Review

The cardiac ryanodine receptor (calcium release channel): Emerging role in heart failure and arrhythmia pathogenesis

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Received 6 May 2002; accepted 2 July 2002

Abstract

The cardiac sarcoplasmic reticulum calcium release channel, commonly referred to as the ryanodine receptor, is a key component in cardiac excitation–contraction coupling, where it is responsible for the release of calcium from the sarcoplasmic reticulum. As our knowledge of the ryanodine receptor has advanced an appreciation that this key E–C coupling component may have a role in the pathogenesis of human cardiac disease has emerged. Heart failure and arrhythmia generation are both pathophysiological states that can result from deranged excitation–contraction coupling. Evidence is now emerging that hyperphosphorylation of the cardiac ryanodine receptor is an important event in chronic heart failure, contributing to impaired contraction and the generation of triggered ventricular arrhythmias. Furthermore the therapeutic benefits of β blockers in heart failure appear to be partly explained through a reversal of this phenomenon. Two rare inherited arrhythmogenic conditions, which can cause sudden death in children, have also been shown to result from mutations in the cardiac ryanodine receptor. These conditions, catecholaminergic polymorphic ventricular tachycardia and arrhythmogenic right ventricular cardiomyopathy (subtype 2), further implicate the ryanodine receptor as a potentially arrhythmogenic substrate and suggest that this channel may offer a new therapeutic target in the treatment of both cardiac arrhythmias and heart failure. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Arrhythmia (mechanisms); Ca-channel; Contractile function; e–c coupling; Heart failure; SR (function)

1. The cardiac ryanodine receptor and excitation–contraction coupling

It is widely accepted that Ca\(^{2+}\) plays a key role in the heart with regards to excitation–contraction (E–C) coupling and crucial to this is the Ca\(^{2+}\) release channel, commonly referred to as the ryanodine receptor (RyR) [1–3]. RyR is a large conductance cation-selective ion channel found within the sarcoplasmic reticulum (SR) membrane where it is responsible for the release of Ca\(^{2+}\) from this intracellular storage organelle [2]. Although much is known about its structure and functional properties, only recently has attention turned to its potential role in cardiac disease. It is not surprising that an ion channel with a key role in the intracellular pathways of Ca\(^{2+}\) movement should be implicated in the pathogenesis of heart failure and cardiac arrhythmias, as Ca\(^{2+}\) is required to maintain the inotropic and electrical properties of the heart. In this article we will review recent work that has provided an insight into how altered RyR function may contribute to the pathogenesis of heart failure and cardiac arrhythmias.

1.1. RyR: structure and nomenclature

Three mammalian isoforms of RyR have been identified and cloned [2]. Isoform 1 (RyR1) is found within skeletal muscle, isoform 2 (RyR2) is the predominant form in cardiac muscle and isoform 3 (RyR3) is expressed at low levels in various tissues. The channel is a high molecular weight homotetramer that traverses the SR membrane. Its function is to provide a pathway for the release of stored Ca\(^{2+}\) from the SR lumen into the cytosol [4] where it can...
then be utilised in various Ca\(^{2+}\) signalling processes such as muscle contraction. Each monomer contains approximately 5000 amino acids and has a molecular weight of 565 kDa [5]. The majority of RyR2 channels in cardiomyocytes are distributed in areas of the SR membrane that lie in proximity to the T-tubule invaginations of the sarcosomal membrane [6]. Here they are closely associated with the L-type voltage-dependent Ca\(^{2+}\) channel and this spatial association of the two channels is key to the signal amplification process that underlies cardiac E–C coupling (Fig. 1).

1.2. Cardiac excitation–contraction coupling

Cardiac E–C coupling is the process where by excitation of the cardiomyocyte, in the form of membrane depolarisation, is used as a stimulus for the movement of Ca\(^{2+}\) around the cell to bring about contraction. During depolarisation Ca\(^{2+}\) enters the cell through L-type voltage-dependent channels as an inward Ca\(^{2+}\) current (cardiac action potential phase 2). The spatial organisation of RyR, adjacent to L-type voltage-dependent channels is vital in that this inward Ca\(^{2+}\) current activates adjacent RyRs to release further Ca\(^{2+}\) from the SR where it is stored, buffered by calsequestrin. This so-called ‘calcium induced calcium release’ [7] raises cytosolic Ca\(^{2+}\) to the level required for contraction. Ca\(^{2+}\) is released in a synchronised manner from individual RyR2 populations and this phenomenon is directly visible using confocal microscopy and has been termed a Ca\(^{2+}\) spark [8]. Depolarisation leads to a co-ordinated release from multiple spark sites, producing a global Ca\(^{2+}\) transient that allows sufficient Ca\(^{2+}\) to bind with troponin C, initiating contraction.

