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Myocardial calcium signalling and arrhythmia pathogenesis

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Abstract

Myocardial calcium signalling is a vital component of the normal physiological function of the heart. Key amongst the many roles calcium plays is its use as the primary signalling component of excitation–contraction coupling, the intracellular process that links cardiomyocyte depolarisation to contraction. Defective cellular calcium handling, due to abnormalities of the various components which mediate and control excitation–contraction coupling, is widely recognised as a significant patho-physiological event in the contractile dysfunction of the failing heart. In addition, similar defects also appear to be increasingly recognised as mediators of certain forms of cardiac arrhythmias. Such defects include single gene defects in excitation–contraction coupling components that lead to inherited sudden death arrhythmia syndromes. Alternatively, arrhythmogenesis occurring within the context of acquired cardiac disease, in particular heart failure, also appears to be highly dependent on abnormal calcium homeostasis. In this article we review the defects in cardiomyocyte calcium homeostasis that lead to particular pro-arrhythmogenic phenomena and discuss recent insights gained into a variety of inherited and acquired arrhythmia syndromes that appear to involve defective calcium signalling as a central component of their patho-physiology. Potential opportunities for new anti arrhythmic therapeutic strategies based on these recent insights are also discussed.

Introduction

Calcium signalling in the heart

Ionised calcium (Ca^{2+}) is required for a range of intracellular and extracellular processes as diverse as blood coagulation, nerve conduction, and muscle contraction. It is also the most common element used by living cells for intracellular signal transduction mechanisms [1] including the activation and regulation of various enzymes, metabolic pathways, and the control of gene transcription factors. In addition, the heart uses Ca^{2+} to achieve a synchronised cellular depolarisation and subsequent activation of contractile proteins, via the physiological mechanism of excitation–contraction coupling (EC coupling). To facilitate this process intracellular Ca^{2+} homeostasis must be carefully regulated to ensure that depolarisation and contraction occur in a synchronised time-dependent fashion during the systolic-diastolic cycle of the heart. As Ca^{2+} cannot be metabolised its total intracellular concentration (and indeed local concentration within defined spatial regions of the cell) is tightly regulated via specific binding and transport proteins. Defects of these processes are increasingly being identified and are providing an insight into the patho-physiological disruption of intracellular Ca^{2+} homeostasis in the heart. We are now in a position to unravel the mechanisms that govern Ca^{2+} dependent arrhythmogenesis.

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The emerging link between myocardial calcium signalling and cardiac arrhythmias

It is not surprising that a physiological mechanism such as EC coupling, which utilises Ca^{2+} as a second messenger, should be implicated in both arrhythmia and heart failure pathogenesis. Although it has been long appreciated that pathological cellular Ca^{2+} overload can lead to a pro-arrhythmogenic state it is only recently that a clearer understanding of the importance of defective Ca^{2+} signalling in arrhythmia pathogenesis has emerged. As a result little progress has been made in the development of new pharmacological agents that mediate an anti-arrhythmic action via modulation of Ca^{2+} signalling pathways. Although the widely used Ca^{2+} channel blocking drugs, such as verapamil, have a known anti-arrhythmic action, especially in the context of supraventricular arrhythmias, this effect is primarily related to their blockade of AV nodal conduction rather than a direct molecular modification of defective Ca^{2+} signalling pathways. In conjunction with the emerging role of defective EC coupling in heart failure, recent work has begun to reveal the role played by Ca^{2+} in the pathogenesis of various inherited and acquired arrhythmia syndromes. In this article we review the mechanisms of defective Ca^{2+} signalling within the heart, how these defects are believed to precipitate cardiac arrhythmias and whether they hold any potential for credible therapeutic targets. Prior to this we briefly review the process of cardiac EC coupling and the clinical electrophysiological characteristics of Ca^{2+} dependent arrhythmia mechanisms.

Cardiac excitation–contraction coupling is the key myocardial calcium signalling process

Cardiac EC coupling refers to the co-ordinated cellular depolarisation and movement of intracellular Ca^{2+} around the cell in order to bring about contraction. It is the key Ca^{2+} signalling process within the heart and its cellular components and other key elements relevant to discussions in this article are outlined in Fig. 1. More in-depth reviews concerning the basic physiology of this process can be found elsewhere [2–5], a brief overview is presented here.

When the myocyte depolarises extracellular Ca^{2+} enters the cell, primarily through the sarcolemmal L-type voltage dependent (dihydropyridine sensitive) Ca^{2+} channel (DHPR). Additional potential routes of Ca^{2+} entry exist including the sarcolemmal Na^{+}/Ca^{2+} exchanger (NCX) and the T-type voltage dependent Ca^{2+} channel, although these are felt to be less important, or in the case of the T type channel confined to specialist pacemaker cells and conducting tissue. The inward Ca^{2+} current (I_{Ca}) through DHPR is, on its own, insufficient to bring about the required conformational change in troponin needed for contraction to occur. Additional Ca^{2+} is required and this is obtained from a pool of stored Ca^{2+} within the sarcoplasmic reticulum (SR) of the cell. The initial inward movement of Ca^{2+} acts as an amplification signal for the release of this stored pool of SR

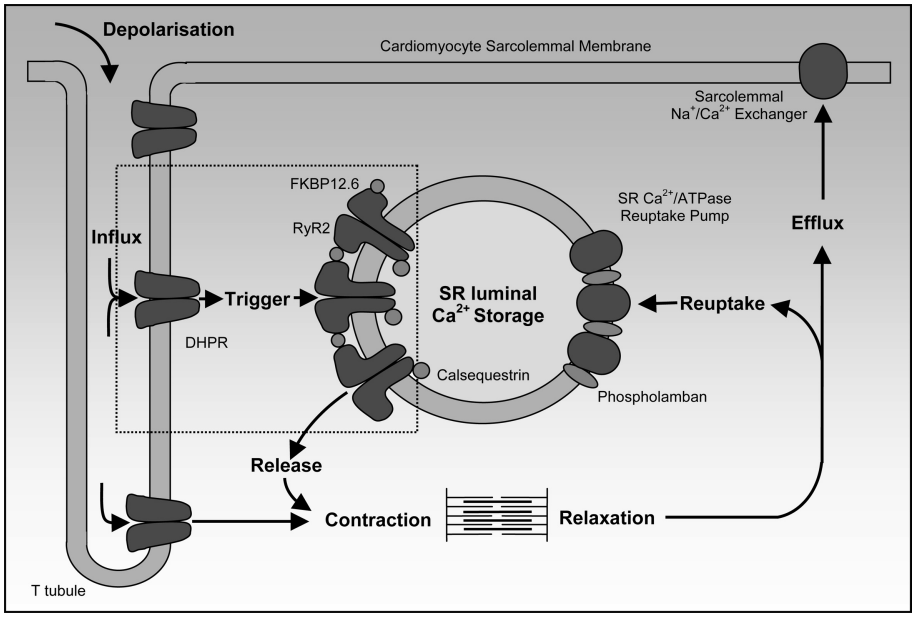

Fig. 1. Key components of cardiac EC coupling. Components of cardiac EC coupling and intracellular Ca^{2+} movement during the contraction–relaxation cycle of ventricular cardiomyocytes. Note (1) close association between DHPR and RyR2 (dashed box) which facilitates Ca^{2+} induced Ca^{2+} release and (2) FKBP 12.6 which has been proposed to couple the gating of adjacent RyR2 channels. The movement of Ca^{2+} is shown in bold text/arrows. For further description and abbreviations see text.

Ca^{2+}. This process, known as "calcium induced calcium release" [6], occurs through a SR membrane ion channel known as the cardiac/isoform 2 ryanodine receptor (RyR2) [7]. Individual populations of RyR2 localise in areas of the SR membrane, adjacent to DHPRs within the T tubules of the sarcolemma [8]. Cytosolic Ca^{2+} is itself the primary ligand that activates RyR2, thus influx of Ca^{2+} through DHPR activates its associated local population of RyR2 channels causing a synchronised release of SR Ca^{2+} known as a Ca^{2+} spark [9]. This synchronised opening of adjacent RyR2 is also facilitated by FK 506 binding protein 12.6 (FKBP12.6), the cardiac isoform of a regulatory protein which appears to mediate coupled gating between neighbouring RyR2 [10,11]. The synchronised release of multiple Ca^{2+} sparks throughout the cell following depolarisation creates a global intracellular Ca^{2+} transient of sufficient magnitude to bring about contraction.

Myocyte relaxation conversely results from closure of RyR2 and the rapid removal of cytosolic Ca^{2+}, either by re-uptake into the SR through the SR Ca^{2+}/ATPase pump (SERCA), where it is buffered by calsequestrin 2 (CASQ2), or by its removal from the cell through the sarcolemmal NCX, operating in forward (Ca^{2+} efflux) mode. These two processes balance systolic cellular influx and SR release of Ca^{2+} such that there is no net gain or loss of cellular Ca^{2+} with each contraction–relaxation cycle. An important observation at this time is that in addition to activation and opening by cytosolic Ca^{2+}, RyR2 gating can also be controlled in a concentration dependent fashion by SR luminal free Ca^{2+} [12]. Furthermore, it also appears that as SR Ca^{2+} content increases a greater proportion of this Ca^{2+} pool will be released for any given trigger [13–15]. Spontaneous Ca^{2+} release from the SR, independent of I_{Ca} mediated release, also occurs during normal cellular physiology, although the frequency of Ca^{2+} sparks is low and not sufficient to precipitate either a significant change in the cellular membrane potential or the activation of contractile proteins. In situations of SR Ca^{2+} overload however the frequency of spontaneous Ca^{2+} sparks is markedly increased [9,16], corresponding to a probable activation of RyR2 by an increased luminal free [Ca^{2+}]. This correlation between SR Ca^{2+} load and spontaneous SR Ca^{2+} release is a key property in the development of Ca^{2+} dependent arrhythmias.

