that is seen between the LTCCs and the RyRs of the SR.[17] A detailed image of the dyad can be seen in **Figure 3**, where the close association of the RyR and LTCCs is evident in regard to the location of the SR and the T-tubules.

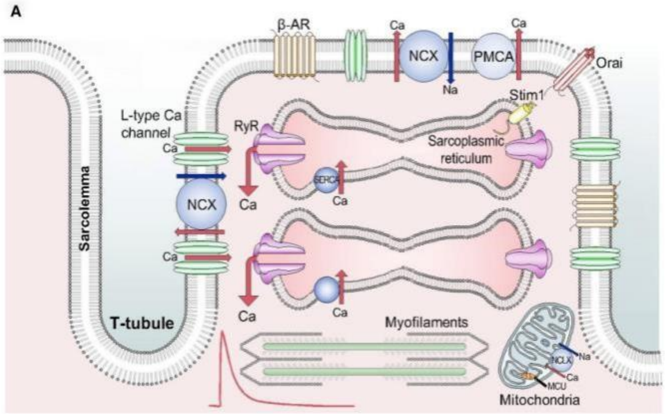

**Figure 3**: The organization of the cardiomyocyte. Key features such as the SR, RyR, LTCCs, and NCX are localized to small spaces where efficient CICR can be carried out. *Eisner et al. 2017.*

### *Molecular Coordination of the Dyad*

Throughout the surface of the T-tubules are L-type calcium channels (LTCCs). These calcium channels are voltagegated calcium channels which facilitate a slow influx of calcium into a cell, making them useful for the transmission of cardiac contraction and action potential transmission in the nervous system. These channels are heteromultimeric structures which respond to changes in voltage from the sino-atrial (SA) node and atrio-ventricular (AV) node. The SA node is a bundle of cardiac tissue that acts as the pacemaker of the heart, while the AV node transmits SA node signals to the ventricles to ensure proper filling of the heart. Responses to these action potentials could not occur without the use of voltage gated ion channels like the LTCC. LTCCs are also essential for the process of calcium induced calcium release (CICR). The initial release of calcium and depolarization causes the ryanodine receptor channels (RyRs) of the SR to release, opening the gates for more calcium to be released into the cytosol from the SR. As mentioned previously, the SR is directly impacted by downstream signaling of adreno-receptor stimulation via PKA. This also applies to RyRs. RyRs are macromolecular structures that are positioned on the junctional face of the SR where it faces towards the LTCCs. The RyRs are located specifically in the membrane junctional face of the SR, and along with other proteins such as calsequestrin, acts to keep calcium inside the SR, and respond to depolarization of the cell as a result of initial calcium that was released by the LTCCs.[18] When exposed to the downstream stimulatory impacts of adrenergic receptor stimulation, RyRs respond faster to depolarization, reducing the latency between LTCC and RyR coordination. Additionally, synchronization occurs between the RyRs, which makes contractions more efficient and stronger.[34] All of this is able to occur because of the careful organization in the dyadic space. Therefore, the close association of the SR (where the RyRs are located) with the LTCCs is necessary for healthy cardiomyocyte contraction. Increased PKA signaling from adrenergic stimulation causes the more rapid triggering of LTCCs as well, with decreased rates of random activation and aberrant signaling. The compartmentalization of all of these proteins is a pattern that is also understood as beneficial to $\boldsymbol{\beta}1$ and $\boldsymbol{\beta}2$AR assisting in signaling. The sequestering of $\boldsymbol{\beta}1$AR has been shown to impact its activation of cAMP and downstream signaling of PKA.[31] The action potential that is initiated by the electrical impulses of the heart activate the LTCCs, causing the release of calcium into the cytosol where it triggers the release of a much larger quantity of calcium from the SR. In a cycle termed excitation-contraction coupling (EC), the calcium induced to release from the SR initiates various cellular processes in the cytosol which causes a contraction.[13] By allowing a small influx of calcium into the cell via the LTCC, a larger amount of calcium is released from the SR as the RyRs respond to the depolarizing environment. The progression of this cycle relies on the affinity change of the troponin I to actin from Troponin C. This causes crossbridge formation, and the hydrolysis of ATP. The force generated by this conformational change pulls the actin filament along the myosin filament.[11] A visual of this process can be seen in **Figure 4** with the depiction of the canonical myosin-actin interactions that takes place in the sarcomeres.