For relaxation to occur Ca\(^{2+}\) must dissociate from troponin C and this requires a reduction in cytosolic Ca\(^{2+}\). The sarcosomal Na\(^{+}\)/Ca\(^{2+}\) exchanger transports Ca\(^{2+}\) out of the cell and the SR Ca\(^{2+}\)/ATPase pump actively replenishes SR Ca\(^{2+}\) stores [9]. Furthermore SR Ca\(^{2+}\) release must be stopped and RyR2 closed. The mechanisms that bring about this are still debated and a combination of factors including stochastic attrition, RyR2 adaptation [10], RyR2 inactivation [11] and SR Ca\(^{2+}\) depletion may all contribute, possibly in a synergistic manner [10]. To maintain cellular steady state conditions the Ca\(^{2+}\) influx into the cell must be balanced by efflux through the sarcosomal Na\(^{+}\)/Ca\(^{2+}\) exchanger and at the level of the SR the amount of Ca\(^{2+}\) release through RyR2 must be balanced by SR Ca\(^{2+}\)/ATPase pump re-uptake.

Fig. 1. The movement of Ca\(^{2+}\) around the cell is shown (arrows), along with the key ion channels and pumps which orchestrate this process. Note also the close association of SR RyR2s with sarcosomal L-type voltage dependent channels, allowing localised Ca\(^{2+}\) induced Ca\(^{2+}\) release events to take place.
1.3. Catecholaminergic modulation of E–C coupling

One important influence on myocardial Ca$^{2+}$ cycling is β$_i$ adrenergic receptor stimulation mediated by sympathetic nerves and circulating catecholamines. Activation of these receptors can alter the properties of E–C coupling components through signal amplification cascades that results in phosphorylation of either the ion channels and pumps themselves, or the regulatory proteins attached to them (Fig. 2). It has been appreciated for several years that the amplitude of the Ca$^{2+}$ transient generated by release of SR Ca$^{2+}$ into the cytosol determines the contractile force (inotropy) of the heart. Catecholamine mediated phosphorylation will increase (1) the inward Ca$^{2+}$ current, (2) SR Ca$^{2+}$ loading and (3) its release into the cytosol, thereby enhancing contractile force. Although these pathways are a normal physiological process along the theme of the ‘fight and flight response’, catecholaminergic input may also modify the function of RyR2 and other E–C coupling components, resulting in changes, which far from being helpful may actually contribute to the pathogenesis of cardiac disease.

2. Cardiac excitation-contraction coupling in heart failure

2.1. Calcium cycling in heart failure

Any detrimental alteration to E–C coupling is a potential factor in the pathogenesis of chronic heart failure. Individual components have been studied in detail and one consistent finding is that SR Ca$^{2+}$ load is reduced in heart failure [12,13]. SR Ca$^{2+}$ load is a key factor in determining the amount of SR Ca$^{2+}$ release each inward Ca$^{2+}$ current generates. This is not just a reflection of the available Ca$^{2+}$ which can be released. In addition SR luminal Ca$^{2+}$ is believed to be an important ligand that regulates RyR2 gating [14,15]. SR luminal Ca$^{2+}$, binding to RyR2, will increase the gain of E–C coupling by allowing a greater release of Ca$^{2+}$ for any given trigger, thus amplifying myocyte contraction. As SR [Ca$^{2+}$] rises may also modify the function of RyR2 and other E–C coupling components, resulting in changes, which far from being helpful may actually contribute to the pathogenesis of cardiac disease.

Fig. 2. Activation of β$_i$ adrenergic receptors by circulating catecholamines or sympathetic nerves results in the activation of various signal amplification cascades and the phosphorylation of target proteins (G protein cascade and protein kinase A activation illustrated here as a primary example). Augmented sequele include (1) Ca$^{2+}$ influx into the cell, (2) SR Ca$^{2+}$ release, (3) SR Ca$^{2+}$ uptake and (4) dissociation of Ca$^{2+}$ from myofilament proteins. Although SR Ca$^{2+}$ uptake has an initial lusitropic effect, accelerating relaxation, it also enhances the next contraction cycle by increasing the SR Ca$^{2+}$ content available for subsequent release.
heart failure not only reduces the total Ca$^{2+}$ load available for release but the reduction of its direct influence on RyR2 gating also reduces the channel’s open probability, further impairing Ca$^{2+}$ release. Thus for every cardiomyocyte depolarisation in heart failure where SR [Ca$^{2+}$] load is reduced, SR Ca$^{2+}$ release is curtailed further and reduced contractile force is generated.

Two states that appear to bring about reduced SR Ca$^{2+}$ loading in heart failure are an up-regulation of the sarcolemmal Na$^+$/Ca$^{2+}$ exchanger [18] and a reduced activity of the SR Ca$^{2+}$/ATPase pump [19]. Both these states could impair the restoration of SR Ca$^{2+}$ stores during diastole, hindering the cells ability to make available the required Ca$^{2+}$ for the next wave of depolarisation. This is believed to be a primary factor in the defective E–C coupling process in heart failure [13].