Catecholaminergic signal transduction pathways and phosphorylation regulate cardiac EC coupling

The various channels and pumps which co-ordinate cardiac EC coupling interact with a variety of structural and regulatory proteins. Of particular importance amongst these are the enzymes which mediate phosphorylation of both the channels/pumps and other regulatory elements attached to them. Such phosphorylation is the final event in a signal amplification cascade that begins with β-adrenergic receptor activation, either by sympa-

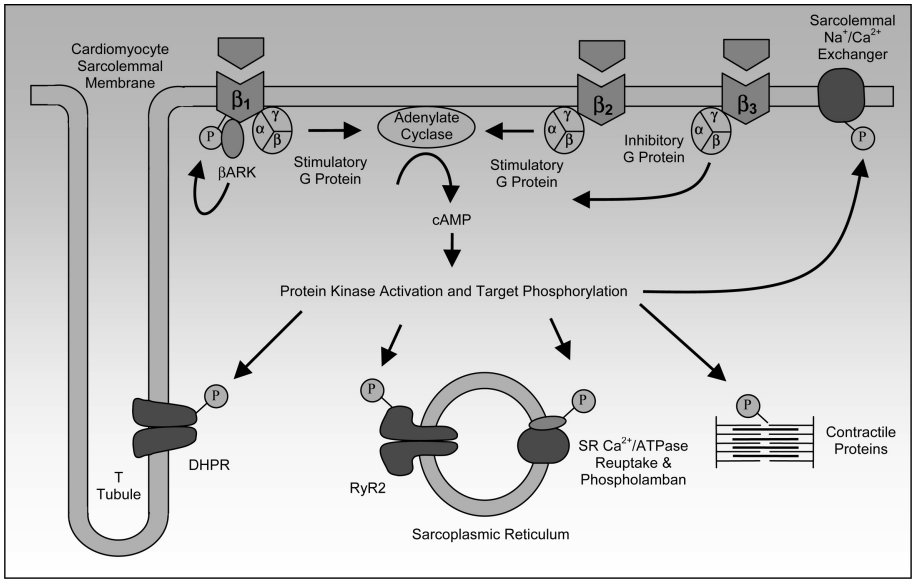

Fig. 2. β-Adrenergic dependent EC coupling augmentation in the heart. In the human heart β_1 and β_2-adrenergic receptor signal transduction pathways activate protein kinase A, causing target protein phosphorylation, which enhances both inotropic and lusitropic aspects of EC coupling. β_3-Adrenergic receptor activation inhibits protein kinase activation. Phosphorylation of β-adrenergic receptors via β-adrenergic receptor kinase (βARK) uncouples the receptor from its signal transduction pathways, acting as a negative feedback mechanism in situations of hyper-catecholaminergic drive. Experience with positive inotropic drugs in heart failure demonstrates that maximal functional augmentation via this process is rapidly achieved. Ongoing β-adrenergic receptor activation produces no further functional improvement, rather detrimental functional states are induced which lead to contractile dysfunction and arrhythmogenesis.

thetic nerves or circulating catecholamines. This subsequently allows the activation of adenylate cyclase and the generation of cyclic AMP, which in turn switches on phosphorylation enzymes such as protein kinase A (PKA). In this way the activity of cardiac EC coupling can be modified by adrenergic input and this process underlies the improvements in haemodynamic parameters seen with positive inotropic drugs and the fight and flight response of the heart. Important functional consequences of phosphorylation include a greater influx of Ca^{2+} through DHPR and a greater release of SR Ca^{2+} through RyR2 [2]. These direct inotropic consequences are balanced by the lusitropic effects of greater SR Ca^{2+} re-uptake through SERCA (as a result of phosphorylation of its regulatory protein phospholamban), the dissociation of Ca^{2+} from troponin, and a greater efflux of Ca^{2+} through NCX. Although these lusitropic actions enhance myocyte relaxation they are nevertheless key to ensuring sufficient SR Ca^{2+} is available for the next cellular depolarisation and thus also contribute to the overall gain in cardiac EC coupling that adrenergic stimulation mediates. β-Adrenergic activation of the heart is a pro-arrhythmogenic event known to increase SR Ca^{2+} load and the frequency of spontaneous SR Ca^{2+} release [17]. It appears that many of the Ca^{2+} mediated arrhythmia syndromes share a common theme of being precipitated by situations of stress, exercise, and emotion when catecholaminergic drive is high. As such it appears that catecholaminergic phosphorylation of cardiac EC coupling components and the subsequent consequences, such as increased loading of the SR with Ca^{2+}, may be a key factor in the development of Ca^{2+} mediated arrhythmias. Catecholaminergic modulation of cardiac EC coupling is summarised in Fig. 2.

Calcium dependent arrhythmias and clinical electrocardiography

Cellular, and in particular SR Ca^{2+} overload, is undoubtedly a substrate for arrhythmia generation,

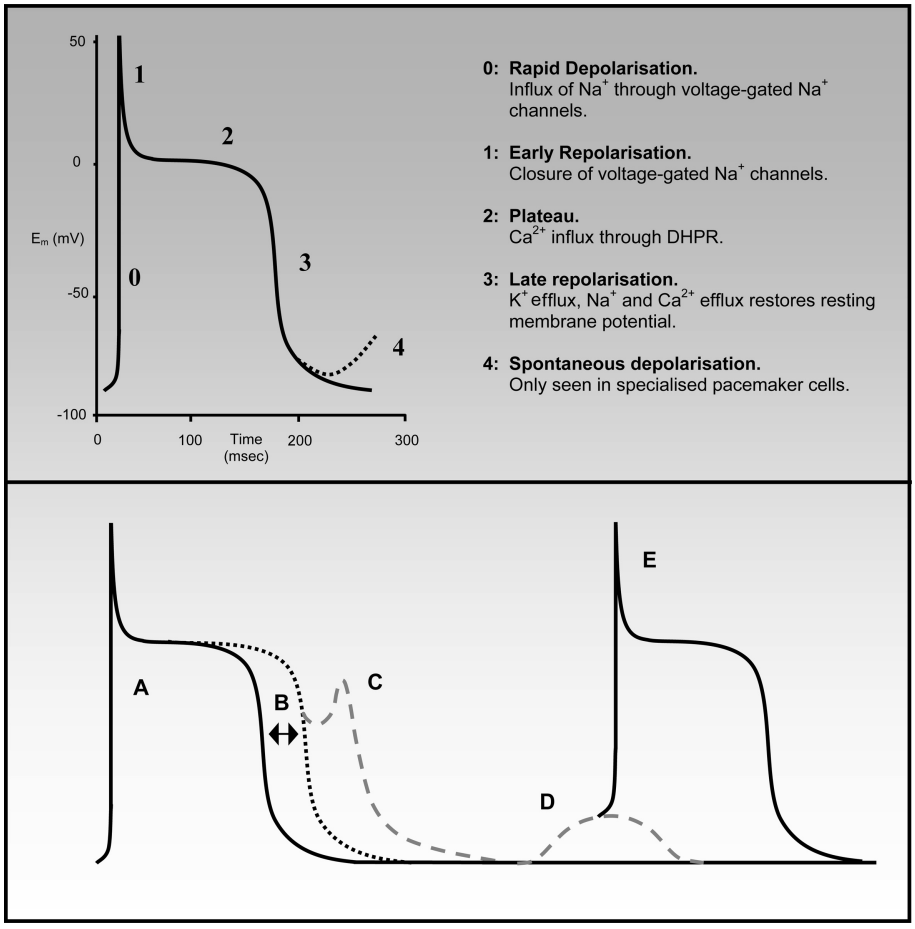

Fig. 3. Schematic representation of early and delayed afterdepolarisations with respect to the normal cardiac action potential. Phases and predominant ion fluxes of normal cardiac action potential (upper window). Timing and morphology of early and delayed afterdepolarisations (lower window). A normal action potential: (A) may be extended in duration (B) such that an early afterdepolarisation is generated (C) during the repolarisation phase. Dispersion of depolarisation time throughout ventricle promotes re-entrant arrhythmia generation. A delayed afterdepolarisations (D) only occurs after a normal action potential (A) is completed and the cell has returned to the resting membrane potential. If the DAD is of sufficient magnitude to reach a depolarising threshold a new action potential (E) can occur which itself can initiate a triggered arrhythmia.

however due to the highly effective intracellular buffering systems, such as sequestration of excess cytosolic Ca^{2+} within the SR by CASQ2, even profound hypercalcaemia is rarely sufficient to initiate arrhythmias, in fact the only common manifestation of hypercalcaemia on the surface 12 lead electrocardiogram is a minor shortening of the QT interval. More profound ECG manifestations which are believed to arise as a result of Ca^{2+} dependent mechanisms include premature ectopic beats (extrasystoles), monomorphic and polymorphic ventricular tachycardia, bidirectional tachycardia, electrical alternans, and atrial fibrillation. The slowly conducted Ca^{2+} dependent action potential through the atrio-ventricular (AV) node is also likely to facilitate re-entrant AV nodal arrhythmias [18], however unlike the examples above the primary defect here is not Ca^{2+} signalling per se, indeed this is normal, rather the arrhythmia simply represents normal Ca^{2+} signalling pathways within an abnormal anatomical re entry arrhythmia substrate [19]. At the level of the cardiac action potential Ca^{2+} currents are vital in the heart for pacemaker depolarisation, conduction through the AV node, and individual myocyte depolarisations that initiate contraction. The cardiac action potential is itself a product of specific ion currents, activated and deactivated in a time dependent manner as shown in Fig. 3, the unique phase 2 plateau being a manifestation of I_{Ca}, primarily through the DHPR.

Most calcium dependent arrhythmias occur in the context of normocalcaemia

When SR Ca^{2+} load is increased, either experimentally or as a consequence of disease, spontaneous release of Ca^{2+} occurs from an overloaded SR [20]. It is the appearance of an elevated cytosolic Ca^{2+} within the myocyte at a point outside of its normal EC coupling time window that appears to underlie the majority of Ca^{2+} dependent arrhythmias. This primarily represents the appearance of excess cytosolic Ca^{2+} during the repolarisation phase of the cardiac action potential when Ca^{2+} is normally being removed from the cytosol. More important than hypercalcaemia as a precipitating cause for such arrhythmia inducing conditions appear to be specific defects in the normal structure and function of various Ca^{2+} handling proteins and ion channels. Such defects appear to be induced by a variety of mechanisms independent of extracellular [Ca^{2+}], hence the majority of Ca^{2+} mediated arrhythmias occur in the context of normocalcaemia. This abnormal cellular handling of Ca^{2+} may arise from genetic defects in Ca^{2+} ion channels, pharmacological modification of EC coupling function (e.g., cardiac glycosides) or altered Ca^{2+} homeostasis induced by other cardiac patho-physiological states such as cardiomyopathy and heart failure. Although the underlying causes of Ca^{2+} induced arrhythmias may be diverse, the common final pathway of such defects is likely to be an increase in cytosolic Ca^{2+} which has the potential to induce a cellular depolarisation, at a time during the normal cardiac action potential cycle where one would not usually be expected, typically during repolarisation. Depending on the repolarisation state of neighbouring cells the depolarisation may propagate locally and ultimately throughout the whole heart. The vast majority of abnormal depolarisations are likely to terminate locally due to surrounding refractory cells, which are unable to propagate the depolarisation wave further. A smaller number may spread to the whole heart precipitating a premature ectopic beat (extrasystole) and a smaller number still may initiate ventricular tachycardia (VT). A further recent observation is that an arrhythmogenic and abnormally high cytosolic Ca^{2+} may spread to surrounding cells, not simply via a high velocity membrane depolarisation signal mediated by Na^{+} channel currents, but by the physical diffusion of Ca^{2+} into neighbouring cells via gap junctions, leading to further spontaneous Ca^{2+} release [21]. This propagation has been termed a "triggered propagated contraction." Its significance has yet to be established but it does offer an alternative mechanism whereby abnormal Ca^{2+} signals may be propagated more widely from an initial small focus of abnormal cells.

