Targeting calcium handling in arrhythmias

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Received 30 June 2008; accepted after revision 8 September 2008; online publish-ahead-of-print 9 October 2008

Abnormal calcium (Ca) handling can contribute to arrhythmogenesis directly by triggering abnormal depolarizations and indirectly by modulating action potential time course and duration. Recent data have shown the importance of these mechanisms in rare genetic diseases but also in more common conditions such as heart failure. Modulating Ca release from the sarcoplasmic reticulum via the ryanodine receptor, Ca uptake via sarcoplasmic reticulum Ca ATPase or Ca removal from the cell via the Na/Ca exchanger, are potential approaches to reduce arrhythmias. New tools allow exploring these ideas. The principles underlying this approach and the first results are critically reviewed.

KEYWORDS
Calcium; Cardiac myocyte; Sarcoplasmic reticulum; Na/Ca exchange; Arrhythmias; Ryanodine receptor; SERCA

Introduction
A first link between abnormal calcium (Ca) handling in cardiac myocytes and ventricular tachyarrhythmias was established many years ago in the pathogenesis of digitalis intoxication, a currently rare event. More recently Ca has been found to play a major role in arrhythmias that occur in more common diseases such as heart failure (HF), as well as in a number of congenital arrhythmia syndromes. In addition, several new approaches have been developed that have the potential to target arrhythmias through Ca handling. The cellular and subcellular mechanisms linking Ca to arrhythmias have been reviewed recently in extenso.1 The purpose of the current mini-review is to re-iterate briefly these mechanisms to next review critically the available data on novel Ca-targeted approaches to treat arrhythmias.

Calcium and arrhythmogenesis
The role of Ca in arrhythmogenesis broadly falls into two categories: a direct role, generating afterdepolarizations and a more indirect role, modulating action potential (AP) duration and time course. The major pathways are illustrated in Figure 1. Afterdepolarizations are carried by a net inward current and can occur within the plateau or repolarization phase of the AP (early afterdepolarizations, EAD), or when repolarization has been completed (delayed afterdepolarizations, DAD). DADs can directly trigger AP but can initiate arrhythmias through local re-entry and further contribute to maintenance by increasing temporal and spatial heterogeneity. Although there is a gap in evidence between cellular data and arrhythmogenesis in vivo, studies using monophasic AP recordings in vivo as well as mapping studies in isolated hearts support a link between the cellular phenomena and the triggering of arrhythmias. Purkinje cells may be especially prone to generating afterdepolarizations.4,5 DADs are typically related to conditions of cellular Ca overload, as with digitalis toxicity, or during ischaemia reperfusion, when high intracellular Na promotes Ca influx. When the Ca content of the sarcoplasmic reticulum (SR) exceeds a threshold, spontaneous Ca release occurs (reviewed in6). Typically this occurs in one or a few foci within the cells and propagates across the cell as a Ca wave. This spontaneous release increases forward Na/Ca exchange (NCX), removing Ca from the cell. Because NCX is electrogenic it produces an inward current which depolarizes the membrane and causes a DAD. The classic conditions of SR Ca overload are not a prerequisite for spontaneous Ca release and DADs. More recently, DADs have been proposed as a trigger for arrhythmias associated with mutations in the SR ryanodine receptors (RyR), such as catecholaminergic polymorphic ventricular tachycardia (CPVT).

Under those conditions, a decrease in the threshold for opening of the RyR reduces the amount of SR Ca loading that is required to induce spontaneous release. In HF, hyperphosphorylation of RyR could also contribute to DADs by increasing the RyR open probability.8 As in the case of RyR mutations, an additional challenge such as adrenergic stimulation, to increase SR Ca loading is likely to be necessary. This relation...
between changes in RyR threshold and SR Ca content was recently highlighted by Venetucci et al. 6