2.2. Does RyR2 dysfunction contribute to deranged E–C coupling in heart failure?

It is logical to ask if RyR2 contributes to reduced SR Ca$^{2+}$ release in heart failure, or whether this is simply a function of impaired SR loading. Biological plausibility for RyR2 dysfunction being a potential causative mechanism in the development of heart failure is obtained from studies using the administration of ryanodine, which binds to RyR and dramatically modifies channel function, inducing a state of reduced open probability and modified Ca$^{2+}$ release. The administration of ryanodine would thus be expected to sufficiently compromise normal in vivo RyR2 function to the extent that its administration gives an indication as to the pathological consequences of RyR2 dysfunction and reduced SR Ca$^{2+}$ release. Ryanodine administration does indeed depress both systolic and diastolic cardiac function [20,21] and this observation is in keeping with the hypothesis that a decline in either the number or function of RyR channels would contribute to the pathogenesis of heart failure.

An alternative view is that if SR Ca$^{2+}$ load was reduced in heart failure any compensatory mechanism affecting RyR function would increase the channel’s open probability in order to maximise SR Ca$^{2+}$ release. Evidence for this is now emerging. Using cardiomyocytes isolated from a canine heart failure model, Yamamota et al. [22] showed that, as expected, SR Ca$^{2+}$ uptake and release was reduced, resulting in a reduced intracellular Ca$^{2+}$ transient and reduced contraction. They also demonstrated however, that the fraction of total SR Ca$^{2+}$ released at any given SR [Ca$^{2+}$] was higher in the heart failure model compared to controls. These observations imply that compensatory modulation of RyR function exists in heart failure to overcome reduced SR Ca$^{2+}$ release by increasing the channel’s open probability.

Many investigators have attempted to gain an insight into the altered dynamics of heart failure E–C coupling by measuring changes in the number of ion channels present or in the levels of relevant gene expression. Unfortunately the wide range of experimental models and protocols employed in such studies have resulted in conflicting results which make the development of firm conclusions difficult. As an illustration of this ryanodine binding studies, which provide both quantitative and qualitative information about the number of RyR2 channels present within the myocardium, have shown the number of functionally active channels (which can assume an open configuration for ryanodine to bind with) to be unchanged [23], increased [24] and decreased [25] in a variety of animal models. Left ventricular hypertrophy and cardiomyopathy models of heart failure in particular have produced conflicting results concerning the numbers of RyR channels present and the relevant levels of RyR2 gene expression. Several studies suggest RyR numbers are initially preserved but gradually fall as heart failure advances [26], where as others suggest relative differences between different channels are of more importance than simply considering the numbers of RyR channels in isolation. Milnes and MacLeod, using a LVH model showed that the number of RyR channels relative to L type voltage-dependent channels was reduced causing a functional uncoupling of the two channels and a defective release of Ca$^{2+}$ from the SR as a result [27]. Gomez et al. [28] also identified a deficit in the normal functional coupling of these two channels, although in their experiments the numbers of channels relative to each other remained unchanged. They demonstrated that the ability of the inward Ca$^{2+}$ transient to trigger SR Ca$^{2+}$ release was curtailed in models of both heart failure and left ventricular hypertrophy. This functional uncoupling of the two channels was reversed by $\beta_1$ adrenergic stimulation in left ventricular hypertrophy but not in heart failure, suggesting that progression from hypertrophy to failure was marked by a loss of this compensatory mechanism. A more in-depth discussion of the conflicting studies relating to levels of gene expression and receptor numbers is available [29].

2.3. RyR2 function in human heart failure

The practical limitations of studying RyR2 function in human heart failure are considerable. What data is available supports the view that RyR2 function is unchanged and/or unimportant in heart failure E–C coupling. Holmberg and Williams [30], using end-stage failing hearts removed at transplantation, were able to carry out a functional assessment of extracted RyR2 channels, reconstituted into artificial lipid bilayers. They could find no significant difference in the properties of such channels, compared with channels from normal sheep hearts, supporting the view that RyR2 function is preserved even in end-stage human heart failure. Furthermore Lindner et al. [13] showed that the depressed systolic Ca$^{2+}$ transients observed in myocytes from failing human hearts could be explained solely through depressed SR Ca$^{2+}$/ATPase
pump activity, with no additional RyR2 dysfunction needed to produce the observed depressed Ca\(^{2+}\) transient. In summary it would appear that in severe heart failure RyR2 remains functional and as demonstrated by Yamamota et al. [22] may even have augmented functional characteristics. As alluded to in Section 1 the compensatory mechanisms that augment RyR2 function may actually have an adverse effect on myocardial function, rather than a helpful one.

2.4. Catecholaminergic modulation of RyR2 in heart failure

Excessive activity of the sympathetic nervous system and adrenal gland produces a hyper-adrenergic state in heart failure to maintain cardiac output and blood pressure. Although β adrenergic receptors are actually down-regulated in heart failure, catecholaminergic modification of E–C coupling is believed, nevertheless, to exert an important effect on this pathophysiological state.

It is well established that phosphorylation of phospholamban increases the uptake of Ca\(^{2+}\) into the SR through the SR Ca\(^{2+}/\)ATPase pump [31], a process inhibited by phospholamban in the non-phosphorylated state. This compensatory mechanism is unable to restore SR Ca\(^{2+}\) levels to normal in heart failure, but appears to provide a mechanism whereby deranged Ca\(^{2+}\) cycling is at least limited. Investigators have also studied RyR2 with respect to phosphorylation and asked whether it too can be modified to maximise myocyte contraction in heart failure. There is certainly circumstantial evidence for this. Multiple phosphorylation sites have been identified on RyR2 and the channel is known to co-assemble with a variety of enzymes that mediate this modification [32]. Hain et al. [33] showed that RyR2 must be phosphorylated in order to be active at physiological [Mg\(^{2+}\)] and studies have reported that phosphorylation increases the open probability of RyR2 and therefore enhances SR Ca\(^{2+}\) release. Valdivia et al. [34] demonstrated that an abrupt [Ca\(^{2+}\)]\(_{\text{cyt}}\) rise caused an initial transient RyR2 opening if the channel is phosphorylated and Marks et al. showed that phosphorylation increased the steady-state open probability of the channel [35]. Such compensatory modulation of RyR2 function would be helpful in heart failure where SR Ca\(^{2+}\) release is impaired.

Although phosphorylation may alter the functional characteristics of RyR2 there is evidence in the non-failing heart that, despite this, its significance as a mechanism for regulating myocyte Ca\(^{2+}\) cycling and SR Ca\(^{2+}\) load is minimal. Li et al. [36] have reported that protein kinase A mediated increases in Ca\(^{2+}\) spark frequency are entirely attributable to phospholamban phosphorylation and enhanced SR Ca\(^{2+}\) loading, with RyR2 phosphorylation having no effect on Ca\(^{2+}\) spark frequency. Furthermore a series of investigations by Eisner [37] argue strongly that RyR2 has only a minimal role in the regulation of steady-state myocyte contraction in the non-failing heart, with SR Ca\(^{2+}/\)ATPase pump activity being the more important regulatory component. They have shown that when there is an abrupt increase in RyR2 opening, much of the additional Ca\(^{2+}\) released will be rapidly removed from the myocyte by the Na\(^+)/Ca\(^{2+}\) exchanger, thereby reducing the pool of cytosolic Ca\(^{2+}\) available to replenish SR Ca\(^{2+}\) stores during diastole. As a result the subsequent reduced SR Ca\(^{2+}\) content rapidly offsets the effects of increased RyR2 opening such that the amplitude of the Ca\(^{2+}\) transient and myocyte contraction remains unchanged.

Although controversy remains regarding the importance of RyR2 phosphorylation under normal physiological conditions there seems little doubt that catecholamine-mediated phosphorylation of E–C components is a compensatory response to deranged Ca\(^{2+}\) cycling in heart failure. This is believed to underlie the cellular mechanisms by which positive inotropic drugs transiently improve cardiac function. Long term such drugs actually worsen mortality in chronic heart failure despite the short-term improvements in a variety of haemodynamic parameters [38]. This discrepancy could be explained by the fact that in chronic heart failure long-term activation of β\(_1\) adrenoceptors and subsequent phosphorylation of E–C coupling components, such as RyR2, has unwanted deleterious effects on cardiomyocyte function, cell repair and apoptosis, which increase mortality rather than improve it.

2.5. RyR2 hyperphosphorylation in heart failure

In a series of experiments Marks and co-workers have shown that RyR2 becomes hyperphosphorylated in chronic heart failure [35,39,40]. Observed consequences of this are a dissociation of the regulatory protein FKBP12.6 (which is proposed to play a role in orchestrating coupled gating between adjacent channels [41]) and a functional change in RyR2’s characteristics allowing an increased leak of Ca\(^{2+}\) out of the SR, particularly during diastole. This ‘diastolic Ca\(^{2+}\) leak’ further depletes SR Ca\(^{2+}\) load and serves as a substrate for delayed afterdepolarisations, which can lead to triggered ventricular arrhythmias and sudden death [40,42]. They propose that although phosphorylation of RyR2 occurs as a compensatory response in failing hearts, its continuation for a prolonged period will eventually become counterproductive to cardiac function.

Further evidence supporting this hypothesis is provided by observations that oral β blockers reverse RyR2 hyperphosphorylation, restore the stoichiometry of the RyR2-FKBP 12.6 complex and normalise RyR2 function [43,44]. This is particularly interesting as the cellular mechanisms by which β blockers enhance heart failure survival, yet at the same time hinder contraction in normal hearts, have never been adequately explained.