Triggered activity underlies the majority of calcium mediated arrhythmias

Cardiac arrhythmias can arise due to a variety of mechanisms such as re-entry, excessive automaticity or triggered activity, the latter two being representative mechanisms of de novo abnormal impulse generations within individual myocytes [22]. Non-re-entrant mechanisms are by no means insignificant; three-dimensional mapping suggests that most VT in the context of non-ischaemic heart failure is initiated by non-re-entrant mechanisms [23–25]. Automaticity refers to the ability of myocardial cells to initiate spontaneous depolarisation during the diastolic interval. More recently, a second mechanism of impulse generation termed triggered activity has been described and it is this that appears to be most closely linked to Ca^{2+} mediated arrhythmogenesis [26]. Triggered activity is so called because unlike the spontaneous depolarisations associated with automaticity the impulse can only occur if it follows a previous action potential, i.e., it is said to be triggered by the previous impulse. Triggered activity arises following the development of sub-threshold membrane depolarisations, termed afterdepolarisations, which follow the previous action potential. These afterdepolarisations can occur during the repolarisation of the previous impulse where they are called early afterdepolarisations (EADs), or they can occur after repolarisation is complete where

they are known as delayed afterdepolarisations (DADs). A representation of these phenomena with respect to the normal cardiac action potential is outlined in Fig. 3. These various arrhythmia mechanisms are not mutually exclusive, for example, a triggered depolarisation may itself initiate a re-entrant arrhythmia if the particular anatomical and electrical properties of the ventricle are present to sustain it. In fact, although DADs appear to induce triggered activity which directly leads to sustained arrhythmias, EADs appear to initiate arrhythmias primarily through a dispersion of repolarisation, which actually leads to re-entry arrhythmias [24].

Delayed afterdepolarisations are a manifestation of SR Ca^{2+} overload and spontaneous Ca^{2+} sparks

Considerable evidence exists to suggest that DAD generation is the primary mechanism by which most ventricular Ca^{2+} dependent arrhythmias occur. DADs are believed to result from a transient Ca^{2+} activated inward current (I_{TI}) evoked by spontaneous Ca^{2+} release from the SR under conditions that favour accumulation of intracellular Ca^{2+} [22,25,27–29]. The role of SR Ca^{2+} release through RyR2 is emphasised by studies using ryanodine that dramatically disrupts the normal gating properties of RyR2 and blocks DAD formation [30,31]. The major component of the I_{TI} current itself however appears to be NCX [25,32]. Upon release of SR Ca^{2+}, NCX removes excess Ca^{2+} in exchange for an inward depolarising movement of Na^+. If this inward Na^+ current were sufficient to cause a DAD amplitude in excess of the threshold potential of the cell, a new cellular depolarisation will occur, which may propagate throughout the heart causing extrasystoles and ventricular tachycardia. Other possible mechanisms may also be involved in the generation of I_{TI} such as a Ca^{2+} activated chloride current or a reduced inward rectifier potassium current (I_{K1}). The latter allows a greater depolarization for any given NCX current and hence a greater likelihood that the threshold for a triggered action potential occurs [33]. Several factors have been shown under experimental conditions to increase the amplitude of DADs and hence the probability that the cellular depolarising threshold potential will be reached. These include: (1) increasing intracellular Ca^{2+} load, for example via a pharmacological effect induced by drugs such as cardiac glycosides (which are a well-known inducer of DADs) [34] and (2) increasing heart rate, for example via the use of catecholamines [17,35,36].

Early afterdepolarisations may also result from Ca^{2+} dependent mechanisms

The underlying ionic basis for EADs appears to be somewhat more complex and heterogeneous than the role played by Ca^{2+} overload in DAD formation and more detailed discussions may be found elsewhere [19,20,37]. EADs have been demonstrated in various conditions, however they are particularly associated with circumstances where the action potential duration and hence surface ECG QT interval is prolonged. As a consequence, they are also closely associated with the development of polymorphic torsades de pointes ventricular tachycardia in both congenital and acquired long QT syndromes. Until recently all cases of inherited long QT syndrome were linked to primary defects in ion channel function which resulted in an impaired outward K^+ current or an enhanced inward Na^+ current (both of which reduce the net outward current, delaying repolarisation). Although defects in Na^+ and K^+ channels may be the primary event in prolonging AP duration as a prerequisite for EAD generation it is believed that the upstroke of the EAD itself is initiated by an inward depolarising Ca^{2+} current, through DHPR [37–39]. If the action potential duration is prolonged, DHPR can recover whilst the cell still remains at a depolarised membrane potential, thereby allowing local reactivation and a further depolarising upstroke before the cell has fully repolarised [19,37]. The EAD upstroke therefore further prolongs the total depolarised phase of the action potential. This may lead to a dispersion of action potential duration within the myocardium, thereby producing a pro-arrhythmogenic state within the ventricle that favours the development of re-entry arrhythmias [24].

Recent experimental evidence is emerging to suggest that cellular Ca^{2+} overload and SR Ca^{2+} release can also result in the generation of EADs [20,40,41]. In line with this hypothesis and as outlined above with respect to DAD formation a Ca^{2+} activated NCX inward Na^+ current could contribute to the genesis of the EAD [20]. Despite such possible similar origins there do appear to be some quantitative differences in the mechanisms underlying Ca^{2+} overload induced EADs and DADs, for example EADs appear to result from a synchronised release of Ca^{2+} throughout the cell whereas DAD inducing Ca^{2+} release appears to be localised within the centre of the myocyte [42,43]. Furthermore, EADs are more likely to occur within the context of bradycardia, where action potential duration is increased, conversely DADs are more likely at catecholamine driven increased heart rates [25].

Inherited calcium dependent arrhythmia syndromes

Mutations in the cardiac ryanodine receptor underlie inherited exercise induced sudden cardiac death syndromes

The identification of genes underlying inherited arrhythmogenic syndromes has greatly enhanced our understanding of the substrate for arrhythmia

development in these conditions [44]. Syndromes such as the long QT syndrome [45–48], Brugarda syndrome [49], Anderson syndrome [50], and certain familial cases of Wolff Parkinson White syndrome [51] have now been shown to arise from specific mutations in ion channels or ion channel associated proteins. For some time however no monogenic arrhythmogenic disorder had been directly linked to a specific defect in myocardial Ca^{2+} handling, despite this being recognised as a theoretical cause of arrhythmogenesis [52]. The past few years however have seen a dramatic advance in the recognition of the role played by inherited Ca^{2+} handling defects in arrhythmia generation.

Recent work has revealed an ever-increasing number of RyR2 mutations, identified independently by several groups [53–56], as the underlying cause of two inherited forms of cardiac arrhythmia which are associated with sudden death in children and young adults. In a recent review over 21 such mutations were listed [57]. Three further mutations have since emerged [58], and more will undoubtedly follow in due course. Current arrhythmogenic RyR2 mutations are summarised in Fig. 4. Both of these conditions, catecholaminergic/polymorphic ventricular tachycardia (CPVT) and the type 2 subtype of arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC2), are now known to arise from autosomal dominant inherited missense mutations in RyR2 and both share the common clinical feature of exercise and stress induced ventricular arrhythmias.

ARVC is an acronym used to describe a genetically heterogeneous group of cardiomyopathies, first described in 1977 [59] and characterised by structural and functional abnormalities of the right ventricle and in particular progressive replacement of the right ventri-

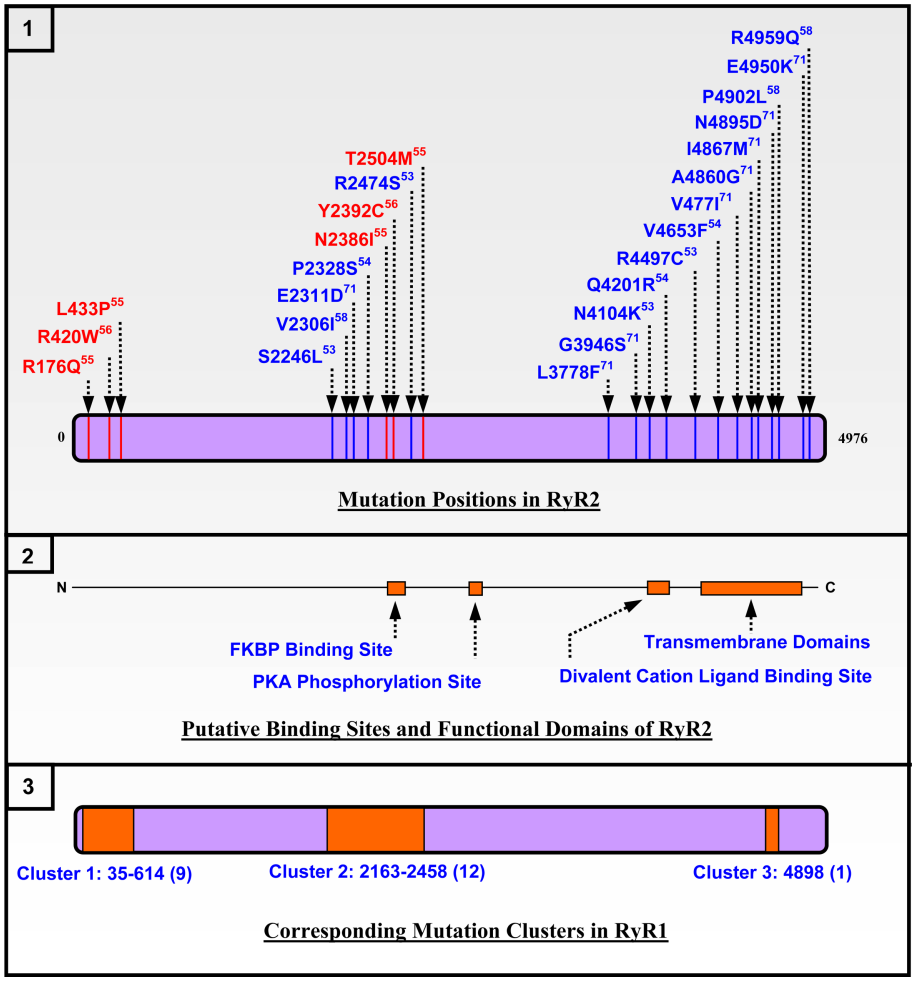

Fig. 4. Identified arrhythmogenic mutations in RyR2 and corresponding positions of RyR1 mutation cluster regions. (1) Identified arrhythmogenic mutations in RyR2 gene reading frame cluster in three evolutionary conserved regions. ARVC2 mutations shown in (red) and CPVT mutations shown in blue. Reference indicates first literature report of mutation. (2) Important functional domains and putative binding sites within RyR2. Several mutations encompass the FKBP 12.6 binding site, a putative cytosolic divalent cation binding site, and the important transmembrane domains (which include the ion channel pore and selectivity filter). No mutation has yet been identified in the protein kinase A (PKA) phosphorylation site on human RyR2 (S2809). (3) Disease causing RyR1 mutation clusters as outlined by McCarthy et al. [77], show a general correlation with arrhythmogenic RyR2 mutation cluster regions.

cle free wall myocardium by fibrous and fatty tissue [60–62]. It usually presents with arrhythmias of right ventricular origin including ventricular tachycardia and sudden death. Linkage studies have identified at least seven candidate chromosomal loci in different families affected by the disease. ARVC type 2, first described in 1988 [63] and characterised by its association with exercise, stress, and effort induced arrhythmias, was linked to the RyR2 chromosomal loci 1q42–43 [64,65]. Recently Tiso et al. [55] identified four RyR2 missense mutations in families with this condition.