Less well studied is the role of (spontaneous) SR Ca release in the initiation of EADs. This type of triggered activity is responsible for most of the long QT syndromes (LQTS), either inherited or acquired in nature. The inherited forms are usually associated with mutations of a single ion channel; these include loss-of-function mutations of K channels, and mutations in Na channels resulting in increased channel activity (see 9 for overview). In Timothy syndrome, mutation of the L-type Ca channel with reduced inactivation of Ca current results in multi-system disorder, including arrhythmias and QT prolongation. 10 Although cellular data are limited, computer modelling suggests that the arrhythmias could be related to DADs resulting from Ca overload, distinguishing this from other forms of congenital LQTS. The more recently described Ca channel mutation with loss-of-function leading to short QT is also associated with arrhythmias, 11 most likely unrelated to the afterdepolarizations discussed here. The acquired forms of LQTS include a reduction in repolarization reserve induced by drugs, usually block of the human ether-a-go-go related gene channel, or remodelling processes as in hypertrophy and HF (reviewed in 12). Reactivation of the L-type Ca current (\( I_{CaL} \)), or of the Na current under conditions of abnormal Na channel activity, has been identified as the inward current underlying the upstroke of EADs. Prolonged repolarization allows sufficient time for the current to recover from inactivation. A role for SR Ca release is most prominent under \( \beta \)-adrenergic stimulation. 13 EADs can result from spontaneous SR Ca release resembling DADs in their subcellular mechanism. In addition, the enhanced Ca release promotes Ca removal on NCX during the plateau or early repolarization phase of the AP, producing a more prominent ‘conditioning’ inward current upon which a Ca window current can develop more readily. With the enhanced SR Ca re-uptake, Ca currents have a fast recovery from release-dependent inactivation and Ca channels are readily available for re-opening, contributing to EADs. 14

Classic approaches to reduce Ca-dependent arrhythmias are Ca channel antagonists and \( \beta \)-blockers. Especially the latter have proven their use in CPVT. 15 The benefit of \( \beta \)-blockers in HF is well established and includes a reduction in sudden, presumed arrhythmic death. 16

After some disappointing results with ion channel blockers, and with the success of device therapy, there was a quiet period in anti-arrhythmic drug development. However, we are now entering a new period with drugs in the pipeline that are targeting specific Ca handling proteins: RyR, sarco-plasmic reticulum Ca ATPase (SERCA), and NCX. The steps at which these drugs would act to interrupt arrhythmogenic events are indicated in Figure 1. This is still at a very early stage, and here we review the current status.

**Targeting the RyR**

The study by Marks and co-authors 8 set the stage for targeting RyR, as subsequently developed. The working hypothesis is that hyperphosphorylation of the RyR dissociates FKBP12.6 (subsequently renamed calstabin2) with a concomitant increase of RyR open probability. This would cause a diastolic Ca leak, depleting the SR of Ca and triggering an arrhythmogenic current upon activation of NCX. Inhibition of the RyR leak could thus be an attractive anti-arrhythmic target, at the same time preventing diastolic Ca leak which would also enhance SR Ca loading. This view is not without controversy with regard to the presence of hyperphosphorylation and its functional impact. 17 There are theoretical considerations against the approach as well. Inhibition of RyR may not necessarily improve contractile function as less Ca may be released from the SR during systole and as it may lead to conditions of SR Ca overload. This was tested recently. 18 Unexpectedly, under adrenergic...
stimulation with spontaneous Ca waves, decreasing the open probability of RyR actually increased the amplitude of the systolic Ca transient and simultaneously abolished arrhythmogenic Ca waves. This study supported the idea of RyR inhibition and its potential benefits in arrhythmogenesis, but the RyR inhibitor used (tetracaine) is unsuitable for clinical use. Studies are however underway with K201, a drug initially introduced as a cardioprotective agent and formerly known as JTV519. This drug was shown to block multiple ion channels: it inhibits Na and Ca currents, and delayed and inward rectifier K channels, \( I_{\text{Kr}} \) and \( I_{\text{Kv}} \). Most interestingly, K201 reduces Ca leak from SR, first shown in vesicles. Whether this occurs by stabilizing the binding of calstabin2 to the receptor, or by restoring defective interdomain interactions irrespective of calstabin2 remains an issue of debate. In mouse models, K201 has been proven successful against CPVT related to altered RyR gating after knockout of calstabin2, and in CPVT related to some RyR mutations, but not all. Although the anti-arrhythmic potential of K201 in these settings has been ascribed to RyR inhibition, it should be emphasized that its beneficial effect could be related to concomitant block of Ca and Na currents. This may be protective against DADs, first, by reducing the Ca load of the cell, and secondly, by less Na channels being available for activation. This increases the threshold for a DAD to trigger an AP. The \( I_{\text{Kr}} \) blocking properties of K201 will presumably offset the latter by destabilizing the resting membrane potential. A recent study by Loughrey et al. reported suppression of arrhythmogenic Ca waves which was ascribed to a reduction in both Sr Ca release and Sr Ca uptake. The latter effect may be proarrhythmic as well. K201 has to our knowledge not yet been tested in models of CPVT related to mutations in calsequstrin that also lead to enhanced probability of spontaneous Ca release, but given its multiple actions this drug may be of interest.