As more evidence emerges to clarify the role of RyR2 hyperphosphorylation and its consequences in heart failure,
new therapeutic interventions which target RyR2 and influence its phosphorylation state may emerge.

2.6. FKBP12.6 in heart failure

FKBP 12.6 is the specific isoform of an important regulatory protein that closely associates with RyR2. A stoichiometry of four FKBP 12.6 to one RyR2 tetramer exists in the normal physiological state. Its long suspected function in the regulation of RyR2 gating has recently been established by the development of FKBP 12.6 null mice which are seen to demonstrate increased duration and amplitude of Ca\(^{2+}\) sparks and increased Ca\(^{2+}\) induced Ca\(^{2+}\) release gain [45]. This observed functional deficit is in keeping with previous work that has suggested dissociation of FKBP 12.6 from RyR2 was associated with an increased channel open probability [46]. It thus appears that FKBP 12.6 serves to modulate channel function and prevent excessive non-physiological opening of the channel and a subsequent detrimental SR Ca\(^{2+}\) leak. Several studies have shown that FKBP 12.6 levels are significantly reduced in heart failure models [47,48] and this observation is believed, at least in part, to explain the abnormal SR Ca\(^{2+}\) leak observed in these models [47]. Such studies point to a decrease in FKBP 12.6 numbers or function as an important pathophysiological development in heart failure in its own right. This may be in addition to the functional uncoupling of RyR2 from FKBP 12.6 as demonstrated by Marks and co-workers as a consequence of RyR2 hyperphosphorylation.

The recent unexpected findings of cardiac hypertrophy in FKBP 12.6 null mice and the protecting influence on the development of this phenotype by oestrogen [45] has certainly raised the profile of this RyR2 associated protein and more studies clarifying its precise role and importance in cardiac disease states can be expected.

3. Cardiac arrhythmias and RyR2 dysfunction

Two forms of sudden cardiac death in children have recently been shown to result from autosomal dominant mutations in RyR2. Both these conditions, catecholaminergic/polymorphic ventricular tachycardia (CPVT) and arrhythmogenic right ventricular cardiomyopathy (ARVC) subtype 2 share the common clinical feature of exercise and stress induced ventricular arrhythmias.

3.1. Arrhythmogenic right ventricular cardiomyopathy

ARVC is an acronym for a genetically heterogeneous group of cardiomyopathies, characterised by structural and functional abnormalities of the right ventricle and progressive replacement of the right ventricle free wall with fibrous and fatty tissue [49,50]. It presents with arrhythmias of right ventricular origin and if sudden death is avoided in the early stages right ventricular failure develops, eventually progressing to biventricular failure which mimics dilated cardiomyopathy [50].

Linkage studies have identified seven different candidate chromosomal loci in families affected by the disease. With the exception of one rare autosomal recessive form (Naxos Syndrome), the condition appears to display an autosomal dominant inheritance pattern with variable penetrance and incomplete expression [51]. Of the six autosomal dominant loci discovered only RyR2 has been identified as a specific causative gene.

3.1.1. Mutations in RyR2 and ARVC

One form of ARVC, classified subtype 2, is characterised by an association with exercise-induced arrhythmias, a high penetrance and an equal male/female ratio (other forms tend to have a male predilection). It was first described in 1988 [52] and following its linkage to the RyR2 chromosomal loci 1q42-43 [53,54] Tiso et al. recently identified four missense mutations of RyR2 in four independent affected families [55]. All four mutations were mapped to two highly conserved regions of RyR2 that code for cytoplasmic domains of the channel and correspond to similar regions of the RyR1 gene where mutations underlying malignant hyperthermia and central core disease are found [56] (Fig. 3). These RyR1 mutations have been proposed to cause an increased leak of Ca\(^{2+}\) from the SR [57] and Tiso et al. speculate that their ARVC RyR2 mutations will have a similar effect. Although this may help explain the association of ARVC type 2 with exercise-induced arrhythmias the mechanisms behind observed structural abnormalities in ARVC is less clear. Nevertheless the identification of a specific genetic cause for ARVC will assist in familial screening and diagnosis and this is particularly important in a condition where the first presentation can be sudden death and where normal non-invasive investigations do not exclude the diagnosis.

3.2. Catecholaminergic polymorphic ventricular tachycardia

CPVT is characterised by adrenergic induced arrhythmias in the form of bi-directional and polymorphic ventricular tachycardia [42,58,59]. It is one of a small group of rare inherited disorders including the congenital long QT and Brugada syndromes that can cause malignant ventricular arrhythmias in the absence of structural heart disease [60]. It is a rare disorder however it has a high mortality rate (up to 50% by the age of 30 [61]) and can present as sudden cardiac death without warning in children and young adults. For these reasons all physicians dealing with childhood syncope and collapse should be aware of its existence.