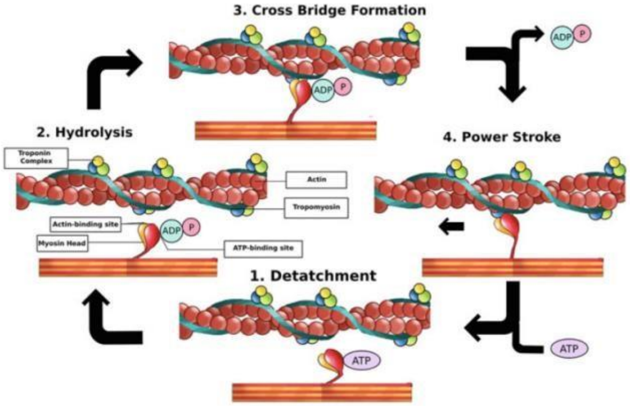

**Figure 4**: A basic model of how contraction within the sarcomere is carried out. Dissociation of ADP and inorganic phosphate causes the power stroke, which pulls the actin along the myosin. *Kartha et al. 2021*

### *Relaxation Kinetics of the Heart*

Importantly, the relaxation kinetics and physiology of cardiomyocyte contractility are equally necessary for healthy heart function. While heart failure typically results from the lack of left ventricular ability to contract forcefully enough (HFrEF), it is the relaxation of left ventricular filling that is dysregulated in heart failure with preserved ejection fraction (HFpEF).[15] Several proteins play key roles in the relaxation (or diastolic phase) of the heart during the contractile cycle. For relaxation to occur, the calcium that was pumped into the cytosol must be removed so that the cell can repolarize. The first key protein is the sarcoendoplasmic reticulum calcium ATPase (SERCA), which pumps calcium released from the SR back into the SR. SERCA is regulated by a protein known as phospholamban which typically acts to inhibit SERCA in its dephosphorylated state. When phosphorylation occurs, the inhibition of SERCA is removed and the calcium can rush back into the SR. This phosphorylation occurs via PKA, which would be activated when a large influx of calcium occurs, representing an efficient negative feedback loop. This occurs due to the increased affinity that SERCA gains for calcium when phospholamban is phosphorylated.[19] This prevents the sequestration of calcium back into the SR, which ensures that contraction happens for a sufficient amount of time. Phospholamban is specifically phosphorylated by calcium/calmodulin-dependent protein kinase II (CaMKII), which is a protein kinase that plays a large role in many calcium dependent processes, such as cardiac function and synaptic pruning.[26] Further calcium in the cytosol may also be released out of the cell entirely by the sodium-calcium exchanger (NCX).[16] The NCX is an antiporter that acts to maintain ion concentrations inside and outside of the cell by transporting 3 sodium in one direction, and a single calcium ion in another direction.

