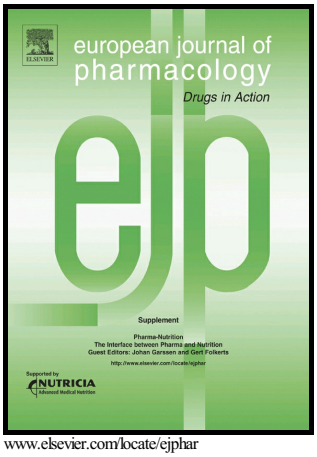

www.elsevier.com/locate/ejphar

Role of the dysfunctional ryanodine receptor - Na^{+}-Ca^{2+}exchanger axis in progression of cardiovascular diseases: what we can learn from pharmacological studies?

Károly Acsai, Balázs Ördög, András Varró, Péter P. Nánási

PII: S0014-2999(16)30137-6
DOI: http://dx.doi.org/10.1016/j.ejphar.2016.03.016
Reference: EJP70518

To appear in: *European Journal of Pharmacology*

Received date: 27 October 2015
Revised date: 4 March 2016
Accepted date: 7 March 2016

Cite this article as: Károly Acsai, Balázs Ördög, András Varró and Péter P. Nánási, Role of the dysfunctional ryanodine receptor - Na^{+}-Ca^{2+}exchanger axi in progression of cardiovascular diseases: what we can learn fron pharmacological studies?, *European Journal of Pharmacology* http://dx.doi.org/10.1016/j.ejphar.2016.03.016

Role of the dysfunctional ryanodine receptor - Na^+-Ca^{2+} exchanger axis in progression of cardiovascular diseases: what we can learn from pharmacological studies?

Károly Acsai[a], Balázs Ördög[b], András Varró[a,b], Péter P. Nánási[c,d]

[a]MTA-SZTE Research Group of Cardiovascular Pharmacology, Szeged, Hungary
[b] Department of Pharmacology and Pharmacotherapy, University of Szeged, Faculty of Medicine, Szeged, Hungary
[c]Department of Physiology, University of Debrecen, Debrecen, Hungary
[d]Department of Dentistry, University of Debrecen, Debrecen, Hungary

Correspondence:
Péter P. Nánási
Department of Physiology, University of Debrecen, H-4012 Debrecen, Nagyerdei krt 98, Hungary

Phone: 36-52-255575
FAX: 36-52-255116
E-mail: nanasi.peter@med.unideb.hu

Abstract

Abnormal Ca^{2+} homeostasis is often associated with chronic cardiovascular diseases, such as hypertension, heart failure or cardiac arrhythmias, and typically contributes to the basic ethiology of the disease. Pharmacological targeting of cardiac Ca^{2+} handling has great therapeutic potential offering invaluable options for the prevention, slowing down the progression or suppression of the harmful outcomes like life threatening cardiac arrhythmias. In this review we outline the existing knowledge on the involvement of malfunction of the ryanodine receptor and the Na^{+}-Ca^{2+} exchanger in disturbances of Ca^{2+} homeostasis and discuss important proof of concept pharmacological studies targeting these mechanisms in context of hypertension, heart failure, atrial fibrillation and ventricular arrhythmias. We emphasize the promising results of preclinical studies underpinning the potential benefits of the therapeutic strategies based on ryanodine receptor or Na^{+}-Ca^{2+} exchanger inhibition.

Keywords: Ca^{2+} homeostasis, intracellular Ca^{2+} concentration, Na^{+}-Ca^{2+} exchanger, ryanodine receptor, heart failure, cardiac arrhythmia.

Abbreviations

RyR2: ryanodine receptor Ca^{2+}-release channel type 2

NCX1: Na^{+}-Ca^{2+}-exchanger type 1

SR: sarco(endo)plasmic reticulum

TRPC6: Transient receptor potential-like channel 6

NKA: Na^{+}-K^{+}-ATP-ase

ATP: adenosine-triphosphate

CamKII: Ca^{2+}/calmodulin-dependent protein kinase II

I_{Ca} : L-type Ca^{2+}-current

EAD: Early afterdepolarization

DAD: Delayed afterdepolarization

TdP: torsades de pointes tachycardia

1. Introduction

Ca^{2+} as a universal messenger is involved in the regulation of many physiological processes. In the heart Ca^{2+} plays a central role in the excitation-contraction coupling and also in the diastolic and systolic function of cardiomyocytes. In addition, Ca^{2+} signals are important in orchestrating the development, growth and differentiation of cardiac cells. Proper Ca^{2+} handling, therefore, is critically important in the maintenance of normal physiological function. Gene expression levels and activity of kinases and phosphatases, ion channels, exchangers and transporters are typically modulated by Ca^{2+} signals. High dynamic gain, the ability of rapid propagation and the precise spatial and temporal regulation are unique features of these Ca^{2+} signals, but at the same time, they also represent important requirements for Ca^{2+} homeostasis to be meet in order to maintain normal function. Disturbance to any aspect of Ca^{2+} handling, on the other hand, leads to suboptimal function and is often associated with chronic diseases, such as hypertension, vascular and cardiac hypertophy, or heart failure. While the mechanistic details of Ca^{2+} homeostasis are being unraveled, pharmacological targeting of malfunctional Ca^{2+} handling has been considered to be a very attractive and promising therapeutic strategy for a long time.

In this review, we focus on two key components of the Ca^{2+} handling: the cardiac-specific isoform of the ryanodine-receptor-Ca^{2+} release complex (RyR2), an important source of Ca^{2+} from the sarco-endoplasmic reticulum (SR) and the Na^{+}-Ca^{2+} exchanger type 1 (NCX1), which is abundantly expressed in the cardiovascular system. NCX1 is the major Ca^{2+} removal mechanism when operating in forward mode, while it is also involved in regulatory Ca^{2+} influx through its reverse mode activity. We will show that dysregulation of the RyR-NCX axis plays a central role in cardiovascular pathology, leading to Ca^{2+} mishandling and generation of pathological Ca^{2+} signals, which may further worsen the diseased state. Although the experimental pharmacology is quite promising in this field, the question whether pharmacological targeting of RyR or NCX function might be beneficial in the human therapy remains to be clarified. By discussing important proof of concept studies, we aim to evaluate the therapeutic potential of NCX and RyR inhibition in cardiovascular diseases.

2. Role of NCX in the development of hypertension

Hypertension is one of the most prevalent cardiovascular diseases. Its most dangerous features are that its damaging effect on various organs often remains hidden for a long period of time and it represents an important risk factor for the development of many cardiovascular diseases, including atherosclerosis, ischemic heart disease, atrial fibrillation, heart failure and renal diseases. In spite of the multitude of anti-hypertension drugs, many hypertensive patients are left uncontrolled. Therefore, the identification of novel compounds and novel therapeutic targets is still a priority.

Involvement of the reverse NCX1 activity as a Ca^{2+}entry mechanism in maintaining the vascular tone has long been recognized (Slodzinski and Blaustein, 1998). Indeed, through co-localization and/or functional coupling with membrane receptors, ion channels and intracellular messengers, NCX appears to play a role in integrating the different vasoactive neurohormonal signals (Syyong et al., 2007). Co-localization of different transporters in functional micro- or nanodomains allows the Ca^{2+} influx or efflux via NCX to respond to the local changes in submembrane Ca^{2+} and Na^{+} concentration (Lynch et al., 2008; Poburko et al., 2008). For example, NCX is co-localized with the transient receptor potential-like channel 6 (TRPC6), a non-specific cation channel, through which the Na^{+} influx and the elevated local Na^{+} concentration can increase the reverse mode NCX activity, resulting in net Ca^{2+} influx (Pulina et al., 2013). A further peculiarity of this pathway is the proximity of NCX and the sarco(endo)plasmic reticulum Ca^{2+}-ATP-ase, that finally allows the Na^{+} influx to regulate the SR Ca^{2+} content (Kuszczak et al., 2010). Similar coupling has been described between NCX and the alpha2 isoform of the Na^{+}-K^{+}-ATP-ase (NKA) (Zhang et al., 2005) (Hauck and Frishman, 2012), which has been shown to participate in the regulation of vascular tone and development of hypertension induced by endogenous ouabain (Blaustein et al., 2012).

The role of the subcellular anatomical and functional coupling between the Na^{+} and Ca^{2+} handling transporters appears to be augmented during the development of hypertension. Hamlyn et al. showed that the increased ouabain level may contribute to the chronic hypertensive effect of angiotensin-II, by increasing NCX and TRPC6 expression, which in turn shifts vascular ionic homeostasis towards a higher vascular tone and responsiveness (Hamlyn et al., 2014). In line with this, elevated expression of NCX and other ion channels involved in Ca^{2+} homeostasis, such

as TRPC6, and the concomitant augmentation of Ca^{2+} signaling has been documented in animal models of hypertension (Taniguchi et al., 2004; Zulian et al., 2010).

Thus, based on the central role of NCX, decoupling of the pathological neurohormonal activation and the intracellular Ca^{2+} signaling by inhibition of the Ca^{2+} influx via reverse NCX seems to be a logical way to prevent or treat hypertension.

3. Pharmacological evidence for the antihypertensive effect of NCX inhibition

As we have seen above, increased NCX activity is an inevitable factor in hypertension, which makes evaluation of pharmacological NCX inhibition in antihypertensive therapy logical. Because of the multiple roles of NCX in the vascular system, possible antihypertensive effect of pharmacological NCX inhibition can only be evaluated by *in vivo* trials. However, the number of studies addressing this issue are surprisingly sparse, although even studies with negative results would be equally important for the antihypertensive research.

The first study showing pharmacological evidence for the involvement of reverse NCX in hypertension has been done using a rodent model of Cushing's syndrome. In this work the adrenocorticotropic hormone-induced hypertension has been effectively prevented by chronic administration of KB-R7943, a reverse NCX inhibitor (Dostanic-Larson et al., 2005).

Excessive dietary salt intake is known to be an important factor in the development of hypertension (Blaustein et al., 2012). In this case the increased Ca^{2+} influx to the smooth muscle cell results from the increased Na^{+} load-induced shift of NCX function towards its reverse-mode activity. Thus, its pharmacological inhibition should be logically antihypertensive. Indeed, selective knock-out of NCX in smooth muscle has been shown to prevent the development of angiotensin-II-dependent salt-induced hypertension (Wang et al., 2015). In spite of the clear-cut pathology in this case, only a few pharmacological studies attempted to explore this therapeutic possibility. Chronic administration of SEA0400 exerted a significant antihypertensive effect in different models of salt-dependent hypertensive rats, including deoxycorticosterone acetate-salt hypertensive, salt-loaded Dahl salt-sensitive, and salt-loaded spontaneously hypertensive rats (Iwamoto et al., 2004). Furthermore SEA0400 was able to reduce vascular hypertrophy and renal dysfunction, factors that exacerbate the progression of hypertension. In acute experiments SEA0400 also displayed a marked vasodilatation when infused into the systemic circulation

suggesting again the crucial role of NCX in maintenance of the abnormal vascular tone. However, SEA0400 was not able to modulate the blood pressure in normotensive rats or salt-independent hypertensive animals.

The different response to SEA0400, which depends on blood pressure and its salt-dependency, may be related to the fact that the effect SEA400 apparently depends on $[Na^+]_i$, since it facilitates the Na^+-dependent inactivation of NCX, which is sufficiently augmented by the higher Na^+ influx in the case of salt-dependent hypertension (Iwamoto and Kita, 2006). It appears, therefore, that SEA400 can be therapeutically effective in salt-dependent hypertension only. Thus, novel NCX inhibitors with different mechanism of action are required to study the potential antihypertensive effect of NCX inhibition in other types of hypertension.

4. Altered NCX function and RyR dysfunction in heart failure

Incidence of heart failure is continuously increasing in the developed world. There are a number of predisposing conditions promoting heart failure, such as hypertension and ischemic heart disease. Heart failure may have multiple origin and divers manifestations with distinct ethiologies (e.g. familiar, diastolic or systolic heart failure, etc.), but it can be considered as a common outcome of various cardiovascular diseases. Modern pharmacological therapies can significantly slow the progression of heart failure by suppressing the sympathetic tone with beta blockers or by inhibiting the pathologically activated renin-angiotensine-system with and ACE-inhibitors. Still, heart failure mortality is high, with cardiac arrhythmias being many times the direct cause of death. It would therefore be highly beneficial to focus on prevention and further slowing of disease progression, potentially by using novel pharmacological targets.

Heart failure is characterized by insufficient pump function and electrical remodeling with cellular basis involving altered regulation of Ca^{2+} handling (Ottolia et al., 2013). One of the most important functional changes is the increased $[Na^+]_i$, with a consequently increased reverse mode NCX activity (Despa and Bers, 2013). On the other hand, elevated expression level of NCX has also been observed in many models in the state of established heart failure (Lugenbiel et al., 2015; Mishra et al., 2005), and increased NCX expression appears to be a key alteration during the transition to heart failure, foregoing the changes in the expression of other Ca^{2+} handling proteins like SERCA (Rodriguez et al., 2014). Increased reverse mode NCX activity may

contribute to the maintenance of contractility by two distinct mechanisms. First, the elevated Ca influx supports Ca^{2+} filling of the SR. Second, upregulated reverse mode NCX may also increase the Ca^{2+} cycling by directly facilitating the Ca^{2+}-induced Ca^{2+} release from the SR (Hobai and O'Rourke, 2000). However, increased Ca^{2+} efflux *via* forward mode NCX favors Ca^{2+} loss resulting in a reduced net SR Ca^{2+} content.

Another critical alteration in heart failure is the Ca^{2+} leak from the SR, due to increased phosphorylation of RyR by the calcium/calmodulin-dependent protein kinase II (CaMKII). CaMKII phosphorylation destabilizes RyR, which results in spontaneous Ca^{2+} release independent of the Ca^{2+} stimulus (Marx and Marks, 2013). On one hand, SR Ca^{2+} leak tends to lower the SR Ca^{2+} content with a consequent reduction of systolic Ca^{2+} transient and contractile force. Furthermore, the uncontrolled Ca^{2+} release is an important arrhythmogenic factor in heart failure (see below). It must be noted that development of heart failure always results from complex structural and electrophysiological changes, associated with altered gene expression pattern (Zarain-Herzberg et al., 2011). However, recent studies suggest that pharmacological modulation of the function of NCX and RyR, the two key players in Ca^{2+} handling, can potentially be beneficial in the treatment of heart failure.

5. Possible improvement by pharmacological NCX inhibition of the defective Ca^{2+} cycling in heart failure

Upon the recognition of the role of NCX in heart failure pathophysiology, the potential beneficial effect of NCX inhibition in correcting the low SR Ca^{2+} load has been emphasized by several review papers. The available pharmacological evidence, however, is still sparse. Regarding the malfunctional Ca^{2+} cycling, NCX inhibition (based on the suppressed forward mode activity) could theoretically cause a positive inotropic response, since the weaker Ca^{2+} extrusion favors the Ca^{2+} uptake into SR by SERCA, resulting in an increased SR Ca^{2+} load. However, as long as selective inhibition of forward mode NCX activity is not achieved, NCX inhibition will decrease reverse mode NCX activity as well, neutralizing the stimulating effect of reverse mode NCX.

In addition, some studies suggest that the first candidate of selective NCX inhibitors, SEA0400 has been shown to be more effective on the reverse than on the forward mode activity

of NCX (Lee et al., 2004). It is not surprising, therefore, that the putative positive inotropic effect was detected neither in heart failure animal models, nor in normal control animals. Under physiological conditions SEA0400 was not able to increase contractions either in myocytes isolated from canine hearts, or in hearts isolated from rabbits and guinea pigs (Birinyi et al., 2008; Farkas et al., 2008; Szentandrassy et al., 2008). In addition, SEA0400 appeared to block L-type Ca^{2+} current (I_{Ca}) too, which may also limit the putative positive inotropic action (Birinyi et al., 2005). These results were further supported by the finding that SEA0400 was able to prevent the reverse NCX-mediated positive inotropy when $[Na^+]_i$ was elevated, but it failed to affect cell shortening under normal conditions in canine myocytes (Nagy et al., 2014).

However, positive inotropic effect of NCX inhibition by SEA0400 can also be seen under experimental conditions where reverse NCX does not play a substantial role in ECC. Application of KB-R7943, a relatively selective reverse mode NCX inhibitor, did not result in negative inotropic effect in rat myocytes, in line with the fact that Ca^{2+} influx *via* reverse mode NCX does not contribute to the Ca^{2+}-induced Ca^{2+} release in this species (Satoh et al., 2000). In accordance with this, inhibition of the Ca^{2+} efflux mode of NCX by SEA0400 was able to cause a significant positive inotropy in rat ventricular myocytes (Acsai et al., 2007).

In some animal models of heart failure, NCX inhibition by SEA0400 has been reported to have different outcomes regarding the inotropic effect, depending on the experimental model used. SEA0400 increased contractility in myocytes isolated from mice with transverse aortic constriction, but not in myocytes from MLP -/- mice (Ozdemir et al., 2008). This apparent controversy suggests that the outcome of NCX inhibition is essentially dependent on the various concomitant Ca^{2+} handling alterations. Another NCX inhibitor, SN-6 has been found to evoke negative inotropic action in normal and also in failing cardiomyocytes, supposedly because of the substantial inhibitory effect on I_{Ca} (Gandhi et al., 2013).

Generally, the lack of positive inotropic effect has been explained on the basis of simultaneous inhibition of Ca^{2+} influx *via* the reverse mode NCX and I_{Ca} (Bourgonje et al., 2013). Thus, the putative improvement of systolic Ca^{2+} in heart failure requires further studies, ideally with application of new, more selective forward mode NCX blockers (i.e. with less effect on I_{Ca} and more pronounced inhibition on forward mode NCX).

6. Pharmacological stabilization of RyR improves defective Ca^{2+} cycling in heart failure

Recent studies show that cessation of the abnormal Ca^{2+} signaling by inhibiting CamKII activity may ameliorate RyR hyperphosphorylation and the consequent abnormal RyR activity, resulting in normalized Ca^{2+} handling (Tzimas et al., 2015; Westenbrink et al., 2013), but the pharmacology of this strategy is still in its infancy. However, cessation of the abnormal signaling can also be achieved by application of dantrolene, an old drug used as a skeletal muscle relaxant agent against spastic diseases and malignant hyperthermia in human clinical practice. Dantrolene appears to act specifically on dysfunctional RyR, and although it inhibits other ion channels, like some K^+ and Ca^{2+} channels (Acsai et al., 2015), its stabilizing effect on dysfunctional RyR surpasses these non-specific side effects. Therefore, dantrolene can be considered to be disease-specific (Maxwell et al., 2012).

Application of dantrolene has been proved to alleviate the abnormal RyR activity and restore normal Ca^{2+} cycling. It suppressed the aftercontractions caused by Ca^{2+} overload, increased SR Ca^{2+} load, exerted positive inotropy and improved diastolic relaxation in isolated hamster heart (Satoh et al., 1997). Similar effects of dantrolene have been found in isolated rat papillary muscles (Meissner et al., 1996). More importantly, dantrolene displayed similar beneficial effects in cardiac preparations from patients with end-stage heart failure (Meissner et al., 1999). It also improved cardiac function *in vivo* in rats with ischemic heart failure (Min et al., 2003), increased SR Ca^{2+} content and exerted positive inotropic effect in chronic heart failure models of dogs (Kobayashi et al., 2009) and rabbits (Maxwell et al., 2012). All these beneficial effects on Ca^{2+} handling may be based on the inhibition of SR Ca^{2+} leak by stabilizing the RyR suggesting that multiple aspects of the cardiac dysfunction can be improved by restoration of normal RyR function. Thus, it can be concluded that dantrolene, although characterized as a RyR inhibitor, displays specificity for dysfunctional RyR, inhibiting the aberrant RyR opening but leaving its normal function relatively unchanged. This unique feature of dantrolene makes it potentially useful in the case of the destabilized RyR in heart failure, where suppression of abnormal function is as important as maintenance of normal activity.

7. Myocardial remodeling is closely associated with abnormal Ca^{2+} handling

Another important aspect of heart failure is the development of structural remodeling in chronic cases. This involves hypertrophy of cardiomyocytes and increased tissue fibrosis. At early stages, myocardial remodeling can be a helpful compensatory response to the increased load, which helps to maintain the compensated stage of the disease. However, it is associated with increased risk of arrhythmias by presenting the structural substrate for arrhythmogenesis.

Recent studies indicate that abnormal Ca^{2+} signaling and Ca^{2+} handling play a crucial role in the induction and progression of adverse remodeling in myocardial cells as well as in cardiac fibroblasts (Chen et al., 2010). Again, CamKII-dependent signaling is implicated in connecting the increased neurohormonal activation to the abnormal Ca^{2+}-dependent regulation of cell function (Mollova et al., 2015). Accordingly, involvement of NCX upregulation and altered phosphorylation status play a key function in remodeling (Gupta et al., 2009; Katanosaka et al., 2007; Valdivia et al., 2015). Similar role of NCX in pathogenesis of atrial remodeling associated with atrial fibrillation has also been reported (see below). It is worth to note that the well known anti-remodeling effect of the angiotensin-II type 1 receptor blocker drugs in heart failure appears to be associated with the restoration of the physiological status of Ca^{2+} signaling and Ca^{2+} handling proteins, including the lowering of the increased NCX expression (Ferreira et al., 2011). On the other hand, abnormal NCX function can also be activated directly by the abnormal neurohormonal regulation. Endogenous ouabain is increased in many patients, which is known to contribute to the development of fibrosis by induction of collagen production in fibroblasts (Fedorova et al., 2010). Since the primary action of digitalis-like factors is the inhibition of NKA with the consequent increase of $[Na^+]_i$ and activation of the reverse mode NCX (resulting in Ca^{2+} influx), it seems logical to speculate that the increased Ca^{2+} signaling due to reverse mode NCX activity may contribute to induction of remodeling.

This latter hypothesis has been addressed by Kamimura et al.using SEA0400 as a pharmacological tool. They found that chronic administration of SEA0400 at doses not affecting hypertension was able to reduce the myocardial remodeling in Dahl salt-sensitive hypertensive rats, and consequently improved the survival of the animals (Kamimura et al., 2012). This beneficial effect was attributed to the inhibition of fibrosis development by blocking the reverse mode NCX, since SEA0400 also suppressed the increased activity of cardiac fibroblasts.

Another study used KB-R7943 to provide evidence for the role of reverse mode NCX in mediating Ca^{2+} signals associated with induction of human cardiac fibroblast proliferation through different pathways (Ikeda et al., 2013), but to our best knowledge the study of Kamimura et al. is the only one demonstrating that NCX inhibition may be promising to delay the adverse remodeling associated with heart failure in a chronic pharmacological experiment.

It is also worth to mention that the Ca^{2+} sensitizer levosimendan, used in the human therapy, has been reported to decrease the hypertension-induced cardiac remodeling in hypertensive Dahl/Rapp rats, resulting improved survival of the animals (Louhelainen et al., 2007). This seems to be particularly interesting in the light of the newly discovered NCX inhibitory effect of levosimendan (Li et al., 2014). Li et al. have shown that levosimendan inhibits the membrane trafficking of NCX, and consequently its activity in cardiomyocytes derived from human cardiomyocyte progenitor cells. Although these observations need to be confirmed in more complex experimental systems, they strongly suggest that the NCX inhibitory effect may contribute to the anti-remodeling activity of levosimendan.

SR Ca^{2+} signaling through RyR also appears to play a central role in remodeling. On the one hand, regulation of RyR by CamKII has been implicated in cardiac remodeling and heart failure (Chakraborty et al., 2014). On the other hand, RyR activity itself seems to affect other pathways and gene expressions involved in hypertrophy (Zou et al., 2011). Pharmacological studies support this central role, since inhibition of aberrant RyR activity has been shown to ameliorate remodeling in different models. Chronic treatment of dogs with JTV519 (K201), a nonspecific RyR stabilizer, was able to prevent left ventricular remodeling in a pacing-induced canine heart failure model and ameliorated the severity of heart failure (Yano et al., 2003). The authors also provided molecular evidence for the protection against RyR hyperphosphorylation and they have shown in acute experiments that JTV519 suppressed the SR Ca^{2+} leak.

Another drug which is useful to study the anti-remodeling consequences of RyR inhibition is dantrolene. The RyR stabilizer effect of this agent has been demonstrated in several in vitro studies that simulated the abnormal inter-domain interaction within the RyR complex (Gangopadhyay and Ikemoto, 2010; Hamada et al., 2009). These studies have shown that application of dantrolene effectively inhibited the hypertrophic alterations induced by endothelin-1 or DPc10 (a destabilizer of the RyR) in neonatal rat cardiac myocytes. Furthermore, protective action of dantrolene has also been demonstrated under *in vivo* conditions. Chronic

dantrolene treatment of hypertensive rats with aortic constriction effectively inhibited the development of myocardial hypertrophy (Ohkusa et al., 1998), increased survial, prevented the structural and molecular remodeling and also the functional deterioration of the hearts in rats with doxorubicin-induced cardiomyopathy (Campos et al., 2011).

We can conclude therefore that according to the available pharmacological evidence, inhibition of both NCX and RyR may display beneficial effects being promising in heart failure to improve contractile dysfunction and to prevent myocardial remodeling.

8. Abnormal Ca^{2+} handling as a possible antiarrhythmic target

Onset of cardiac arrhythmias belongs to the most common complications and causes of death during the time course of the cardiovascular diseases. Several recent review papers discuss the arrhythmogenic mechanisms associated with Ca^{2+} handling abnormalities occurring in various cardiac diseases (Driessen et al., 2014; Wagner et al., 2015). RyR and NCX play a crucial role in the disease-related Ca^{2+} signaling defect, and their involvement in the arrhythmogenesis is based on the functional coupling between the submembrane Ca^{2+} level and the transmembrane potential. NCX activity is highly dependent on both $[Na^+]_i$ and $[Ca^{2+}]_i$, and because of the 1:3 stoichiometry of the Ca^{2+}-Na^+ antiport, it generates inward current upon removing Ca^{2+} from the cytosol. In addition, along the T-tubule membrane of the ventricular myocytes, NCX molecules are expressed in close proximity with junctional SR, from which Ca^{2+} is released through RyR. Thus, Ca^{2+} release from SR and part of Ca^{2+} removal through the NCX occur in the same functional space called local Ca^{2+} microdomain, in which Ca^{2+} level is at least ten times higher than in the bulk phase of cytoplasm (Acsai et al., 2011). Once activated by increased local $[Ca^{2+}]_i$, NCX generates an inward current contributing to the generation of early (EAD) and delayed (DAD) afterdepolarizations. These elementary electrical events can promote ectopic beats and triggered activity, potentially leading to life threatening arrhythmias. Since the introduction of this concept (January and Fozzard, 1988) several studies demonstrated the arrhythmogenic consequences of the increased NCX activity associated with heart failure and atrial fibrillation, and pharmacological modulation of NCX became generally accepted as a possible new antiarrhythmic intervention (Antoons et al., 2012). However, the lack of potent and

selective NCX blockers obviously hampered the pharmacological verification of this concept, especially in *in vivo* animal models.

On the other hand, there are some antiarrhythmic drugs in the current clinical practice, including bepridil, aprindine, amiodarone, dronedarone and cibenzoline that are known to inhibit NCX to a certain degree (Watanabe et al., 2004; Watanabe et al., 2002; Watanabe and Kimura, 2000, 2001, 2010). Since their primary electrophysiological actions are more pronounced, these drugs are not suitable to study the potential protective effects of NCX inhibition *per se*. However, the concomitant NCX inhibition is likely to contribute to the beneficial effects of these compounds observed e.g. in atrial fibrillation (Nakatani et al., 2015).

Investigation of the putative antiarrhythmic effects of NCX inhibition became possible with the development of relatively specific and selective NCX blockers. Based on the available evidence, NCX inhibition seems to affect arrhythmia generation differentially depending on the experimental conditions and the actual mechanism of arrhythmias.

9. Efficacy of NCX inhibition against DAD and triggered arrhythmias induced by Ca^{2+}-leak

The first experiments showing the efficacy of NCX inhibition by SEA0400 against Ca^{2+}-overload related arrhythmia generation was carried out in canine cardiac preparations (Nagy et al., 2004). In this study SEA0400 effectively suppressed DAD generation and triggered arrhythmias induced by strophantidine and fast pacing in canine Purkinje fibers. Similar results have been obtained in guinea pig papillary muscles showing that SEA0400 decreased the ouabain-mediated positive inotropy and arrhythmias (Tanaka et al., 2007). This was further supported in a study using *in vivo* canine model where the antiarrhythmic effect of SEA0400 was convincing against digitalis-induced tachyarrhythmias (Nagasawa et al., 2005).

Since digitalis induced positive inotropy is known to be mediated by increased Ca^{2+} influx *via* reverse mode NCX, the above finding raises the possibility that the inhibition of NCX may reduce the incidence of Ca^{2+}-dependent arrhythmias not only by reducing the $[Ca^{2+}]_i$-voltage gain, but also by ameliorating the Ca^{2+} load of the cell. This is in line with the results obtained in single canine myocytes, where NCX inhibition completely prevented and reversed the elevated $[Na^{+}]_i$-induced positive inotropic effect (Nagy et al., 2014).

These basic studies have been followed by experiments using more sophisticated techniques in isolated heart. Fujiwara et al. have studied the link between proarrhythmic Ca^{2+} waves and membrane potential oscillations using confocal imaging in isolated rat hearts (Fujiwara et al., 2008). Again, SEA0400 effectively decreased the arrhythmogenic membrane depolarizations. Furthermore, it is important to note that these authors also applied ryanodine to inhibit RyR, an intervention that was also able to suppress arrhythmogenesis. Thus, this study may underline the perspective of the potential synergistic effect between the suppression of both RyR and NCX.

Involvement of the elementary electrical events in generation of disease-associated arrhythmias has also been demonstrated in a model of Andersen-Tawil syndrome (Radwanski and Poelzing, 2011). In this study KB-R7943 has been successfully applied to lower the incidence of tachycardia, while the enhancement of NCX activity in Ca^{2+} removal increased the arrhythmia occurrence, further demonstrating the pathogenic role of NCX in arrhythmias.

However, there are some conflicting results in the literature. It has been shown for instance that aconitine-induced arrhythmias are also based on Ca^{2+} overload, DAD and triggered activity (Sun et al., 2014; Zhou et al., 2013), but interestingly, SEA0400 was not effective in those experiments where arrhythmias were evoked by aconitine (Amran et al., 2004). This may suggest that in more complex situations other factors than NCX (e.g. EAD generation) may contribute to development of arrhythmias, thus the antiarrhythmic action of NCX inhibition may not always be manifested.

It must be noted regarding the above experiments that both KB-R7943 and SEA0400 can inhibit I_{Ca} in the cardiac cells, which raises the possibility tha the antiarrhythmic activity can also be attributed to the reduced Ca^{2+} load of the myocytes. However, ORM-10103, a novel and selective NCX inhibitor with no direct effect on I_{Ca}, also effectively reduced the incidence of DADs and aftercontractions under conditions of intracellular Ca^{2+} overload both in multicellular cardiac preparations and single cells (Jost et al., 2013; Nagy et al., 2014). These results strongly suggest that inhibition of NCX alone is able to reduce the Ca^{2+}-dependent arrhythmia propensity, and the concomitant I_{Ca} inhibition is not necessary for the manifest antiarrhythmic effect.

10. Effect of NCX inhibition on EAD related arrhythmias

Another potential mechanism to reduce arrhythmia propensity by NCX inhibition is suppression of EADs. EADs can be evoked by decreasing the repolarization power, which lengthens action potential duration (APD), increases beat-to-beat variability of APD and often leads to the development of torsades de pointes (TdP) tachycardia (Oros et al., 2010). By generating inward current upon Ca^{2+} removal, NCX is thought to play an important role in evoking EADs either directly by depolarizing the membrane, or indirectly by allowing the reactivation of Ca^{2+} and Na^{+} currents due to the slower rate of repolarization (Volders et al., 2000). Indeed, pharmacological studies investigating the role of NCX in EAD generation have shown that EAD can be effectively attenuated by pharmacological NCX inhibition. In isolated canine ventricular preparations, both SEA0400 and ORM-10103 significantly reduced the incidence of EADs (Jost et al., 2013; Nagy et al., 2004), and EADs have been completely suppressed by SEA0400 in isolated canine myocytes (Johnson et al., 2010). These findings were also supported by results obtained in a more complex model of the long QT syndrome and heart failure in intact rabbit heart, where NCX inhibition by SEA0400 prevented EADs by reducing AP prolongation, dispersion of repolarization, and thereby suppressed TdP types arrhythmias (Milberg et al., 2008; Milberg et al., 2012). Furthermore, in a study investigating the putative antiarrhythmic effect of SEA0400 in dogs with chronic atrioventricular block, a model of compensated cardiac hypertrophy, SEA0400 was able to moderate the dofetilide-induced increased action potential variability and TdP, supporting the role of NCX current in the short term variability and EAD generation (Bourgonje et al., 2013). However, since SEA0400 was shown to block I_{Ca} at the concentrations used, the degree to which NCX inhibition contributes to the observed effects is not obvious. Nevertheless, it appears that modulation of the interaction between SR Ca^{2+} release and I_{Ca} occurring in the subsarcolemmal Ca^{2+} microdomain may be involved in the antiarrhythmic effect of NCX inhibition (Antoons et al., 2015).

It must be noted however, that other study using SEA0400 in isolated rabbit hearts failed to find any evidence for the contribution of NCX to generation of EADs and TdP by dofetilide (Farkas et al., 2009). Thus, the detailed mechanism of the antiarrhythmic action of NCX inhibition requires further studies.

11. Ischemia-reperfusion induced arrhythmias

As we have shown above, the pharmacological NCX inhibition may be a useful antiarrhythmic strategy, although the exact electrophysiological explanation for this beneficial effect may be difficult even in relatively simple arrhythmogenic conditions. Furthermore, the situation becomes more complicated when different arrhythmogenic mechanisms can exist at the same time. Arrhythmias generated by an ischemia-repefusion protocol are good examples for this complexity. Change in action potential morphology, action potential heterogeneity, conduction delay and its heterogeneity and Ca^{2+} overload may exist in parallel or after each other, making any inferences about the putative antiarrhythmic efficacy of NCX inhibition very difficult in these situations. On the other hand, the pathogenetic role of NCX in mediating ischemia-reperfusion induced cell death and myocardial injury is well established (Baczko et al., 2003, 2004) and indeed pharmacological NCX inhibition has been shown to be protective against ischemia-reperfusion injury (Namekata et al., 2005). Therefore, NCX inhibition may also modulate the arrhythmia outcome indirectly by ameliorating the injury of the myocardium.

The first study reporting the antiarrhythmic potential of NCX inhibition was performed using an *in vivo* rat model (Takahashi et al., 2003). These authors applied regional ischemia followed by reperfusion, and found that SEA0400 decreased the incidence of ventricular fibrillation. However, despite of the extensively documented cardioprotective effect of NCX inhibition seen in ischemic-reperfused myocardium, further studies were unable to demonstrate the protection against ventricular arrhythmias under these conditions. In Langendorff-perfused rat hearts SEA0400 even increased the incidence and duration of ventricular arrhythmias induced by reperfusion, in spite of the observed protection of myocardium (Feng et al., 2006), while others failed to demonstrate any significant protection against cardiac arrhythmias associated with regional ischemia-reperfusion injuries in dogs *in vivo* (Nagasawa et al., 2005). Interestingly, the antiarrhythmic effect of SEA0400 was convincing against digitalis-induced tachyarrhythmias in the same study, thus the authors concluded that NCX inhibition can be effective only in Ca^{2+}-dependent arrhythmias by reducing the depolarizing NCX current. Similar conclusion has been made in a recent study, which compared the antiarrhythmic effect of SEA0400 and ORM10103 in isolated Langendorff-perfused rat hearts (Szepesi et al., 2015). Both compounds appeared to reduce the incidences of extra beats, but not the duration and incidences of ventricular tachyarrhythmias, suggesting again that NCX inhibition may be effective against EAD and DAD generation, but not in reducing reentry-based sustained arrhythmias. Similarly, in a chronic

model of myocardial infarction in rabbit, KB-R7943 suppressed afterdepolarizations and the concomitant spontaneous tachyarrhythmias, but the inducibility of tachyarrhythmias was increased at the same time (Chang et al., 2015b), presenting again an example for the difficulties arising from the coexistence of various arrhythmogenic conditions.

12. Antiarrhythmic effect of RyR inhibition

Inhibition of pathological RyR activity is an emerging antiarrhythmic mechanism. The protective effect of dantrolene against dysrhythmias associated with serious conditions like malignant hyperthermia has been attributed historically to its inhibitory effect on the Ca^{2+} and K^{+} currents (Acsai et al., 2015). However, recent studies have shown that the underlying mechanism is more specific and is related to inhibition of the uncontrolled Ca^{2+} release by stabilization of the RyR complex. Based on this effect, dantrolene was shown to effectively reduce the arrhythmia incidence in various cardiac situations like ischemia-reperfusion, heart failure or catecholaminergic polymorphic ventricular tachycardia (CPVT). The antiarrhythmic effect of dantrolene observed in experimental models of heart failure has been attributed to the normalized Ca^{2+} handling (Kobayashi et al., 2009; Maxwell et al., 2012), which was reflected by the reduced spontaneous Ca^{2+} spark frequency and consequently the arrhythmogenic membrane potential oscillations. Furthermore, in the whole heart level the inhibition of the cellular arrhythmogenic events has been manifested as an effective protection against ventricular ectopic activity in isolated failing rabbit hearts , (Chou et al., 2013).

CPVT is an inherited genetic disorder with potentially lethal outcome. It is caused by different mutations in the RyR gene, which results in uncontrolled Ca^{2+} release upon adrenergic stimulation leading to afterdepolarizations and VT. Application of dantrolene or other drugs with RyR inhibitory effect may be therefore a logical choice against CPVT. Indeed, when patients responded surprisingly well to Na^{+} channel inhibitors like flecainide, it turned out that this drug has an inhibitory effect on RyR (Hilliard et al., 2010). However, suppression of Ca^{2+} release events can also be explained on the basis of Na^{+} channel inhibition per se (Radwanski et al., 2015), thus the current debate regarding the contribution of Na^{+} channel blockade and RyR stabilization to the beneficial effect reflects the difficulties arising from the lack of selectivity. Nevertheless, dantrolene with its primary effect on RyR has been proved to be very efficient in

pharmacological studies. In an *in vivo* mouse model of the disease, chronic treatment with dantrolene effectively reduced the occurrence of VT induced by epinephrine or physical exercise (Kobayashi et al., 2010). Cellular studies have also supported these findings, by showing that the elevated Ca^{2+} spark frequency displayed by mutant cells in response to isoproterenol could be corrected with acute application of dantrolene (Suetomi et al., 2011; Xu et al., 2010).

Ventricular fibrillation (VF) is the most serious arrhythmia leading to death within minutes, and represents the most frequent form of the sudden cardiac death. Despite of the effective resuscitation techniques, the mortality rate of VF is extremely high, and this may occur at any stage during the time course of heart failure. VF is characterized by fast, repetitive activation of myocytes due to development of reentrant circuits within the electrical conduction pathway. Because of these fast repetitive activations, intracellular Na^{+} and Ca^{2+} overload occurs contributing to Ca^{2+} oscillations, the consequent electrical instability and myocardial dysfunction even after successful defibrillation (Zaugg et al., 1998). An early study showing the increased fibrillation threshold after dantrolene administration in rats (Brooks et al., 1989) has been followed by a recent study demonstrating that cessation of the abnormal Ca^{2+} cycling by applying dantrolene to inhibit RyR effectively ameliorated the postfibrillatory ventricular dysfunction in pigs *in vivo* (Zamiri et al., 2014). In addition, dantrolene facilitated the success of defibrillation and decreased the number of refibrillation episodes. Although these observations may represent a promising new strategy against VF, it must be noted that the dantrolene-induced elongation of action potential duration might also be involved in the protective effect. Therefore further studies are required to confer the antifibrillatory action of RyR inhibition - ideally using a compound with a selective RyR stabilizer effect. S107 is a new agent with apparently more selective RyR inhibitory effect (Mei et al., 2013), although it is not widely used at present. It is also important to note that because of the well established role of NCX in electrical instability, a synergistic interaction between inhibition of RyR and NCX can be anticipated against VF, but this requires further support by detailed studies.

Finally, it is also known that Ca^{2+} overload of the myocyte developed during an ishemia-reperfusion episode profoundly contributes to the cellular dysfunction and arrhythmia generation. However, since ischemia-reperfusion presents a complex scenario regarding the arrhythmogenic mechanisms, it is not surprising that there are conflicting results with use of

dantrolene, with some studies well demonstrating its antiarrhythmic action (Brooks et al., 1989), while others could not find any protection (Pelleg et al., 1985; Preckel et al., 2000).

In conclusion, several studies demonstrated that both NCX and RyR inhibition display direct antiarrhythmic actions under various conditions. In addition, the antiremodeling effects of NCX and RyR inhibitors (see above) may also reduce the arrhythmia propensity indirectly by reducing cardiac fibrosis (Morita et al., 2014).

13. Pathological Ca^{2+} mishandling in atrial fibrillation

Atrial fibrillation belongs to the most common cardiac arrhythmias which represents an increased risk for stroke. Conversion to sinus rhythm is often impossible because of the fast development of structural and electrical remodeling. Electrical remodeling occurring in atrial fibrillation involves alterations primarily in the repolarizing K^+ currents, but abnormal changes in Ca^{2+} handling transporters may also play a prominent role (Lugenbiel et al., 2015). Increased expression and activity of NCX appears to be a key factor also in atrial fibrillation associated with non-steroidal anti-inflammatory drugs (Chang et al., 2015a) and taurocholic acid (Rainer et al., 2013). Also, increased NCX activity has been reported to exert fibrillatory effects in atrial fibrillation induced by endothelin-1 and angiotensin-II in atrial myocytes (Chen et al., 2006; Li et al., 2005). Indeed, angiotensin-II induced EADs could be prevented by using SEA0400 to block NCX in cultured human atrial myocytes (Tsai et al., 2011).

As a consequence of dysfunctional RyR activity, Ca^{2+} leak from the SR plays also a central role in atrial fibrillation. Abnormal RyR activity can be caused by several factors. For example, reduced atrial junctiophilin-2 level or loss of function mutation may lead to SR Ca^{2+} leak due to the hampered junctiophilin-2 mediated RyR stabilization (Beavers et al., 2013). Hyperphosphorylation of RyR by activated CaMKII also may promote SR Ca^{2+} leak (Voigt et al., 2012). Whatever the cause should be, the abnormal Ca^{2+} release is immediately coupled to the development of DADs through forward mode NCX activity (see above), which may evoke triggered activity in patients with chronic atrial fibrillation. The $[Ca^{2+}]$i-voltage gain is also increased as a consequence of the elevated NCX expression, promoting further the generation of DADs (Voigt et al., 2012).

The correlation between the occurrence of afterdepolarizations the increased NCX expression has been elegantly demonstrated in a recent study by showing that EADs and DADs have more frequently developed in regions with higher NCX expression level (Chang et al., 2013). Notably, this study also demonstrated that inhibition of NCX was able to suppress atrial fibrillation. The arrhythmogenic coupling between aberrant SR Ca^{2+} leak and NCX is also present in pulmonary vein myocytes, the ectopic activity of which is an important cause of atrial fibrillation. KB-R7943 was shown to reduce this arrhythmogenic activity in pulmonary vein myocytes, underlying again the causal role of NCX in generation of ectopic activity (Wongcharoen et al., 2006). It can be concluded that although the involvement of NCX in many pathological situations leading to atrial fibrillation seems to be convincing, and NCX inhibition appears to reduce the elementary arrhythmogenic events at the cellular level, *in vivo* pharmacological studies designed to show the putative beneficial effects on longer time scale are still missing.

14. Conclusions

Significance of pharmacological studies designed to assess the therapeutic value of NCX and RyR modulation in cardiovascular diseases were discussed in this review. While both RyR and NCX inhibition have beneficial effects reflected in improvements of many aspects of cardiac disease, and despite the high need for a more successful therapy urged by the high prevalence and mortality in cardiovascular diseases, a relatively limited number of compounds are available for these targets. Therefore, in order to a successful transition from the preclinical studies to the clinical setting, pharmacology of RyR and NXC inhibition should progress in the direction of developing drugs with higher specificity and in case of NCX inhibition, with mode-specific effect, which could offer more opportunities for the disease-specific adaptation of therapy.

Pharmacological modulation of NCX is indeed a unique possibility, since by its bimodal function it can also increase and decrease SR Ca^{2+} load. NCX inhibition can therefore be beneficial depending on the efficacy of inhibition of the forward and reverse mode activity of NCX. As a specific example, although the inhibition of forward mode NCX can be antiarrhythmic by reducing Ca^{2+}- induced depolarization, it may also result in cellular Ca^{2+} gain, and in turn in an increased diastolic SR Ca^{2+} leak. In this case the simultaneous inhibition of

reverse-mode NCX may help to avoid excess Ca^{2+} load, but at the same time, it may weaken the efficacy of ECC, particularly in heart failure, where relative contribution of NCX to ECC is increased. Detailed studies are therefore needed to explore the potential benefit of NCX inhibition in pathological situations with different Ca^{2+} handling disturbances.

Further improvement of therapeutic efficacy using the existing compounds seems to be possible as well. RyR and NCX inhibition theoretically could have synergistic effects, which could be beneficial under certain conditions such as atrial fibrillation or CPVT. Blockade on RyR can weaken the uncontrolled Ca^{2+} release from the SR. On the other hand, arrhythmogenic potential of SR Ca^{2+} leak can also be attenuated by inhibition of forward mode NCX and thereby suppressing the EAD- and/or DAD-induced triggered activity. Combination of these strategies could potentially result in improved outcome, however, experimental validation of this approach is still required.

Despite the promising results of preclinical studies, concerns must be taken into account regarding the safety of chronic application of drugs interacting with the cellular Ca^{2+} handling. Dantrolene is being used as a muscle relaxant in the clinical practice and safety margins are relatively well known for this drug. To our best knowledge, the long-term safety of NCX inhibition, however, has not been evaluated yet. The novel, highly specific ORM compounds, however, may have great potential in this regard as well.

In summary, Ca^{2+} handling has been considered in the context of pathological cardiovascular conditions, such as hypertension, heart failure and the most important fatal consequence, cardiac arrhythmias, for a long time. Dysregulated RyR and NCX as key players in the Ca^{2+} mishandling have been emerging pharmacological targets with an enormous therapeutic potential. Perfecting pharmacological tools in this field could provide us with novel avenues for drug treatment which could prevent the development, slow down the progression of the disease, and suppress fatal arrhythmias.

Acknowledgements

This work was supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP-4.2.4.A/ 2-11/1-2012-0001 'National Excellence Program' for KA and BO; the Hungarian Scientific Research Fund [OTKA ANN-113273 and K115397].

References

Acsai, K., Antoons, G., Livshitz, L., Rudy, Y., Sipido, K.R., 2011. Microdomain [Ca(2)(+)] near ryanodine receptors as reported by L-type Ca(2)(+) and Na+/Ca(2)(+) exchange currents. The Journal of physiology 589, 2569-2583.

Acsai, K., Kun, A., Farkas, A.S., Fulop, F., Nagy, N., Balazs, M., Szentandrassy, N., Nanasi, P.P., Papp, J.G., Varro, A., Toth, A., 2007. Effect of partial blockade of the Na(+)/Ca(2+)-exchanger on Ca(2+) handling in isolated rat ventricular myocytes. European journal of pharmacology 576, 1-6.

Acsai, K., Nagy, N., Marton, Z., Oravecz, K., Varro, A., 2015. Antiarrhythmic potential of drugs targeting the cardiac ryanodine receptor Ca2+ release channel: case study of dantrolene. Current pharmaceutical design 21, 1062-1072.

Amran, M.S., Hashimoto, K., Homma, N., 2004. Effects of sodium-calcium exchange inhibitors, KB-R7943 and SEA0400, on aconitine-induced arrhythmias in guinea pigs in vivo, in vitro, and in computer simulation studies. The Journal of pharmacology and experimental therapeutics 310, 83-89.

Antoons, G., Johnson, D.M., Dries, E., Santiago, D.J., Ozdemir, S., Lenaerts, I., Beekman, J.D., Houtman, M.J., Sipido, K., Vos, M.A., 2015. Calcium release near l-type calcium channels promotes beat-to-beat variability in ventricular myocytes from the chronic AV block dog. Journal of molecular and cellular cardiology.

Antoons, G., Willems, R., Sipido, K.R., 2012. Alternative strategies in arrhythmia therapy: evaluation of Na/Ca exchange as an anti-arrhythmic target. Pharmacology & therapeutics 134, 26-42.

Baczko, I., Giles, W.R., Light, P.E., 2003. Resting membrane potential regulates Na(+)-Ca2+ exchange-mediated Ca2+ overload during hypoxia-reoxygenation in rat ventricular myocytes. The Journal of physiology 550, 889-898.

Baczko, I., Giles, W.R., Light, P.E., 2004. Pharmacological activation of plasma-membrane KATP channels reduces reoxygenation-induced Ca(2+) overload in cardiac myocytes via modulation of the diastolic membrane potential. British journal of pharmacology 141, 1059-1067.

Beavers, D.L., Wang, W., Ather, S., Voigt, N., Garbino, A., Dixit, S.S., Landstrom, A.P., Li, N., Wang, Q., Olivotto, I., Dobrev, D., Ackerman, M.J., Wehrens, X.H., 2013. Mutation E169K in junctophilin-2 causes atrial fibrillation due to impaired RyR2 stabilization. Journal of the American College of Cardiology 62, 2010-2019.

Birinyi, P., Acsai, K., Banyasz, T., Toth, A., Horvath, B., Virag, L., Szentandrassy, N., Magyar, J., Varro, A., Fulop, F., Nanasi, P.P., 2005. Effects of SEA0400 and KB-R7943 on Na+/Ca2+ exchange current and L-type Ca2+ current in canine ventricular cardiomyocytes. Naunyn-Schmiedeberg's archives of pharmacology 372, 63-70.

Birinyi, P., Toth, A., Jona, I., Acsai, K., Almassy, J., Nagy, N., Prorok, J., Gherasim, I., Papp, Z., Hertelendi, Z., Szentandrassy, N., Banyasz, T., Fulop, F., Papp, J.G., Varro, A., Nanasi, P.P., Magyar, J., 2008. The Na+/Ca2+ exchange blocker SEA0400 fails to enhance cytosolic Ca2+ transient and contractility in canine ventricular cardiomyocytes. Cardiovascular research 78, 476-484.

Blaustein, M.P., Leenen, F.H., Chen, L., Golovina, V.A., Hamlyn, J.M., Pallone, T.L., Van Huysse, J.W., Zhang, J., Wier, W.G., 2012. How NaCl raises blood pressure: a new paradigm for the pathogenesis of salt-dependent hypertension. American journal of physiology. Heart and circulatory physiology 302, H1031-1049.

Bourgonje, V.J., Vos, M.A., Ozdemir, S., Doisne, N., Acsai, K., Varro, A., Sztojkov-Ivanov, A., Zupko, I., Rauch, E., Kattner, L., Bito, V., Houtman, M., van der Nagel, R., Beekman, J.D., van Veen, T.A., Sipido, K.R., Antoons, G., 2013. Combined Na(+)/Ca(2+) exchanger and L-type calcium channel block as a potential strategy to suppress arrhythmias and maintain ventricular function. Circulation. Arrhythmia and electrophysiology 6, 371-379.

Brooks, R.R., Carpenter, J.F., Jones, S.M., Gregory, C.M., 1989. Effects of dantrolene sodium in rodent models of cardiac arrhythmia. European journal of pharmacology 164, 521-530.

Campos, E.C., O'Connell, J.L., Malvestio, L.M., Romano, M.M., Ramos, S.G., Celes, M.R., Prado, C.M., Simoes, M.V., Rossi, M.A., 2011. Calpain-mediated dystrophin disruption may be a potential structural culprit behind chronic doxorubicin-induced cardiomyopathy. European journal of pharmacology 670, 541-553.

Chakraborty, A., Pasek, D.A., Huang, T.Q., Gomez, A.C., Yamaguchi, N., Anderson, M.E., Meissner, G., 2014. Inhibition of CaMKII does not attenuate cardiac hypertrophy in mice with dysfunctional ryanodine receptor. PloS one 9, e104338.

Chang, C.J., Cheng, C.C., Yang, T.F., Chen, Y.C., Lin, Y.K., Chen, S.A., Chen, Y.J., 2015a. Selective and non-selective non-steroidal anti-inflammatory drugs differentially regulate pulmonary vein and atrial arrhythmogenesis. International journal of cardiology 184, 559-567.

Chang, P.C., Wo, H.T., Lee, H.L., Wen, M.S., Chou, C.C., 2015b. Paradoxical effects of KB-R7943 on arrhythmogenicity in a chronic myocardial infarction rabbit model. Journal of cardiology 66, 80-87.

Chang, S.L., Chen, Y.C., Hsu, C.P., Kao, Y.H., Lin, Y.K., Lin, Y.J., Wu, T.J., Chen, S.A., Chen, Y.J., 2013. Electrophysiological characteristics of complex fractionated electrograms and high frequency activity in atrial fibrillation. International journal of cardiology 168, 2289-2299.

Chen, J.B., Tao, R., Sun, H.Y., Tse, H.F., Lau, C.P., Li, G.R., 2010. Multiple Ca2+ signaling pathways regulate intracellular Ca2+ activity in human cardiac fibroblasts. Journal of cellular physiology 223, 68-75.

Chen, Y.J., Chen, Y.C., Tai, C.T., Yeh, H.I., Lin, C.I., Chen, S.A., 2006. Angiotensin II and angiotensin II receptor blocker modulate the arrhythmogenic activity of pulmonary veins. British journal of pharmacology 147, 12-22.

Chou, C.C., Wen, M.S., Lee, H.L., Chang, P.C., Wo, H.T., Yeh, S.J., Wu, D., 2013. Dantrolene Suppresses Ventricular Ectopy and Arrhythmogenicity with Acute Myocardial Infarction in a Langendorff-Perfused Pacing-Induced Heart Failure Rabbit Model. Journal of cardiovascular electrophysiology.

Despa, S., Bers, D.M., 2013. Na(+) transport in the normal and failing heart - remember the balance. Journal of molecular and cellular cardiology 61, 2-10.

Dostanic-Larson, I., Van Huysse, J.W., Lorenz, J.N., Lingrel, J.B., 2005. The highly conserved cardiac glycoside binding site of Na,K-ATPase plays a role in blood pressure regulation. Proceedings of the National Academy of Sciences of the United States of America 102, 15845-15850.

Driessen, H.E., Bourgonje, V.J., van Veen, T.A., Vos, M.A., 2014. New antiarrhythmic targets to control intracellular calcium handling. Netherlands heart journal : monthly journal of the Netherlands Society of Cardiology and the Netherlands Heart Foundation 22, 198-213.

Farkas, A.S., Acsai, K., Nagy, N., Toth, A., Fulop, F., Seprenyi, G., Birinyi, P., Nanasi, P.P., Forster, T., Csanady, M., Papp, J.G., Varro, A., Farkas, A., 2008. Na(+)/Ca(2+) exchanger inhibition exerts a positive inotropic effect in the rat heart, but fails to influence the contractility of the rabbit heart. British journal of pharmacology 154, 93-104.
Farkas, A.S., Makra, P., Csik, N., Orosz, S., Shattock, M.J., Fulop, F., Forster, T., Csanady, M., Papp, J.G., Varro, A., Farkas, A., 2009. The role of the Na+/Ca2+ exchanger, I(Na) and I(CaL) in the genesis of dofetilide-induced torsades de pointes in isolated, AV-blocked rabbit hearts. British journal of pharmacology 156, 920-932.
Fedorova, O.V., Shapiro, J.I., Bagrov, A.Y., 2010. Endogenous cardiotonic steroids and salt-sensitive hypertension. Biochimica et biophysica acta 1802, 1230-1236.
Feng, N.C., Satoh, H., Urushida, T., Katoh, H., Terada, H., Watanabe, Y., Hayashi, H., 2006. A selective inhibitor of Na+/Ca2+ exchanger, SEA0400, preserves cardiac function and high-energy phosphates against ischemia/reperfusion injury. Journal of cardiovascular pharmacology 47, 263-270.
Ferreira, J.C., Moreira, J.B., Campos, J.C., Pereira, M.G., Mattos, K.C., Coelho, M.A., Brum, P.C., 2011. Angiotensin receptor blockade improves the net balance of cardiac Ca(2+) handling-related proteins in sympathetic hyperactivity-induced heart failure. Life sciences 88, 578-585.
Fujiwara, K., Tanaka, H., Mani, H., Nakagami, T., Takamatsu, T., 2008. Burst emergence of intracellular Ca2+ waves evokes arrhythmogenic oscillatory depolarization via the Na+-Ca2+ exchanger: simultaneous confocal recording of membrane potential and intracellular Ca2+ in the heart. Circulation research 103, 509-518.
Gandhi, A., Siedlecka, U., Shah, A.P., Navaratnarajah, M., Yacoub, M.H., Terracciano, C.M., 2013. The effect of SN-6, a novel sodium-calcium exchange inhibitor, on contractility and calcium handling in isolated failing rat ventricular myocytes. Cardiovascular therapeutics 31, e115-124.
Gangopadhyay, J.P., Ikemoto, N., 2010. Intracellular translocation of calmodulin and Ca2+/calmodulin-dependent protein kinase II during the development of hypertrophy in neonatal cardiomyocytes. Biochemical and biophysical research communications 396, 515-521.
Gupta, R.C., Mishra, S., Wang, M., Jiang, A., Rastogi, S., Rousso, B., Mika, Y., Sabbah, H.N., 2009. Cardiac contractility modulation electrical signals normalize activity, expression, and phosphorylation of the Na+-Ca2+ exchanger in heart failure. Journal of cardiac failure 15, 48-56.
Hamada, T., Gangopadhyay, J.P., Mandl, A., Erhardt, P., Ikemoto, N., 2009. Defective regulation of the ryanodine receptor induces hypertrophy in cardiomyocytes. Biochemical and biophysical research communications 380, 493-497.
Hamlyn, J.M., Linde, C.I., Gao, J., Huang, B.S., Golovina, V.A., Blaustein, M.P., Leenen, F.H., 2014. Neuroendocrine humoral and vascular components in the pressor pathway for brain angiotensin II: a new axis in long term blood pressure control. PloS one 9, e108916.
Hauck, C., Frishman, W.H., 2012. Systemic hypertension: the roles of salt, vascular Na+/K+ ATPase and the endogenous glycosides, ouabain and marinobufagenin. Cardiology in review 20, 130-138.
Hilliard, F.A., Steele, D.S., Laver, D., Yang, Z., Le Marchand, S.J., Chopra, N., Piston, D.W., Huke, S., Knollmann, B.C., 2010. Flecainide inhibits arrhythmogenic Ca2+ waves by open state block of ryanodine receptor Ca2+ release channels and reduction of Ca2+ spark mass. Journal of molecular and cellular cardiology 48, 293-301.
Hobai, I.A., O'Rourke, B., 2000. Enhanced Ca(2+)-activated Na(+)-Ca(2+) exchange activity in canine pacing-induced heart failure. Circulation research 87, 690-698.

Ikeda, K., Nakajima, T., Yamamoto, Y., Takano, N., Tanaka, T., Kikuchi, H., Oguri, G., Morita, T., Nakamura, F., Komuro, I., 2013. Roles of transient receptor potential canonical (TRPC) channels and reverse-mode Na+/Ca2+ exchanger on cell proliferation in human cardiac fibroblasts: effects of transforming growth factor beta1. Cell calcium 54, 213-225.
Iwamoto, T., Kita, S., 2006. Hypertension, Na+/Ca2+ exchanger, and Na+, K+-ATPase. Kidney international 69, 2148-2154.
Iwamoto, T., Kita, S., Zhang, J., Blaustein, M.P., Arai, Y., Yoshida, S., Wakimoto, K., Komuro, I., Katsuragi, T., 2004. Salt-sensitive hypertension is triggered by Ca2+ entry via Na+/Ca2+ exchanger type-1 in vascular smooth muscle. Nature medicine 10, 1193-1199.
January, C.T., Fozzard, H.A., 1988. Delayed afterdepolarizations in heart muscle: mechanisms and relevance. Pharmacological reviews 40, 219-227.
Johnson, D.M., Heijman, J., Pollard, C.E., