New antiarrhythmic targets to control intracellular calcium handling

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Abstract Sudden cardiac death due to ventricular arrhythmias is a major problem. Drug therapies to prevent SCD do not provide satisfying results, leading to the demand for new antiarrhythmic strategies. New targets include Ca\(^{2+}\)/Calmodulin-dependent protein kinase II (CaMKII), the Na/Ca exchanger (NCX), the Ryanodine receptor (RyR, and its associated protein FKBP12.6 (Calstabin)) and the late component of the sodium current (\(I_{\text{Na-Late}}\)), all related to intracellular calcium (Ca\(^{2+}\)) handling. In this review, drugs interfering with these targets (SEA-0400, K201, KN-93, W7, ranolazine, sophocarpine, and GS-967) are evaluated and their future as clinical compounds is considered. These new targets prove to be interesting; however more insight into long-term drug effects is necessary before clinical applicability becomes reality.

Keywords Arrhythmia · Calcium · Drugs · Treatment

Introduction

Sudden cardiac death (SCD) due to arrhythmias is a major problem in the Western population [1]. SCD occurs when, due to a trigger, sinus rhythm lapses into ventricular tachycardia (VT) which can deteriorate into ventricular fibrillation. At that stage of rhythm disorder, the contractile performance of the heart is severely compromised and eventually results in asystole and, as such, arrest of effective circulation [2]. Conditions such as congenital heart disease, cardiomyopathies and risk factors (e.g. smoking or hypertension) can be the underlying mechanisms of SCD [2]. In patients with dilated cardiomyopathy 30 % will suffer from SCD [3]. SCD in patients with end-stage heart failure is mostly due to mechanical dysfunction based on structural and contractile changes [3]. Prevention strategies focus on antiarrhythmic drugs and implantation of a cardiac defibrillator. Current drug therapies, though effective, also have major drawbacks (as discussed below) and the results have partially been disappointing [4].

Besides the classical modulation of sarcolemmal ion channels by drugs (including both activation and block), new antiarrhythmic targets emerge that can be of great interest in pharmacological treatment of VT in cardiac disease. Especially arrhythmias that occur in hypertrophy and heart failure have our attention, because of the difficulty in treating these patients effectively. Besides disappointing data concerning anti-arrhythmic efficacy, current therapies such as blockade of the late-type calcium current (\(I_{\text{Ca-L}}\)), are known to affect haemodynamics negatively.

These new targets include Ca\(^{2+}\)/Calmodulin-dependent protein kinase II (CaMKII), the Na/Ca exchanger (NCX), the Ryanodine receptor (RyR, and its associated protein FKBP12.6) and the late component of the sodium current (\(I_{\text{Na-Late}}\)), all related to intracellular calcium (Ca\(^{2+}\)) handling of the cardiac myocyte. With this development, emphasis of antiarrhythmic drugs seems to shift therapy more in the direction of focal arrhythmias, which can be caused by delayed (DAD) or early after depolarisations (EAD), a logical step knowing that triggered activity-related arrhythmias are becoming more and more common in hypertrophy and heart failure [5].

Impulse propagation

For the heart to function properly, excitation and contraction of all myocytes in the heart needs to be coordinated and...
balanced. Therefore the electrical impulse that initiates excitation moves throughout the heart via a specific route starting in the sinoatrial node. Next the atria are activated, after which the electrical signal passes the atrioventricular node and travels down through the bundle of His and the bundle branches towards the apex of the heart. From there it activates the ventricular myocytes from apex to base via the network of Purkinje fibres and this is further supported by the anisotropic fibre structure. This sequence leads to a coordinated contraction of the ventricles. For the signal to travel from myocyte to myocyte they need to be coupled electrically. This electrical and metabolic coupling of myocytes is facilitated by gap junctions that are built from connexin proteins (Cx). In the heart three isoforms of Cx are present. Cx40 is mainly expressed in the atria and throughout the conduction system. Cx43 is the most abundant Cx isoform in both the atria and ventricles but is also found in the distal conduction pathway. Finally, Cx45 is only found in the nodes, the His bundle, and bundle branches [6]. For a detailed review of cardiac connexins see Jansen et al. [6]. The Cxs are localised in the intercalated disks between the myocytes. It is this localisation that facilitates that conduction is anisotropic with a faster conduction along the fibre length (longitudinal conduction) compared with the conduction perpendicular to that given orientation (transverse conduction).

Remodelling of the highly homogeneous expression pattern of Cxs during disease may contribute to generation of a proarrhythmic substrate which may increase the propensity for re-entry based arrhythmias. Re-entry based arrhythmias are, however, beyond the scope of this review. In addition, focal uncoupling of the electrical syncytium may favour the occurrence of ectopic activity. The mechanisms that underlie ectopic activity will be further discussed and provide potential new targets for antiarrhythmic interference.

**Impulse generation and contraction**

Ions → action potential duration (APD)

The action potential (AP) is generated via a complex interaction of ion channels, and membrane voltage [7], and is generally divided into five phases. They are established through a fine-tuned interaction of sodium (Na⁺), potassium (K⁺), and Ca²⁺ currents. The inward Na⁺ current (\(I_{\text{Na}}\)) is responsible for the upstroke during phase 0 (Fig. 1, phase 0). The total Na⁺ current is formed by the peak and late \(I_{\text{Na}}\); the latter contributes to depolarisation currents during the plateau phase. During phase 1, the Na⁺ channel inactivates considerably and at the same time \(I_{\text{Na}}\) and \(I_{\text{Na}2}\) (transient outward currents) create outward currents of K⁺ and chloride, respectively, to form the notch (Fig. 1, phase 1). Subsequently, Ca²⁺ enters the cell through voltage-gated Ca²⁺ channels (L-type calcium channels, LTCC) (\(I_{Ca,L}\)) and is involved in creating the plateau phase of the AP since inward movement of Ca²⁺ is counterbalanced by outward K⁺ flow driven by the delayed rectifier potassium currents \(I_{Ks}\) and \(I_{Kr}\) (Fig. 1, phase 2). The plateau phase delays repolarisation of the AP creating time for contraction and relaxation of the cardiomyocytes in between action potentials. Full repolarisation occurs when the LTCC closes and \(I_{Ks}\) and \(I_{Kr}\) take over dominantly (Fig. 1, phase 3). Finally, K⁺ restores the negative membrane potential via the \(I_{Kr}\) current (Fig. 1, phase 4) [7].