### ***β**-Adrenergic Receptors*

When looking at the overall contractility and calcium signaling of the heart, adrenergic receptors prevail as the most important and abundant class of protein that initiate and carry out this process. ***β***ARs carry out several functions within the heart alone, including the regulation of heart rate, contractility, and other intracellular processes.[29] Under healthy conditions in the heart, the $G\alpha_S$ GPCR, which is present at the highest concentration in the ventricles, activates adenylyl cyclase VI, which is closely associated with ***β***-1 adreno- receptors (***β***1AR) present on the surface of the cardiomyocytes. The $G\alpha_S$ system is one of the most abundant G proteins for initiating signaling. β1ARs largely mediate the activity of protein kinase A (PKA), which is commonly activated by $G\alpha_S$. Adenylyl cyclase activates second messengers such as cAMP, which can bind to PKA. Several types of βAR receptors are found within the heart and transmit important signals such as sympathetic nervous system arousal signals (i.e. adrenaline). β1ARs and β2AR activate L-type calcium channels (LTCCs) in different ways as well. β1ARs activate LTCCs across the eternity of the myocyte via the action of PKA. LTCCs are voltage gated ion channels important for transmitting calcium induced signals and will be expanded on in depth later. For contractile function and rate regulation, the β1AR are the most important class of adrenergic receptors, and couple directly with the $G\alpha_S$ GPCR. β1ARs are commonly thought to initiate the contraction of the cardiac muscle over larger tissue areas. On the other hand, β2Ars are only able to activate LTCCs in their local vicinity.[25] Instead of activating contraction of cardiac tissue, β2ARs initiate the relaxation of smooth muscle such as vessels.[29] Furthermore, the downstream signaling effects of β1 and β2ARs vary in cardiac muscle as well. β2ARs have been noted to initiate a larger quantity of cAMP, despite the larger observed functional impact that β1ARs have on the heart.[30] This is likely due to the former activating cAMP on a wider and greater area, while the latter have more specified regions where they activate cAMP. The process via which this pathway takes place can be seen in **Figure 5,** where the downstream signaling of adrenergic receptors is depicted as connected to RyR and LTCC signaling via PKA and calcium movement. βARs have a direct and observable effect on calcium loading in the sarcoplasmic reticulum (SR), which is a membrane bound smooth type of ER which stores intracellular calcium. Stimulation of β1ARs is observed to have a direct increase on the amount of calcium released into the cell during systole.[34] Because localization of calcium is important to the transmission of signals in the heart, the modeling of the locations of the SR and LTCCs are directly related to the transmission of calcium signals by adrenergic receptors.

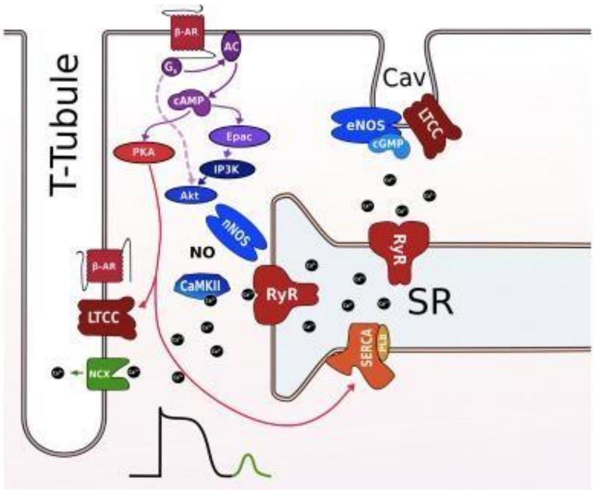

**Figure 5**: The canonical beta-adrenergic receptor stimulation pathway as seen within the dyadic space of the cardiomyocyte. The functional units within the cardiomyocytes are closely grouped together in order to maximize the efficacy of CICR. *Johnson et al. 2018.*

### *Activation of Canonical GPCR Signaling Pathways*

βAR and sympathetic nervous system arousal have been linked to the progression of the cardiac symptoms seen in HF. The chronic stimulation of βARs as a compensatory mechanism in order to regain the perfusion of tissues around the body leads to desensitization of the receptors. In addition to this, many βARs become decoupled to their G protein counterparts as a result of increased Gi signaling.[27] The close relationship between β-adrenergic receptors and the $G\alpha_i$ and $G\alpha_S$ pathways has been closely documented. Both β1 and β2ARs couple to the $G\alpha_S$ pathway, with this specific G protein acting as the downstream signaling pathway initiator during catecholamine stimulation of the adrenergic receptors.[30] Therefore, the importance of these two pathways cannot be understated in looking at cardiac contractility and the progression of pathology. Both of these pathways operate through activation of inhibition of PKA, which explains the ways in which they activate downstream signaling pathways through phosphorylation.