CPVT was first reported as a case of exercise induced bi-directional tachycardia in the early 1970s [62]. Numer-
ous short series and case reports followed [63] yet it was not until Leenhart et al. reported a 10 year follow-up of 21 children with the disorder in 1995 [59] that it became established as a distinct clinical entity. Cases have been reported throughout the world and both sexes appear to be susceptible. Its key features include a childhood onset of syncope and collapse brought on by exercise and other stressful scenarios, with reproducible polymorphic and/or bi-directional ventricular tachycardia demonstrated during exercise testing and catecholamine infusion. Cardiac investigations show no evidence of structural heart disease and the resting 12-lead electrocardiogram is unremarkable with a normal QT interval.

3.3. The genetic basis of CPVT

CPVT shows a clear familial tendency with an autosomal dominant inheritance pattern. In 1999 Swan et al. reported that the disorder mapped to the RyR2 gene locus in two Finnish families [58] and following on from this work two groups recently identified a total of seven missense mutations in RyR2 as being responsible for the disorder. Priori et al. [64] identified four mutations, three apparently sporadic in nature and a fourth occurring in five clinically affected members of the same family. Laitinen et al. [65] identified three mutations in three unrelated families. Along with the four ARVD mutations already mentioned this brings the total number of missense mutations identified in RyR2 and associated with exercise induced arrhythmic disorders to 11 (Fig. 3). These mutations cluster in three regions of the RyR2 gene that are comparable to three regions in RyR1 where mutations underlying malignant hyperthermia and central core disease are found. This would suggest that these are highly conserved regions of both the RyR1 and RyR2 genes, which have an important role in regulating channel function.

An autosomal recessive form of CPVT also exists and the genetic basis of this form has been pinpointed to a highly conserved region of the calsequestrin 2 gene by Lahat and co-workers [66]. The mutation appears to disrupt Ca\(^{2+}\) binding to calsequestrin, and Lahat speculates that this may allow an unwanted leak of free Ca\(^{2+}\) out of the SR during exercise when catecholaminergic drive predisposes to RyR2 opening.

3.4. The pathogenesis of CPVT caused by mutations in RyR2

The identification of RyR2 mutations in CPVT suggests that a disruption of SR Ca\(^{2+}\) release underlies the disease. Furthermore the nature of CPVT would suggest that these mutations alter the normal physiological response of the channel to increased catecholaminergic drive during exercise. Although characterisation of these mutations is not yet available it is possible to gain some insight into the
likely pathophysiological mechanisms behind CPVT by considering the peculiar arrhythmia strongly associated with it, namely bi-directional ventricular tachycardia.

3.5. Bi-directional ventricular tachycardia: a marker of abnormal RyR function?

Bi-directional ventricular tachycardia is a rare and unusual arrhythmia first described in 1922 [67]. Although it is consistently associated with CPVT it is better known for its association with digoxin toxicity [68]. It is a broad complex tachycardia with a curious alternating QRS complex polarity and its origins remained unclear for many years until Cohen and co-workers [69] finally demonstrated that it was in fact a ventricular arrhythmia originating close to the common undivided part of the left bundle branch. They showed that its unique ECG morphology resulted from conduction within the left ventricle alternating between the anterior and posterior fascicle of the bundle, due to an alternating refractoriness in the other fascicle (Fig. 4).

Abnormal impulse generations that can initiate cardiac arrhythmias are generally believed to originate from either automaticity or triggered activity [70]. Triggered activity is so-called because unlike the spontaneous depolarisations of 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 impulses result from sub-threshold membrane depolarisations, termed afterdepolarisations, which follow the previous action potential. They can occur during 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) (Fig. 5).

EADs occur when the action potential duration is extended. Reviews of their importance and electrochemical generation can be found elsewhere [70]. DADs, which are

Fig. 4. The normal impulse conduction of sinus rhythm (A) is compared with bi-directional ventricular tachycardia (B) where the arrhythmogenic focus arises within the left ventricle and propagates into the common left bundle branch. The alternating complex appearance results from (B1) a right axis morphology complex where conduction occurs down the anterior division with blocked conduction in the posterior division followed in the next complex by (B2) a left axis morphology resulting from conduction down the posterior division with blocked conduction down a refractory anterior division. Adapted from Ref. [69].