Excitation-contraction coupling

Of the ions involved in the activation of the heart, Ca²⁺ plays a key role in excitation-contraction. As mentioned, Ca²⁺ has effects on the membrane potential during the AP plateau via LTCC. LTCC is activated upon depolarisation of the sarcolemma due to a local increase of positive charge that is brought about through influx of [Na⁺], while LTCC is inactivated by local [Ca²⁺] via calmodulin (CaM) binding on the C-terminus of the channel. The initial Ca²⁺ influx via the LTCC leads to Ca²⁺ induced calcium release (CICR) from the sarcoplasmic reticulum (SR), which is mediated by the RyRs (reviewed by Bers [8]). When RyR is activated, this leads to Ca²⁺ extrusion from the SR thereby increasing [Ca²⁺], but this increase in Ca²⁺ also triggers inactivation of the LTCC. The RyR is a channel, but also a scaffolding protein that clusters proteins such as CaM (exerts Ca²⁺ dependent modulation of RyR and LTCC function, see below), protein kinase A (PKA, which can alter RyR and \(I_{Ca}\) gating), and sorcin (which connects RyRs and LTCCs) near the Ca²⁺ release complex. Subsequently, Ca²⁺ released from the SR binds to troponin to facilitate contraction of the sarcomere, the contractile element of the myocyte. Thus, Ca²⁺ links the electrical activation of cardiomyocytes to mechanical contraction: excitation-contraction coupling (Fig. 2).

During relaxation, free cytoplasmic Ca²⁺ must decline to allow Ca²⁺ to dissociate from troponin leading to relaxation of the contractile element. This Ca²⁺ transport is facilitated by a Ca²⁺-ATPase (SERCA) on the SR which transports Ca²⁺ back into the SR and the NCX on the sarcolemma [8]. SERCA is an active Ca²⁺ pump whose activity is controlled by the phosphorylation status of phospholamban (PLN). When certain residues on PLN are not phosphorylated, SERCA activity is inhibited but this inhibition is relieved when PLN becomes phosphorylated by PKA. Activators of PKA, such as β-adrenergic stimulation, can therefore play a role in relaxation, as more Ca²⁺ is restored in the SR because of higher SERCA activity. This, in turn, renders more Ca²⁺ available for CICR in the subsequent beats which results in a stronger force of contraction.
The NCX on the sarcolemma exchanges three Na\(^+\) ions for one Ca\(^{2+}\) ion. This exchange generates an electrical current that can go in both directions and is dependent on the [Na\(^+\)] and [Ca\(^{2+}\)] across the sarcolemma as well as the membrane potential. Whether the current is in the forward or reversed mode depends on the driving force for NCX. High [Ca\(^{2+}\)]
 favours forward \(I_{Na/Ca}\) whereas high [Na\(^+\)] and positive membrane potential favours reversed \(I_{Na/Ca}\) \[8\].
**Arrhythmias: abnormal excitation**

Triggered arrhythmias

In hypertrophy and heart failure, $\text{Ca}^{2+}$ handling is disturbed. As has been shown in several models, functional expression of SERCA is reduced whereas activity of the NCX is increased $[5, 8]$. Moreover, kinetics of RyR openings are also changed, leading to unanticipated $\text{Ca}^{2+}$ releases that can initiate EADs and DADs (Fig. 3). They are defined as: oscillations that attend (EADs) or follow (DADs) the cardiac AP and respond to preceding activation for their manifestation $[9]$. When the amplitude of the depolarisation reaches threshold, triggered activity in the form of ectopic beats occurs.

**DADs**

DADs arise after full repolarisation of the myocyte. $\text{Ca}^{2+}$ release from the SR is triggered when free SR $[\text{Ca}^{2+}]$, $([\text{Ca}^{2+}]_{\text{SR}})$, reaches a certain threshold leading to opening of RyRs (Fig. 4) $[10]$. $[\text{Ca}^{2+}]_{\text{SR}}$ is influenced by total $[\text{Ca}^{2+}]$, and the activity of SERCA. Increased $[\text{Ca}^{2+}]_{\text{SR}}$ load due to higher SERCA activity, for example via $\beta$-adrenergic stimulation, brings the $[\text{Ca}^{2+}]_{\text{SR}}$ closer to the threshold for SR leak. Furthermore, the threshold is affected by the RyR open probability: if the open probability increases, the threshold lowers. The open probability of RyR is modulated by $[\text{Ca}^{2+}]_{\text{SR}}$, $[\text{Ca}^{2+}]$, AP, RyR phosphorylation and the stabilising protein FKBP12.6 (Calstabin) $[11, 12]$. RyR phosphorylation is among others executed by CaMKII, and this increases the open probability of RyR $[13, 14]$. Opening of multiple RyRs creates $\text{Ca}^{2+}$ sparks, which can lead to DADs via creation of the transient inward current ($I_{\text{it}}$) by the NCX (Fig. 3) $[15]$. If the DADs reach threshold a new AP arises. In heart failure, $[\text{Ca}^{2+}]_{\text{SR}}$ and threshold are both lowered (Fig. 4) yet threshold is affected more than $[\text{Ca}^{2+}]_{\text{SR}}$ leading to a higher occurrence of triggered arrhythmias in these patients $[16]$. 