CPVT in contrast shows no evidence of structural heart disease at either gross anatomical or histological level and was traditionally grouped with other rare inherited sudden death syndromes that occur in a structurally normal heart, such as the congenital long QT syndromes, Brugada syndrome, and idiopathic familial VF [66]. CPVT is characterised by a normal resting ECG that gives way to ventricular arrhythmias at the time of adrenergic activation, such as stress, emotion, and exercise. These arrhythmias take the form of bidirectional and/or polymorphic ventricular tachycardia (BDT/PVT), both of which are capable of degenerating into ventricular fibrillation and sudden death [67–71]. Although it is undeniably a rare disorder, it is nevertheless important as it has a high mortality rate (30–50% by the age of 30 [72]) and can present as sudden cardiac death in children without warning. Despite several early case reports and short series of exercise induced BDT/PVT [73,74] it was not until Leenhart *et al* reported a 10-year followup of 21 children with the disorder in 1995 [69], that the arrhythmia became established as a distinct clinical entity.

CPVT shows a familial tendency suggestive of an autosomal dominant inheritance pattern. In 1999 Swan et al. [67] reported that the disorder mapped to the RyR2 gene locus in two Finnish families. Priori et al. [53] and Laitenen et al. [54] subsequently identified a total of seven missense mutations in RyR2 as being responsible for the disorder in several families attending their centres. These identified arrhythmogenic mutations in RyR2 (both CPVT and ARVC2) appear to cluster in three highly conserved regions of the RyR2 gene, which include domains of known important functional significance within the RyR2 reading frame. These include residues that are likely to encompass the FKBP 12.6 binding site, potential cytosolic divalent cation binding site(s), and the important trans membrane domains, which encompass the channel pore and selectivity filter [75,76]. Also of interest is the observation that these mutation cluster regions correspond with similar regions in the skeletal muscle ryanodine receptor (RyR1) gene where mutations in RyR1 underlying malignant hyperthermia and central core disease are located [77]. Although functional characterisation of arrhythmogenic RyR2 mutations is at an early stage, considerable data are available concerning these RyR1 mutations. In general, such studies appear to show that RyR1 mutations affect Ca^{2+} dependent regulation, leading to increased channel opening [78–80]. Single channel studies have suggested increased sensitivity to activation by Ca^{2+} and a decreased sensitivity to inhibition by Mg^{2+}, both of which are properties which could lead to SR Ca^{2+} leak [81]. Early reports of arrhythmogenic RyR2 mutations suggested that such mutations were most likely to result in a channel more sensitive to opening stimuli, also resulting in a gain in function, which could then be amplified by catecholamine induced SR Ca^{2+} release during exercise [55].

The identification of mutations in RyR2 would logically suggest that a disruption of the normal physiological release of Ca^{2+} from the SR during EC coupling underlies these arrhythmogenic syndromes and that the mutations alter the normal physiological response of the channel to catecholaminergic input. This hypothesis is supported by a consideration of the clinical electrophysiological properties of bidirectional tachycardia (BDT), a rare and unusual arrhythmia associated with CPVT in ~31% [82] and 46% [71] of cases in two recent series. BDT was first described in 1922 [83] and is better known for its association with digoxin toxicity [84]. Cardiac glycosides are known to lead to intracellular Ca^{2+} overload and DAD generation within cardiomyocytes [34,85] and there is now also direct evidence from electrophysiological studies during arrhythmia induction with isoproterenol infusion in CPVT that DAD generation occurs in vivo as a result of this stimulus and does indeed result in the generation of BDT [86]. These parallel observations suggest that BDT is a triggered arrhythmia resulting from DADs, secondary to intracellular Ca^{2+} overload and spontaneous SR Ca^{2+} release. Interestingly, although cardiac glycosides are thought to produce arrhythmogenic DAD inducing intracellular Ca^{2+} overload, via a compensatory reverse mode influx of Ca^{2+} through NCX, itself a consequence of increased inward Na^+ influx through the Na^+/K^+ ATPase [85], their direct effect on RyR2 may also have a role. Cardiac glycosides are known to increase the open probability of RyR2 [87–89] and the revelation that CPVT mutations are associated with BDT suggests that BDT arising from glycoside toxicity may result from direct pharmacological activation of RyR2 and a subsequent DAD inducing SR Ca^{2+} release.

On the basis of pre-existing observations concerning BDT, DAD, and RyR1 mutations it would seem that CPVT results from so-called "gain in function mutations" which alter the channel properties and mediate an excessive SR Ca^{2+} release, in particular during diastole where the channel would normally be closed. A further consideration is how do these mutations result in a gain in function that is only revealed in the context of a catecholaminergic stimulus? One possible answer

appears from the analogue of maladaptive hyperphosphorylation in chronic heart failure, which appears to occur secondary to hypercatecholaminergic drive and alters RyR2 function, causing depletion of SR Ca^{2+} via a diastolic leak that can initiate DADs and triggered arrhythmias [90–92]. Extrapolation of this hypothesis to CPVT would suggest that during exercise, increased sympathetic drive would lead to RyR2 phosphorylation, thereby activating and opening the channel. If the threshold for channel activation and Ca^{2+} release as a result of catecholaminergic induced phosphorylation is lowered by these mutations it could indeed bring about a transient set of conditions that allowed DADs and triggered arrhythmias to develop, and crucially explain why under normal circumstances, where there is no excessive sympathetic drive, the mutations have no effect on channel function and remain silent. The additional histological abnormalities of ARVC2 are also intriguing, suggesting the presence of additional patho-physiology such as apoptosis, necrosis, inflammation, and fibrosis. Can these structural defects also be linked directly to RyR2 mutation effects? It is well established that defects in intracellular Ca^{2+} homeostasis can have fatal consequences on cellular function and indeed expression of recombinant RyR2 in stable cell lines with no endogenous RyR2 expression is problematic, leading to cellular toxicity, reduced viability, and a premature cell death, presumably due to deranged intracellular Ca^{2+} homeostasis [93]. Tiso et al. [94] demonstrated that ARVC2 RyR2 mutations resulted in a decreased affinity of RyR2 for FKBP 12.6, causing intracellular Ca^{2+} overload, apoptosis, and cell death, whereas mutations linked to CPVT were associated with an increased affinity for FKBP 12.6 and a lack of structural derangements. This suggests that particular ARVC2 RyR2 mutations are associated with a specific disruption of the intracellular environment, mediated by derangement of RyR2s' interaction with FKBP 12.6, which can produce regional fibrosis, apoptosis or cell death. In support of this it has previously been noted that cellular toxicity mediated by expressed recombinant RyR2 in cell culture systems can be prevented by the co-expression of FKBP 12.6 [93]. The association of ARVC2 and CPVT with RyR2 mutations and exercise-induced arrhythmias could suggest a single underlying genetic disease mechanism whose varying clinical phenotype could simply represent variable expression and penetrance. The above work however suggests that ARVC2 associated mutations, whilst likely sharing the same arrhythmogenic mechanism as CPVT, have additional and distinct functional consequences that lead to structural defects.

In addition to the functional work from Tiso quoted above more studies have recently emerged to suggest how altered RyR2 function causes CPVT. Wehrens et al. [95] also showed a vital link between the mechanism of CPVT and FKBP 12.6. They demonstrated that FKBP 12.6 was dissociated from RyR2 during exercise in association with PKA mediated RyR2 phosphorylation and that this led to increased SR Ca^{2+} release. Furthermore, FKBP 12.6 null mice consistently demonstrated DADs and exercise induced VT. The single channel properties of three CPVT mutations were also assessed by expression of mutant containing recombinant RyR2. Each of these mutants demonstrated reduced affinity for FKBP 12.6 and an increased RyR2 open probability. These defects were only seen after PKA phosphorylation. This work suggests that catecholamine/PKA mediated RyR2 phosphorylation in CPVT causes dissociation of FKBP 12.6 and that this subsequently induces abnormal channel function, allowing RyR2 opening, aberrant SR Ca^{2+} release, DADs, and VT.

George et al. [96], using recombinant human RyR2 containing CPVT mutations co-expressed in a cardiomyocyte cell line, demonstrated equivalent interaction between mutant and wild type human RyR2 and FKBP 12.6 at rest. Following catecholamine stimulation they showed dramatic disruption of the association between RyR2 and FKBP 12.6, however this effect was seen in both wild type and mutant transfected myocytes. Consistent with the clinical phenotype the resting properties of these cells were not altered, however following RyR2 activation by adrenergic stimulation, augmented Ca^{2+} release was seen in only mutant RyR2 transfected myocytes, even though levels of wild type and mutant hyperphosphorylation were the same. In conclusion, these data show that following catecholamine stimulation and RyR2 hyperphosphorylation the physiologically important RyR2:FKBP 12.6 interaction is disrupted and in the presence of RyR2 mutations this causes augmented SR Ca^{2+} release.

Jiang et al. [97] showed that recombinant expressed mouse RyR2 containing a CPVT mutant had a higher open probability than wild type channels at low cytosolic Ca^{2+} where open probability would normally be very low. Cells transfected with CPVT mutant recombinant RyR2 DNA showed this increased basal activity as spontaneous $[Ca^{2+}]_i$ oscillations, resulting from a propensity for spontaneous SR Ca^{2+} release. Again such data are consistent with a gain in function effect. These authors also demonstrated that further amino acid manipulation of this CPVT mutation residue (human equivalent R4497C), specifically the insertion of a more negatively charged residue, further enhanced this increased basal activity, suggesting that alteration of amino acid charge within a specific domain of the channel may be the mechanism underlying functional defects.

Although there are some discrepancies and contradictions between the above outlined functional analysis of CPVT/ARVC2 RyR2 mutations some general themes are emerging. There is now increasing evidence that

CPVT is mediated by gain in function mutations that are associated with altered characteristics of channel gating. These altered mechanisms may involve altered sensitivity to Ca^{2+} activation and a disruption of the channel's normal interaction with FKBP 12.6. Such defects appear to be minimal or of limited functional significance at rest. The link between the arrhythmia onset and catecholaminergic drive appears to be that PKA phosphorylation is required to "unmask" the full consequences of these altered characteristics, thereby initiating spontaneous Ca^{2+} release and DAD generation. A summary of likely mechanisms is outlined in Fig. 5. Further work and clarification will undoubtedly follow and questions still remain. For example in normal EC coupling, the intrinsic regulation of RyR2 gating may on its own have only minor and transient influence on the amount of Ca^{2+} released from the SR, with the Ca^{2+} concentration of the SR (itself a function of influx into the SR through SERCA) being the key determining factor [98,99]. Therefore, in addition to a direct phosphorylation-dependent effect on RyR2 gating, the possibility that catecholaminergic mediated loading of the SR through SERCA is important in producing conditions that favour spontaneous SR Ca^{2+} release and hence DAD dependent triggered arrhythmias during exercise should also be considered.

Empirical therapy with β-blockers and/or implantable defibrillators are the current mainstay of CPVT treatment. There is evidence that new pharmacological strategies directed specifically against the functional RyR2 defects seen in CPVT may soon be available. In particular, RyR2s' interaction with FKBP 12.6 appears to be a promising therapeutic target. The benzodiazepine derivative JTV519 appears to prevent the dissociation of FKBP12.6 from RyR2 and has been shown to reverse RyR2 functional defects seen in heart failure [100–102]. Application of such agents may have implications for the future treatment of CPVT as both conditions appear to share some similarities in their patho-physiology [102,103] and the above functional analysis studies have suggested that stabilization of the RyR2:FKBP12.6 complex may be a potential therapeutic strategy. At present, RyR2 mutation identification in CPVT is primarily used for familial screening and risk stratification. Priori et al. [71] showed that RyR2 positive CPVT cases were associated with a more severe clinical phenotype, higher risk of sudden death, and an earlier age of onset, especially in male patients.

Mutations in calsequestrin are a further cause of catecholaminergic polymorphic ventricular tachycardia

An emerging picture of genetic screening for CPVT mutations is that not all cases can be linked to RyR2. In two recent studies RyR2 mutations were identified in only 14 of 30 (47%) CPVT probands [71] and 6 out of 16 (38%) families [58]. This observation suggests that mutations in other Ca^{2+} signalling components may also be responsible for this clinical phenotype. In contrast to the consistent autosomal dominant inheritance pattern seen in RyR2 CPVT families, an autosomal recessive CPVT inheritance pattern was seen in several Bedouin tribe families in Israel and linkage analysis mapped this disorder to chromosome

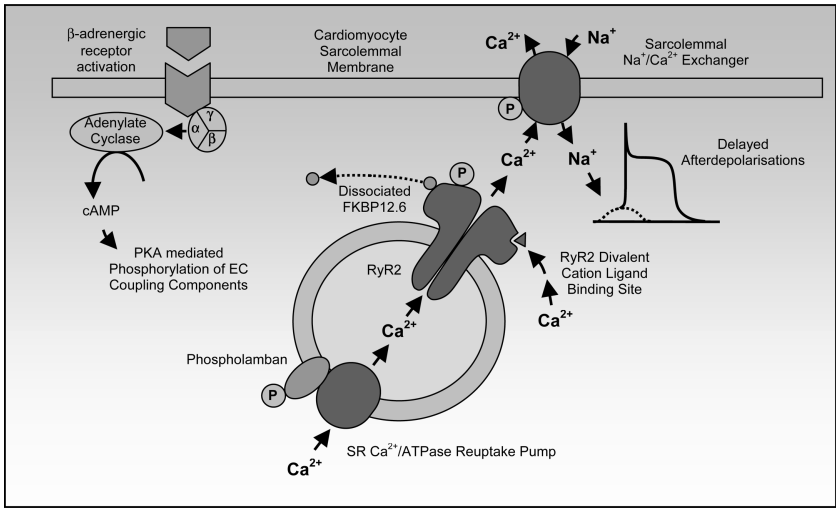

Fig. 5. Proposed mechanism of catecholaminergic ventricular tachycardia resulting from "gain in function" mutations in RyR2. A summary of possible mechanisms leading to arrhythmogenesis in CPVT and ARVC2. β-Adrenergic receptor activation leads to phosphorylation of RyR2, SERCA/phospholamban, and NCX. Increased SR Ca^{2+} loading provides a substrate for arrhythmogenesis and the potential for spontaneous Ca^{2+} release. RyR2 phosphorylation leads to dissociation of FKBP12.6, which in conjunction with increased basal sensitivity to Ca^{2+} activation promotes diastolic opening of RyR2 and Ca^{2+} release. Diastolic SR Ca^{2+} release is removed from the cytosol by forward mode NCX Ca^{2+} efflux, which in turn produces a delayed afterdepolarisation inducing inward Na^{+} current.

1p13–21 [104]. Despite this apparent difference in genetic aetiology, the clinical phenotype was strikingly similar to RyR2 associated CPVT. The disorder was subsequently identified as a missense mutation in a highly conserved region of the calsequestrin 2 (*CASQ2*) gene [105,106], the cardiac isoform of a high capacity Ca^{2+} binding protein whose primary function appears to be the buffering of Ca^{2+} within the SR [2,3,107]. CASQ2 also has a potential role in the regulation of SR Ca^{2+} release as it is one factor that controls local SR luminal [Ca^{2+}], which in turn can modify RyR2 gating. In fact, it has been proposed that *CASQ2* could function as a local luminal Ca^{2+} sensor for RyR2 [108] and it does indeed closely associate with the RyR2 macromolecular complex, where it is linked to RyR2 via the structural proteins junctin and triadin [109,110]. The identified missense mutation in *CASQ2* results in the substitution of a negatively charged amino acid (aspartate) for a positively charged amino acid (histidine), within a highly conserved and negatively charged domain of the protein. Total levels of expressed *CASQ2* were unaffected. The authors of this work suggested that an alteration in charge could disrupt Ca^{2+} binding, although how this led to catecholamine dependent arrhythmogenesis remained unclear. Nevertheless, the identification of this mutation led to recommendations that all CPVT patients should be screened for *CASQ2* mutations as well as RyR2 [106,111]. Subsequent genetic screening of three further RyR2 negative CPVT families (geographically and ethnically distinct from the Bedouin CPVT families) revealed three new nonsense *CASQ2* mutations, two with an autosomal recessive inheritance pattern and one appearing to show autosomal dominant inheritance [112]. Despite having severe CPVT if untreated, autosomal recessive homozygotes reveal that the complete absence of normal functional *CASQ2* is still compatible with life (compared with RyR2 knockout mice, which die in embryonic life [113]), suggesting that SR Ca^{2+} buffering must occur by other means in the absence of *CASQ2*. This work indicates that multiple *CASQ2* mutations underlie a clinically similar phenotype of CPVT and that *CASQ2* mutations may be a more widespread and common basis for CPVT than originally thought, although this latter point is not suggested by all data [58].

Further functional studies have now emerged which offer insights into how *CASQ2* mutations cause CPVT. These studies have also provided useful information on the precise role of *CASQ2* within the SR RyR2 macromolecular complex. Experimental reduction in *CASQ2* expression in isolated rat myocytes (via adenovirus mediated anti sense transduction) has revealed an important role for *CASQ2* in the control of SR Ca^{2+} release [114]. In such cells SR Ca^{2+} storage capacity was reduced and the duration of RyR2 mediated Ca^{2+} release was shortened. Furthermore, there was a premature recovery of RyR2 from a previous luminal [Ca^{2+}] dependent refractory state during diastole, i.e., as Ca^{2+} release occurs and luminal Ca^{2+} falls it eventually reaches a level which promotes RyR2 closure, preventing further SR Ca^{2+} release and heralding the onset of diastole. Subsequently SR Ca^{2+} refilling occurs via SERCA, but in the absence of a competent SR Ca^{2+} buffering system luminal free [Ca^{2+}] rises more rapidly to a level that can mediate RyR2 opening. Thus, RyR2 is ''re-primed'' for opening and the release of further Ca^{2+} at an earlier stage than would normally be the case. The subsequent application of isoproterenol to these cells was shown to cause oscillations in $[Ca^{2+}]_i$, presumably due to the spontaneous discharge of SR Ca^{2+} through a prematurely opening RyR2. As discussed previously this appears to be the cellular basis for DADs and hence suggests a mechanism by which these *CASQ2* mutations could cause arrhythmias. These observations were supported by recent adenovirus mediated expression of a known CPVT *CASQ2* mutation in adult rat myocytes [115]. This mutant was associated with a reduced Ca^{2+} storage capacity of the SR, which could be restored by artificially loading the SR with alternative Ca^{2+} buffers such as citrate. It was again observed that in the absence of *CASQ2* buffering the free luminal SR [Ca^{2+}] necessary to activate RyR2 was reached faster, thus resulting in an enhanced and premature functional restitution of RyR2. Furthermore, rapid pacing and catecholamine stimulation in this model caused abnormal $[Ca^{2+}]_i$ oscillations and DADs. A suggested basis for this observation and thus the link between *CASQ2* mutations and catecholamine dependent arrhythmias is that catecholaminergic induced phosphorylation of phospholamban leads to amplified SERCA reuptake of Ca^{2+} and thus an even faster achievement of functional RyR2 restitution along with an overload of free luminal Ca^{2+} which can no longer be buffered. This combination leads to spontaneous SR Ca^{2+} release during diastole and the potential for DADs. The identification of mutations that disrupt function has highlighted the important role played by *CASQ2* in regulating SR Ca^{2+} release and its potential for causing arrhythmogenesis, as outlined in Fig. 6.

Mutations in ankyrin lead to abnormal myocardial Ca^{2+} signalling and underlie a variant of the long QT syndrome

A specific form of long QT Syndrome identified in a large French family [116] has been designated long QT syndrome type 4. This clinical syndrome is characterised by an autosomal dominant inheritance pattern, prolonged QT interval, sinus node dysfunction (bradycardia), atrial fibrillation, and a high incidence of arrhythmogenic sudden death after exercise and emotional stress. The genetic defect was mapped to chromo-