## *Impacts of MI on Adrenergic Receptors*

Post-MI, many β1ARs are uncoupled from their G proteins, leading to loss of signal transduction and the formation of orphaned receptors. Many receptors may become internalized or degraded in the early stages post-MI as well, with expression profiles showing a stark drop in βAR in the house after MI. Free radical formation also causes the destruction of the protein adenylyl cyclase during the ischemic attack, leading to further disruption of GPCR signaling.[4] The decoupling of GPCRs that has been observed in MI and post-MI has been linked to aberrant calcium signaling within the heart. Because calcium signaling controls the contractility of cardiac muscles, its mishandling within the cell leads to decreased cardiac output and susceptibility to arrhythmia later in life.[7] While decoupling continues, the expression of adrenergic receptors on the cardiac surface is seen to increase as a compensatory mechanism. However, this is typically unable to rebound the EF loss post-ischemia.[4] In addition to this, there is an increase in Gi signaling, the inhibitory pathway that takes place as the GPCRs are being uncoupled. This leads to lower basal activation of adenylyl cyclase in the event of ischemia. Further post-translational modifications may take place that impact adenylyl cyclase activity, as its expression is independent of the overall impacts seen to GPCRs in ischemia.[9] All of these factors contribute to the dissociation of the cardiac dyad and the decoupling of the RyR to the LTCC, leaving much calcium free-floating. This impacts the downstream signaling that calcium initiates, as well as the action potentials spread around the heart becoming dysregulated from remodeling. The stimulation of βARs to counteract this leads to desensitization and the eventual drop in EF, causing the clinical presentation of HF symptoms. Therefore, understanding of this relationship is critical to developing treatment options.

## *Pathological Cardiac Modeling and Hypertrophy*

Heart failure (HF) is a condition marked by the lack of the ability of the heart to sufficiently supply the body with oxygenated blood. Left-sided heart failure (often marked as HFrEF) is when the left ventricle is unable to sufficiently pump oxygenated blood into the blood stream due to the hypertrophic remodeling of the ventricle.[8] In this type of heart failure, the ejection fraction is reduced to below 40%.[3] In order to compensate for the decreases in EF, sympathetic nervous system arousal is a common symptom that accompanies HFrEF patients. An increased risk of HF can result from previous cardiac events, such as myocardial infarction (MI). When analyzing the overall physiological implications of HF, it is important to begin by looking at the most basic units of the heart. The cardiomyocyte is the basic contractile cellular unit of cardiac tissue. Similar to other (skeletal) muscle tissues, it is striated with sarcomeres and uninucleated. The remodeling of cardiomyocytes takes place when the $G\alpha_S$ pathway is disrupted in pathology, typically by increased stimulation from adrenergic receptors. During the compensatory phases of post-MI adrenergic receptor stimulation, chronic activation of the $G\alpha_S$ pathway occurs, leading to elevated heart rate and pressure within the heart. Over time, this leads to the hypertrophy of the left ventricle due to the increased load being placed on the heart.

Another major pathway that leads to hypertrophic effects in HF is the $G\alpha_q$ pathway. When observed in HF, there is an increase in the expression of the $G\alpha_q$ pathway, which leads to the downstream activation of PLC, IP3, DAG, and subsequently PKC. While this can cause the activation of processes such as cardiac contractility, it also promotes the synthesis of various proteins that lead to hypertrophy. Namely, through PKC activation of the mitogen activated protein kinase (MAPK) pathway. This pathway leads to the activation of transcription factors that induce hypertrophy.[28] A significant number of changes occur to the physiology of cardiomyocytes under pathological conditions. Namely in heart failure from past ischemic events, the contractile ability of the heart is impacted due to the death of cardiac tissue. A combination of complex metabolic changes that lead to the preference of glucose for energy, increased reactive oxygenated species (ROS), and decreased high energy phosphate groups present (such as on ATP or phosphocreatine) leads to decreased EF and cardiac output.[10] In the early stages following an event of ischemia, other physiological changes that take place in the heart are made to compensate for the loss of EF and cardiac output.[21] Under pathological conditions, calcium uptake that is typically mediated by SERCA back into the SR is impaired due to aberrations in the RyRs. An important protein that is responsible for the remodeling of cardiomyocytes in hypertrophy is stromal interaction molecule 1 (STIM-1), which is associated with the calcium release channels ORAI 1/3.[16] Due to the expansion of cardiomyocytes in hypertrophy, hypertrophy causes increased calcium load into the cell via ORAI channels. In heart failure conditions, hypertrophy refers to the abnormal muscular enlargement of the heart. This typically occurs with the left ventricle, which works to pump a large volume of blood out of the heart and into circulation, therefore pushing against all of the blood circulating around the body. This stretching of the cardiomyocytes leads to the end-to-end association of sarcomeres, which leads to decreased contractile force over time.[22]