**EADs**

EADs are generated in the ventricle during phase 2 or phase 3 of the AP, and can occur during prolongation of AP duration (APD) via a window current of the LTCC (Fig. 3) $[9, 17]$. However, also NCX plays a role in EAD formation in a mode...
which is comparable with that as described for DADs. This indicates that EAD formation can occur via two routes, namely a sarcolemmal (LTCC) and an SR (RyR and NCX) dependent mechanism [18]. Whether the LTCC window or NCX has a dominant role in creating EADs depends on the disease setting; it has been suggested that when oxygen radicals are involved LTCC seems dominant, whereas in a β-adrenergic setting NCX may be dominant [18]. Either way NCX and LTCC are both important players in the generation of EADs. In addition, an increase in \( \text{INa-Late} \) leads to higher \([\text{Na}^+]_i\), which pushes NCX in its reverse mode and \( \text{Na}^+ \) is transported out and \( \text{Ca}^{2+} \) into the cell, also leading to increased \([\text{Ca}^{2+}]_i\) [19]. Via the aforementioned mechanism, this in turn can lead to EAD formation again, as is shown in Fig. 3.

**Old strategies**

Antiarrhythmic drugs are still classified according to the Vaughan Williams classification. The underlying molecular strategy includes class I: \( \text{Na}^+ \) channel block; class II: \( \beta \)-adrenergic receptor block; class III: \( \text{K}^+ \) channel block; class IV: \( \text{Ca}^{2+} \) channel block; and class V: \( \text{Na}^+/\text{K}^+ \) ATPase block [20]. Drugs categorised into these various classes all have the capability of terminating arrhythmias via different strategies. These strategies are reviewed elsewhere [21]. Only class II antiarrhythmic drugs, the \( \beta \)-adrenergic receptor blockers, have proved to reduce mortality [21, 22]. One of the drawbacks of the existing drugs is that most of them temper cardiac performance leading to a negative haemodynamic effect in patients. For example, class I drugs such as flecainide are used in supraventricular arrhythmias but are negative inotropic and also have proarrhythmic effects, which are likely due to excessive conduction slowing and reentry like tachyarrhythmias [20, 21]. Class III drugs prolong the AP, thereby increasing the refractory period, meanwhile terminating arrhythmias based on short APDs such as atrial fibrillation. On the other hand, APD prolongation is a well-established risk factor for long-QT arrhythmias and can lead to ectopic activity through EADs (Fig. 3). Class IV agents, due to their \( \text{Ca}^{2+} \) channel blocking properties, have negatively inotropic, chronotropic, and dromotropic effects. Class V agents such as digitalis lead to high \([\text{Na}^+]_i\), which will activate NCX thereby triggering \( \text{Ca}^{2+} \) overload, and associated ectopic activity (Fig. 3). New antiarrhythmic strategies attempt to find ways to increase antiarrhythmic efficacy while relieving or omitting the adverse side effects.

**New targets for antiarrhythmic treatment**

Drugs that interfere with CaMKII, NCX, RyR and its associated stabilising protein FKBP12.6, and the \( I_{\text{Na-Late}} \) are potential targets that are currently being investigated in order to
achieve clinical applicability. The focus is on interfering with calcium handling of the cardiomyocytes and to become active in the prevention or suppression of VTs. To date, interference on SERCA has not resulted in drug candidates with potential clinical applicability. Since SERCA function in heart failure is considered to be depressed (as observed in several preclinical models of heart failure), it is to note that a non-pharmacological, but viral approach is currently under consideration to restore SERCA function under conditions of heart failure [23].

Acute modulation of antiarrhythmic targets

NCX

NCX and SERCA are the most important players during Ca\(^{2+}\) removal from the cytoplasm of the cardiomyocyte. During depolarisation under physiological conditions, NCX contributes marginally to the total inward Ca\(^{2+}\) current but most of the time the channel is in its forward mode extruding Ca\(^{2+}\) from the cell creating a \(I_n\) (proarrhythmic) [24].

NCX inhibition

Known inhibitors of NCX are KB-R7943 and SEA-0400. However, KB-R7943 also inhibits LTCC, \(I_K\), and \(I_K1\) [25, 26], and thereby KB-R7943 is less specific than SEA-0400 (Table 1) [26]. Blocking NCX theoretically leads to Ca\(^{2+}\) accumulation and increased SR Ca\(^{2+}\) load, which in turn could lead to adverse effects such as Ca\(^{2+}\) sparks [28]. Nonetheless, due to the fact that SEA-0400 probably simultaneously inhibits LTCC, thereby inducing negative inotropic effects, this counteraction possibly preserves cardiac output. Moreover, NCX has a profound role in EAD formation (see section above). Therefore, blocking of NCX potentially exerts antiarrhythmic effects [27]. Recently, our group showed that SEA-0400 effectively prohibited torsades des pointes (TdP) arrhythmia and EAD formation and, as important, without the occurrence of the negative inotropic effects that are typically observed when LTCC is blocked alone [27].

Antiarrhythmic approaches using NCX block do not, however, provide a uniform and straightforward answer about the effectiveness of this strategy (Table 1). In ventricular myocytes SEA-0400 showed different effects in EADs in two different models, although it efficiently inhibited EADs in both settings [18]. In another study, using canine isolated Purkinje fibres, EAD amplitude and DAD incidence were also suppressed by SEA-0400 [30]. Similarly, in Langendorff perfused rabbit hearts, SEA-0400 administration (in the presence of sotalol or verapamil in order to induce TdP) shortened APD and the incidence of EADs [32], thereby reducing the incidence of arrhythmias [33].

In contrast, in Langendorff perfused rabbit hearts, Farkas et al. observed no antiarrhythmic effects when TdPs where induced using dofetilide [31]. In guinea pigs, no effect of SEA-0400 on arrhythmogenicity was found, and no effect on AP configuration was noticed [35]. On the other hand, in dogs, SEA-0400 did not change heart rate or blood pressure, which are promising findings [27]. Moreover, a 2007 study showed that SEA-0400 had no effects on inotropy and could reverse the positive inotropic effects of Ouabain in isolated guinea pig myocardium [33]. Nevertheless, no antiarrhythmic outcome was found in ischaemia-reperfusion induced arrhythmias.

Concerning safety, SEA-0400 decreased arrhythmias based on intoxication with digitals, but also caused AV block and cardiac arrest in a small number of the dogs [34]. Due to lack of consensus found in these results more research should be performed before it can be concluded whether or not NCX block has potency in clinical use.

RyR

RyR, and its function in cardiac physiology, has been extensively reviewed by Kushnir and Marks [37–39]. Whether or not RyR opens to release Ca\(^{2+}\) depends on the open probability of RyR and the SR Ca\(^{2+}\) load, which are influenced via various mechanisms, as described earlier.

RyR inhibition

Spontaneous release of Ca\(^{2+}\) by RyR is involved in the generation of triggered activity (Fig. 3a), and as such RyR is an interesting target in antiarrhythmic therapy that would be based on a decreased risk of DAD formation. This spontaneous release of Ca\(^{2+}\) is causative for arrhythmias as found in a disease named catecholaminergic polymorphic ventricular tachycardia (CPVT). Mutations in RyR can enhance the susceptibility for Ca\(^{2+}\) leak, especially under conditions with an increased adrenergic drive. Flecainide, a well-established class-Ic antiarrhythmic, showed additional and remarkable efficacy to suppress these arrhythmias both in mice and humans through enhancement of the threshold for triggered activity [40, 41]. It remains to be discussed what the exact contribution is of sodium channel block and RyR inhibition in this preventive effect.

K201 (JTV-519) is a more recent compound that has been tested as a potential inhibitor of RyR. It decreased spontaneous Ca\(^{2+}\) release at 1 μM by binding to FKBP12.6, thereby increasing its affinity for RyR and stabilisation of the closed conformation of RyRs [42, 43]. K201 has, however, various other effects on different sarcolemmal ion channels (a multi-channel blocker) [1], see Table 2. Previous experiments have not been able to confirm consistent antiarrhythmic effects of K201 (Table 2). Of the positive studies, K201 prevented the
| Target  | Action   | Model                      | Dose       | Block        | Author                    |
|---------|----------|----------------------------|------------|--------------|---------------------------|
| ICX     | Inhibition | Ventricular myocytes       | 1 μM       | Forward 82.5% | Tanaka et al. 2002 [25]  |
| ICX     | Inhibition | CAVB myocytes              | 1 μM       | Forward 50%  | Bourgonje et al. 2013 [27]|
| ICX     | Inhibition | Ventricular myocytes       | 0.3–1 μM  | Forward 50%  | Ozdemir et al. 2008 [28] |
| ICX     | Inhibition | Ventricular myocytes       | 1 μM       | Reverse 60%  | Birinyi et al. 2005 [29] |
| ICa,L   | Inhibition | Cardiac tissue             | 1 μM       | 3%           | Nagy et al. 2004 [30]    |
| ICa,L   | Inhibition | Ventricular myocytes       | 0.3–1 μM  | 25%          | Ozdemir et al. 2008 [28] |
| ICa,L   | Inhibition | Ventricular myocytes       | 1 μM       | 9%           | Tanaka et al. 2002 [25]  |
| ICa,L   | Inhibition | CAVB myocytes              | 1 μM       | 33%          | Bourgonje et al. 2013 [27]|

Model

| In vivo animal model (CAVB dog) | SEA-0400 | After challenge | 0.4 and 0.8 mg/kg | 0.4 mg/kg decreased TdP episodes 7±4 → 3±4 0.8 abolished TdP incidence | Bourgonje et al. 2013 [27] |
| Single rabbit ventricular myocytes | SEA-0400 | After challenge | 2 μM       | Abolished EADs | Zhao et al. 2012 [18] |
| Langendorff perfused rabbit hearts (dofetilide induced arrhythmias) | SEA-0400 | After challenge | 1 μM       | No effect on TdP incidence | Farkas et al. 2009 [31] |
| Langendorff perfused rabbit hearts (sotalol/veratridine induced arrhythmias) | SEA-0400 | After challenge | 1 μM       | TdP incidence ↓ (16/18 → 1/18, sotalol, 6/13 →0/13 veratridine) | Milberg et al. 2008 [32] |
| Isolated guinea pig myocardium (Ouabain induced arrhythmias) | SEA-0400 | Co-administration | 1 μM       | Arrhythmic contractions ↓ (19/26 → 12/26) | Tanaka et al. 2007 [33] |
| In vivo animal model (dog ischaemia/reperfusion model and digitalis induced arrhythmias) | SEA-0400 | Prior and co-administration (I/R) and After challenge (Digitalis) | 0.3–3 mg/Kg | Did not change haemodynamics. No antiarrhythmic effect (I/R). Arrhythmic ratio ↓ (digitalis) | Nagasawa et al. 2005 [34] |
| Isolated dog Purkinje fibres (dofetilide induced arrhythmias) | SEA-0400 | After challenge | 1 μM       | EAD amplitude ↓ (26.6±2.5 → 14.8±1.8 mV) DAD incidence ↓ (6/6 → 3/6) | Nagy et al. 2004 [30] |
| In vivo animal model, guinea pigs (aconitine induced arrhythmias) | SEA-0400 | Prior to challenge | 1–10 mg/Kg | Ineffective in suppressing triggered activity | Amran et al. 2004 [35] |

