Targeting Ca\textsuperscript{2+} Handling Proteins for the Treatment of Heart Failure and Arrhythmias

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Diseases of the heart, such as heart failure and cardiac arrhythmias, are a growing socio-economic burden. Calcium (Ca\textsuperscript{2+}) dysregulation is key hallmark of the failing myocardium and has long been touted as a potential therapeutic target in the treatment of a variety of cardiovascular diseases (CVD). In the heart, Ca\textsuperscript{2+} is essential for maintaining normal cardiac function through the generation of the cardiac action potential and its involvement in excitation-contraction coupling. As such, the proteins which regulate Ca\textsuperscript{2+} cycling and signaling play a vital role in maintaining Ca\textsuperscript{2+} homeostasis. Changes to the expression levels and function of Ca\textsuperscript{2+}-channels, pumps and associated intracellular handling proteins contribute to altered Ca\textsuperscript{2+} homeostasis in CVD. The remodeling of Ca\textsuperscript{2+}-handling proteins therefore results in impaired Ca\textsuperscript{2+} cycling, Ca\textsuperscript{2+} leak from the sarcoplasmic reticulum and reduced Ca\textsuperscript{2+} clearance, all of which contributes to increased intracellular Ca\textsuperscript{2+}. Currently, approved treatments for targeting Ca\textsuperscript{2+} handling dysfunction in CVD are focused on Ca\textsuperscript{2+} channel blockers. However, whilst Ca\textsuperscript{2+} channel blockers have been successful in the treatment of some arrhythmic disorders, they are not universally prescribed to heart failure patients owing to their ability to depress cardiac function. Despite the progress in CVD treatments, there remains a clear need for novel therapeutic approaches which are able to reverse pathophysiology associated with heart failure and arrhythmias. Given that heart failure and cardiac arrhythmias are closely associated with altered Ca\textsuperscript{2+} homeostasis, this review will address the molecular changes to proteins associated with both Ca\textsuperscript{2+}- handling and -signaling; their potential as novel therapeutic targets will be discussed in the context of pre-clinical and, where available, clinical data.

Keywords: cardiovascular disease, heart failure, arrhythmia, calcium dysregulation, calcium

Abbreviations: AC, adenylyl cyclase; AC6, adenylyl cyclase 6; ACE, angiotensin-converting enzyme; ACEI, ACE inhibitors; AF, atrial fibrillation; ANG-II, angiotensin II; AP, action potential; ARB, angiotensin receptor blocker; AVN, atrioventricular node; Ca\textsuperscript{2+}, calcium; CaM, calmodulin; CaMKII, Ca\textsuperscript{2+}/calmodulin-dependent protein kinase; CPVT, catecholaminergic polymorphic ventricular tachycardia; CVD, cardiovascular disease; DAD, delayed after depolarization; EAD, early after depolarization; ECC, excitation contraction coupling; GWAS, genome wide association studies; HF, heart failure; HFmEF, HF with mid-range ejection fraction; HFrEF, HF with preserved ejection fraction; HFrEF, HF with reduced ejection fraction; HNO/NO, nitroxyl; I_{CaL}, Ca\textsuperscript{2+} current; I_{CaT}, L-type Ca\textsuperscript{2+} current; I_{K1}, K\textsuperscript{+} current; I_{Na}, Na\textsuperscript{+} current; I_{NCX}, NCX current; I_{out}, transient outward K\textsuperscript{+} current; I_{SR}, ischaemia/reperfusion; LQT, long QT syndrome; LTCC, L-type Ca\textsuperscript{2+} channel; MAPK, mitogen activated protein kinase; MCU, mitochondrial Ca\textsuperscript{2+} uniporter; MI, myocardial infarction; NCX, Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger; NFAT, nuclear factor of activated T-cell; PKA, protein kinase A; PLN, phospholamban; PMCA, plasma membrane Ca\textsuperscript{2+} ATPase; PPy-3, pyrazole-3; RAAS, renin-angiotensin–aldosterone signaling; ROS, reactive oxygen species; RR, rubidium red; RyR, ryanodine receptor; SAN, sino-atrial node; SERCA, SR Ca\textsuperscript{2+} ATPase; SNPs, single nucleotide polymorphisms; SR, sarcoplasmic reticulum; TAC, transverse aortic constriction; TdP, Torsades de Pointes; TRIC, trimeric intracellular cation.
INTRODUCTION

Cardiovascular diseases, such as ischemic heart disease, stroke, and arrhythmias, are the leading cause of death globally (Roth et al., 2017); with over 17 million fatalities attributed annually to CVD (Collaborators GBDeCoD, 2018). Acquired CVD are thought to arise due to a combination of modifiable environmental causes and underlying genetic pre-dispositions (Kannel et al., 1961; Khot et al., 2003). CVDs lead to both functional and structural changes to the vasculature and myocardium. In the heart, cardiac dysfunction encompassing cardiac decompensation, arrhythmogenesis and impaired contractility are all closely associated with changes to calcium (Ca\(^{2+}\)) handling proteins, Ca\(^{2+}\) channels and Ca\(^{2+}\) pumps (Lou et al., 2012). Determining the full scope of changes to Ca\(^{2+}\)-handling proteins and mechanisms will aid in the development of novel therapeutics to reverse and/or prevent CVD. This review will focus on how changes to Ca\(^{2+}\)-handling proteins observed during cardiac arrhythmogenesis and HF have been therapeutically targeted, with emphasis placed on recent developments in the search for novel therapies.

Ca\(^{2+}\) AND Ca\(^{2+}\)-HANDLING PROTEINS IN THE HEART

Maintenance of Ca\(^{2+}\) Homeostasis

The initiation and control of each heartbeat is achieved by the cardiac conduction system consisting of the SAN, AVN, and His-Purkinje system. Electrical impulses, termed cardiac AP arise from the SAN, often referred to as the pacemaker of heart, and propagate throughout the heart (Boyett, 2009). During the cardiac AP, the electrical membrane potential rapidly rises and falls, referred to as depolarization and repolarization respectively, triggering ECC. The changes in membrane potential are driven by the movement of ions across the cell membrane (Grant, 2009). Generally, the cardiac AP is divided into five phases (Figure 1): (Phase 0) involves rapid depolarization of the membrane potential which is key to the propagation of cardiac impulse in the heart; (Phase 1) is a brief interval of rapid depolarization; (Phase 2) known as the plateau phase of the cardiac AP in which Ca\(^{2+}\) enters the cell to trigger ECC; (Phase 3) rapid repolarization restores the membrane potential; and (Phase 4) the membrane potential is stable at the resting potential (Grant, 2009). Following an AP, the cell enters a recovery state termed the refractory period. Here, the cell is unable to initiate an AP which allows the heart to prepare for the next contraction (Ramza et al., 1990). The key ionic currents involved in the generation and propagation of the cardiac AP are the Ca\(^{2+}\) (I\(_{Ca}\)), sodium (Na\(^{+}\), I\(_{Na}\)), and potassium (K\(^{+}\), I\(_{K}\)) currents (Grant, 2009). It is worth noting that different cell types within the cardiac conduction system exhibit specific cardiac AP relating to their function. Differences in ion channel expression results in regional specific APs (Monfredi et al., 2010).

In the heart, Ca\(^{2+}\) has an indispensable role in cardiomyocyte ECC; in addition, as a signaling molecule, Ca\(^{2+}\) also acts as an intracellular second messenger in cardiomyocytes and other cell types. ECC results in cardiac contraction and helps to maintain diastolic and systolic cardiac function (Lou et al., 2012). During ECC, four steps involving Ca\(^{2+}\) trigger cardiomyocyte contraction: (1) membrane depolarization causes a rapid influx of Ca\(^{2+}\) through the LTCC located on transverse (T)-tubules, which results in an intracellular Ca\(^{2+}\) gradient in the junctional zone; (2) Ca\(^{2+}\) diffusion activates RyR located on the SR resulting in Ca\(^{2+}\) sparks and an amplification of the Ca\(^{2+}\) signal in a process termed Ca\(^{2+}\)-induced Ca\(^{2+}\) release; (3) Ca\(^{2+}\) release via RyR from internal SR stores binds to troponin-C to cause contraction through actin-myosin filament crossbridge formation; (4) Ca\(^{2+}\) is transported out of the cardiomyocyte through NCX and PMCA or back into internal stores through SERCA and MCU (Bers, 2002; Lou et al., 2012) (Figure 2). The function of SERCA is tightly regulated by PLN. In its dephosphorylated form, PLN inhibits SERCA2a but when its phosphorylated at Ser10, Ser16 or Thr17, the inhibition of SERCA2 is relieved (Mattiazzi et al., 2005). Such is the importance of PLN-mediated SERCA2a regulation that PLN ablation induces a hypercontractile cardiac phenotype (Luo et al., 1994). Ca\(^{2+}\) homeostasis is also maintained through additional Ca\(^{2+}\) channels located on the plasma membrane (Figure 2). The transient receptor potential channels (TRP) are a large family of non-selective cation permeable channels which, depending on the family, permit the influx of Ca\(^{2+}\), along with Mg\(^{2+}\), Zn\(^{2+}\), and Na\(^{+}\) (Falcon et al., 2019). The TRP family can be further divided into subfamilies, including TRP-canonical (TRPC), TRP vallinoid-related (TRPV), and TRP melastatin-related (TRPM) (Freichel et al., 2017). The ECC Ca\(^{2+}\) cycle is tightly regulated by protein kinases and accessory proteins which modify the activity and expression of ECC Ca\(^{2+}\) handling proteins. Functional changes to proteins involved in ECC and Ca\(^{2+}\) homeostasis can impair contractility and contribute to cardiac dysfunction. Furthermore, changes to Ca\(^{2+}\) handling can also impair cardiac conductivity, causing fatal arrhythmias. Altered Ca\(^{2+}\) homeostasis can result in abnormal automaticity, triggered activity, and can provide a substrate for re-entry arrhythmias (Landstrom et al., 2017). The contributions these Ca\(^{2+}\) handling proteins make to the pathophysiology of cardiac hypertrophy, HF, and cardiac arrhythmias are discussed in detail later in this review.

Current Therapeutic Approaches

Targeting HF and Cardiac Arrhythmia and Their Impact on Ca\(^{2+}\)-Handling Proteins

Treatment of HF and cardiac arrhythmias can be surgical (including implantation of a pacemaker or implantable cardioverter defibrillator) and/or pharmacological. Primary drug-based therapies for these disorders include targeting (1) the RAAS signaling system, (2) β-adrenergic hyperactivation, and (3) aberrant ion channel activation (Ca\(^{2+}\), Na\(^{+}\), and K\(^{+}\)). As the focus of this review concerns how Ca\(^{2+}\) handling proteins present a novel therapeutic approach, we discuss the current
pharmacological therapies mentioned above in brief, including any known impact they have on Ca\textsuperscript{2+} handling in the heart.

The Renin–Angiotensin–Aldosterone System
Renin–angiotensin–aldosterone signaling is elevated in patients with HF and reduced ejection fraction (HFrEF, ejection fraction < 40%) (Watkins et al., 1976) and in advanced HF, the levels of plasma renin activity and aldosterone are increased (Dzau et al., 1981). Furthermore, aldosterone secretion is enhanced in response to ANG-II signaling and its receptor is upregulated in cardiomyocytes from HFrEF patients (Yoshida et al., 2005). Aldosterone alone is pro-hypertrophic, pro-fibrotic and pro-inflammatory (Pinilla-Vera et al., 2019). Increased neurohormonal signaling can activate the MAPK which, in turn, upregulates pro-hypertrophic gene transcription (Zhang et al., 2003; Nakamura and Sadoshima, 2018). The MAPK signaling cascade comprises four main ‘typical’ branches; ERK1/2, JNK, p38 and ERK5, all with distinct upstream regulators (Rose et al., 2010) and activation of the ERK1/2 signaling cascade may determine whether concentric or eccentric cardiomyocyte growth and remodeling occurs (Bueno et al., 2000; Lorenz et al., 2009; Kehat et al., 2011). Activation of RAAS can also promote cardiac arrhythmias including AF and ventricular tachyarrhythmias, partly due to the formation of a pro-arrhythmia substrate in cardiac hypertrophy and HF. In addition, RAAS is also known to directly affect cardiac ion channels. Angiotensin and aldosterone have been shown to attenuate the transient outward K\textsuperscript{+} current (\textit{I}_{\text{to}}) and enhance \textit{I}_{\text{CaL}}, impacting AP duration and repolarization. Whilst oxidative stress triggered by RAAS activation is reported to affect cardiac ion currents and several Ca\textsuperscript{2+} handling proteins (Iravanian and Dudley, 2008).

The maladaptive effects associated with over-activation of the RAAS is one of main focuses of current therapeutics; both ACEI and ARBs are prescribed to patients with HF to
help improve cardiac function and survival (McMurray, 2004; Demers et al., 2005). Several clinical trials have shown that ACEI and ARBs reduce mortality in patients with HFrEF (Kjekshus et al., 1992; Maggioni et al., 2002; McMurray et al., 2003). In addition, further inhibition of RAAS is achieved through aldosterone antagonism. Aldosterone receptor blockade with an agent such as eplerenone has been shown to reduce the risk of death and hospitalization events in patients with HFrEF (Pitt et al., 2003; Zannad et al., 2011). However, the absolute beneficial use of aldosterone agonists in patients with HF and preserved ejection fraction (HFpEF) is still yet to be determined (Pitt et al., 2014). Studies have also aimed to dissect the use of RAAS inhibitors in patients with cardiac arrhythmias (Iravanian and Dudley, 2008). ACEI and ARBs have been shown to reduce the recurrence rate in patients with existing AF (Madrid et al., 2002; Ueng et al., 2003), and reduce the incidence of new onset AF in hypertensive (Wachtell et al., 2005)- and post-MI (Pedersen et al., 1999) patients.