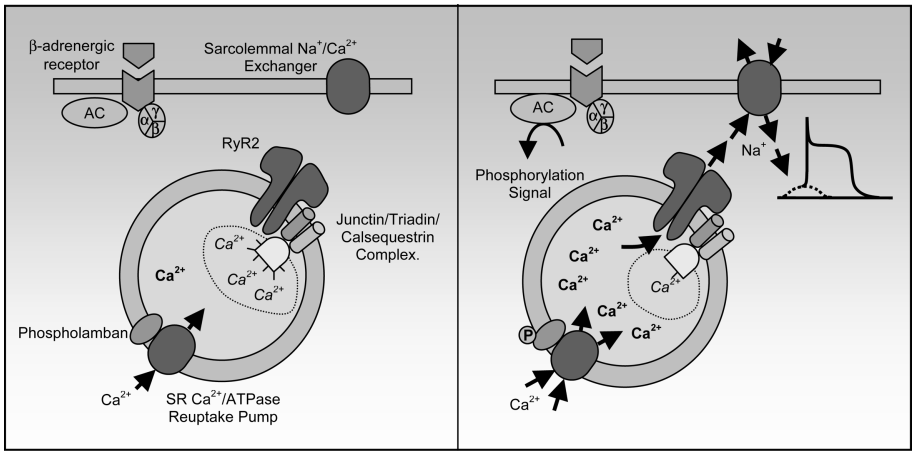

Fig. 6. Luminal control of RyR2 gating: role of calsequestrin 2 (CASQ2) and likely defective mechanism in CPVT arrhythmogenesis. Left-hand window: Normal EC coupling physiology at rest during diastole. Luminal free Ca^{2+} (bold) is low and RyR2 is closed. SERCA mediated reuptake of Ca^{2+} into SR occurs. Calsequestrin buffers Ca^{2+} (italics) such that luminal free Ca^{2+} remains low, preventing opening of RyR2 and spontaneous SR Ca^{2+} release. β-Adrenergic signal transduction pathways are not excessively activated. Right-hand window: EC coupling during diastole in the presence of defective calsequestrin and stress induced adrenergic signalling pathway activation. Phospholamban phosphorylation increases SERCA-mediated reuptake of Ca^{2+} into SR. The abnormally low buffering capacity of mutant calsequestrin is rapidly exceeded allowing luminal free Ca^{2+} to rise, exceeding the threshold for RyR2 opening. Diastolic SR Ca^{2+} release occurs leading to delayed afterdepolarisations.

some 4q25–27 and has recently been identified as a single amino acid substitution in the gene for ankyrin B (glutamate to glycine at amino acid 1425) [117], which appears to disrupt the normal function of this protein. The function of ankyrin proteins is to recognise specific ion transporters and ensure they are correctly targeted and inserted within precise spatial domains of the sarcolemmal/t tubule and SR membrane network [118]. Ankyrin B within the heart appears to be particularly associated with the correct positioning of the sarcolemmal voltage gated Na^+ channel, Na^+/K^+/ATPase, NCX, and the SR inositol 1,4,5-trisphosphate Ca^{2+} release channel. It appears that correct DHPR and RyR2 localisation is not dependent on ankyrin mediated targeting. A mouse model heterozygous for a null mutation in ankyrin B has demonstrated features consistent with human Long QT syndrome type 4 and has helped in the elucidation of the mechanisms whereby ankyrin loss of function mutations causes this syndrome. Analysis of myocytes from this animal model has revealed several defects of intracellular Ca^{2+} handling. These include a significant increase in the duration and peak of the systolic intracellular Ca^{2+} transient, abnormal $[Ca^{2+}]_i$ oscillations, and extrasystoles induced by both EAD and DADs following the application of isoproterenol. Consistent with the human clinical phenotype over 50% of study animals died from PVT after exercise or catecholamine administration. The authors speculate that the primary underlying defect is a loss of function in the sarcolemmal Na^+/K^+/ATPase, causing an accumulation of intracellular Na^+ which gives rise to increased Na^+ extrusion/Ca^{2+} influx through NCX (reverse mode). The range of ion channels and pumps which ankyrin influences may also explain the wide spectrum of electrocardiographic abnormalities seen in long QT type 4 syndrome which, unlike the appearance of stress induced EADs, DADs, and PVT, are not all easily explained by defects in Ca^{2+} handling. For example, it may well be that the loss of ankyrin targeting of voltage gated Na^+ channels may be responsible for the sinus node dysfunction and prolonged QT interval seen in this syndrome [119]. Nevertheless, abnormal Ca^{2+} signalling is clearly a component of the pathophysiology of this condition, in particular with respect to the specific arrhythmias that occur during stress and exercise. This mutation not only represents a completely new mechanism for long QT syndrome, distinct from the direct Na^+ and K^+ channel mutations identified in other long QT syndrome families, but significantly it also represents an inherited arrhythmogenic syndrome whose genetic basis is not secondary to a primary defect of a specific ion channel or pump. This raises the possibility that other targeting proteins, for example proteins which link specific kinases and phosphatase enzymes to ion channels, may also underlie some inherited and acquired arrhythmias and that these may ultimately offer alternative therapeutic targets more attractive than the current complement of anti-arrhythmic drugs which target ion channels and pumps and which are generally of limited efficacy [120].

Cardiac troponin T mutations also cause stress induced ventricular tachycardia due to abnormal myocardial calcium handling

Ca^{2+} binding to the troponin myofilament complex represents an important intracellular buffer for this cation, indeed it represents the largest component of

dynamic Ca^{2+} buffering during the cardiac cycle [3]. Increased troponin Ca^{2+} sensitivity is known to increase myocardial contractility as the binding of Ca^{2+} to troponin is a key initial step in contraction and this property underlies the therapeutic basis of myofilament Ca^{2+} sensitising drugs, such as levosimendan, which were developed as highly targeted positive inotropic agents to improve contractile performance [121,122]. All forms of hypertrophic obstructive cardiomyopathy (HOCM) are associated with an increased risk of cardiac arrhythmias and sudden death and mutations in cardiac troponin are known to be responsible for some familial cases of HOCM [123]. One particular mutation (isoleucine to asparagine at amino acid 79) is notable in that sudden cardiac death is particularly prominent in situations of stress and occurs even when hypertrophy and fibrosis are minimal or even non-existent [124,125]. Such observations have led to the hypothesis that arrhythmogenic sudden cardiac death occurs as a result of a mutation dependent defect in the Ca^{2+} handling characteristics of troponin. Recent work with transgenic mice possessing this mutation has indeed supported this proposal [126]. These animals have increased troponin Ca^{2+} sensitivity and a subsequent increased contractility. The animals were particularly prone to develop ventricular arrhythmias, especially in situations of stress and catecholamine stimulation. A characteristic observation was the remodelling of the cardiac action potential (in particular, a prolonged decay period). It appears that initially more Ca^{2+} is bound to troponin due to its increased Ca^{2+} sensitivity and buffering capacity, hence the intracellular systolic Ca^{2+} transient was initially reduced. Subsequently as myofilament relaxation occurs this additional buffered Ca^{2+} is released from troponin producing a slower decay of the intracellular $[Ca^{2+}]_i$ transient and a relative elevation in diastolic $[Ca^{2+}]_i$. These quantitative differences were exacerbated by the effects of stress and catecholamine stimulation. For example, ventricular ectopy was seen in isolated hearts subjected to isoproterenol perfusion and non-sustained ventricular tachycardia was seen in freely moving animals exposed to an exogenous stress. Precisely how remodelling of the intracellular Ca^{2+} transient and cardiac action potential results in stress induced arrhythmias in this case remains to be established. Possible mechanisms include activation of Ca^{2+}/calmodulin kinase II by the slowed Ca^{2+} transient decay, which has been shown to lead to EAD generation [127] and also activates RyR2, leading to channel opening [128]; alternatively the slow decay in $[Ca^{2+}]_i$ may leave $[Ca^{2+}]_i$ sufficiently high to initiate DADs directly, especially if amplified by catecholamines [37]. Regardless of the precise mechanism of arrhythmia generation this example represents yet another link between defects in Ca^{2+} signalling components and stress/catecholamine induced arrhythmogenesis.

Acquired cardiac arrhythmia syndromes and calcium signalling defects

Mechanical alternans is linked to arrhythmogenic sudden death and may represent a defect of intracellular calcium homeostasis

Mechanical alternans is a regular biphasic beat to beat oscillation in the strength of cardiac muscle contraction whilst at constant heart rate [129]. The phenomenon, when seen as a regular biphasic variation in the surface ECG waveform, has been termed electrical alternans and at the cellular level alternans is observed as a biphasic variation in the peak amplitude of the systolic intracellular Ca^{2+} transient. Alternans is seen in a diverse range of cardiac patho-physiology including heart failure and ischaemia and its occurrence is usually viewed as a marker of poor prognosis and advanced disease. It appears however that this association does not simply represent a reduced cardiac output and impaired contractile function. Rather alternans itself may be linked directly with the onset of both atrial and ventricular arrhythmias, including ventricular fibrillation [130–132].

Although the association between mechanical alternans and a corresponding biphasic Ca^{2+} transient alternans has been known for some time the precise mechanism behind the onset of alternans has remained unclear. It seems logical to suspect that a defect in intracellular Ca^{2+} handling, specifically one that involves the production of the Ca^{2+} transient, is key to its pathophysiology. This hypothesis is supported by various studies that have demonstrated the importance of SR Ca^{2+} release to this phenomenon. Abolishing SR Ca^{2+} release can directly terminate and prevent electro-mechanical alternans [133] and increases in sarcolemmal Ca^{2+} influx and/or SR Ca^{2+} load and release can reverse established alternans [134,135]. An initial hypothesis suggested that there were two compartments of Ca^{2+} recycling within the SR, one for Ca^{2+} reuptake and one for subsequent release, with the lower amplitude transient occurring when the majority of Ca^{2+} used in the preceding transient, despite having been taken back into the SR, had yet to be fed back through to the compartment of the SR where it could be released. This theory was supported by observations that alternans was more likely at increased heart rate, when there was reduced diastolic time for the recycling of Ca^{2+} (for further discussion see [129]).

Further information has been provided by recent work, which has demonstrated that alternans can also occur at a subcellular level, as a result of spatial and temporal desynchronisation of SR Ca^{2+} release within an individual cell. More precisely subcellular alternans reflects local Ca^{2+} transients within two neighbouring regions of the cell alternating out of phase with each

other and producing corresponding alternating local large and small amplitude Ca^{2+} transients [136,137]. Again these observations were made following disruption of SR Ca^{2+} release by inhibition of normal RyR2 function, either with pharmacological blockade, intracellular acidosis or by inhibiting energy production pathways. Furthermore, both the large and small amplitude Ca^{2+} transients observed have been shown to arise specifically from SR Ca^{2+} release [137]. Significantly these subcellular phenomena occur with minimal overall change in global cellular Ca^{2+} influx and efflux and previous studies have shown that I_{Ca} does not alternate significantly during alternans [135]. Such observations all point to a defect at the level of SR Ca^{2+} release and RyR2 function as the primary abnormality of intracellular Ca^{2+} handling that allows alternans to develop. Specifically Diaz et al. [137] suggest that subcellular alternans arises due to an initial activation of RyR2 by DHPR-mediated Ca^{2+} influx which produces the large amplitude Ca^{2+} transient. The second, lower amplitude transient occurs due to a propagating Ca^{2+} wave, resulting from subsequent activation of further RyR, which are themselves either spatially or functionally uncoupled from DHPR mediated CICR, and hence not activated at the time of the first Ca^{2+} transient. The second smaller amplitude Ca^{2+} transient then occurs but the majority of DHPR linked RyR2 channels that gave rise to the first transient are at this stage refractory to further opening, hence there is dispersion of RyR2 (and hence CICR) refractoriness between neighbouring regions of the cell.

Diaz et al. [138] have also recently shown that alternans induced by an alternative method (repeated small depolarising pulses) produces alternans that is homogeneous throughout the cell, as opposed to the inhomogeneous subcellular alternans described above. They also directly demonstrated that alternans in this model was dependent on alternating SR Ca^{2+} content. SR Ca^{2+} content was large before a large $[Ca^{2+}]_i$ transient and small before the subsequent smaller transient. During alternans both the amplitude of the Ca^{2+} transient and Ca^{2+} efflux via NCX displayed a steeper dependence on SR Ca^{2+} content. In contrast to the studies described in the preceding paragraph there was no direct evidence that RyR function was abnormal or the primary abnormality underlying alternans. Nevertheless, it does reinforce the suggestion that SR Ca^{2+} content and the regulation of Ca^{2+} release from this intracellular compartment are key elements of alternans [139].

It remains to be seen precisely how such subcellular and cellular alternans become amplified to induce a more widespread and co-ordinated alternans phenomenon throughout the heart. It is also important to ask how alternans at the subcellular and cellular level, can be linked to the onset of life-threatening arrhythmias. One possibility is that a delayed propagating Ca^{2+} wave could activate an inward Na^+ current through NCX, which is an afterdepolarising phenomenon. Furthermore, as alternans has been observed in atrial as well as ventricular myocytes, abnormalities associated with this phenomenon may represent an important and novel mechanism for the initiation of atrial arrhythmias [136] as well as more life-threatening ventricular arrhythmias.

Calcium dependent arrhythmogenesis is increasingly implicated as a cause of sudden death in heart failure

Sudden arrhythmogenic death in heart failure causes a significant proportion of the total mortality associated with this syndrome [140,141]. A further observation and paradox is that sudden death, presumably arrhythmogenic, is more common as a cause of death in New York Heart Association Class I and II heart failure than it is in class III and IV, (50–60% vs. 20–30% of deaths, respectively). In more severe heart failure death from progressive pump failure seems to predominate [142]. Furthermore, although sudden death primarily equates to arrhythmias with a ventricular origin it should also be remembered that atrial arrhythmias, in particular atrial fibrillation, are also much more common in heart failure and are responsible for considerable morbidity [143,144].

Heart failure arrhythmia pathogenesis is likely to be more heterogeneous than the well-defined and emerging mechanisms of single gene defects relating to inherited sudden cardiac death syndromes. Multiple pathological processes occur within the heart failure syndrome [145] and the variety of underlying aetiologies, animal models, and experimental techniques used for analysis can result in a confusing and conflicting picture of the defects in intracellular processes, ionic currents, and gene/protein expression which contribute to heart failure arrhythmogenesis. This makes interpretation and generalisations difficult. Undoubtedly a significant proportion of arrhythmias are mediated by defects that do not centre on Ca^{2+} handling abnormalities. For example, heart failure is associated with prolongation of action potential duration and this appears to be secondary to a reduction in Ca^{2+} independent repolarising outward K^+ currents [146,147]. Furthermore in ischaemic heart failure re-entry arrhythmia mechanisms appear to predominate. Nevertheless, triggered arrhythmias (and hence Ca^{2+} dependent arrhythmia mechanisms) are also involved in heart failure arrhythmogenesis and may be responsible for up to 50% of ischaemic and 100% of non-ischaemic cardiomyopathy associated ventricular arrhythmias (for review see [25]).

Heart failure is a well-described hypercatecholaminergic state and indeed levels of circulating catecholamines are closely correlated with severity and prognosis in heart failure [148,149]. Possibly as a response to this there is a downregulation of β-adrenergic receptors in the heart, however, significantly it appears

that with the exception of severe end stage heart failure residual β-adrenergic responsiveness is retained [33] and the function of various components of cardiac EC coupling, such as RyR [150], DHPR [151], and NCX [152], is augmented by β-adrenergic dependent increases in phosphorylation. Specific defects of EC Coupling in heart failure are summarised in Fig. 7. These include upregulation of NCX, downregulation of SERCA, increased SR Ca^{2+} leak through RyR2, and a reduced SR Ca^{2+} load and depressed $[Ca^{2+}]_i$ transient (for review see [2,153,154]). Although these defects may have a primary role in reducing contractile performance (despite PKA/phosphorylation-mediated augmentation of function), they may also have a key role in the development of Ca^{2+}-induced arrhythmias in heart failure. This is despite the paradox that SR Ca^{2+} overload has until now been considered as a central mechanism underlying Ca^{2+}-mediated triggered arrhythmias. A key observation in most models of heart failure is an upregulation of the NCX [155–157]. This, in conjunction with its hyperphosphorylated state and augmented function, may mean that NCX competes more effectively with SERCA (which is itself expressed at a reduced level in heart failure) for diastolic Ca^{2+}, such that less SR Ca^{2+} reuptake occurs. As a result SR load is reduced and the systolic Ca^{2+} transient and contractile function fall. It now appears that the increased NCX density and function is key to initiating arrhythmogenic DADs in heart failure. Pogwizd et al. [33] showed that the residual β-adrenergic receptor responsiveness that persisted in all but extreme heart failure was sufficient to load the SR with enough Ca^{2+} to reach the threshold needed to initiate spontaneous Ca^{2+} release. As NCX is upregulated in heart failure, for every given SR Ca^{2+} release there is a greater efflux of Ca^{2+} via NCX and an inward arrhythmogenic Na^+ current causing DADs. In more severe heart failure worsening contractile function continues and β-adrenergic responses are finally lost. In this situation SR Ca^{2+} loading cannot be driven to the required threshold for spontaneous Ca^{2+} release and hence DADs and arrhythmias are not seen, explaining the paradox that arrhythmogenic death is less common in severe heart failure.

β-Blockers appear to have a specific effect in reducing sudden death in heart failure, in addition to their observed effects in reducing all cause mortality [158,159]. This effect may be partially explained by a reduction in heart rate, which itself is protective against DAD generation [36]. However, even when data are corrected for this effect it appears that β-blockers exert a further beneficial action on survival via other mechanisms [160]. As β-adrenergic signalling pathways hyperphosphorylate EC coupling components one possible additional effect may be through a reduction in this phenomenon. In a series of experiments involving various models of heart failure it has been demonstrated that FKBP 12.6 becomes dissociated from RyR2. This observation has been proposed as the specific cause for an arrhythmogenic diastolic Ca^{2+} leak that not only precipitates DADs but also lowers SR Ca^{2+} content and therefore

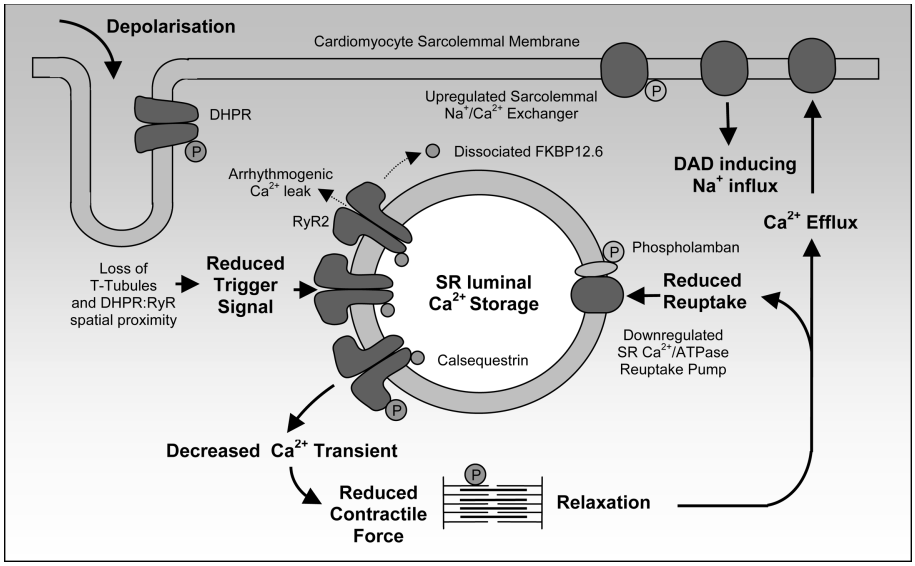

Fig. 7. Defective cardiac excitation–contraction coupling in heart failure and possible arrhythmogenic mechanisms. Identified structural and functional abnormalities in heart failure which impact on cardiomyocyte excitation–contraction coupling (contrast with Fig. 1). Loss of T tubule density removes close spatial proximity between RyR2 and DHPR, which is needed for Ca^{2+} induced Ca^{2+} release. Downregulated SERCA and upregulated NCX lowers intracellular Ca^{2+} available for Ca^{2+} transient generation. As a result subsequent contractile force is impaired. Despite this residual SR Ca^{2+} loading is sufficient to reach spontaneous diastolic Ca^{2+} release threshold. This release is facilitated by RyR2 hyperphosphorylation and dissociation of FKBP 12.6, which also promotes RyR2 opening and results in loss of coupled RyR2 gating. Upregulated and functionally augmented NCX operating in forward mode results in Ca^{2+} efflux and an arrhythmogenic depolarising Na^+ influx.

impacts on the magnitude of systolic Ca^{2+} transients and contraction [90–92,103,150,161,162]. It has also been demonstrated that these effects occurred within the context of PKA hyperphosphorylation of RyR2. This possible arrhythmogenic mechanism has already been strongly implicated in CPVT as outlined in Section 2 of this review. Furthermore, it has been shown that β-blockers restore these structural and functional defects in RyR2, in association with an improvement of cardiac function [163–165]. Also new strategies to reverse FKBP 12.6 dissociation with novel pharmacological agents do appear to improve RyR2 function in heart failure [100–102]. Recent work has also suggested that PKA-mediated hyperphosphorylation of RyR2, FKBP 12.6 dissociation, and defective RyR2 function in heart failure could also be directly prevented by treatment with the angiotensin II antagonist valsartan [166]. Furthermore, this agent also resulted in restoration of normal SERCA expression and SR Ca^{2+} uptake within the failing heart. By correcting these various Ca^{2+} handling abnormalities, which may contribute to arrhythmia pathogenesis as well as contractile failure, such drugs may have a direct anti-arrhythmic action due to beneficial effects on EC coupling, ventricular remodelling, and contractile dysfunction. It remains to be established whether the beneficial effects of valsartan on EC coupling are mediated through a direct effect of the drug on the heart or reduced angiotensin II mediated activation of catecholaminergic sympathetic nerves.

The various studies outlined above which suggest a role for RyR2 hyperphosphorylation-mediated dysfunction in heart failure have been challenged by other experimental work that has either failed to reproduce key findings or has produced contradictory results [167–169]. As a result the RyR2 hyperphosphorylation hypothesis remains an area of intense debate [153,170]. Nevertheless, there is no doubt that this hypothesis offers a reproducible and biologically plausible mechanism for the generation of DAD-induced triggered arrhythmias in heart failure. What is certainly clear to date is that Ca^{2+} mediated mechanisms appear to cause a significant proportion of heart failure associated arrhythmias and that this represents an as yet untapped aspect of possible anti-arrhythmic drug development which will undoubtedly expand as the precise defects in myocardial Ca^{2+} handling are revealed [171]. Unfortunately pharmacological targeting of specific components involved in DAD generation, such as ion fluxes through RyR and NCX, may, in turn be limited by detrimental effects on contractile function.