Overview of studies on isolated cardiomyocytes, tissue preparations, whole hearts, and intact animals. EAD early after depolarisation. TdP torsade de pointes arrhythmia. I/R ischaemia reperfusion model. DAD delayed after depolarisation.
Table 2  
K201 effects on ion currents in cardiomyocytes and experimental models focusing on antiarrhythmic properties of K201s

| Target | Action | Model | Dose          | Block | Author                      |
|--------|--------|-------|---------------|-------|-----------------------------|
| I₅Na  | Inhibition | Ventricular cardiomyocytes (guinea pig) | 1.2 μM | 50 % | Kimura et al. 1999 [44]     |
| I₅K₁  | Inhibition | Ventricular cardiomyocytes (guinea pig) | 5 μM | 50 % | Kimura et al. 1999 [44]     |
| I₅Ca  | Inhibition | Ventricular cardiomyocytes (guinea pig) | 3 μM | 50 % | Kimura et al. 1999 [44]     |
| I₅Ca  | Inhibition | Ventricular cardiomyocytes (rabbit)    | 3 μM | 34 % | Loughrey et al. 2007 [43]   |
| I₅Kr  | Inhibition | Ventricular cardiomyocytes (guinea pig) | 1.2 μM | 50 % | Kiriyama et al. 2000 [45]   |
| I₅Kr  | Inhibition | Atrial cardiomyocytes (guinea pig)     | 1 μM | 50 % | Nakaya et al. 2000 [46]     |
| I₅-ACH| Inhibition | Atrial cardiomyocytes (guinea pig)     | 0.12 μM | 50 % | Nakaya et al. 2000 [46]     |

| Model | Inhibitor | Inhibitor administration | Dose | Effect | Author                      |
|-------|-----------|--------------------------|------|--------|-----------------------------|
| In vivo rat isoproterenol or I/R induced | K201 | Prior to challenge       | 1 mg/kg | Incidence of arrhythmia ↓ 9/10 → 2/10 (isoproterenol) 14/14 → 7/15 (I/R) | Ouni et al. 2013 [47] |
| In vivo dog (CAVB dog) | K201 | Prior to and during challenge | 0.1 and 0.3 mg/kg/2 min followed by 0.01 and 0.03 mg/kg/30 min iv | No significant anti-arrhythmic but pro-arrhythmic effects were observed | Stams et al. 2011 [48] |
| RyR<sup>R4496C</sup> myocytes +/- ouabain | K201 | Acutely upon DAD incidence, and prior to challenge | 1 μmol/L | No effect on DADs under baseline conditions. But decreased incidence of spontaneous APs during ouabain challenge | Selej et al. 2010 [49] |
| Pulmonary cardiomyocytes, isoprenaline (rabbit) | K201 | Prior to challenge       | 0.3 μM | Reduction in spontaneous activity | Chen et al. 2008 [50] |
| In vivo rabbit methoxamine and clofilium induced | K201 | After challenge          | 50, 200, 400 μg/kg/min | TdP incidence ↓ 6/6 → 4/6, 2/5, 0/6 (dose dependent) | Hasumi et al. 2007 [51] |
| RyR<sup>R4496C</sup> myocytes + in vivo mouse (RyR 4496C<sup>+/−</sup>) | K201 | Prior to challenge       | 1 μmol-10 μmol 18 mg/Kg per day | Failed to limit DADs or arrhythmias | Liu et al. 2006 [52] |
| Mouse whole animal (FKBP12.6<sup>−/−</sup> en FKBP12.6<sup>+/−</sup>) | K201 | Prior to challenge       | 0.5 mg/kg per hour | Prevention of arrhythmias and SCD in FKBP12.6<sup>+/−</sup> but not FKBP12.6<sup>−/−</sup> mice. | Wehrens et al. 2004 [42] |
| In vivo dog pacing induced | K201 | Prior to challenge       | 0.03 mg/kg/min | AF episodes ↓ 4.2±2.9 → 0±0 | Kumagai et al. 2003 [53] |
| Isolated guinea pig heart | K201 | After challenge          | 0.3 μM and 1 μM | Incidence AF ↓ 2/5 → 0/6 | Nakaya et al. 2000 [46] |

*DADs* delayed after depolarisations, *AP* action potential, *SR* sarcoplasmic reticulum, *SCD* sudden cardiac death. Adapted from Currie et al. [1]
frequency of spontaneous APs after Ouabain in mice with the RyR R4496C+/− mutation [49]. Wehrens et al. showed that when FKBP12.6+/− mice were treated with K201 no arrhythmias could be recorded, which was in contrast to non-treated animals that regularly showed SCD [42]. On the other hand, experiments on isolated cardiomyocytes or in intact RyR2 R4496C knock-in mice, elicited no decrease in DAD incidence upon K201 administration [52]. In the CAVB (chronic AV block) dog, K201 was not able to decrease the incidence nor the severity of dofetilide-induced TdP. In addition, K201 prolonged repolarisation and slowed heart rate, although no negative inotropic effects were observed [48]. These divergent results are in contrast with the well-established molecular role of RyR in the incidence of Ca2+ sparks and DADs and its suppression by K201.

In the mentioned dog study, our group showed that at a relatively high dose (1.5–2 μM), even proarrhythmic effects of the drug were observed [48]. Although K201 has already been used in clinical trials for treatment of atrial fibrillation (AF), the results seem disappointing: only one has been completed without publication of the results, and two other trials were prematurely terminated.

Other drugs are currently being tested or designed to allow continuation of targeting RyR. Carvedilol, a registered β-blocker, also blocks RyR and prevents spontaneous Ca2+ release at doses higher than needed for its β-blocking activity. In this respect, the development of the carvedilol analogue VK-II-86 has to be mentioned since it shows an enhanced blocking capacity compared with a potent β-blocker, this could be a promising antiarrhythmic approach although thus far no further studies have been published using these compounds in order to test their efficacy [54].