### *Metabolic Changes and ROS*

Alterations in the physiology of mitochondria, such as in processes like fission, mitophagy, and fusion, causes a switch from fatty-acid oxidation and carbohydrate metabolism to anaerobic energy breakdown. Further dysfunction is seen in mitochondria during HF with an increased number of ROS. ROS are generated in the electron transport chain and are harmful free radicals that can cause oxidative stress in the cell. In the failing heart, ATP-binding cassette transporters (ABCs), which regulate the redox state of the mitochondria using iron heme groups are decreased, which increases oxidative stress.[23] Prolonged oxidative stress can cause damage to DNA, cell membranes, and other organelles as the antioxidant mechanisms of the cells are overcome with free radicals. This adds another level of complexity to the stress already placed on cardiomyocytes, leading to further dysfunction.

### *Electrochemical Alterations in the Heart*

As previously mentioned, a compensatory mechanism seen in HF is the arousal of the sympathetic nervous system. Chronic stimulation of cardiomyocytes with catecholamines such as adrenaline have been shown to alter the metabolic responses that cells have. Previous studies such as those performed by *Zhou et al. 2009* have studied this compensatory mechanism using the catecholamine agonist isoproterenol. When β-ARs are stimulated using isoproterenol, the coupling of LTCCs and RyRs is significantly impacted. Modulation of RyRs has a significant impact on EC coupling within the heart as a result of physiological rearrangement. Namely, the synchronization of RyRs is impacted. More RyRs are recruited in order to carry out a contraction during catecholamine stimulation. The use of loose-patch confocal imaging allows for the visualization of a single LTCC and RyR that are coupled in the dyadic space. When treated with isoproterenol, the latency of the depolarization signal sent between the LTCC and RyR is seen to become altered when compared to healthy controls. These conditions represent the early stages of HF soon after an ischemic event, where the body is compensating for a reduced EF by utilizing sympathetic nervous system arousal. This is evident in the decreased latency seen between LTCC and RyR sparks, where there is a more efficient transmission and increased heart rate and blood pressure as a result.[34] However, in the later stages of HF, there is seen to be a less efficient transmission of signals between these two structures. The formation of so-called "orphaned" or "leaky" channels causes an increase of free calcium within the cell, leading to aberrant signaling and furthering dysfunction.[35] This process is largely believed to be mediated by PKA, whose activation can again be linked to adrenergic receptor stimulation. Previous studies have shown that through the use of knock-in mice which express higher than normal levels of RyRs, this same pattern of hyperphosphorylation is evident.[36] These same studies linked ECC deficiencies to the dissociation of RyRs from the LTCCs. Specific binding proteins that are key in anchoring the RyRs close to LTCCs are critical for maintaining the integrity of the dyadic space. However, hyperphosphorylation via PKA leads to their dissociation, which causes the formation of leaky receptors.