Defective cellular calcium handling appears to mediate atrial remodelling in chronic atrial fibrillation

Atrial fibrillation (AF) is the most common cardiac arrhythmia in clinical practice. It is responsible for much morbidity, an increased risk of thrombo-embolic stroke and its treatment is often problematic. The patho-physiology of atrial fibrillation at a macroscopic level has undergone re-evaluation over the past 5–10 years and has recently been reviewed [172]. The cellular electrophysiology of atrial fibrillation has also been the subject of much research; however, AF is an especially complex subject at the cellular and molecular levels. A general overview may be found elsewhere [173]. For example, factors involved in the initiation of AF may be quite different from those which are important in its propagation and maintenance. Furthermore, chronic AF is characterised by the process of "electrical remodelling" [174], whereby AF itself induces changes in atrial cellular electrophysiology which promote the ongoing maintenance of the arrhythmia or its early re-initiation should sinus rhythm be restored.

The role of defective atrial myocyte Ca^{2+} handling in the patho-physiology of AF has been examined in various studies and although identified defects cannot explain the full spectrum of abnormalities in the cellular electrophysiology of AF there is evidence that they play a significant role. In particular, it appears that atrial myocyte Ca^{2+} overload is an important factor in the mechanism of early electrical and structural atrial remodelling. The onset of AF (and indeed all atrial tachycardias) is characterised by a significant increase in the rate of atrial depolarisation, which is associated with an increase in cellular Ca^{2+} entry via the I_{Ca} current and a subsequent intracellular Ca^{2+} overload [175–177]. Initially cells show features of Ca^{2+} overload including organelle swelling and cytoskeleton damage, and it is likely that Ca^{2+} activated proteases are involved in this disruption and structural remodelling [178]. Compensatory mechanisms to overcome Ca^{2+} overload and protect cellular integrity are subsequently induced and these lead to electrical remodelling. The key component of this appears to be an inactivation of the DHPR-mediated Ca^{2+} influx and a reduced DHPR expression. Consequently I_{Ca} influx is reduced and this is manifest as a shortening of the cardiac action potential phase 2 plateau. However, shortening of the action potential duration leads to a corresponding shortening of the cellular refractory period and this subsequently promotes the further induction and maintenance of AF by allowing multiple circuit re-entry mechanisms [179]. DHPR blockers can prevent short-term remodelling [176,180–182] however they are not effective at preventing long-term remodelling from occurring [183,184], indicating the above mechanism is by no means the only intracellular event involved in this process. For example, in AF associated with heart failure, as opposed to tachycardia pacing, action potential duration is not significantly reduced and reductions in I_{Ca} are less severe [185]. Also within the context of heart failure, NCX expression is increased promoting an

afterdepolarising inward current which can initiate atrial extrasystoles and induce AF [186]. More recently re-initiation of AF after its previous termination in a paced model was shown to result from EAD induced triggered activity extrasystoles. Furthermore, these EADs were prevented by ryanodine, suggesting that tachycardia resulted in cellular and SR Ca^{2+} overload and that after its termination the high SR Ca^{2+} load is released and this re-initiates AF [187].

Several studies have looked specifically at the expression levels of various Ca^{2+} handling proteins in human atrial tissue following AF. Unfortunately there is some inconsistency in the results, making generalised interpretation difficult. For example, Ohkusa et al. [188] found that there was a significant reduction in the maximum number of functional RyR2 in atrial tissue taken from mitral valve disease patients with chronic atrial fibrillation (assessed by [^{3}H]ryanodine binding studies). They also demonstrated a reduction in the mRNA levels for both RyR2 and SERCA. Lai et al. [189] also looked at the levels of expression of several Ca^{2+} handling proteins in atrial tissue from chronic atrial fibrillation patients. They found no significant changes in the level of RyR2, calsequestrin, and phospholamban expression whilst demonstrating that DHPR and SERCA expression was reduced. Rundle et al. [190] also reported a reduction in DHPR and SERCA expression but found no change in RyR2, NCX, and phospholamban expression. Although some of these observations, such as reduced DHPR expression, are consistent with models of AF patho-physiology as outlined above, the significance of other observations remains to be determined.

The myocardial inositol 1,4,5-trisphosphate receptor: an emerging mediator of cardiac arrhythmogenesis?

Inositol 1,4,5-trisphosphate (IP_3) receptors are a further mediator of SR Ca^{2+} release in addition to RyR2. They appear to be the primary mediator of SR Ca^{2+} release in non-excitable cells however their expression level in the heart, and in particular, in the ventricles is several orders of magnitude lower than that of RyR2 [191]. The role of IP_3 receptors in EC coupling and cardiac contractile function is controversial and poorly defined compared with RyR2 and in general their role, if any, in Ca^{2+}-mediated arrhythmogenesis within the ventricles is unknown. Although IP_3 receptor-mediated Ca^{2+} release may be involved in ventricular arrhythmias, for example in ischaemic reperfusion [192], the higher levels of IP_3 receptor expression within atrial tissue [193,194], have led to the suggestion of a more likely role for IP_3 receptors in precipitating atrial arrhythmias [194,195]. Furthermore, there is evidence that upregulation of the IP_3 receptor occurs in atria displaying arrhythmias, including atrial fibrillation [196]. Again it is unclear whether this has a causative role in AF initiation, maintenance, and/or remodelling. It has been shown that endothelin-1, which is a potent arrhythmogenic agent and an activator of IP_3 receptor-mediated Ca^{2+} release, increases both intracellular Ca^{2+} levels and systolic Ca^{2+} transients within atrial myocytes and results in cellular alternans and spontaneous SR Ca^{2+} release. All of these effects were prevented by pharmacological blockade of the IP_3 receptor and were observed in the presence of RyR2 blockade, suggesting SR Ca^{2+} release through RyR2 was not involved [195]. Furthermore, Mackenzie et al. [194] demonstrated that IP_3 receptor-mediated Ca^{2+} release was vital in the generation of endothelin-1-mediated DADs and arrhythmogenic triggered activity in rat atrial myocytes. Such work suggests that the atrial IP_3 receptor may be a promising new target for understanding and treating atrial arrhythmias.

Myocardial calcium signalling pathway defects and ischemia/reperfusion arrhythmias

Arrhythmias occurring in association with acute myocardial ischemia and infarction have not generally been strongly associated with defective Ca^{2+} signalling as a primary abnormality in their pathogenesis. Ischemia is associated with changes in the cardiac action potential that favour the development of re-entrant arrhythmias [197,198]. Regional and transmural heterogeneity of these defects within the myocardium also predisposes to re-entrant arrhythmias. In contrast, arrhythmias occurring during reperfusion injury may be more strongly associated with defects in cellular Ca^{2+} handling. During ischemia decreased intracellular pH leads to activation of the sarcolemmal Na^+/H^+ exchanger and an inward Na^+ influx [199,200]. Upon reperfusion this defect is partially overcome by reverse mode activation of NCX and a resulting inward Ca^{2+} flux [201]. Using a specific inhibitor of reverse mode NCX Elias et al. [202] demonstrated a complete prevention of reperfusion-mediated arrhythmias, including both ventricular tachycardia and atrial fibrillation, when rabbit hearts were pre-treated with this agent. They argue that this suggests a crucial role for reverse mode NCX function in the pathogenesis of such arrhythmias. Woodcock et al. have previously demonstrated that in reperfusion, norepinephrine release, acting on cardiac α_1-adrenoceptors, causes a rapid generation of IP_3. This acute rise in IP_3 was associated with the onset of arrhythmias that were terminated by inhibitors of IP_3 generation (for review see [192]). It is logical to suggest that IP_3 generation could lead to activation of SR IP_3 receptors and an arrhythmogenic release of SR Ca^{2+}, however it has recently been shown that this IP_3 rise and arrhythmia onset also require reverse mode activation of NCX [203] adding further strength to the possibility of using reverse mode NCX activity as an anti-arrhythmic therapeutic target during reperfusion.

del Monte et al. [204] have also recently shown that overexpression of SERCA2a has an anti-arrhythmic action in the context of cellular Ca^{2+} overload during ischemia and reperfusion. Overexpression of SERCA2a has already been proposed as a mechanism of targeted inotropic therapy that delivers increased ventricular performance without an associated increased mortality [205,206]. A theoretical problem with this strategy is that SERCA2a overexpression could lead to an increase in SR Ca^{2+} load and a subsequent pro-arrhythmogenic spontaneous SR Ca^{2+} release. Such a phenomenon would be considered even more likely during ischemia and reperfusion where cellular Ca^{2+} overload is known to occur. In fact, del Monte et al. demonstrated that rather than increasing arrhythmia risk in ischemia and reperfusion, overexpression of SERCA2a significantly reduced the risk of ventricular arrhythmias. A possible explanation for these observations is that SR Ca^{2+} uptake is protective against arrhythmogenic afterdepolarisations, which are induced by the increased cellular Ca^{2+} influx in ischemia/reperfusion. This anti arrhythmic benefit must outweigh the pro arrhythmic risk of a subsequent increased spontaneous SR Ca^{2+} release.

Future developments and conclusions

The past decade has seen great advances in our understanding of the role played by Ca^{2+} signalling mechanisms within the heart, both during normal physiology and in the context of disease patho-physiology. Furthermore, specific functional defects such as those seen in single gene defect cardiac disease states offer insights into the normal physiological function of the heart. The inherited arrhythmia syndromes that disrupt normal cardiac Ca^{2+} signalling are illustrative examples of this point.

Further work is still required however to establish the significance of disruptions in Ca^{2+} handling in causing arrhythmias. We must also be aware of the difficulties associated with a wealth of conflicting data obtained from studies of both human tissue and various animal models, in particular, extrapolation of findings in the latter to human patho-physiology must be made with extreme caution. Improved experimental analysis will be needed to establish important parameters such as SR Ca^{2+} load and the role of Ca^{2+} signalling within defined spatial microdomains of the cell. In addition, observations in highly artificial laboratory environments need to be confirmed in vivo. It also remains to be established whether the Ca^{2+} handling defects outlined above impact on other aspects of Ca^{2+} signalling which are not unique to excitable cells, such as the regulation of gene transcription factors.

Ca^{2+} signalling defects undoubtedly have a role in human arrhythmia generation. Sudden cardiac death in children and young adults, secondary to inherited Ca^{2+} signalling defects, is fortunately rare, yet when they occur they can be utterly devastating in their outcome. Furthermore, Ca^{2+}-mediated arrhythmogenesis in heart failure and atrial fibrillation impacts on society due to the prevalence of these disease states in the population. The widely publicised beneficial actions of β-blockers appear to result, at least in part, from a reversal of Ca^{2+}-mediated signalling defects. This offers an insight into the potential therapeutic benefit that could result from a new era of targeted strategies directed against the Ca^{2+} signalling defects outlined in this article.

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