**CaMKII**

Besides its activation under pathological conditions through oxidation by reactive oxygen species (ROS), activation of CaMKII is mainly facilitated by CaM, a small cytoplasmatic protein. CaM needs to bind cytosolic Ca2+ before CaMKII can be activated [55]. For that, CaMKII activation is indirectly dependent on [Ca2+]i, but due to its capability of autophosphorylation it is not solely dependent on the rise and fall in [Ca2+]i. When phosphorylation of CaMKII has been accomplished, the enzyme becomes persistently active and therefore the natural beat-to-beat fall in [Ca2+]i will not immediately affect the enzymes’ activity. CaMKII has a central role in Ca2+ handling, influencing RyR, LTCC, and SERCA (Fig. 2). By phosphorylating RyR, CaMKII increases the open probability [12, 56]. LTCC phosphorylation by CaMKII leads to faster recovery from inactivation [57], whereas the effect of CaMKII on SERCA (via phosphorylation of PLN) leads to an increased SR Ca2+ load. CaMKII activation leads to an increase in [Ca2+]i and is therefore able to influence NCX by pushing it into the forward mode which results in a depolarising current. All of the above-mentioned actions are under physiological conditions. During cardiac pathology CaMKII expression and function is upregulated, which can trigger proarrhythmia via induction of ectopic activity [58–60]. This makes it an interesting target for antiarrhythmic intervention.

**CaMKII inhibition**

Up till now two CaMKII inhibitors are known, W7 and KN-93. KN-93 is a compound that competes with CaM for the binding site on CaMKII, and through this mode it inhibits activation of CaMKII, with an IC50 of 370 nM [61]. However, KN-93 also appears to act as a multi-channel blocker in cardiomyocytes as is depicted in Table 3. W7 is actually an inhibitor of CaM [74], and therefore considered to be an indirect inhibitor of CaMKII as well as of other targets of CaM (e.g. RyR, LTCC).

So far, CaMKII antagonists show promising experimental results (Table 3). In genetically engineered Langendorff perfused mouse hearts, W7 and KN-93 suppressed VTs in almost all mice. However, W7 appeared to prolong APD to a higher degree than KN-93 which renders the use of the more specific inhibitor KN-93 favourable in this setting [71]. In Langendorff perfused rat hearts, KN-93 effectively suppressed ventricular fibrillation (VF) without affecting APD [67], and also lowered the incidence of premature beats occurring after ischaemia reperfusion interventions [66]. Similarly, VF induced by angiotensin II was prevented by KN-93 in 75% of the hearts [65]. Furthermore, KN-93 also prevented the occurrence of EADs in Langendorff perfused rabbit hearts [62], while W-7 decreased EAD as well as TdP incidence, even though W-7 was not able to counteract the sotalol-induced APD prolongation [70].

Looking at intact animal studies, CaMKII inhibition by KN-93 was able to prevent isoproterenol-induced arrhythmias [69], as well as in other experimental setups in mice, such as aortic banding, where KN-93 also proved to be successful in preventing VTs [68]. In rabbits, pretreatment with W-7 prevented methoxamine-induced TdP [72, 73], and had no undesirable effects on haemodynamics [73]. In CAVB dogs, W-7 was successful in terminating dofetilide-induced TdP in the majority of dogs [36]. Given its antiarrhythmic potency, the minimal effects on APD and the fact that haemodynamics were virtually unaffected, CaMKII inhibition might be a favourable approach when compared with the old antiarrhythmic strategies.

**Late inward sodium current**

The total inward sodium current is composed of a peak and late component and is provided through voltage-gated Na
### Table 3

KN-93 effects on ion currents in cardiomyocytes and overview of antiarrhythmic experiments with CaMKII inhibitors W7 and KN-93

| Target  | Action | Model                          | Dose     | Block  | Author                  |
|---------|--------|---------------------------------|----------|--------|-------------------------|
| $I_{ca}$ | Inhibition | Rabbit cardiomyocytes | 1 μM     | 41 %   | Anderson et al. 1998 [62] |
| $I_K$  | Inhibition | HEK cells overexpressing Kv1.5 | 3 μM     | 50 %   | Rezazadeh et al. 2006 [63] |

| Model                          | Inhibitor | Inhibitor administration | Dose      | Effect                          | Author                  |
|--------------------------------|-----------|---------------------------|-----------|---------------------------------|-------------------------|
| In vivo animal model (mdx mice) | KN-93     | After challenge           | 1 μM      | VT incidence ↓ in presence of KN-93 (7/14 → 2/14) | Ather 2013 [64]          |
| CAVB dog (dofetilide induced)  | W7        | After challenge           | 18.87 mg/kg/5 min | Abolished almost all TdPs         | Bourgonje et al. 2012 [36] |
| Langendorff perfused rat heart (AngII induced arrhythmia) | KN-93     | Prior to challenge        | 2 μM      | VF incidence ↓ in presence of KN-93 (4/4 → 1/4) | Bapat et al. 2012 [65]   |
| Langendorff perfused rat heart (glycolytic inhibition induced arrhythmia) | KN-93     | Prior to challenge        | 2.5 μM    | Incidence of premature beats ↓ (71.5 %) | Saíd et al. 2011 [66]    |
| Whole animal (mouse RyR2-S2814D knock in and aortic banding) | KN-93     | Prior to challenge        | 30 μmol/kg | Incidence of VT ↓ (6/12 → 0/12) | Lui et al. 2011 [68]     |
| Whole animal (heart failure mouse, iso induced arrhythmia) | KN-93     | Prior to challenge        | 20 μmol/L/kg | Incidence of arrhythmias ↓ (5/6 → 0/4) | Sag et al. 2009 [69]     |
| Langendorff perfused rabbit heart | W7        | Co-administration with challenge | 20, 50, 100 μM | EAD incidence ↓ (9/9 → 1/9) TdP incidence ↓ (7/9 → 1/9) | Pu et al. 2005 [70]      |
| Langendorff perfused mouse heart | W7, KN-93 | After challenge           | W7: 25 μM KN-93: 2 μM | W7: pVT incidence ↓ (7/11 → 1/11) KN93: pVT incidence ↓ (5/8 → 1/8) | Kirchhof et al. 2004 [71] |
| In vivo animal model (methoxamine rabbit model) | W7        | Prior to challenge        | 50 μM/kg  | TdP incidence ↓ (12/14 → 1/11) | Gbadebo et al. 2002 [72] |
| In vivo animal model (methoxamine rabbit model) | W7        | Prior to challenge        | 25, 50 μM/kg | TdP incidence ↓ (6/8 → 1/7) | Mazur et al. 1999 [73]   |
| Langendorff perfused rabbit heart | KN-93     | Prior to challenge        | 0.5 μM    | EAD Incidence ↓ (8/8 → 4/10) | Anderson et al. 1998 [62] |

AV = atrioventricular node; TdP = torsades des pointes; AngII = angiotensin II; VT = ventricular tachycardia; VF = ventricular fibrillation; iso = isoproterenol; EAD = early after depolarisation; pVT = polymorphic VT
channels in the sarcolemma, which are rapidly activated and inactivated. Under physiological conditions, the $I_{\text{Na-Late}}$ has a much smaller amplitude than peak $I_{\text{Na}}$. Under pathological circumstances, however, the $I_{\text{Na-Late}}$ can be elevated up to 5 times, and substantially affect total $I_{\text{Na}}$, leading to accumulation of Na+ in the cytosol [75]. On its own, increased [Na+]i leads to APD prolongation, and by pushing NCX in the reverse mode it can lead to increased [Ca$^{2+}$]i, and triggered arrhythmias.

Late $I_{\text{Na}}$ inhibition

Due to its previously described role in arrhythmias, inhibition of $I_{\text{Na-Late}}$ is a topic of interest because it can possibly prevent EAD and DAD triggered activity. Ranolazine is a well-known inhibitor of $I_{\text{Na-Late}}$ but its actions are not restricted to this channel as it evokes multiple other effects (Table 4). In Table 5, we have listed its antiarrhythmic properties. In Langendorff perfused guinea pig hearts, ranolazine abolished and prevented ATX-II induced (pro) arrhythmic features on APD, EADs and VTs in the ‘LQT-3 syndrome’ [84]. In guinea pig isolated ventricular myocytes, ranolazine was able to counteract the arrhythmic effects, such as APD prolongation and subsequent EAD formation [82, 86]. In canine wedge preparations, ranolazine also effectively prevented TdP and EADs [76], and in rats it showed various antiarrhythmic properties [77, 80, 85]. Even in clinical trials, ranolazine showed its capability of reducing arrhythmic events [81]. Ranolazine is already being prescribed to treat patients with chronic angina and, importantly, in a clinical trial a significant decrease in the occurrence of ventricular arrhythmias was observed compared to controls [81].

Recently, data have been published which suggest that sophocarpine also possesses $I_{\text{Na-Late}}$ inhibiting potency next to its effect on other currents ($I_{\text{Kc}}, I_{\text{Ca}}$) [90], GS-967 is another new compound that is proposed to inhibit late $I_{\text{Na,Late}}$ specifically (it affects peak $I_{\text{Na}}$ and $I_{\text{Kc}}$, only weakly at much higher doses). This new compound has been tested in animal models with positive findings [87, 88], which makes it an interesting and potentially antiarrhythmic compound for future testing in a clinical setting.

### Table 4 Ranolazine effects on ion currents in cardiomyocytes

| Target | Action | Model           | Dose    | Block       | Author               |
|--------|--------|-----------------|---------|-------------|----------------------|
| $I_{\text{Na}}$ | Inhibition | Canine wedge preparations | 6 μmol/L | 50 % | Antzelevitch 2004 [76] |
| $I_{\text{Ca}}$ | Inhibition | Canine wedge preparations | 2–6 μmol/L | 25–30 % | Antzelevitch 2004 [76] |
| $I_{\text{Kc}}$ | Inhibition | Canine wedge preparations | 20 μmol/L | 17 % | Antzelevitch 2004 [76] |
| $I_{\text{Na-Ca}}$ | Inhibition | Canine wedge preparations | 50 μmol/L | 50 % | Antzelevitch 2004 [76] |
| $I_{\text{K}}$ | Inhibition | Canine wedge preparations | 12 μmol/L | 50 % | Antzelevitch 2004 [76] |

**Conclusion**

Old antiarrhythmic strategies, though proven clinically effective, have important drawbacks such as negative haemodynamic effects and a small safety margin, thereby prohibiting the use of higher dosages [21]. To improve specificity and efficacy of pharmacological intervention, there is a continuous quest for new pharmacological targets. Calcium handling within the cardiomyocytes is such an important target since disturbed calcium handling has a maladaptive and dual effect that leads to both an increased propensity to develop arrhythmias and to induction of contractile dysfunction. In the current study we have reviewed the recent investments made to target NCX, RyR, CaMKII and the late sodium current, all being involved in proarrhythmia due to disturbed calcium handling. Figure 5 provides a schematic overview of the current knowledge regarding the potency and potential efficacy of drugs affecting these new antiarrhythmic targets.

NCX So far, blocking of NCX, using SEA-0400, appears to be neutral on inotropy due to the counteracting effects of 1) anticipated positive inotropy by NCX block alone and 2) negative haemodynamics by its additional inhibition of LTCC [27, 36]. Taking into account that this compound is possibly totally specific for NCX, it shows a neutral haemodynamic effect but so far did not prove to be antiarrhythmic in all experimental studies. The pharmacological industry has no plans for further exploration in this direction and therefore the future of this drug as an antiarrhythmic agent is uncertain. Development of new NCX blockers is, however, foreseen and could become tools for future clinical applicability.

RyR The antiarrhythmic potential of RyR block remains debatable, as positive results of K201 are scarce and counteracted by negative results (Table 2). Together with the possible proarrhythmic effects at the high dosage that is required for treatment of atrium fibrillation, the lack of convincing antiarrhythmic results, and various effects on other ion currents, K201 may not be the best suitable future antiarrhythmic agent. For this reason, development of a more selective inhibitor is required to assess its antiarrhythmic properties.
| Model | Inhibitor | Inhibitor administration | Dose | Effect | Author |
|-------|-----------|--------------------------|------|--------|--------|
| Langendorff perfused rat hearts (rapid pacing induced VF and oxidative stress induced VF) | Ranolazine | Prior to challenge | 10 μM | Pacing induced VF shortening > 3 min → 12±6 s Oxidative stress induced VF termination and suppression | Morita 2011 [77] |
| Transgenic CaMKII mice papillary muscles | Ranolazine | After challenge | 5 μmol/L | | |
| CAVB dog, dofetilide induced | Ranolazine | After challenge | 4 mg/kg/0.5 min + 0.225 mg/kg/min | Termination of premature arrhythmogenic contractions | Sossalla 2011 [78] |
| In vivo animal model (rats I/R induced arrhythmias and ischemia induced arrhythmias) | Ranolazine | After challenge (I/R) | 10 mg/kg iv bolus (I/R) | Sustained VT incidence ↓ 9/12 vs. 1/11 (I/R) VF incidence ↓ 10/12, 8/12, 5/10, 4/12 (control, 2, 8, 10 μM Ranolazine resp.) | Dhalla 2009 [80] |
| Clinical trial | Ranolazine | Prior to challenge | Reduced the incidence of VT vs placebo | Scirica 2007 [81] |
| Rabbit and guinea pig isolated ventricular myocytes H2O2 challenge | Ranolazine | After challenge | 10 μM | Suppression of APD prolongation and EAD formation | Song 2006 [82] |
| Canine myocytes of normal and HF dogs | Ranolazine | After challenge | 5, 10, 20 μM | Shortening of APD and suppression of EADs | Undrovinas 2006 [83] |
| Langendorff perfused guinea pig hearts. ATX-II induced arrhythmias | Ranolazine | Both | 5 μM | Ranolazine abolished ATX-II induced EADs/VTs and prevented ATX-II induced EADs/VTs in pretreated hearts | Wu 2004 [84] |
| Langendorff perfused rat hearts I/R ATX-II challenge | Ranolazine | Prior to challenge | 4 μM, 9 μM in perfusate | Reduced Ca2+ overload and LV mechanical dysfunction | Fraser 2006 [85] |
| Isolated canine wedge preparations, M cells and Purkinje fibres | Ranolazine | Prior to challenge | 1–100 μmol/L | Abolished TdP and EADs | Antzleveitch 2004 [76] |
| Isolated guinea pig ventricular myocytes ATX-II challenge | Ranolazine | After challenge | 0.1–30 μmol/L | Reduced ATX-II induced EADs | Song 2004 [86] |
| Canine Purkinje fibres E-4031, ATX-II and high Ca+ isoproterenol induction | GS-967 | After challenge | 30 nM/100 nM | EAD and DAD incidence ↓ EAD 4/4 → 2/5 + 0.5 (E-4031) EAD 4/4 → 1/4 → 0/4 (ATX-II) DAD 4/4 → 2/4 → 0/5 (high Ca + isoproterenol) | Sicouri et al. 2013 [87] |
| Langendorff perfused rabbit heart ATX-II and E-4031 induction | GS-967 | After challenge | 100 and 600 nM/100 nM (ATX-II and E-4031 resp.) | Incidence of VT ↓ 6/11 → 0/11 (ATX-II) 5/5 → 0/5 (E-4031) | Belardinelli et al. 2013 [88] |
| In vivo animal model (rabbits clofilium/methoxamine and ischaemia induced) | GS-967 | Prior to challenge | 60 μg/kg bolus + 16 μg/kg/min (clofilium) | Incidence VT ↓ 5/6 → 1/6 (clofilium) 5/10 → 2/8 (ischemia) | Belardinelli et al. 2013 [88] |
| Langendorff perfused guinea pig heart isoprenaline induction | Sophocarpine | After challenge | 300 μmol/L | Incidence VT ↓ 6/6 → 0/6 | Yang et al. 2011 [89] |

EAD early after depolarisation. TdP torsade de pointes arrhythmia. I/R ischaemia reperfusion model. VT ventricular tachycardia. A H2O2 challenge mimics oxidative stress.
CaMKII In Table 3, studies on CaMKII inhibition using either W-7 or KN-93 in intact animals and Langendorff perfused hearts are summarised, mostly showing a profound antiarrhythmic effect of the drugs. Also in various experimental settings using isolated cardiomyocytes, the antiarrhythmic properties of CaMKII inhibition are readily observed [36, 91]. Additionally important, blocking CaMKII provides antihypertrophic effects and maintenance of LV function [1]. The clinical use of W-7 and KN-93 is still under consideration. The currently used and obviously attractive drugs are not completely target specific, and long-term inhibition of CaMKII with these drugs induces neurotoxicity [92] which makes them less favourable [93]. Current development of new entities by several industries is expectantly awaited by the scientific community.

Late INa inhibition The use of ranolazine has also shown positive results: prevention and suppression of EADs, and VT/VF in several experimental approaches. Important aspect is that the drug is already registered and clinically applicable. Its antiarrhythmic efficacy is not complete, which opens the road for improvement using this approach. In this line of intervention, promising new drugs such as sophocarpine and GS-967 are being investigated.

Overall, regardless of the current possibilities for pharmacological interventions, it is clear that new strategies will be
welcome to prevent SCD due to arrhythmias. The new targets discussed here prove to be interesting strategies for the future and provide food for thought. Obviously, more research with respect to the long-term effects of chronically blocking each of the discussed targets is essential before research can proceed to a more clinical setting.

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