## TECHNIQUES:

### *Fluorescence and Loose-Patch Confocal Imaging*

In order to more accurately visualize the impacts of cardiac remodeling and how this connects to the larger idea of calcium dysregulation via beta adrenergic receptors, it is necessary to utilize imaging techniques that can track calcium movement throughout the cell. A major method to analyze this is through the use of patch clamp electrophysiology. Patch clamp electrophysiology allows for the measurement of voltage changes across cell membranes to track when cells depolarize and repolarize, such as with cardiomyocytes during the contractile cycle.[33] This method operates through the use of a pipet tip that is in contact with the membrane of the cell being measured. A tight seal is formed between the pipet and the cell, and the voltage changes in this part of the cell are analyzed. In the context of cardiomyocytes, the depolarization of sodium channels and the repolarization by potassium channels can be tracked. Loose-patch confocal imaging differs from regular confocal imaging in that the normally tight seal that is formed between the pipet and the cell is not formed. This is typically performed for the purpose of not disturbing the local environments of the cell. Measuring the dyadic space of the cardiomyocytes is critical to understanding the method for hypertrophic development and altered calcium signaling. If the environment of the cell was disturbed too much, it would lead to possible artificial alterations to the cellular environment.[34] In addition to tracking calcium changes due to remodeling impacts, analyzing the general flow of calcium and other ions is critical to understanding cardiac pathology. Confocal fluorescence imaging is a common technique used for this purpose, which utilizes a dye that can be excited with a laser. Dyes such as Fura2 are used to bind free calcium within cells, and the confocal microscope is able to take excitation-emission data and generate graphs known as calcium transients. These graphs display what the course of calcium release and reuptake looks like based on fluorescence data. This can be seen in **Figure 6,** where the coupling latency between the LTCC calcium release and RyR calcium release is measured using a patch clamp pipet for voltage and confocal imaging for calcium sparks. When combined with techniques like loose-patch confocal imaging, the behavior of ion channels like the LTCC can be directly observed. Using this method, there was found to be a 62% higher amplitude after isoproterenol stimulation with calcium sparks. Additionally, ryanodine receptors are pulled away from the LTCCs, and their activation is decreased by around 2 ms. RyRs are also activated by depolarization less often and are more often activated by the synchronization of surrounding RyRs, making action potentials less likely. This is modulated by PKA. Another major finding was that these changes are independent of the concentration of calcium within the SR. While initially increased due to βAR stimulation, loose-patch confocal imaging reveals that this concentration did not stay increased significantly over time.[34]

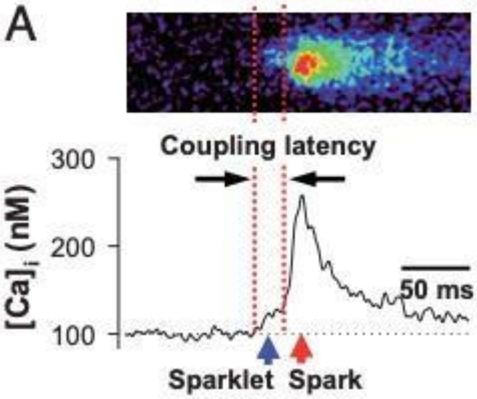

**Figure 6***:* Graph of βAR stimulation impacts on the kinetics of LTCC and RyR coupling. There is a delay noted between the opening of the LTCC (sparklet) and the activation of the RyR (spark). This can be seen in the trace, while the top image shows membrane depolarization delay, as the coupling latency is increased. *Zhou et al 2009.*

### *Therapeutic Approaches for HF*

Because there is a diverse array of clinical symptoms presented with HF, the number of possible approaches to treatment are numerous. Hypertrophy of the heart as the post-ischemic pathology continues in the years leading to heart failure represents the most immediate threat to life for those afflicted. Angiotensin receptor blockers and angiotensin converting enzyme inhibitors are used to prevent cardiac remodeling and stop hypertrophy.[25] The reninangiotensin-aldosterone pathway is an important, multisystem mediated pathway that works to maintain stable blood pressure and sodium absorption. The final product of this pathway, angiotensin II, acts on blood vessels and causes them to constrict, which increases blood pressure. In the context of pathological heart failure, this would have negative impacts on the heart. Increased blood pressure leads to hypertrophy over time, due to the harder contraction required by the left ventricle. Angiotensin II also signals vascular smooth muscle contraction.[25] Chronic stimulation with Angiotensin II as a compensatory mechanism in early heart failure causes signaling of the angiotensin II type 1 receptor (AT1R), which is seen to be downregulated in the end stages of HF. Therefore, the use of ACE inhibitors and angiotensin receptor blockers is necessary to slow the remodeling effects of HF. Additionally, blood pressure medications such as beta blockers work to directly counteract the compensatory mechanisms of the heart in HF. Beta blockers negate the effects of catecholamines like adrenaline by blocking binding sites of βARs. This helps to relax blood vessels and lower blood pressure. Because of the reduced ejection fraction of the heart and its reduced ability to efficiently manage fluids within the body, medications like diuretics may also be prescribed. Diuretics work to clear excess fluid that may have invaded the interstitial space via the lymphatic system. This causes the collection of water at the lower parts of the body, causing massive inflammation in a condition known as edema. A medication complementary to this are SGLT2 inhibitors. These inhibit the sodium-glucose cotransporter 2 (SGLT2) proteins that are present in the proximal convoluted tubule of the nephron, which prevents the reabsorption of glucose and other ions. Because water follows a solute gradient, it causes water to be excreted in larger volumes, which lessens the fluid load that the heart must pump. These medications represent ways to manage symptoms, while no current operations are able to reverse the effects of HF. SGLT2 inhibitors work directly to counteract the activity of the sympathetic nervous system, counteracting the effects of βARs. Exploring these avenues of treatment are necessary for the suppression of HF symptoms management of chronic βAR stimulation.

## CONCLUSION:

Cardiovascular disease, especially that of heart failure, represents a major growing threat to the elderly population, as well as those with pre-existing conditions. During the early stages of HF, chronic stimulation of βAR acts as a compensatory mechanism, activating downstream signaling pathways that are impacted by remodeling of the vasculature after ischemic cell death. The role of LTCCs and RyRs can be directly connected to the depolarization that is initiated by $\boldsymbol{\beta}$AR stimulation, leading to impaired contractile mechanisms within cardiomyocytes. While the pathology of HF has been extensively studied, there are still many unanswered questions in regard to the dyadic space and remodeling of the cardiomyocyte. For example, while much of the downstream signaling present can be attributed to the action of PKA, the exact mechanisms by which RyRs are found to lose their function is currently unknown. Additionally, many studies load calcium into the SR with manual loading buffers. The loading of calcium within the SR is an independent mechanism and cannot be directly studied when the cell is manually loaded with calcium. Therefore, this limits the actual clinical application that can be applied in this instance. For further clinical translation, this type of research has many applications. The physiological changes that take place during HF significantly lower the quality of life for those affected. Therefore, understanding the compensatory mechanisms that the body tries to employ but ultimately end up being detrimental to cardiac function are critical to mitigating the wide array of symptoms. The heart is arguably one of if not the most vital organ of the body, and it is often represented in culture and society as being the core of human beings, mediating love and feelings of connectedness and kindness. Therefore, understanding how to mend and fix the failing heart is integral to the human experience.

# AUTHOR INFORMATION:

**Liam Gannon**

Temple University, Philadelphia, Pennsylvania 19122, United States

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### ABBREVIATIONS

$\boldsymbol{\beta}$AR, $\boldsymbol{\beta}$-adrenergic receptor. RyR, ryanodine receptor. LTCC, L-type calcium channel. GPCR, G-protein coupled receptor. SR, sarcoplasmic reticulum. NCX, sodium calcium exchanger. PKA, protein kinase A. ACE, angiotensin converting enzyme. ROS, reactive oxygen species. HF, heart failure. LV, left ventricle. EF, ejection fraction. SERCA, SRER-calcium ATPase. EC, excitation- contraction coupling. CICR, calcium induced calcium release. DAG, diacylglycerol. MI, myocardial infarction.

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