K201 has also been tested in models of proarrhythmia where the arrhythmogenic mechanism does not involve defective RyR function. In a rabbit model with combined \( I_{\text{Kr}} \) block and \( \alpha \)-adrenergic stimulation, K201 prevented torsades de pointes. The mechanism by which \( \alpha \)-adrenergic agonists make these hearts more vulnerable to \( I_{\text{Kr}} \) inhibition and Tdp is not clear, but presumably involves changes in Ca. The antiarrhythmic actions of K201 under these conditions could be related to its effects on Ca handling, including \( I_{\text{CaL}} \) block, but also \( \alpha \)-adrenergic block. K201 has been proven successful as well in a canine model of atrial fibrillation, where it prolongs the effective refractory period of atrial conduction. This may be explained by inhibition of K currents and prolongation of the atrial AP duration. In these settings, its inhibitory effect on RyR may offer additional benefit, since hyperphosphorylation and decreased calstabin binding to the receptor have been documented in patients with atrial fibrillation. At the moment, the importance of this mechanism for the initiation and/or maintenance of atrial fibrillation is not clear. K201 is currently in phase 2 of a clinical trial for testing against atrial fibrillation (RESTORATION trial), and new derivates are being developed.

**Targeting the NCX**

The potential of NCX blockers was reviewed extensively recently. NCX is the main pathway for removing Ca out of the cell. Because the exchanger is electrogenic, Ca removal through the forward mode produces an inward current; the reverse mode bringing Ca into the cell produces an outward current. Its mode of action depends on Ca, Na, and membrane potential. These parameters are dynamically changing during a single cardiac cycle, and therefore NCX can operate in both modes and having depolarizing and repolarizing effects during the AP. Under normal conditions, the predominant and net NCX activity is the forward mode, removing Ca that has entered through Ca channels. This is required for maintaining the Ca balance of the cell and the inward current is a normal part of the many currents that shape the AP late plateau, repolarization, and diastolic potential. The reverse mode may become more prominent with high Na loads, or reduced Ca release and prolonged AP, as in HF.

NCX has a dual role in arrhythmogenesis. First, in conditions of high Na, the reverse mode of the exchanger is the principal pathway that loads the cell with Ca and causes Ca overload. Secondly, the forward mode carries the inward current of a DAD following spontaneous release outside the normal cycle of excitation–contraction coupling. Under conditions of high Ca loading, inward NCX during the AP may also facilitate EADs. Thus, inhibition of the reverse mode is of particular strategic interest for arrhythmias related to Ca overload, when NCX provides the pathway for excessive Ca loading. Inhibition of forward NCX may also have potential benefits from an electrical point of view as less inward current will be generated for a given Ca wave. The challenge is to do so without enhancing Ca overload and arrhythmias. On the other hand increasing Sr Ca loading may actually be of benefit in HF.

Theoretically, the unwanted effects of blocking Ca efflux on NCX could be limited by predominant inhibition of the reverse mode over the forward mode. KBR-7943 was the first drug of a generation of NCX blockers with reported mode-selectivity, later followed by SEA-0400, more potent and more selective for NCX, and most recently SN-6. The intrinsic mode dependence of NCX blockers has been questioned in studies in more physiological conditions and is from a biophysical point of view difficult to understand. However, drug binding/activity could be dependent on the prevailing Na and Ca concentrations, and thus be more effective under conditions where reverse mode is dominant. With bidirectional block under physiological conditions, block of NCX is expected to raise the cellular and SR Ca load, as observed in mice and pigs, and also in the dog with HF. If no effect is seen, as reported in the two studies in the dog and rabbit using SEA-0400, this could be explained by a concomitant block of \( I_{\text{Cal}} \). The latter has been reported for SEA-0400 at concentrations close to the \( IC_{50} \) values for NCX inhibition. The concomitant block of \( I_{\text{Cal}} \) may render these drugs less useful as a tool for studying NCX function, but from a clinical perspective, it may be advantageous and part of an anti-arrhythmic strategy. \( I_{\text{Cal}} \) block likely contributed to some of the beneficial effects that have been observed in a number of animal studies.