Treatment with ACEI has also been associated with a reduced risk of sudden cardiac death in HF patients (Heart Outcomes Prevention Evaluation Study Investigator, Yusuf et al., 2000).

The β-Adrenergic Pathway
In HF, elevated sympathetic activity increases blood catecholamines; an increase which inversely correlates with survival (Lymeropoulos et al., 2013). The initial elevation in sympathetic activation is beneficial as cardiac contractility is enhanced to maintain function; however, chronic activation of β-adrenergic receptors (β-AR) promotes cardiac dysfunction through increased cardiomyocyte hypertrophy (Lohse et al., 2003). Catecholamine binding to β-AR activates AC and subsequently leads to an increase in cAMP levels; cAMP activates PKA which phosphorylates proteins associated with Ca^{2+} handling, including LTCC (Schroder et al., 1998), RyR2 (Wehrens et al., 2006), and PLN (Antos et al., 2001; Nakamura and Sadushima, 2018), together culminating in increased levels of cytosolic Ca^{2+} (Port and Bristow, 2001;
Nakamura and Sadoshima, 2018). In addition to phosphorylation of key Ca\(^{2+}\) handling proteins, \(\beta\)-AR signaling also results in the s-nitrosylation of many Ca\(^{2+}\) handling proteins in cardiomyocytes, such as PLN and cardiac troponin C (Irie et al., 2015). Furthermore, activation of \(\beta\)-AR is the primary mechanism underlying sympathetic control of heart rhythm, with many of the cardiac ionic currents responsive to \(\beta\)-adrenergic stimulation (Gardner et al., 2016). Unsurprisingly, enhanced adrenergic activity has been identified in arrhythmic conditions, including AF, and results in enhanced automaticity and triggered activity (Workman, 2010; Antzelevitch and Burashnikov, 2011).

Therapeutic targeting of this excessive \(\beta\)-adrenergic response is achieved using \(\beta\)-blockers. \(\beta\)-blockers are deployed to improve cardiac function, reduce incidence of arrhythmias, and decrease mortality associated with HF by diminishing the hyperactivity of the \(\beta\)-adrenergic pathway. Improvements in mortality rates in patients with HFpEF have been recorded, irrespective of the type of \(\beta\)-blocker (Chatterjee et al., 2013; Pinilla-Vera et al., 2019), and large randomized trials have provided further evidence of the advantageous effects of single \(\beta\)-blockers agents in the treatment of HFpEF (CIBIS-II Investigators and Committees, 1999; Tepper, 1999; Packer et al., 2002). Furthermore, a meta-analysis of all clinical trials involving the use of \(\beta\)-blockers in HFpEF patients suggested the use of \(\beta\)-blockers may reduce mortality by 22% (Fukuta et al., 2017). Interestingly, through their mechanism of action, \(\beta\)-blockers also inhibit PKA hyperphosphorylation of the RyR2 channel in both human and animal models of HF (Reiken et al., 2001, 2003); the proposed mechanism by which \(\beta\)-blockers improve cardiac function in HF is by preventing PKA hyperphosphorylation of RyR2 Ser2808 and subsequent receptor destabilization (Shan et al., 2016). The aim of Na\(^{+}\) channel blockers target the voltage-gated Na\(^{+}\) channel, Na\(_{\text{hA}1.5}\), which drives the inward Na\(^{+}\) current responsible for the rapid depolarization at the start of the cardiac AP (Lei et al., 2018). The aim of Na\(^{+}\) channel blockers is therefore to reduce the rate of the AP upstroke and reduce AP conduction in the atria, ventricle, and Purkinje fiber tissue (where Na\(_{\text{hA}1.5}\) is preferentially expressed). Class I Na\(^{+}\) channel blockers are further subclassified based on their molecular properties and interaction with the Na\(_{\text{hA}1.5}\) (Lei et al., 2018;
Li et al., 2019). Class Ia (quinidine, ajmaline, disopyramide) and Ib (lidocaine, mexiletine) Na\(^+\) channel blockers are used to treat patients with sustained ventricular tachycardia and ventricular fibrillation. In addition, quinidine has been indicated for short QT syndrome and Brugada syndrome and mexiletine suggested for the treatment of premature ventricular complexes, LQT3, and ventricular arrhythmias after MI (Al-Khatib et al., 2018; Lei et al., 2018). Class Ic agents (flecainide, propafenone) are used for ventricular tachycardia, premature ventricular complexes, and AF with flecainide also having a role in the treatment of CPVT (January et al., 2014; Al-Khatib et al., 2018). However, Class Ic agents should not be used in patients with underlying structural heart disease (January et al., 2014). Ranolazine, a Class Id agent, is used to treat ventricular tachycardia and differs from Class Ia-Ic agents by inhibiting the late Na\(^+\) current (Al-Khatib et al., 2018; Lei et al., 2018). Despite Na\(^+\) channel blockers being identified as an effective antiarrhythmic treatment; they have also been shown to be proarrhythmic. The Cardiac Arrhythmia Suppression Trial (CAST) in the late 1980s studied the effect of Na\(^+\) channel blockers on patients with ventricular ectopic activity up to 2 years following a MI. The study revealed a higher rate of death in patients receiving flecainide or encaïnide compared to the placebo group (Cardiac Arrhythmia Suppression Trial I, 1989). Na\(^+\) channel blockers are therefore generally limited to patients who do not have structural heart disease.

The Class III K\(^+\) channel blockers work by binding to K\(^+\) channels involved in Phase 3 repolarization, and thus delay repolarization and increase AP duration (Lei et al., 2018). Class IIIa K\(^+\) blockers can be divided based on their K\(^+\) channel target. Non-selective K\(^+\) channel blockers (ambisubilide, amiodarone, dronedarone) and K\(_{r}11.1\) blockers (dofetilide, ibutilide, sotolol) have been indicated for the treatment of ventricular tachycardia in patients with structural heart disease or with remote MI, tachyarrhythmias in patients with Wolf-Parkinson-White syndrome, rate control in AF with amiodarone the most effective antiarrhythmic drug for maintenance of sinus rhythm in patients with persistent or paroxysmal AF (January et al., 2014), ventricular fibrillation and premature ventricular contraction, and supraventricular tachyarrhythmias (January et al., 2014; Al-Khatib et al., 2018; Lei et al., 2018). Another type of K\(^+\) channel blocker which targets K\(_{r}1.5\) (vernakalant) has been suggested for the treatment of AF (Lei et al., 2018). Similar to Na\(^+\) channel blockers, K\(^+\) blockers can exhibit proarhythmic effects. QT interval prolongation caused by K\(^+\) channel blockers has been shown to predispose patients to ventricular arrhythmias and TdP (McCauley et al., 2016). Due to the AP prolongation effect, K\(^+\) channel blockers should not be given to patients with LQTS, those being treated with QT-prolonging drugs, or with comorbidities associated with an increased risk for TdP (Dan et al., 2018). In addition, co-administration of amiodarone with other cardiac therapeutics including β-blockers, can result in bradycardia and atrioventricular block (Dan et al., 2018). As well as K\(^+\) channel blockers, Class IIIb drugs are metabolically dependent K\(^+\) channel openers and target K\(_{r}6.2\) channels (although these have not been indicated in the treatment of arrhythmias) (Lei et al., 2018). A recent updated classification determined a Class IIIc group made up of transmitter dependent K\(^+\) channel blockers with BMS 914392 currently under review for the management of AF (Lei et al., 2018).

### PATHOLOGICAL CARDIAC HYPERTROPHY AND HEART FAILURE

Cardiac hypertrophy occurs under both physiological and pathological conditions. Hypertrophy itself is an adaptive cardiac response which arises due to either pressure- or volume-cardiac overload. Typically, in physiological hypertrophy, the increase in cardiomyocyte size is initially due to the addition of sarcomeres in parallel (eccentric hypertrophy) which increases both ventricular volume and relative wall thickness, resulting in increased contractility which preserves cardiac output (Nakamura and Sadoshima, 2018). Conversely, in pathological hypertrophy cardiomyocytes are added in series (concentric hypertrophy) in order to compensate for the increased cardiac stress, this type of hypertrophy results in maintained or reduced ventricle volume but an increased relative wall thickness (Sawada and Kawamura, 1991; Lazzeroni et al., 2016; Nakamura and Sadoshima, 2018). Furthermore, persistent maladaptive remodeling can eventually lead to ventricular dilatation (eccentric hypertrophy), contractile dysfunction and impaired cardiac output (Sawada and Kawamura, 1991; Lazzeroni et al., 2016; Nakamura and Sadoshima, 2018).

It is now widely believed that each form of hypertrophy is regulated by distinct molecular mechanisms that drive the differing cardiac phenotypes and overall prognosis. Despite the activation of initial compensatory hypertrophic mechanisms, maladaptive remodeling occurs when the following processes occur: cell death (apoptosis, necrosis, autophagy), deposition of fibrosis throughout the myocardium, impaired mitochondrial function and regulation, dysregulation of Ca\(^{2+}\)-handling proteins, re-expression of fetal genes, changes to sarcomere structure and rarefaction of the cardiac microvasculature (Nakamura and Sadoshima, 2018). Chronic maladaptive remodeling witnessed during the pathological response to hypertrophy often develops into HF with the diagnosis of HF itself rapidly becoming a universal healthcare and financial burden. HF is an end-stage CVD which frequently occurs secondary to many different aetiologies (Ziaeian and Fonarow, 2016). Physiologically, HF can be defined as (1) insufficient cardiac output to meet metabolic demands or (2) adequate cardiac output with increased left ventricular filling pressure through activation of compensatory neurohormonal pathways (Ponikowski et al., 2016). HF can be further classified into one of three subtypes: HFpEF, HFrEF or HfmrEF (Ponikowski et al., 2016; Rastogi et al., 2017). These subclassifications are divided based on quantitation of ejection fraction; HFrEF < 40%, HfmrEF > 40% but < 50% and HFPpEF > 50% (Ponikowski et al., 2016; Ziaeian and Fonarow, 2016; Rastogi et al., 2017).

The presence of altered Ca\(^{2+}\) transients in HF has been well-documented since the late 1980s. These early studies were amongst the first to show that cardiomyocytes isolated from the failing human heart exhibit reduced Ca\(^{2+}\) transient amplitudes,
fewer actin-myosin crossbridge formations, a slower rate of Ca\(^{2+}\) removal and increased resting intracellular Ca\(^{2+}\) when compared to healthy cardiomyocytes (Gwathmey et al., 1987, 1991; Beuckelmann et al., 1992; Hasenfuss et al., 1993). In broad terms, the failing cardiomyocyte shows impaired Ca\(^{2+}\) cycling, Ca\(^{2+}\) release from the SR and Ca\(^{2+}\) removal. Therefore, altering Ca\(^{2+}\) in HF through modulation of Ca\(^{2+}\) handling proteins has long been investigated as a therapeutic target.

CALCIUM HANDLING PROTEINS IN CARDIAC HYPERTROPHY AND HF AND THEIR POTENTIAL AS NOVEL THERAPEUTIC TARGETS

The National Institute of Clinical Excellence (NICE, United Kingdom) and other regulatory bodies such as the European Society of Cardiology recommends ACEIs and β-blockers as first-line treatment for HF (Al-Mohammad et al., 2010; Ponikowski et al., 2016); however, dysregulation of Ca\(^{2+}\) handling proteins occurs exclusively during pathological hypertrophy (and not physiological hypertrophy) which suggests altered Ca\(^{2+}\) cycling and signaling precedes Ca\(^{2+}\) dysregulation observed in HF. The following section of this review will focus on how proteins associated with Ca\(^{2+}\)-handling, encompassing Ca\(^{2+}\) signaling proteins and Ca\(^{2+}\) channels and pumps, are altered in cardiac hypertrophy and HF.

Ca\(^{2+}\) Homeostasis and Signaling in Hypertrophy and HF

Given the importance of Ca\(^{2+}\)-dependent signaling pathways in the development of hypertrophy and HF, there is scope for developing therapies which target their aberrant expression and activity. Three such potential targets are calmodulin (CaM), calcineurin and CaMKII which are all activated following the release of Ca\(^{2+}\) from internal stores. CaMKII signaling is upregulated in human HF (Anderson, 2009). Evidence from animal studies suggests that CaMKII-δ is important in the cardiac stress response as CaMKII-δ null mice show no overt cardiac phenotype under physiological conditions but have an attenuated response to pressure-overload induced hypertrophy (Backs et al., 2009; Ling et al., 2009). CaMKII-δ may influence cardiac hypertrophy through two distinct mechanisms which are mediated by different splice variants; CaMKII-δC regulates phosphorylation of RyR and PLN resulting in increased Ca\(^{2+}\) spark frequency and decreased SR Ca\(^{2+}\) content whereas both CaMKII-δC and CaMKII-δB variants mediate pro-hypertrophic gene transcription (Zhang et al., 2007). Despite this data to suggest CaMKII plays an important role in HF, no clinically available compounds are currently in clinical trials. However, a recently developed, novel ATP-competitive CaMKII-δ and CaMKII-γ inhibitor, RA306, has shown potential in animal studies (Beauverger et al., 2020) (Figure 3 and Table 1). RA306 prevented phosphorylation of PLN (Thr17) in vitro and in vivo following stimulation with isoproterenol (Beauverger et al., 2020). Furthermore, twice-daily RA306 treatment was sufficient to increase ejection fraction in a mouse model of dilated cardiomyopathy; importantly, the study used aged mice with advanced cardiac disease to demonstrate the ‘curative’ ability of RA306 (Beauverger et al., 2020).

The use of gene therapy to treat CVD has garnered much attention in recent years and has been used as a tool to alter expression of a wide variety of Ca\(^{2+}\)-handling proteins. Recent work has been undertaken to trial a triple gene plasmid construct INXN-4001 in patients with an outpatient left ventricular assist...
Gene therapy has also been utilized to overexpress AC6 (Kieserman et al., 2019) (been developed for use in clinical trials. However, gene therapy using S100A1 alone has not yet ability to rescue cardiac function in mouse, rat, and pig models of MI-induced HF (Most et al., 2004; Pleger et al., 2005, 2007, 2011; Kraus et al., 2009). Importantly, S100A1 gene transfer alone can restore Ca^{2+} homeostasis in acute and chronic models of HF (Most et al., 2004; Pleger et al., 2005, 2007, 2011; Kraus et al., 2009). However, gene therapy using S100A1 alone has not yet been developed for use in clinical trials.

Gene therapy has also been utilized to overexpress AC6 (Kieserman et al., 2019) (Figure 3 and Table 1). Plasmid INXN-4001 contains human VEGF165, S100A1 and stromal cell-derived factor 1; expression of these three proteins has been designed to target fibrosis, angiogenesis and impaired cardiac contractility (Jaruga-Killeen et al., 2019). To date there are no reported adverse cardiac-specific safety effects after one infusion of INXN-4001 (Jaruga-Killeen et al., 2019). Of interest to this review is the expression of constitutively active S100A1 protein within the INXN-4001 plasmid. S100A1 is a Ca^{2+} binding protein which controls Ca^{2+}-dependent networks in cardiomyocytes through, for example, modulation of RyR2 and SERCA2a activity (Ritterhoff and Most, 2012). S100A1 protein levels are reduced in cardiomyocytes from both human and animal model-derived failing myocardium and levels of reduced S100A1 are associated with poorer contractile function (Du et al., 2002; Most et al., 2006). Adenoviral-based delivery of S100A1 is able to rescue cardiac function in mouse, rat, and pig models of MI-induced HF (Most et al., 2004; Pleger et al., 2005, 2007, 2011; Kraus et al., 2009). Importantly, S100A1 gene transfer alone can restore Ca^{2+} homeostasis in acute and chronic models of HF (Most et al., 2004; Pleger et al., 2005, 2007, 2011; Kraus et al., 2009). However, gene therapy using S100A1 alone has not yet been developed for use in clinical trials.

Gene therapy has also been utilized to overexpress AC6 (Kieserman et al., 2019) (Figure 3 and Table 1). The primary role of AC6 is to catalyze the conversion of ATP to cAMP; however, AC6 can also downregulate the expression of PLN, independently of cAMP concentration (Gao et al., 2004). Adenoviral transfer of AC6 (Ad.AC6) in a porcine model of congestive HF successfully improved LV function (Lai et al., 2004). The promising outcome of Ad.AC6 treatment in a large animal models of HF led to its development for a first in man clinical trial (Ad5.hAC6, also known as RT-100), beginning in 2010 (Hammond et al., 2016; Penny et al., 2018). Results from this placebo-controlled, randomized clinical trial of 56 HFrEF patients confirmed the safety of Ad5.hAC6 (clinicaltrials.gov Identifier NCT00787059) (Hammond et al., 2016). Furthermore, patients receiving the two highest doses (out of five possible different doses) of Ad5.hAC6 showed an acute improvement in EF, assessed 4 weeks post-viral delivery (Hammond et al., 2016). The promising results from this Phase II clinical trial paved the way for the ‘FLOURISH’ trial, a Phase III follow-up (clinicaltrials.gov Identifier NCT03360448) (Penny et al., 2018). However, the FLOURISH study was withdrawn prior to patient recruitment owing to a change of clinical development plans. Therefore, the clinical benefit of Ad5.hAC6 for the treatment of HF still requires investigation in a large, multi-centre clinical trial.

### Ca^{2+} Handling Proteins During Hypertrophy and HF; Ca^{2+} Channels, Pumps and Exchangers

**LTCC**

Currently, LTCC Ca^{2+} blockers are used with caution in patients with HF; however, there may be some therapeutic potential in targeting a subset of LTCC which have been shown to specifically localize to caveolae and do not present as ‘typical’ LTCC which reside on T-tubules (Makarewich et al., 2012). Within caveolae membrane invaginations, Ca^{2+} entry through LTCC may activate pro-hypertrophic calcineurin/NFAT signaling without contributing to ECC (Makarewich et al., 2012). Interestingly, the calcineurin/NFAT signaling axis only contributes to pathological and not physiological hypertrophy (Wilkins et al., 2004). Inhibition of LTCC with channel blocking Rem proteins specifically targeted to Cav-3 caveolae membranes attenuates the inward Ca^{2+} current and prevents pro-hypertrophic gene activation without affecting contractility of feline cardiomyocytes (Makarewich et al., 2012). However,

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**Table 1: Therapeutic targeting of Ca^{2+}-handling proteins in cardiac hypertrophy, HF and arrhythmia.**

| Target  | Pre-clinical | FDA approved | Clinical trial identifier | Status | Results |
|---------|--------------|--------------|---------------------------|--------|---------|
| S100A1  | INXN-4001    | NCT03409627  | A                         | N/A    |         |
| CaM     | W-7          | Pre-clinical | N/A                       | N/A    |         |
| CaMKII  | RA306        | Pre-clinical | N/A                       | N/A    |         |
|         | KN-93        | Pre-clinical | N/A                       | N/A    |         |
|         | AAV.GFP.AIP  | Pre-clinical | N/A                       | N/A    |         |
| AC6     | Ad5.hAC6     | NCT00787059  | C                         | Safe, tolerable (Hammond et al., 2016) |
|         |              | NCT00360448  | W                         | N/A    |         |
| PLN     | BMS-986231   | NCT03016325  | C                         | Safe, well-tolerated; adverse effects included headaches (Cowart et al., 2019) |
|         |              | NCT02157506  | C                         | Increased treatment-emergent adverse events in treatment group (<55% in all three regimes vs. 25% in placebo) |
|         |              | NCT03016325  | C                         | None posted |
|         |              | NCT03357731  | C                         | None posted |
|         |              | NCT03730961  | C                         | None posted |

For each therapy listed in Figure 3, their status as either pre-clinical or FDA-approved is listed in the table. In addition, any clinical trial information available for the therapies listed in the table is also presented, where NCT denotes the number given to clinical trials on clinicaltrials.gov. Therapies which have yet to enter registered clinical trials are shown as pre-clinical. A brief description on the in human safety and tolerability of each therapy is also shown where available. A, active; C, completed; N/A, not applicable; W, withdrawn.
these findings were not replicated a murine model of cardiac hypertrophy in vivo (Correll et al., 2017). Therefore, the direct contribution of LTCC to the development of cardiac hypertrophy remains inconclusive and requires further investigation in different pre-clinical models of HF.

**SERCA2**

In failing human cardiomyocytes and in animal models of hypertrophy and HF, the levels of SERCA2 are reduced (Komuro et al., 1989; Nagai et al., 1989; Mercadier et al., 1990; Lipskaia et al., 2014). Downregulation of SERCA2 and therefore a reduction in SERCA2 activity are concordantly associated with both systolic and diastolic dysfunction (Periasamy et al., 1999). The importance of SERCA2 signaling is evidenced by knockout mouse studies. Cardiomyocyte-specific deletion of SERCA2 in adult mice leads to diastolic dysfunction under basal conditions (Andersson et al., 2009). Furthermore, an important feature of Ca\(^{2+}\) dysregulation during cardiac hypertrophy is SR Ca\(^{2+}\) leak and at the molecular level, cardiomyocyte loss of SERCA2 results in changes to SR Ca\(^{2+}\) handling, characterized by slower cytosolic Ca\(^{2+}\) removal, reduced SR content and impaired Ca\(^{2+}\) transient magnitude (Andersson et al., 2009; Louch et al., 2010; Li et al., 2012). SERCA2 activity could also be affected in HF due to changes in the phosphorylation status of PLN (Schwinger et al., 1995; Mattiazi and Kranias, 2014). The reduction in phosphorylation of PLN is partly due to increased activity of calcineurin and protein phosphatase 1, as shown in both human HF and animal models (Neumann et al., 1997; Munch et al., 2002). However, more recent data suggests direct post-transcriptional modifications of SERCA2a contribute to its decreased activity (Kranias and Hajjar, 2012). In HF, SERCA2 can undergo either 'stimulatory' modification by glutathionylation on C674 (Adachi et al., 2004) and/or SUMOylation on K480 and K585 (Kho et al., 2011), and ‘inhibitory’ acetylation of K492 (Gorski et al., 2019). These modifications to SERCA2a and its regulatory proteins provides more scope for enhancing SERCA2 activity in HF patients.

Restoring SERCA2a protein expression levels has been shown by numerous groups to improve cardiac metabolism, including a reduced incidence of arrhythmia and increased coronary blood flow (Hayward et al., 2015; Hulot et al., 2016). However, the benefits of administering SERCA2a were shown to be diminished in a myocardium with a limited energy supply (Pinz et al., 2011), suggesting increasing SERCA2 levels may only be beneficial in non-ischemic tissue. In humans, directly restoring SERCA2 levels has been achieved using gene transfer. Adeno-associated virus 1.SERCA2a (AAV1.SERCA2a) gene transfer successfully restored the Ca\(^{2+}\) transient and contraction in isolated failing human cardiomyocytes (del Monte et al., 1999) and in animal models of HF, SERCA2a gene transfer was shown to increase survival and improve cardiac hemodynamics and function (del Monte et al., 2001, 2004; Kawase et al., 2008) (Figure 4 and Table 2). Given that SERCA2a gene transfer improved outcome in animal models of HF, AAV1.SERCA2a was administered in Phase I and Phase II clinical trials [ClinicalTrials.gov identifiers NCT00534703 and NCT00454818] (Jessup et al., 2011; Zsebo et al., 2014; Greenberg et al., 2016). The results of the initial Phase I and IIa trials suggested that AAV1.SERCA2a treatment reduced the number of cardiac events in HF patients (Jaski et al., 2009; Jessup et al., 2011); however, larger Phase IIb clinical trials failed to show improvement in any primary or secondary endpoints, leading to their early termination (Hulot et al., 2017). The failure of these clinical trials suggests the dosage and delivery method of AAV1.SERCA2a may require optimizing (Hulot et al., 2016) [SERCA2a gene therapy in HF has been extensively reviewed elsewhere (Samuel et al., 2018)]. Despite the lack of promising results for AAV1.SERCA2a, clinical trials are underway for istaroxime, a dual functional luso-inotropic agent which both stimulates SERCA2a and inhibits Na\(^{+}/K\(^{+}\) ATPase (Micheletti et al., 2002) (Figure 4 and Table 2). Istaroxime increases Ca\(^{2+}\) uptake into the SR by relieving PLN-mediated inhibition of SERCA2a through cAMP/PKA-depending mechanism (Ferrandi et al., 2013). In animal models of HF, intravenous istaroxime improved cardiac function without causing any arrhythmic events (Micheletti et al., 2007; Sabbah et al., 2007). Phase I-II clinical trials demonstrated the safety of istaroxime in HF patients and showed beneficial effects on cardiac contractility (Ghali et al., 2007). The follow-up Phase IIa clinical trial, ‘HORIZON-HF’, showed a reduction in diastolic stiffness and increase in contractility in HFrEF patients receiving istaroxime when compared to placebo (Shah et al., 2009). Additional clinical trials for istaroxime are on-going [ClinicalTrials.gov identifiers NCT02617446 and NCT02477449].

Focus for novel SERCA2 therapeutics has also extended to SERCA2 post-translational modifications and mediators of SERCA2 expression. Gene-transfer of SUMO1 and S100A1 which both promote SERCA2 activity were shown to improve cardiac function in rodent and porcine models of HF (Most et al., 2004; Kho et al., 2011; Pleger et al., 2011; Tilemann et al., 2013). Moreover, a small-molecule SUMO1 activator, termed N106, has recently been developed which, through activating the SUMO-activating enzyme, leads to the SUMOylation of SERCA2 (Kho et al., 2015). In addition, microRNAs upregulated in human HF also provide a novel therapeutic target as they can be inhibited using anti-miR's. One such microRNA, miR-25, is upregulated in human HF and application of anti-miR-25 following TAC improved survival and function by restoring SERCA2 activity (Wahlquist et al., 2014). However, none of these SERCA2 modifying mechanisms have been investigated in the clinical setting. SERCA2a and its regulator PLN can be modified by nitroxyl (HNO/NO\(^{•-}\)), the single electron reduced form of nitric oxide (Paolocci et al., 2001; Farah et al., 2018). HNO readily converts negatively charged thiol groups of proteins, such as cysteine, to disulfide residues; a feature which is reversible (Fukuto, 2019; Pinilla-Vera et al., 2019). HNO modifies cysteine residues on SERCA2a and PLN (and RyR2) which enhances Ca\(^{2+}\) cycling through reduced PLN-mediated SERCA2a inhibition (Froehlich et al., 2008; Farah et al., 2018). The original HNO donor used in proof-of-principal experiments, Angeli’s salt, is not stable at room temperature, therefore, first- and second-generation HNO donors have been developed with more favorable drug-like properties. One such second-generation HNO derivative to have undergone clinical testing is BMS-986231 (Figure 3 and Table 1). To date, there
have been eight completed different clinical trials utilizing BMS-986231. Results from a Phase I clinical trial showed that BMS-986231 was well tolerated but that nearly half of healthy volunteers receiving BMS-986231 reported headaches; however, the authors noted that owing to BMS-986231 vasodilatory effects, the presence of headaches was not unexpected (Cowart et al., 2019) [clinicaltrials.gov identifier NCT03016325]. In a separate placebo-controlled, double-blind Phase IIa study, 34 patients were randomly assigned to receive one infusion of BMS-986231 (Tita et al., 2017) [clinicaltrials.gov identifier NCT02157506]. The results from this Phase IIa clinical trial also suggest BMS-986231 is safe and in HFrEF patients, exhibits a favorable hemodynamic profile (Tita et al., 2017). The success of these early Phase I and II clinical trials have led to the development of three further clinical trials to determine the effect of BMS-986231 on (1) symptomatic hypotension in HFrEF patients, (2) cardiac systolic and diastolic function in HFrEF patients, and (3) salt and water-handling in patients with HFrEF and HFmrEF (EF < 45%) [clinicaltrials.gov identifiers NCT03016325, NCT03357731, and NCT03730961, respectively] (Felker et al., 2019). To date, these studies have completed recruitment and are on-going.

NCX

Depending on the membrane potential and intracellular Ca\textsuperscript{2+} and Na\textsuperscript{+} gradients, NCX1 can act in ‘forward’ (Ca\textsuperscript{2+} ion out, 3 Na\textsuperscript{+} ions in) or ‘reverse’ (Ca\textsuperscript{2+} ion in, 3 Na\textsuperscript{+} ions out) mode. In a failing cardiomyocyte, NCX1 has been shown to operate in reverse mode which results in Ca\textsuperscript{2+} entry and a repolarizing current (Mattiello et al., 1998). Furthermore, elevated intracellular Ca\textsuperscript{2+} seen after I/R injury is caused by NCX1 operating in ‘reverse’ mode (Piper et al., 1996; Inserte et al., 2002; Imahashi et al., 2005). NCX1 is upregulated in human and murine models of HF (Hasenfuss et al., 1999; Menick et al., 2007) and over-expression of NCX is sufficient to impair cardiomyocyte function \textit{in vitro} (Schillinger et al., 2000). However, as the function of NCX is also affected by Na\textsuperscript{+}, and intracellular Na\textsuperscript{+} is increased in both human HF and models of HF (Bers and Despa, 2006), Ca\textsuperscript{2+} extrusion through NCX could be impaired due to Na\textsuperscript{+}-loading observed in end stage HF (Louch et al., 2010; Li et al., 2012). Interestingly, following TAC there is an increase in NCX expression but no change in SERCA2a; however, when TAC progresses into HF, there is a downregulation in SERCA2 expression (Wang et al., 2001). Therefore, in HF there is a change in the NCX/SERCA2 ratio which has been implicated in impaired cardiac contraction and conduction (Pogwizd et al., 2001).

Historically, digoxin has been used to inhibit NCX; however, there is no improvement in mortality of patients receiving digoxin (Campbell and MacDonald, 2003; Goldhaber and Hamilton, 2010). It was recently observed that an increase in NCX1 seen in an animal model of HF could be attenuated by neuroglin-1β (Wang et al., 2019) (Figure 4). Neuroglin-1β has been extensively explored as a therapeutic option in HF and expression of recombinant human neuroglin-1β (rhNRG-1/also known as Neucardin) has undergone successful clinical trials; however, its full mechanism of action has not yet been determined (Galindo et al., 2014). Neuroglin-1β is a growth factor which, along with its receptors ErbB2-4, is critical for the cardiac response to stress (Lemmens et al., 2004; Galindo et al., 2014;
### TABLE 2 | Therapeutic targeting of Ca$^{2+}$-channels and pumps in cardiac hypertrophy, HF and cardiac arrhythmia.

| Target            | Pre-clinical | FDA approved | Clinical trial identifier | Status | Results                                      |
|-------------------|--------------|--------------|---------------------------|--------|----------------------------------------------|
| NCX               | Neucardin    |              | NT01251406                | C      | None posted                                  |
|                   |              |              | NT01541202                | T      | N/A                                          |
|                   |              |              | NT039388593               | R      | N/A                                          |
| JK07              |              |              | NT04210375                | NYR    | N/A                                          |
| KB-R7943          |              |              | Pre-clinical              | N/A    | N/A                                          |
| SEA-0400          |              |              | Pre-clinical              | N/A    | N/A                                          |
| SN-6              |              |              | Pre-clinical              | N/A    | N/A                                          |
| ORM-10962         |              |              | Pre-clinical              | N/A    | N/A                                          |
| PMCA4             | ATA          |              | Pre-clinical              | N/A    | N/A                                          |
|                   | Caloxin 1B1  |              | Pre-clinical              | N/A    | N/A                                          |
| PMCA1             | Caloxin 1B3  |              | Pre-clinical              | N/A    | N/A                                          |
| RyR2              | Dantrolene    |              | jRCTs61810059             | R      | N/A                                          |
|                   | Phenytoin     |              | Pre-clinical for HF       | N/A    | N/A                                          |
|                   | JTVS19        |              | NCT00626652               | C      | None posted                                  |
|                   | S107          |              | Pre-clinical              | N/A    | N/A                                          |
|                   | Phlecanide    |              | NCT0117454               | C      | Used with β-blockers, no safety concerns     |
|                   |              |              |                           |        | [Kamraneri et al., 2017]                     |
| SERCA2a           | Istaroxime    |              | NCT02617446               | U      | N/A                                          |
|                   |              |              | NCT02477449               | U      | N/A                                          |
| AAV1.SERCA2a      |              |              | NCT00534703               | T      | Only 5 participants, trial terminated early  |
|                   |              |              | NCT00454818               | C      | No safety concerns in 3-year follow-up       |
|                   | N106          |              | Pre-clinical              | N/A    | N/A                                          |
|                   | Ad.SERCA2a    |              | Pre-clinical              | N/A    | N/A                                          |
| TRPC3             | Pyr3          |              | Pre-clinical              | N/A    | N/A                                          |
| TRPC3/C6          | GSK2333255B   |              | Pre-clinical              | N/A    | N/A                                          |
|                   | GSK2835003A   |              | Pre-clinical              | N/A    | N/A                                          |
| TRPV4             | GSK2798745    |              | NCT02497937               | C      | 1(1/11) incident of a serious adverse event  |
|                   |              |              |                           |        | in treatment group                           |
|                   | HC-067047     |              | NCT02119260               | C      | Safe, well-tolerated [Goyal et al., 2019]     |
| TRPM4             | 9-phenanthrol |              | Pre-clinical              | N/A    | N/A                                          |
| TTCC              | Mibebradil*   |              | Pre-clinical              | N/A    | N/A                                          |
|                   |              |              |                           |        | Initially safe [Bursztyn et al., 1997;     |
|                   |              |              |                           |        | Kobrin et al., 1997] but later withdrawn    |
|                   |              |              |                           |        | due to interference with metabolism of other |
|                   |              |              |                           |        | medicines                                    |
| MCU               | Ruthenium red |              | Pre-clinical              | N/A    | N/A                                          |
|                   | Ru360         |              | Pre-clinical              | N/A    | N/A                                          |

Each therapy listed in Figure 4 is listed as having either pre-clinical or FDA-approved status. Any clinical trial information available, either through publication or as results published, is also presented. Clinical trials accessed through clinicaltrials.gov have an NCT number whereas those obtained through the Japan Registry of Clinical Trials are denoted by a jRCTs number. *Mibebradil was withdrawn from the market following FDA approval. C, completed; N/A, not applicable; NYR, not yet recruiting; R, recruiting; T, terminated; U, unknown.

Following pressure-overload induced cardiac hypertrophy, the expression of ErbB2 and B4 are increased during the cardiac compensation phase and reduced following the transition into HF (Rohrbach et al., 1999). Similarly, in the left ventricle of human failing myocardium, the expression of ErbB2 and B4 is reduced (Rohrbach et al., 2005). Animal models of hypertrophic- and ischemia-induced HF have been used extensively to show the benefit of recombinant neuroglin-1β (Galindo et al., 2014). Treatment with neuroglin-1β for 7 days was sufficient to prevent ventricular remodeling following volume-overload induced cardiac hypertrophy (Wang et al., 2019). At the molecular level, neuroglin-1β partially prevented the decrease in SERCA2a, LTCC and increase in NCX1 associated with HF (Wang et al., 2019). Therefore, neuroglin-1β may confer its cardioprotective effects through regulation of Ca$^{2+}$-handling protein expression. Given the promising effects of recombinant neuroglin-1β treatment in animal models of HF, Phase II and III clinical trials were approved to determine the safety and efficacy of daily infusions of Neucardin (Galindo et al., 2014). The first Phase II clinical trials successfully demonstrated the safety, tolerability and efficacy of rhNRG-1 in patients with chronic HF (Gao et al., 2010; Jabbour et al., 2011) [clinicaltrials.gov identifier NCT01251406 and NCT01541202]. Following on from these early Phase II clinical trials, large-scale, multi-centre
Phase III clinical trials have begun recruiting patients to determine the effect of Neucardin on all-cause mortality after 1 year [clinicaltrials.gov NCT03388593]. Furthermore, a Phase I clinical trial has recently been accepted to test the safety, tolerability and immunogenicity of the drug JK07 in HFrEF patients; JK07 is a human immunoglobulin Ig1 monoclonal antibody containing an active polypeptide fragment of neurogin-1 [clinicaltrials.gov identifier NCT04210375] (Figure 4 and Table 2). Future studies should aim to determine whether neurogin-1β mediates NCX1, SERCA2a and LTCC channel expression in other animal models of HF and whether the efficacious effects of Neucardin in clinical trials is partly due to altered Ca^{2+} dynamics.

**RyR2**

There is a general consensus that SR-located RyR2 contributes to Ca^{2+} leak during HF which can cause (1) cardiac arrhythmias, (2) reduced Ca^{2+} SR content, (3) systolic dysfunction, and (4) altered cardiac energetics (Bers, 2014). The function of RyR2, the major cardiac isoform of the RyR, is tightly regulated though changes to its phosphorylation status, Ca^{2+} activation and inactivation sites and post-translational modifications ( Roe et al., 2015). RyR2 channel gating is regulated by accessory proteins whilst its tetrameric confirmation is stabilized by FK506-binding protein-12.6 (FKBP12.6). Dissociation of FKBP12.6 from RyR2 is observed in some models of HF and is achieved through PKA-mediated hyperphosphorylation of RyR2 at Ser2808 ( Marx et al., 2000). One study has demonstrated that transgenic mice with an absent PKA phosphorylation site on RyR2 are protected against MI-induced HF ( Wehrens et al., 2006); however, others have shown that CaMKII-mediated RyR2 phosphorylation leads to altered Ca^{2+} sparks as opposed to PKA-mediated phosphorylation ( Li et al., 2002; Guo et al., 2006). Such is the importance of CaMKII-mediated phosphorylation of RyR2 that it has been shown to promote the transition from hypertrophy into HF ( Ling et al., 2009; Respress et al., 2012).

Changes to Ca^{2+} transfer between the SR and mitochondria impede mitochondrial function in cardiomyocytes ( Fernandez-Sanz et al., 2014; Ruiz-Meana et al., 2019), potentially conferring to cardiomyocyte deterioration in HF. Recently, a novel mechanism of RyR2 hyperglycation was shown to increase Ca^{2+} leak from the SR in cardiomyocytes of aged mice ( Ruiz-Meana et al., 2019). Aging alone results in advanced glycation of RyR2, leading to increased SR Ca^{2+} leak, mitochondrial Ca^{2+} overload and mitochondrial dysfunction, independently of cardiac disease ( Ruiz-Meana et al., 2019). Importantly, hyperglycation of RyR2 and increased mitochondrial Ca^{2+} was prevalent in atrial appendages from aged patients (75 years or above) and absent in young patients ( Ruiz-Meana et al., 2019). Preventing the glycation of RyR2 by inhibiting dicarbonyl intermediaries or stimulating their detoxification may provide a preventative measure to attenuate mitochondrial damage associated with aged, failing cardiomyocytes.

RyR2 can be inhibited by dantrolene sodium, a derivative of the hydantoin class of compounds ( Maxwell et al., 2012). Application of dantrolene normalizes the diastolic Ca^{2+} leak seen in HF; however, owing to its hepatotoxic side-effect, dantrolene is not suitable for long-term use ( Durham et al., 1984) (Figure 4 and Table 2). Despite this, the ‘SHO-IN’ clinical trial seeks to determine the safety and efficacy of dantrolene on patients with chronic HF [Japanese Registry of Clinical Trials identifier 61180059] ( Kobayashi et al., 2020). Recent work has shown that phenytoin, a member of the same class of hydantoin compounds, shares the same RyR2 inhibitory effects as dantrolene ( Ashna et al., 2020). In vitro application of phenytoin to RyR2 channels isolated from failing human hearts shows that phenytoin reversibly inhibits RyR2 which subsequently inhibits diastolic cytoplasmic, but not systolic, Ca^{2+} release ( Ashna et al., 2020). Interestingly, phenytoin is currently used clinically as a Na^{+} channel blocker to treat epilepsy, therefore there is scope to determine the potential therapeutic use of phenytoin in HF (Figure 4). Designing novel therapeutic targets for RyR2 may, in future, involve RyR2 accessory proteins which modulate its function. TRIC-A and B are SR-located K^{+} channels known to participate in ECC ( Yazawa et al., 2007; Yamazaki et al., 2009b; Yang et al., 2016); both TRIC-A and TRIC-B knockout mice exhibit Ca^{2+} overload in the SR ( Yamazaki et al., 2009a; Zhao et al., 2010). TRIC-A, but not TRIC-B, physically associates with RyR2 to enhance RyR2-dependent Ca^{2+} efflux from the SR; however, there is still a limited understanding of the function of TRIC-A-dependent RyR2 modulation under pathological conditions ( Zhou et al., 2020).

**TRP Channels**

When compared to a healthy myocardium, TRPC1, C5, M4, and M7 are upregulated and TRPC4 and V2 are downregulated in the failing human heart ( Dragun et al., 2019), suggesting each TRP channel may differentially contribute to CVD [reviewed extensively elsewhere ( Falcon et al., 2019; Hof et al., 2019)]. Interestingly, in neonatal rat cardiomyocytes, stimulation with hypertrophy-inducing agents leads to the upregulation and subsequent activation of TRPC1 ( Obha et al., 2007) and C5 ( Sunggip et al., 2018). Furthermore, in mouse models, global deletion of Trpc1 attenuates pressure-overload induced hypertrophy through reduced activation of the calcineurin/NFAT pathway ( Obha et al., 2007; Seth et al., 2009). In addition to TRPC1 and C5, TRPC3 ( Brenner and Dolmetsch, 2007) and C7 ( Satoh et al., 2007) are also upregulated in hypertrophy-stimulated cardiomyocytes and TRPC6 overexpression induces cardiac hypertrophy in mice ( Kuwahara et al., 2006). TRPC3 and C6 have been successfully co-targeted with the thiazole inhibitors GSK2332255B ( GSK255B) and GSK2833503A ( GSK503A), respectively ( Washburn et al., 2013) (Figure 4 and Table 2). When these inhibitors were co-administered following pressure-overload induced hypertrophy in mice, both cardiomyocyte hypertrophy and cardiac fibrosis were reduced ( Seo et al., 2014). Mechanistically, blockade of TRPC3 reduces phosphorylation of Ca^{2+}-handling proteins in mouse embryonic stem cell-derived cardiomyocytes ( Qi et al., 2016), which may alter downstream Ca^{2+}-mediated signaling. More specifically, TRPC3 can be exclusively inhibited by Pyr-3 ( Kiyonaka et al., 2009) (Figure 4 and Table 2). Pyr-3 inhibition of TRPC3 attenuates NFAT-mediated transcription and can prevent hypertrophy in vitro in ANG-II stimulated neonatal rat cardiomyocytes and

| Table 2 | Targeting Ca^{2+} Handling Proteins |
|---------|-----------------------------------|
| Handling Proteins | TRP Channels |
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in vivo following pressure overload-induced hypertrophy in mice (Kiyonaka et al., 2009). Other studies have also shown TRPC1 and TRPC3-C6 upregulation in failing human hearts when compared to non-failing myocardium (Bush et al., 2006; Morine et al., 2016). Interestingly, the same TRPC channels are also upregulated in the border zone of rats hearts after left anterior descending coronary artery ligation-induced MI (Domínguez-Rodríguez et al., 2018). Therefore, it appears that the TRPC family of channels contribute to the cardiac HF phenotype; however, more research is required in order to determine the functional effect of each upregulated TRPC channel in order to progress pre-clinical studies.

The TRPV family have also been extensively investigated; however, as mentioned above, there is no detectable upregulation of TRPV family members in the failing human myocardium (Dragun et al., 2019). Despite this, there is promising data to suggest blockade of TRPV4 may prove beneficial in HF patients; the pulmonary endothelial-localized TRPV4 channel has been shown to play a role in pulmonary venous pressure-induced formation of pulmonary oedema in HF (Thorneloe et al., 2012). In support of this, TRPV4 is upregulated in human lungs derived from patients with congestive HF (Thorneloe et al., 2012). As such, TRPV4 channel blockade with the first in class orally active pharmacuetic GSK2798745 successfully prevents the formation of and reverses established pulmonary oedema in acute and chronic HF models (Thorneloe et al., 2012) (Figure 4 and Table 2). The positive data obtained from TRPV4 inhibition in animal models led to a Phase I clinical trial to investigate the safety and efficacy of inhibiting TRPV4 with GSK2798745 (Goyal et al., 2019). Results of this first in man trial has shown there are no adverse effects or safety issues in both healthy volunteers and patients with mild to moderate HF (Goyal et al., 2019) [clinicaltrials.gov identifiers NCT02497937 and NCT02119260]. In addition, TRPV4 is activated in the murine model of I/R injury (Dong et al., 2017). Mechanistically, activation of TRPV4 following I/R leads to increased Ca$^{2+}$ influx and generation of ROS (Wu et al., 2017). Therefore, chemical inhibition of TRPV4 with the selective antagonist HC-067047 prevents aberrant TRPV4 activation which leads to a reduction in infarct size and improved cardiac function following I/R injury (Dong et al., 2017). Furthermore, both TRPV1 and V2 contribute to the inflammatory response following MI; however, mice harboring a global deletion of TRPV1 show an exacerbated inflammatory response 3- and 7-days post-MI whereas mice with a global knockout of TRPV2 show improved cardiac recovery following MI due to a reduction in peri-infarct macrophages (Huang et al., 2009; Entin-Meer et al., 2017; Falcon et al., 2019).

Some of the TRPM and TRPP channels exhibit the highest levels of expression in basal ventricular myocytes, amongst these are TRPM7, M1 and M4 and TRPP1 and P2 (Chevalier et al., 2018). As mentioned, both TRPM4 and M7 levels are expressed in the failing human ventricle myocardium (Dragun et al., 2019); however, TRPM7 mRNA was shown to be reduced in the left ventricle of patients with ischemic cardiomyopathy (Ortega et al., 2016), therefore the underlying cause of HF may influence the expression pattern of TRP channels. TRPM7 is expressed in the SAN and has a known role in modulation of cardiac automaticity (Sah et al., 2013a,b); however, TRMP7 also promotes cardiomyocyte proliferation during early cardiogenesis (Sah et al., 2013a,b). TRMP7 kinase dead mice exhibit significant cardiac hypertrophy and upregulation of pro-inflammatory mediators, driven by a Mg$^{2+}$- but not a Ca$^{2+}$-sensitive mechanism (Rios et al., 2020). Therefore, TRPM4 may provide a better option to therapeutically target Ca$^{2+}$ mishandling. Interestingly, despite the upregulation of TRPM4 in failing human hearts, the channel itself is impermeable to Ca$^{2+}$; however, it is both Ca$^{2+}$- and voltage-activated (Nilius et al., 2003, 2005; Wang et al., 2018). TRPM4 expression is upregulated by 50-fold in spontaneously hypertensive rats when compared to controls (Guinamard et al., 2006). Furthermore, global deletion of Trpm4 causes eccentric cardiac hypertrophy with aging which is caused by neonatal cardiomyocyte hyperplasia (Demion et al., 2014). In a cardiomyocyte-specific Trpm4 knockout mouse (Trpm4$^{cko}$), induction of cardiac hypertrophy with ANG-II resulted in increased hypertrophy in Trpm4$^{cko}$ mice when compared to controls (Kecskes et al., 2015). TRPM4 activity can be exclusively inhibited by 9-phenanthrol (Guinamard et al., 2014). Most studies using 9-phenanthrol have aimed to determine the contribution of TRMP4 to smooth muscle cell contraction (Guinamard et al., 2014); however, application of 9-phenanthrol to isolated rat hearts in a model of I/R injury reduces infarct size and improves contractility (Wang J. et al., 2013).

**PMCAs**

The PMCAs are rapidly emerging as potential signaling mediators (Armesilla et al., 2004; Oceandy et al., 2007; Cartwright et al., 2011; Stafford et al., 2017); however, to date, only a role for PMCA4 has been elucidated in a mouse model of TAC-induced pressure-overload hypertrophy. In the study by Mohamed et al. (2016), global knockout of Atp2b4 was shown to attenuate the pressure-overload response; however, the anti-hypertrophic effects of PMCA4 knockout could only be replicated in fibroblast-specific and not in cardiomyocyte-specific knockout mouse models (Mohamed et al., 2016; Stafford et al., 2017). Non-myocyte cells such as cardiac fibroblasts and endothelial cells account for around 70% of total cardiac cells (Pinto et al., 2016), and are able to secrete factors which can influence adjacent cardiomyocyte signaling. Interestingly, fibroblast-specific deletion of PMCA4 leads to significant upregulation of secreted frizzled related protein 2 which is thought to confer cardioprotective properties by inhibiting the Wnt pathway in neighboring cells (Mohamed et al., 2016). Despite this role for PMCA4 in cardiac hypertrophy, there is no evidence that human ATP2B4 is associated with CVD; genome-wide association studies (GWAS) have to date been unable to associate SNPs within ATP2B4 with any CVD (Tabara et al., 2010).

In contrast to GWAS data for ATP2B4, SNPs located in ATP2B1, the gene encoding PMCA1, have been strongly associated with CVDs, including hypertension, coronary artery disease and MI (Tabara et al., 2010; Takeuchi et al., 2010; Wang Y. et al., 2013; Xu et al., 2016; Jamshidi et al., 2018; Sombie et al., 2019). The association of ATP2B1 with hypertension has been
shown in numerous studies across different ethnic groups; to date, there are at least six different SNPs within ATP2B1 which are associated with elevated blood pressure (Tabara et al., 2010; Takeuchi et al., 2010; Wang Y. et al., 2013; Xu et al., 2016; Jamshidi et al., 2018; Sombie et al., 2019). In mice, vascular smooth muscle cell-specific PMCA1 knockout mice exhibit elevated blood pressure (Kobayashi et al., 2012). Mechanistically, loss of PMCA1 from vascular smooth muscle cells leads to enhanced expression of LTCC and increased Ca\(^{2+}\) concentration which may affect smooth muscle cell stiffness and subsequently, vascular tone (Okuyama et al., 2018; Zhu et al., 2019). Furthermore, PMCA1 global heterozygous mice have increased hypertension with aging, which is preceded by vascular remodeling (Little et al., 2017). Taken together, PMCA1 appears to play a functional role in the development of hypertension through modulation of Ca\(^{2+}\). Whilst more research is required to determine the contribution of PMCA1 to hypertrophy and HF, there is scope to therapeutically target PMCA1 as a modulator of hypertension to prevent CVD (Little et al., 2016).

The PMCA are typical ATPases and therefore present a suitable novel therapeutic target (Figure 4 and Table 2). ATPases have been clinically targeted to treat hypotension and some cardiac arrhythmias; ouabain inhibits Na\(^+/K^+\)-ATPase and omeprazole inhibits H\(^+\)/K\(^+\)-ATPase. Therapeutically, the two main cardiac isoforms of PMCA have been successfully targeted in vitro; the caloxins, a class of PMCA inhibitors, show differential specificity for each PMCA isoform (Pande et al., 2011). Caloxin 1B3 shows increased specificity for PMCA1 over PMCA4 and results in increased Ca\(^{2+}\) in endothelial cells (Szewczyk et al., 2010; Pande et al., 2011), whereas caloxin 1B1 is predominantly PMCA4-isofrom specific (Pande et al., 2006). PMCA4 has also been successfully targeted with aurintricarboxylic acid (Mohamed et al., 2013a). Application of aurintricarboxylic acid in vivo was able to prevent and also reverse hypertrophy in the murine TAC model of pressure overload (Mohamed et al., 2013b), suggesting inhibition of PMCA4 may provide an interesting therapeutic target.

**MCU**

Ca\(^{2+}\) is also required for effective mitochondrial function. Mitochondrial Ca\(^{2+}\) cycling is achieved through the inner mitochondrial membrane located MCU (Figure 2). Mitochondria are abundant in cardiomyocytes owing to their function in the synthesis of ATP (Kwong, 2017). In the process of ATP generation, mitochondria utilize Ca\(^{2+}\) to activate pyruvate dehydrogenase, ATP synthase and other enzymes involved in the redox reaction, as such [Ca\(^{2+}\)] in the inner matrix is tightly regulated by MCU (Kwong, 2017). MCU levels are elevated in the failing human heart and in animal models of HF (Zaglia et al., 2017; Yu et al., 2018). Under pathological conditions, overload of mitochondrial Ca\(^{2+}\) is associated with reduced cell viability, as well as apoptotic and autophagic cell death (Pinton et al., 2008). Interestingly, recent work suggests MCU is dispensable for normal cardiac function; cardiomyocyte-specific Muc\(^{-/-}\) mice show no overt phenotype under basal conditions, suggesting MCU is not required for maintaining cardiac homeostasis (Luongo et al., 2015). Furthermore, Muc\(^{-/-}\) mice do not experience mitochondrial Ca\(^{2+}\) overload following I/R injury which protects them against cell death and adverse cardiac remodeling (Luongo et al., 2015). Similarly, following TAC-induced pathological cardiac hypertrophy, mice treated with the MCU chemical inhibitor RR show improved survival, cardiac function and maintained mitochondrial integrity (Yu et al., 2018) (Figure 4 and Table 2). Treatment of isoprenaline stressed H9C2 cells with RR in vitro confirmed its ability to increase both autophagy and mitophagy (Yu et al., 2018). Taken together, inhibition or depletion of MCU appears beneficial for attenuating the pathological response to ischemic and hypertrophy stimuli. However, the use of RR has been shown to interfere with Ca\(^{2+}\) flux through mitochondrial-located RyR1 and membrane bound LTCC (Beutner et al., 2001). Therefore, developing more specific inhibitors of MCU to combat HF offers an alternative to targeting the ‘typical’ Ca\(^{2+}\)-handling proteins.

**CARDIAC ARRHYTHMIAS**

Cardiac arrhythmias are a class of conditions describing abnormalities in the heart's normal rhythm. Arrhythmias are a leading cause of morbidity and mortality, and can lead to stroke, deterioration of ventricular function, and sudden cardiac death (Kass and Clancy, 2006). Arrhythmic events can vary in terms of speed, rhythm, duration and location in the heart, and can present in numerous ways. The main types of arrhythmia include bradyarrhythmias (slow but regular heart rhythms) and tachyarrhythmias (faster than normal heart rates), including supraventricular tachycardias (tachycardia originating in the atria). Other common arrhythmias are AF (an irregular, often fast heart rhythm) and heart block (the heart beats slower than normal due to a delay in conduction) (Keating and Sanguinetti, 2001; Kass and Clancy, 2006).

Arrhythmogenesis is complex and multifactorial. Numerous genetic mutations have been shown underlie primary arrhythmia disorders, such as LQTS and CPVT. Within these conditions, the underlying genetic defect determines the arrhythmia phenotype, including the physiological triggers which incite arrhythmic events in these patients (Wilde and Bezzina, 2005). In addition, pathological and physiological factors can influence cardiac electrical remodeling, and therefore trigger arrhythmia development, including HF, hypertension, and coronary heart disease. For example, HF can result in structural and hemodynamic dysfunction, electrical remodeling (including those impacting Ca\(^{2+}\) handling), and metabolic changes which pre-dispose the development of arrhythmias (Masarone et al., 2017). Furthermore, susceptibility to these acquired arrhythmic disorders can be influenced by genetic factors (Wilde and Bezzina, 2005).

**Calcium Handling Proteins in Arrhythmia Development and Their Potential as Novel Therapeutic Targets**

Calcium handling in the myocardium is key to the generation and control of the heartbeat. Intracellular Ca\(^{2+}\) remodeling has been identified as a major factor in the development of arrhythmias...
and current approved therapies for arrhythmic disorders include Ca\(^{2+}\) channel blockers (Deo et al., 2017). Advancements in human genetic studies have highlighted mutations in Ca\(^{2+}\) handling proteins, as well as Ca\(^{2+}\) channels, that can result in the development of arrhythmias. In addition, remodeling of Ca\(^{2+}\) handling proteins has been identified in patients with acquired arrhythmic conditions (Landstrom et al., 2017). Therefore, Ca\(^{2+}\) handling proteins are promising therapeutic targets for the treatment of arrhythmic disorders. In the following section, we will discuss the current research aimed at targeting Ca\(^{2+}\) handling proteins in arrhythmia development.

The Role of Ca\(^{2+}\) in Arrhythmia Development

The mechanisms underlying arrhythmia development are generally divided into three categories: automaticity, triggered activity, and re-entry (Antzelevitch and Burashnikov, 2011). The role of Ca\(^{2+}\) in the generation of arrhythmias has been previously extensively reviewed (see Antzelevitch and Burashnikov, 2011; Kistamas et al., 2020). Automaticity is the ability of a cell to spontaneously depolarize and generate an AP. Under normal conditions, automaticity is restricted to the cardiac conduction system, specifically the SAN pacemaker cells which initiate cardiac contraction (Antzelevitch and Burashnikov, 2011). Abnormal automaticity occurs when the automaticity of the SAN is altered or when other cells begin to fire spontaneously. Spontaneous beating of the SAN is regulated by a coupled system of the surface membrane clock (inactivation and deactivation of cardiac ion channels) and the intracellular Ca\(^{2+}\) clock (rhythmic SR Ca\(^{2+}\) release) (Joung et al., 2011). Unsurprisingly, malfunction of both clocks has been associated with arrhythmia development. In relation to Ca\(^{2+}\) handling, Ca\(^{2+}\) clock remodeling (unresponsiveness to β-adrenergic stimulation and downregulation of RyR2) has been identified in SAN dysfunction (Joung et al., 2011). In addition, triggered oxidation and activation of CaMKII has been shown to result in SAN cell death which may contribute to SAN dysfunction (Huke and Knollmann, 2011).

Triggered activity refers to voltage oscillations termed afterdepolarizations which are often Ca\(^{2+}\)-mediated. Early afterdepolarizations (EADs) occur during the plateau phase of the cardiac AP and are associated with LQTS and ventricular tachyarrhythmias (associated with HF) (Landstrom et al., 2017). They are triggered by a reduction in repolarization reserve as a result of an increase in an inward current or a decrease in an outward current (Huang et al., 2018). A main contributor to EAD initiation is an increase in L-type Ca\(^{2+}\) current (I\(_{\text{Cal}}\)) which drives the EAD upstroke. This can occur due to a reactivation of the current during the repolarization phase caused by a reduction in the outward IK current or by CaMKII-mediated phosphorylation of I\(_{\text{Cal}}\) (Weiss et al., 2010; Landstrom et al., 2017). In addition, increased NCX current (I\(_{\text{NCX}}\)) can contribute to EAD. Spontaneous Ca\(^{2+}\) release events either prior to the completion of repolarization or during the initial phase of EADs can cause an increase in I\(_{\text{NCX}}\) activity which may delay repolarization and allow I\(_{\text{Cal}}\) to reactivate (Zhong et al., 2018).

Delayed afterdepolarizations (DADs) typically occur after repolarization and are caused by Ca\(^{2+}\) overload driving spontaneous Ca\(^{2+}\) release from the SR (Fink et al., 2011). Ca\(^{2+}\) release from the SR activates NCX (Ca\(^{2+}\)-sensitive Cl\(^{-}\)) and non-specific ion currents can also be activated leading to depolarization of the membrane. If the excitation threshold is reached, I\(_{\text{Na}}\) becomes activated and an AP is triggered (Fink et al., 2011). An increase in Ca\(^{2+}\) influx or a decrease in Ca\(^{2+}\) efflux can lead to an increase in SR Ca\(^{2+}\) content and drive the Ca\(^{2+}\) waves that activate NCX. The SR threshold for Ca\(^{2+}\) waves is thought to be dependent on the properties of RyR, with the opening of RyR decreasing the threshold for Ca\(^{2+}\) release (Venetucci et al., 2008). Elevated Ca\(^{2+}\) levels increase the open probability of RyR, making them more sensitive to stochastic Ca\(^{2+}\) release, ultimately leading to Ca\(^{2+}\) release (Fink et al., 2011). Phosphorylation of RyR at the PKA site or the CaMKII site can also increase RyR calcium sensitivity and open probability (Wehrens et al., 2004a). Modulation of RyR has been attributed to arrhythmias associated with HF and CPVT (caused by an underlying RyR mutation) (Venetucci et al., 2008).

Re-entry underlies the formation of arrhythmias due to abnormal impulse conduction. Here an electrical impulse fails to terminate and instead re-excites the myocardium. For re-entry to occur there must be an abnormal electrical circuit, slow conduction and a unidirectional block (Antzelevitch and Burashnikov, 2011). Changes to Ca\(^{2+}\) handling can cause cardiac tissue to become vulnerable to re-entry arrhythmias by increasing the dispersion of refractoriness, leading to unidirectional conduction block, and slowing of conduction (Weiss et al., 2011). Subthreshold EADs and DADs cause electrical homogeneity by increasing dispersion of refractoriness and dispersion of excitability, respectively (Landstrom et al., 2017; Kistamas et al., 2020). In addition, cardiac alternans can act as a substrate for the initiation of re-entry and can arise due to Ca\(^{2+}\) cycling dysfunction. Cardiac alternans present as beat to beat variations in Ca\(^{2+}\) transient amplitude (Ca\(^{2+}\) alternans), AP duration (APD alternans), or contraction amplitude (mechanical alternans) (Kistamas et al., 2020). Changes in the level of SR Ca\(^{2+}\) content, reduced SERCA activity, and changes in the refractoriness have been proposed as mechanisms for the generation of Ca\(^{2+}\)-derived alternans. DADs can also result in Ca\(^{2+}\) alternans and subsequent APD alternans (Kistamas et al., 2020).

Ca\(^{2+}\)-Handling Proteins in Arrhythmia Development; Ca\(^{2+}\) Channels, Pumps and Exchangers

RyR2

Abnormal RyR2-mediated Ca\(^{2+}\) release from the SR can lead to both atrial and ventricular arrhythmias, some primary arrhythmia disorders including CPVT and forms of acquired arrhythmias associated with heart disease (AF during HF) (McCauley and Wehrens, 2011). To diminish arrhythmic events, therapies have been developed to reduce and stabilize abnormal RyR activity, in order to inhibit diastolic SR Ca\(^{2+}\) release whilst maintaining the normal SR Ca\(^{2+}\) release during systole.
Dantrolene sodium is an approved muscle relaxant which is used for the treatment of malignant hyperthermia, caused by mutations in the skeletal RyR (RyR1) (McCausley and Wehrens, 2011). Dantrolene binds to the N-terminal of RyR1 to stabilize the inter-domain interactions required for the closed state of the RyR Ca\(^{2+}\) channel (Kobayashi et al., 2005). Further studies have shown dantrolene binds to RyR2 in a similar manner to improve the function of the receptor, suggesting the use of dantrolene in the treatment of arrhythmias (Figure 4) (Kobayashi et al., 2009). In a study using a knock-in mouse model carrying a human CPVT-RyR2 mutation (R2475S), dantrolene inhibited CPVT-related tachycardia (Kobayashi et al., 2010). Furthermore, in a cohort of CPVT-1 patients, dantrolene had an anti-arrhythmic effect, and reduced the number of premature ventricular complexes. This anti-arrhythmic response was also evident in induced pluriotent stem cell derived CPVT1 cardiomyocytes (Penttinen et al., 2015). In addition to CPVT, dantrolene has been shown to prevent the occurrence of DADs in failing cardiomyocytes isolated from a canine model (Kobayashi et al., 2009) and have a protective effect against arrhythmic events in animal models of I/R (Balam Ortiz et al., 1999; Boys et al., 2010). Together the data suggest dantrolene is a promising therapy to attenuate Ca\(^{2+}\)-mediated arrhythmias associated with HF. An early Phase I clinical trial aimed to assess the feasibility of intravenous delivery of dantrolene to reduce ventricular arrhythmias in patients with structural heart disease [clinicaltrials.gov identifier NCT01434845]. While no results have yet been posted, the study highlights the potential of RyR2 inhibition for the treatment of arrhythmias.

JTV519 (also known as K201) is a 1,4-Benzothiazepines derivative initially identified to suppress sudden cardiac cell death associated with Ca\(^{2+}\)-overload (Kaneko et al., 2009). Further studies identified RyR2 as a target of JTV519, with JTV519 having a protective effect in models of AF as a result of the restoration of RyR function (Figure 4 and Table 2) (Kumagai et al., 2003; Vest et al., 2005). PKA hyperphosphorylation of RyR2 and a reduction in the RyR2 channel-stabilizing subunit calcium2 has been identified in AF. In a canine model of AF, treatment with JTV519 restored calcium2 levels, stabilizing the closed state of RyR2 and reducing Ca\(^{2+}\) leak (Vest et al., 2005). A Phase 2 clinical trial evaluated the effects of JTV519 on sinus rhythm restoration in patients with AF [clinicaltrials.gov identifier NCT00628652], although no data is available. In terms of ventricular arrhythmias, a study using a calstabin2 global heterozygous knockout found pre-treatment with JTV519 prevented exercise-induced ventricular arrhythmias and sudden cardiac death associated with depletion of calstabin2. In the same study, JTV519 had no effect on calstabin2 knockout mice highlighting the requirement of calstabin2 for the antiarrhythmic effect of JTV519 (Wehrens et al., 2004b). Another 1,4-Benzothiazepines derivative is S107 which, like JTV519, can enhance RyR2 and calstabin2 binding to stabilize RyR2. S107 has been shown to be more specific than JTV519, with the latter known to interact with other cardiac ion channels including LTCC (Andersson and Marks, 2010). In a mouse model, S107 increased the binding of calstabin2 to RyRs carrying a CPVT mutation (R2474S) and inhibited associated arrhythmias (Figure 4) (Lehnart et al., 2008). The antiarrhythmic effect of S107 was further identified using a human induced pluripotent stem cell model of CPVT (Sasaki et al., 2016).

Flecainide is a trifluoroethoxybenzamide approved for the treatment of AF and/or supraventricular tachycardia. Importantly, flecainide is not recommended to patients with underlying structural heart disease as the drug can be pro-arrhythmic in this environment (Andrikopulos et al., 2015). While flecainide is classed as a type IC antiarrhythmic drug (a Na\(^{+}\) channel blocker) it is worth noting here the drugs ability to inhibit RyR2 action by reducing the duration of channel opening (Figure 4). Through this role as an RyR inhibitor, flecainide has been shown, in a mouse model of CPVT, to prevent ventricular tachycardia by suppressing spontaneous Ca\(^{2+}\) release. In addition, flecainide prevented exercise-induced ventricular arrhythmias in two patients carrying a CPVT-linked RyR mutation who were refractory to conventional therapy (Watanabe et al., 2009).

NCX
NCX has been identified to have a dual role in arrhythmogenesis. When Na\(^{+}\) levels are high, NCX acts in the reverse mode to bring Ca\(^{2+}\) into the cell which can result in Ca\(^{2+}\) overload. When acting in the forward mode, the inward current formed during Ca\(^{2+}\) release can result in DADs (Antoons and Sipido, 2008). Increased NCX activity has been shown to underlie arrhythmogenesis in numerous conditions including HF and AF, making NCX inhibition an attractive arrhythmia treatment.

First generation NCX blockers such as aprindine and bepridil displayed anti-arrhythmic effects. However, these NCX blockers lacked selectivity and were suggested to only have modest NCX blocking effects at therapeutic concentrations (Hobai and O’Rourke, 2004). KB-R7943 was the first NCX blocker with reported mode-selectivity, shown to be a more specific blocker of NCX in the reverse mode (Eliaz et al., 2001). Animal studies have shown KB-R7943 to be protective against Ca\(^{2+}\)-overload and able to suppress reoxygenation-induced arrhythmias (Figure 4) (Ladilov et al., 1999; Mukai et al., 2000). In addition, KB-R7943 suppressed afterdepolarizations and ventricular tachyarrhythmias in a rabbit MI mode I (Chang et al., 2015). Paradoxically, this study also found KB-R7943 to enhance the inducibility of arrhythmias in this model (Chang et al., 2015), with another study showing KB-R7943 had no effect on I/R arrhythmias (Miyamoto et al., 2002). Further studies have also shown KB-R7943 to inhibit the LTCC current and various K\(^{+}\) currents, which brings into question the use of the drug in the treatment of arrhythmias (Tanaka et al., 2002).

SEA-0400 has been described as the most potent and selective NCX inhibitor compared to KB-R7943 (Tanaka et al., 2002). Like KB-R7943, SEA-0400 has been suggested to have a higher potency for the forward NCX mode (Lee et al., 2004) and has been shown to have a cardioprotective and antiarrhythmic effect, although results vary in different models (Figure 4). In a rat model of myocardial I/R injury, SEA-0400 improved cardiac function, and reduced the incidence of ventricular fibrillation and mortality (Takahashi et al., 2003). In contrast, however, the drug had no effect on the incidence of ventricular tachycardia and fibrillation in a larger animal model of I/R injury.
(Nagasawa et al., 2005). This study also investigated arrhythmias induced by digitalis. Here, SEA-0400 reduced the incidence of induced tachyarrhythmias although 2/8 animals experienced AV block and cardiac standstill (Nagasawa et al., 2005). This differential arrhythmic effect may be dependent on the expression levels of NCX. A study found SEA-0400 to be antiarrhythmic in mouse models with increased NCX expression, such as HF and AF; but in conditions with reduced NCX expression, SEA-0400 was found to increase Ca\(^{2+}\) transient amplitude and spontaneous Ca\(^{2+}\) transients, ultimately acting proarrhythmically (Bogeholz et al., 2017). These findings suggest that in clinical conditions in which NCX function is reduced, such as diabetic cardiomyopathy, further NCX inhibition could trigger arrhythmias by reducing NCX function below the level required for balanced Ca\(^{2+}\) dynamics (Bogeholz et al., 2017).

The antiarrhythmic benefits of blocking NCX with KB-R7943 and SEA-0700 has driven further studies to identify novel NCX inhibitors including SN-6 and ORM-10962 (Figure 4 and Table 2). While SN-6 has been shown to have cardioprotective effects, the potential of the benzyloxyphenyl derivative in treating HF-associated arrhythmias is hindered by its ability to also increase diastolic Ca\(^{2+}\) and block IC\(_{La}\) (Gandhi et al., 2013). ORM-10962 is a potent NCX blocker which can inhibit both forward and reverse NCX currents and has no significant effect on other cardiac ion currents. The drug has been shown to attenuate digoxin-induced DAD in isolated ventricular Purkinje fibers but had no effect on arrhythmias related to I/R injury (Kohajda et al., 2016). The contrasting effects of NCX blockers on arrhythmogenesis under different conditions highlights the need for further studies to explore the use of NCX blockers.

Interestingly, another therapeutic approach has been to stimulate the forward mode of NCX to reduce cytosolic Ca\(^{2+}\) and attenuate Ca\(^{2+}\) overload. Heparin oligosaccharides have been shown to directly interact with NCX and accelerate its activity (de Godoy et al., 2018). In a model of I/R, trisulfated heparin disaccharide and low molecular weight heparins had a significant antiarrhythmic effect, as a result of increased NCX forward activity. While it is worth noting that both agents are known to target LTCC and Na\(^{+}\) channels, the mechanism of NCX acceleration appears to be a promising antiarrhythmic target (de Godoy et al., 2018).

**SERCA**

SERCA and its inhibitory protein PLN have been identified as important regulators of cardiac contractility and therefore potential antiarrhythmic targets (Antoons and Sipido, 2008). Impaired SERCA activity in HF results in dysregulation of systolic and diastolic function which can lead to cardiac arrhythmias. Increasing SERCA activity is a way of improving cardiac function in HF. In terms of arrhythmogenesis an increase in SERCA activity could theoretically result in Ca\(^{2+}\) overload and ultimately be arrhythmogenic (Antoons and Sipido, 2008). Encouragingly however, studies focusing on HF have found an increase in SERCA2 expression to be antiarrhythmic. In preclinical models of HF, increased SERCA2 expression through adenoviral delivery (Ad.SERCA2a) improved intracellular Ca\(^{2+}\) handling, restored contractility and attenuated ventricular arrhythmias (Figure 4) (del Monte et al., 2004; Lyon et al., 2011; Cutler et al., 2012). Other direct therapies to activate SERCA2 or inhibit PLN binding have been studied. Istaroxime, a Na\(^{+}\)/K\(^{+}\) ATPase inhibitor which also stimulates SERCA, has been shown to have a moderate antiarrhythmic effect (Figure 4) (Bossu et al., 2018). Several clinical trials have assessed istaroxime in HF, highlighting the promise of the drug in cardiac disorders. However, it is worth noting is not known if higher doses of istaroxime could stimulate further arrhythmic episodes through Na\(^{+}\)/K\(^{+}\) ATPase inhibition (Bossu et al., 2018).

**T-Type Ca\(^{2+}\) Channel**

As previously described, LTCC blockers are an approved treatment strategy for some arrhythmic conditions. The T-type Ca\(^{2+}\) channel has been also investigated as an antiarrhythmic target. T-type Ca\(^{2+}\) channels are localized in the pacemaker regions of the heart (the SAN, AVN and Purkinje fibers) where they are thought to contribute to SAN pacemaker activity and atrioventricular conduction (Mangoni et al., 2006). Inactivation of T-type Ca\(^{2+}\) channels results in bradycardia and delayed atrioventricular conduction, highlighting the importance of the channel in cardiac rhythm (Mangoni et al., 2006). Several studies have investigated the effect of blocking T-type Ca\(^{2+}\) channels with nifedipine, a tetralol derivative which inhibits T-type Ca\(^{2+}\) channels but is also known to block IC\(_{Cal}\). Treatment with nifedipine results in a dose-dependent decrease in heart rate (Brogden and Markham, 1997), and prevention of ischemia-induced ventricular fibrillation (Figure 4) (Billman and Hamlin, 1996). However, the drug has been reported to result in toxicity, inducing TdP due to QTc prolongation (Glaser et al., 2001).

**CaMKII**

CaMKII dysfunction is a common hallmark of many cardiac pathologies including HF. CaMKII-mediated arrhythmias have been identified in HF, AF, reperfusion injury, and genetic arrhythmogenic conditions including LQTS. Changes in CaMKII expression can influence the function of key cardiac ion channels and result in arrhythmogenesis (Mustroph et al., 2017). CaMKII-mediated hyperphosphorylation of RyR2 leads to spontaneous Ca\(^{2+}\) release events which can be proarrhythmic in disease conditions associated with Ca\(^{2+}\) leak. CaMKII can also drive Ca\(^{2+}\) overload through phosphorylation of Na\(_{v}1.5\) and subsequent reduction in NCX-mediated Ca\(^{2+}\) extrusion. In addition, an increase in CaMKII activity can result in prolonged repolarization through an increase in IC\(_{Cal}\), an increase in Na\(^{+}\) influx, and reduced IK function (Mustroph et al., 2017). This makes the inhibition of CaMKII is a promising antiarrhythmic approach.

KN-93, a CaMKII inhibitor, which works by binding directly to Ca\(^{2+}\)/CaM, disrupts the ability of Ca\(^{2+}\)/CaM to interact with CaMKII and activate the kinase (Wong et al., 2019). Inhibition of CaMKII by KN-93 has been shown to be antiarrhythmic in several models (Figure 3). In a RyR2\(R4496C\) knock-in mouse model of CPVT, KN-93 prevented catecholamine-induced sustained ventricular tachyarrhythmias (Liu et al., 2011). Further studies using induced pluripotent stem cells generated from a CPVT patient carrying a RyR2 mutation, showed KN-93 to
drastically reduce DADs induced by catecholaminergic stress as well as stabilize $Ca^{2+}$ activation under $\beta$-adrenergic stimulation to a pattern seen in normal tissue (Di Pasquale et al., 2013). CaMKII inhibition by KN-93 was also shown to reduce the incidence of stress-induced ventricular tachycardia in a junction knock-out mouse model which presents with a phenotype similar to CPVT (RyR2 channels display CaMKII-dependent hyperphosphorylation). Here, KN-93 also reduced the number of spontaneous $Ca^{2+}$ aftertransients and aftercontractions under stress conditions (Tzimas et al., 2015). It is worth noting that in relation to CPVT, AAV-mediated delivery of a CaMKII peptide inhibitor (autocamtide-2-related inhibitory peptide, AIP) has been shown to suppress ventricular arrhythmias induced by either $\beta$-adrenergic stimulation or programmed ventricular pacing. AAV-GFP-AIP delivery also suppressed abnormal $Ca^{2+}$ release events in induced pluripotent stem cells derived from two patients with two distinct CPVT pathogenic mutations (Bezzerides et al., 2019). The advantage of using a gene therapy approach over classic CaMKII blockers, including KN-93, is that it allows for cardiomyocyte-specific CaMKII inhibition with limited extracardiac effects.

In addition to CPVT, KN-93 has been shown to be effective in several other models of cardiac arrhythmias. Increased CaMKII-dependent SR $Ca^{2+}$ leak is a hallmark of AF. Diastolic SR $Ca^{2+}$ release via leaky RyR2 is hypothesized to contribute to arrhythmogenesis. In $Ryr^{2R176Q/+}$ mice (exhibiting a gain-of-function defect in RyR), CaMKII inhibition prevented arrhythmias induced by rapid atrial pacing (Chelu et al., 2009). Studies have also indicated that KN-93 is effective at reversing the diastolic $Ca^{2+}$ leak and attenuating the frequency of spontaneous $Ca^{2+}$ transients in aged atrial murine myocytes (Guo et al., 2014), which is an important finding given that aging is associated with an increase risk of AF. Finally, CaMKII is also activated in HF and has been linked to HF-associated arrhythmias. In hypertrophic mice, KN-93 suppressed isoproterenol-induced ventricular arrhythmias and reduced isoproterenol-induced QTc prolongation (Feng et al., 2017). Furthermore, KN-93 significantly reduced arrhythmia inducibility and slowed initiation of ventricular tachycardia in a rabbit model of HF (Hoeker et al., 2016), strengthening the notion that CaMKII inhibition may have antiarrhythmic effects in the failing human heart.

W-7 has been identified as a CaMKII inhibitor; as a CaM antagonist it inhibits the $Ca^{2+}$/CaM dependent activation of CaMKII thereby acting as an upstream blocker of CaMKII (Mazur et al., 1999). In a mouse model of cardiac hypertrophy (in which CaMKII expression levels were increased), inhibition of CaMKII activation by W-7 and KN-93 suppressed polymorphic ventricular arrhythmias (Figure 3). Here, W-7 was found to prolong AP duration to a greater extent than that of KN-93 (Kirchhof et al., 2004). W-7 has been shown to suppress TdP induction, without affecting QT duration or heart rate (Gbadebo et al., 2002; Pu et al., 2005). In a canine model of chronic AV block (associated with compensated hypertrophy and an increased risk of TdP) W-7 suppressed drug-induced TdP and shortened AP duration. Additional in vitro experiments found W-7 attenuated the dofetilide-induced enhanced CaMKII phosphorylation (Bourgonje et al., 2012).

TRPC

Transient receptor potential channels may prove to be suitable therapeutic targets for arrhythmic disorders as a number of these channels have been implicated in arrhythmogenesis and they exhibit specific expression patterns in the cardiac conduction system (Hof et al., 2019). Within the SAN, TRP channels have been identified to contribute to both the membrane and $Ca^{2+}$ clock (Hof et al., 2019). TRPC isoforms 1, 2, 3, 4, 6 and 7 have been detected in the mouse SAN, and treatment with the broad TRP channel inhibitor SKF-96365 results in reduced $Ca^{2+}$ influx and a reduction in the spontaneous pacemaker rate (Ju et al., 2007). Store-operated $Ca^{2+}$ entry (SOCE) describes the influx of $Ca^{2+}$ into the cell when $Ca^{2+}$ levels within the SR become depleted, a process which has been implicated in arrhythmia development (Bonilla et al., 2019). TRPC channels have been identified at store-operated transmembrane $Ca^{2+}$ channels (Bonilla et al., 2019). With TRPC3 being the only TRPC channel to be located at the plasma membrane of pacemaker cells, it has been suggested that this channel is the main isofrom to be involved in SOCE in the SAN (Hof et al., 2019). Spontaneous AP events via SOCE-activation were found to be reduced in a TRPC3 knockout mouse model (Ju et al., 2015), while excessive activation of TRPC3 has been shown to result in $Ca^{2+}$ overload and arrhythmias. Under normal conditions, TRPC3 is understood to mediate $Ca^{2+}$ and Na+ entry near NCX1, thereby promoting the forward mode of NCX and driving diastolic depolarization (Doleschal et al., 2015; Hof et al., 2019). However, when TRPC activation is increased, $Ca^{2+}$ overload and spatial uncoupling between TRPC3 and NCX1 occurs, resulting in arrhythmogenesis (Doleschal et al., 2015). This evidence therefore suggests that TRPC3 contributes to SAN pacemaking through the stimulation of the $Ca^{2+}$ clock via SOCE, activation of CaMKII and phosphorylation of $Ca^{2+}$ handling proteins (including PLN and RyR2), and via TRPC3-NCX dynamics (Hof et al., 2019).

TRPC3 expression is upregulated in the atria of AF patients, and in numerous animal models of AF (Harada et al., 2012; Zhao et al., 2013), conversely, when TRPC3 expression is deleted, in a TRPC3 knockout mouse model, pacing-induced AF was significantly reduced (Ju et al., 2015). Further studies have suggested TRPC3 overexpression in AF mediates cardiac fibroblast proliferation and differentiation via the regulation of $Ca^{2+}$ influx and downstream $Ca^{2+}$-mediated signaling (Harada et al., 2012). In a canine model of AF, treatment with the selective TRPC3 blocker Pyr-3 reduced atrial fibroblast proliferation and therefore the development of an AF substrate (Figure 4) (Harada et al., 2012). TRPC3 has also been linked to other arrhythmia conditions in addition to AF. Its activation by GS1702934A has been shown to influence cardiac contractility and favor rhythmic instability (Doleschal et al., 2015), and in a TRPC3 overexpression mouse model, GS1702934A resulted in arrhythmic events including paired ventricular beats, and episodes of atrial tachycardia, ventricular tachycardia and cardiac alternans (Doleschal et al., 2015). TRPC3 overexpressing hearts
were also more susceptible to ANG-II-stimulated arrhythmic events which included atrial or ventricular tachycardia. The interaction between TRPC3 and NCX1 is believed to drive the increased contractility and arrhythmogenesis upon TRPC3 activation (Doleschal et al., 2015). Therefore, inhibiting TRPC3 may be a potential therapeutic approach for arrhythmias arising due to sinus node dysfunction and AF. Pyr-3 has been identified as a selective inhibitor of TRPC3 and has been shown to efficiently suppress physiological events associated with TRPC3, including atrial fibroblast proliferation as highlighted above (Kiyonaka et al., 2009; Harada et al., 2012).

Studies have identified TRPM4 to be involved in SAN pacemaking through modulation of diastolic depolarization, suggesting that therapeutically targeting TRPM4 channels may be useful for heart rate modulation (Hof et al., 2019). Inhibition of TRPM4 by 9-phenanthrol reduced spontaneous AP in isolated mouse and rat atrial preparations and resulted in a frequency-dependent reduction of right atria beating rate (Figure 4 and Table 2). Similar results were evident in rabbit SAN pacemaker cells where inhibition of TRPM4 reduced the diastolic depolarization slope (Hof et al., 2013). The study suggested that TRPM4 may act as a rate accelerator channel in the SAN that becomes activated when heart rate decreases to provide protection against bradycardia (Hof et al., 2013). In addition to TRPM4, TRPM7 has also been detected in the mouse SAN (Hof et al., 2019) and is required for maintaining cardiac automaticity. Deletion of TRPM7 in vitro slows spontaneous Ca$^{2+}$ transient frequency and disrupts cardiac automaticity, while in vivo a loss-of-function zebrafish model displayed a slowing of heart rate (Sah et al., 2013b). In addition, the study of two mouse models of TRPM7 deletion has revealed a role for TRPM7 SAN function: (1) a cardiomyocyte-specific deletion of TRPM7 resulted in sinus pauses and (2) an inducible SAN/AVN TRPM7 knock-out drove atrioventricular block. These phenotypes were associated with a reduction in diastolic depolarization due to a downregulation of HCN4, and $I_f$ as a result of TRPM7 deletion (Sah et al., 2013b).

Both TRPM4 and TRPM7 are also expressed in human atrial cells (Hof et al., 2019). Small animal studies have identified that these TRP channels contribute to the atrial AP (Simard et al., 2013), with inhibition of TRPM4 by 9-phenanthrol leading to a shortening of the atrial AP duration, suggesting it may be a potential target for pharmacological approaches against atrial arrhythmias (Simard et al., 2013). It is proposed that TRPM7 channels are involved in fibrogenesis in human AF, and TRPM7 protein expression is upregulated in atrial specimens from AF patients (Zhang et al., 2012). In terms of ventricle conduction, studies using a knockout TRPM4 mouse model found that its loss increased β-adrenergic inotropic response, with TRPM4$^{-/-}$ ventricular myocytes displaying a faster repolarization phase and a shorter AP waveform compared to controls (Mathar et al., 2014). The study concluded that TRPM4 reduces the driving force of Ca$^{2+}$ influx, and subsequently contractile force, with the action of TRPM4 specifically important during β-adrenergic stimulation when there is an increase in the Ca$^{2+}$ current. TRPM4 channels have also been identified in Purkinje fibers, with mRNA expression most abundant in the Purkinje fibers compared to the atria, ventricle and septum (Hof et al., 2019).

Inhibition of TRPM4 by 9-phenanthrol was identified to shorten the AP of Purkinje fibers isolated from rabbit hearts with no effect on ventricle AP, suggesting TRPM4 is specifically involved in Purkinje fiber AP regulation (Hof et al., 2016).

The clinical importance of TRPM4 in cardiac conduction has been highlighted by studies that have identified TRPM4 mutations in numerous arrhythmic conditions including progressive cardiac bundle branch disease (Kruse et al., 2009), Brugada syndrome (Liu et al., 2013), LQT syndrome (Hof et al., 2017), atrioventricular and right bundle branch block (Stallmeyer et al., 2012), chronic heart block (Bianchi et al., 2018), and ventricular fibrillation (Bianchi et al., 2018). Furthermore, the cardiac phenotype of TRPM4 knockout mice included conduction block with PR ad QRS lengthening and Luciani-Wenckebach atrioventricular block (Demion et al., 2014). Interestingly, the mutations identified in familial arrhythmic conditions included loss- and gain-of function mutations, which suggests targets may have to be specific for the underlying mutation.

As highlighted in the studies above, 9-phenanthrol is a commonly used inhibitor of TRPM4 (Figure 4 and Table 2). The phenanthrene-derivative has been shown to be a specific inhibitor of TRPM4 channels in numerous tissues in the body (Guinamard et al., 2014). Within cardiomyocytes it is worth noting that 9-phenanthrol has been shown to modulate intracellular Ca$^{2+}$ oscillation in HL-1 cells derived from mouse atrial cardiomyocytes, resulting in a transient increase in intracellular Ca$^{2+}$ levels (most likely from mitochondrial Ca$^{2+}$ stores). The study suggested this may be due to a feedback mechanism from inhibition of TRPM4 since it is known to be activated by Ca$^{2+}$ (Burt et al., 2013). While 9-phenanthrol is a strong TRPM4 inhibitor for in vitro and ex vivo studies, the use of the agent in vivo is limited due low solubility and possible toxicity (Guinamard et al., 2014). In terms of TRPM7, pharmacological agents driving specific TRPM7 inhibition within the heart are lacking. TRPM7 is also one of several TRP channels which display broad tissue expression, suggesting targeting of this channel may result in off-target effects (Hof et al., 2019). Despite this, TRPM7 remains a potential therapeutic target for a range of conditions, including cardiac arrhythmias.

In addition to TRPC and TRPM channels, further TRP channels have been implicated in cardiac rhythm. Increased gene expression of various TRP channels (including TRPM, TRPC, TRPV, and TRPP) have been identified in leukocytes of patients with non-valvular AF (Duzen et al., 2017). TRPV channels have been linked to atrial inotropy related to ROS-mediated signaling (Odoshivikina et al., 2015), and in a porcine model, depletion of TRPV1 afferent fibers resulted in a reduction in ventricular arrhythmic events associated with MI (Yoshie et al., 2020). A study has shown that TRPA1 may be a novel therapeutic target for increasing inotropic and lusitropic states in the heart, having demonstrated that stimulation of TRPA1 in isolated murine cardiomyocytes resulted in activation of CaMKII-dependent signaling and an increase in peak intracellular Ca$^{2+}$ levels, driving an increase in cardiomyocyte contractile function (Andrej et al., 2017). Finally, in a zebrafish model, mutations in PKD2 (which encodes for TRPP2) resulted in atrioventricular...
block, with isolated hearts displaying impaired intracellular \( \text{Ca}^{2+} \) cycling and \( \text{Ca}^{2+} \) alternans (Paavola et al., 2013).

**MCU**

It has been suggested that cardiac mitochondria influence arrhythmia development through the regulation of cytoplasmic \( \text{Ca}^{2+} \) fluxes achieved via the uptake of \( \text{Ca}^{2+} \) through MCU (Brown and O’Rourke, 2010). Much of the work focused on targeting MCU as a potential therapeutic approach to arrhythmogenesis has focused on HF models. A study utilizing a knockout MCU mouse model found, in a non-ischemic HF setting, MCU knockout mice display a shorter AP duration and fewer incidences of EADs and ventricular fibrillation when compared to control animals (Xie et al., 2018). Use of the MCU and fewer incidences of EADs and ventricular fibrillation when setting, MCU knockout mice display a shorter AP duration (Brown and O’Rourke, 2010). Much of the work focused on targeting MCU as a potential therapeutic approach to arrhythmia development through the regulation of cytoplasmic \( \text{Ca}^{2+} \) cycling and \( \text{Ca}^{2+} \) alternans (Paavola et al., 2013). However, RR has off-target effects related to \( \text{Ca}^{2+} \) handling, including SR \( \text{Ca}^{2+} \) release and LTCC (Brown and O’Rourke, 2010). Ru360, an RR analog, has been shown to be a more specific MCU inhibitor and able to suppress ventricular fibrillation associated with I/R (Figure 4 and Table 2) (de et al., 2006). Both Ruthenium compounds have also been shown to convert pacing-induced ventricular fibrillation to ventricular tachycardia in isolated rat hearts, highlighting the role of MCU in arrhythmia development (Kawahara et al., 2003). It is worth noting that while Ru360 had no effect on left ventricle pressure, at high concentrations RR reduced left ventricle pressure amplitude (Kawahara et al., 2003).

**DISCUSSION**

\( \text{Ca}^{2+} \) plays a critical role in maintaining cardiac physiology and changes to the proteins which are involved in \( \text{Ca}^{2+} \) handling underly numerous cardiac diseases. Targeting \( \text{Ca}^{2+} \) dysregulation in HF and arrhythmias has long been investigated as a potential therapeutic target and, as is evident from this review, there is considerable current research investigating targeting proteins associated with \( \text{Ca}^{2+} \) handling, including RyR2, NCX, SERCA, and TRP channels, as a therapeutic approach for cardiomyopathy, HF and arrhythmias. The pathogenic remodeling of these \( \text{Ca}^{2+} \) handling proteins has been shown to differ dependent on the underlying pathological mechanism, suggesting a “personalized” approach to targeting \( \text{Ca}^{2+} \) handling proteins may be required for specific cardiac conditions. Furthermore, while several therapeutics targeting \( \text{Ca}^{2+} \) have progressed to clinical trials, several are still in early preclinical stages, demonstrating the need for further research in this area. Altogether, targeting \( \text{Ca}^{2+} \) handling is a promising approach to developing novel and effective therapies for key cardiovascular disorders.

**AUTHOR CONTRIBUTIONS**

EC, CW, and AN contributed conceptually to the manuscript. AN and CW drafted the manuscript. AN contributed the section concerning cardiac hypertrophy and heart failure, and the tables. CW contributed the section regarding cardiac arrhythmias. Both AN and CW contributed to the introduction, the current therapeutic approaches, the discussion, the figures, and the abstract. All authors reviewed the manuscript prior to submission.

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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