3.6. Bi-directional ventricular tachycardia is a triggered arrhythmia resulting from delayed afterdepolarisations secondary to intracellular calcium overload and spontaneous SR calcium release
believed to be the underlying cause of bi-directional ventricular tachycardia [70] and hence arrhythmia generation in CPVT [64], result from a transient inward current evoked by spontaneous Ca\(^{2+}\) release from the SR under conditions that favour accumulation of cellular Ca\(^{2+}\) [70–72]. If this inward current is sufficient to cause a DAD whose amplitude exceeds the threshold potential, depolarisation will occur. The abnormal impulse generated from this can cause a triggered arrhythmia if surrounding polarised cells propagate the depolarisation wave. Several factors increase the amplitude of DADs and hence the probability that the threshold potential will be reached. These include increasing the rate of the triggering action potential (corresponding to an increase in heart rate) and increasing intracellular calcium loading [73]. This latter condition is brought about by cardiac glycoside toxicity [74] and catecholaminergic influence on the heart will increase DAD amplitude by both these mechanisms [75,76]. Hypercalcaemia alone does not cause this particular arrhythmia. Presumably without the additional actions of digoxin or RyR2 mutations any increased Ca\(^{2+}\) influx into the cell is successfully buffered and/or balanced by Ca\(^{2+}\) efflux, such that SR Ca\(^{2+}\) levels remain relatively normal preventing any spontaneous diastolic Ca\(^{2+}\) leak.

Cardiac glycosides have consistently been shown to cause DADs in cardiomyocytes [74,77]. These drugs increase intracellular [Ca\(^{2+}\)], and hence conditions which favour DAD generation, because in an attempt to remove excess cytosolic Na\(^+\) resulting from the drug’s action on the sarcoplasmic Ca\(^{2+}\)/K\(^+\) ATPase pump, Na\(^+\) is removed in exchange for an inward movement of Ca\(^{2+}\) through the sarcoplasmic Na\(^+\)/Ca\(^{2+}\) exchanger [77]. The realisation that RyR2 mutations are associated with bi-directional ventricular tachycardia raises the possibility that the mechanism whereby glycosides bring about this arrhythmia is also linked to a direct action on RyR2. Work in our laboratory [78] and by others [79] has shown that cardiac glycosides do have a direct action on RyR2, leading to an increased open probability of the channel. It is possible that bi-directional ventricular tachycardia is a manifestation of DADs, brought about by an unwanted diastolic release of SR Ca\(^{2+}\), which is itself a manifestation of abnormal RyR2 function secondary to unwanted cardiac glycoside action or CPVT mutations.

A convincing case for the role of DADs in bi-directional ventricular tachycardia is not only supported by work characterising arrhythmia generation secondary to digoxin toxicity. There is now direct evidence from electrophysiological studies during catecholamine infusion in CPVT that DAD generation occurs and does indeed result in the generation of bi-directional ventricular tachycardia [80].
Furthermore it is possible that only a brief period of exercise would be needed to initiate a triggered arrhythmia by the above mechanisms. Once generated the arrhythmia could potentially self perpetuate itself long after the transient conditions which caused it had ceased.

3.7. Functional consequences of RyR2 mutations underlying CPVT

Until results on the functional characterisation of CPVT mutations emerge our knowledge of bi-directional ventricular tachycardia and how DADs are generated allow us to speculate that CPVT results from mutations which allow an excessive Ca\textsuperscript{2+} release from the SR, particularly during diastole when the channel would normally be closed.

One question that needs to be asked 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 may also alter RyR2 function, causing depletion of SR Ca\textsuperscript{2+} via a diastolic leak and as a result triggered arrhythmias. During exercise an increase in sympathetic drive will result in increased RyR2 phosphorylation. If the threshold for channel activation and Ca\textsuperscript{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 such as bi-directional ventricular tachycardia to develop. Such a modification of function would also explain how, under normal circumstances where there is no excessive sympathetic drive, the mutations remain silent having no noticeable effect on channel function.

As with ARVC type 2 we can also gain insight into the functional consequences of these mutations by looking at studies of similar RyR1 mutations which underlie malignant hyperthermia and central core disease and are seen to cluster in three corresponding regions of the RyR1 gene as the identified CPVT mutations in RyR2 (Fig. 3). It has been proposed that these mutations lead to a ‘gain in function’ and increased channel opening [57].

3.8. CPVT may be caused by RyR2 mutations that reduce channel opening

The mechanisms that govern SR Ca\textsuperscript{2+} loading and release are key factors in determining myocardial contraction and the development of pro-arrhythmic states outlined above. These include (1) the magnitude of Ca\textsuperscript{2+} influx into the cell through the sarcolemmal L-type voltage-dependent Ca\textsuperscript{2+} channel, (2) the uptake of Ca\textsuperscript{2+} into the SR through the SR Ca\textsuperscript{2+}/ATPase pump, (3) the Ca\textsuperscript{2+} buffering capacity of the SR and (4) the intrinsic gating properties of RyR2. All of these are targets for phosphorylation-mediated modulation, brought about by increased sympathetic drive. The experimental work of Eisner [37], Li [36] and others, suggests that intrinsic regulation of RyR2 has, on its own, minimal long-term influence on the amount of Ca\textsuperscript{2+} released from the SR. Instead it appears that SR [Ca\textsuperscript{2+}], itself a function of influx into the SR through the SR Ca\textsuperscript{2+}/ATPase pump, is the key determining factor. How does this viewpoint stand up against the observation that arrhythmias in CPVT appear to result from a pathological release of SR Ca\textsuperscript{2+} as a consequence of mutations in RyR2? Surely this implies a regulatory role for RyR2 in the process of SR Ca\textsuperscript{2+} release?

We suggest that these two arguments are not necessarily mutually exclusive and indeed result in an interesting hypothesis in which CPVT mutations in RyR2 actually result in a move in the direction of reduced open probability, as opposed to the ‘gain in function’ mutation theory mentioned above. If mutations in RyR2 promote an environment whereby Ca\textsuperscript{2+} release is curtailed, feedback mechanisms would be expected to promote an increased uptake of Ca\textsuperscript{2+} into the SR, through the Ca\textsuperscript{2+}/ATPase pump, to compensate for this deficient release. This would lead to a steady-state set of conditions where the SR Ca\textsuperscript{2+} content is simply shifted to a higher concentration. Under situations of catecholamine-induced phosphorylation the gain in RyR2 function mediated by this modulation may be enough to overcome the inhibitory effects of the mutations thereby allowing release of what is now an abnormally excessive SR Ca\textsuperscript{2+} load. Furthermore, phosphorylation effects during exercise would promote even further loading of the SR with Ca\textsuperscript{2+}, again working to overcome the inhibitory constraints of the mutations (Fig. 6). The development of transgenic animal models containing CPVT mutations will facilitate the measurement of SR Ca\textsuperscript{2+} content in this condition and help elucidate whether steady-state Ca\textsuperscript{2+} concentration is indeed elevated.

3.9. RyR2 dysfunction and atrial arrhythmias

To our knowledge the only other cardiac arrhythmia that has received attention with regards to RyR2 dysfunction is atrial fibrillation. With respect to this common arrhythmia, studies have attempted to clarify changes in the density and expression levels of various Ca\textsuperscript{2+} regulatory proteins, including RyR2. Ohkusa et al. [81] found that there was a significant reduction in the maximum number of ryanodine binding sites (B\textsubscript{max}) in atrial tissue taken from mitral valve disease patients with chronic atrial fibrillation. Correspondingly they also showed that the expression levels of RyR2 mRNA were also reduced. The authors speculate that these observed abnormalities may have importance in the initiation and/or propagation of atrial fibrillation, although they concede that a specific causative role cannot be inferred from such observations alone. Furthermore Lai et al. [82] also looked at the expression levels of several Ca\textsuperscript{2+} handling genes in atrial tissue from chronic atrial fibrillation patients. They conversely found no significant
changes in the level of RyR2 gene expression and at present the precise role, if any, of RyR2 in the pathogenesis of atrial fibrillation remains to be established.

4. Concluding comments

In this article we have summarised the role played by RyR2 in cardiac E–C coupling. As its role in normal myocardial physiology has become clearer it is not surprising that it has been seen as a potential factor in heart failure and arrhythmia generation, two pathophysiological processes that can occur when E–C coupling is deranged.

There is now clear evidence that RyR2 modulation, either as a primary event or more likely secondary to other pathophysiological processes within the cell, is an important factor in chronic heart failure, regardless of its underlying aetiology. RyR2 dysfunction may not only result in impaired contraction but it may also predispose to intracellular conditions that favour arrhythmia generation. This emerging knowledge raises the possibility that therapeutic manipulation of RyR2 may be a further possible target for drug therapy in heart failure, in fact it is likely that the benefits of β blockers in this condition are, in part, a result of their indirect action on the phosphorylation state of RyR2.
The arrhythmogenic potential of RyR2 is further demonstrated by the identification of mutations that underlie CPVT and ARVC type 2. These interesting but rare disorders are important nonetheless, as they are both causes of preventable sudden death in children and young adults. The discovery of RyR2 mutations now makes possible pre-symptomatic screening and treatment in affected families and as their functional consequences emerge not only will targeted drug strategies become possible but we will also gain a better understanding of precisely how RyR2 works in the human heart.

Note added in proof

The first reported work to provide an insight into the functional characterisation of arrhythmogenic RyR2 mutations has now emerged. Jiang et al. [83], working on the mouse equivalent of the human RyR2 CPVT mutation R4497C, expressed and isolated in human embryonic kidney cells which have no endogenous RyR2 expression, have been able to assess the mutant channels function using ryanodine binding and single channel analysis. Both these techniques have shown that the mutant channel demonstrates enhanced activity, especially at low [Ca\(^{2+}\)], whilst at the same time retaining its normal sensitivity to modulation by other ligands such as caffeine, ATP and Mg\(^{2+}\). This would appear to provide the first piece of evidence to support the hypothesis of increased Ca\(^{2+}\) leak through RyR2 as the underlying mechanism of arrhythmia generation in CPVT. Further functional characterisation of this and other mutations will no doubt soon follow.

Acknowledgements

We thank Philip Poole-Wilson and Ken MacLeod for their helpful comments in reading the manuscript and the British Heart Foundation for ongoing funding of the work in our laboratory.

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