Initial studies on the therapeutic use of NCX blockers focused on cardioprotective effects against ischaemia/reperfusion injury. KBR-7943 and SEA-0400 prevented Ca overload following Na accumulation during an ischaemic event and limited reperfusion injury, but failed to...
reduce the incidence of ventricular fibrillation in ischaemic dogs.\textsuperscript{51,52} These compounds have also proven to be successful against DADs and arrhythmias related to Na overload induced by glycosides,\textsuperscript{53,54} or Na channel openers.\textsuperscript{55} Of interest, SEA-0400 also suppressed EADs induced by K current blockers in cardiac muscle.\textsuperscript{54} This awaits further confirmation in the intact animal and models of proarhythmia, but opens new perspectives for NCX blockers as treatment against arrhythmias related to EADs and LQTS. For HF, the concomitant inotropic effects should be carefully considered. The eventual effect may be dependent on the etiology and concurrent changes in Ca handling as recently shown for two different mouse models of HF.\textsuperscript{43}

**Targeting SR Ca uptake**

The SR Ca ATPase, SERCA, has been intensely studied as a target for treatment of HF, but less for arrhythmias as such. SERCA is the major pathway for removing Ca from the cytosol after SR Ca release in a normal heart beat. It determines the rate of relaxation and hence diastolic function, and it modulates systolic function by reloading the SR with Ca. SERCA is regulated by a small inhibitory protein, phospholamban (PLN); phosphorylation by protein kinase A dissociates PLN from SERCA and relieves its inhibitory action. SERCA and PLN are thus important regulators of cardiac contractility (reviewed in\textsuperscript{64}).

Impaired SERCA function either related to dysfunction of SERCA proper or to altered regulation by PLN, is mainly held responsible for the poor relaxation of the failing heart. Combined with upregulated NCX and ‘leaky’ RyR channels, it further contributes to the depletion of the SR content and low Ca loads. Consequently, improving SERCA function has been considered as an attractive target for restoring cardiac function in a setting of HF (for review of targeting Ca handling proteins in HF, see\textsuperscript{57}). Pharmacological tools for increasing SERCA activity are not (yet) available, and evaluation of this therapeutic strategy has focused on gene transfer. Acute overexpression of SERCA or deletion of PLN through adenoviral gene delivery, improves diastolic and systolic function in cellular and in vivo studies.\textsuperscript{58–60} Improved viral vector design and gene delivery methods have led to rapid advances in the field of gene therapy.\textsuperscript{61,62} Following positive preclinical studies in large animal models, the first clinical trial on gene therapy with SERCA in HF patients with left ventricular assistance devices is under way.\textsuperscript{63}

Could this also help to reduce arrhythmias in HF? Theoretically there is a serious risk that higher SERCA activity increases the risk of Ca overload and would be arrhythmogenic, in particular in diseases other than HF. In practice however, even in the presence of β-adrenergic stimulation, overexpression of SERCA in normal myocytes reduced the propensity of aftercontractions, despite nearly doubling of the SR Ca content.\textsuperscript{64} A possible explanation is given by a study by O’Neill \textit{et al.}\textsuperscript{18} who showed that with a faster SR Ca re-uptake, the propagation of a Ca wave throughout the cell is less efficient. This is consistent with earlier and recent reports that SERCA overexpression is protective against reperfusion arrhythmias.\textsuperscript{65,66}

Despite major efforts, drugs to inhibit PLN or directly increase SERCA activity are not yet available. There are however some very recent data that this may be within reach.\textsuperscript{67}

**Conclusions and perspectives**

A new generation of anti-arrhythmic strategies focusing on Ca handling is under evaluation. Although still in early days, this approach represents a new and exciting field.

Potential targets include the SR Ca release channel, the NCX and SERCA. Very selective drugs are excellent tools for the experimental studies characterizing these targets, but many of the currently available substances have other actions as well. In view of earlier experience with anti-arrhythmic therapy, this may eventually be of benefit.

Inherent to these drugs is their modulation of Ca handling and consequently contractile function. Further cellular and preclinical studies should include models of disease that allow testing in the setting of altered Ca handling to evaluate the full range of effects.

**Funding**

This study was supported by grants to K.R.S from the FWO, the Fund for Scientific Research Flanders (G.0384.07) the European Union (LSHM-CT-2005–018833, EUGeneHeart) and Belgian Science Program IAP6/31.

**Conflict of interest:** none declared.

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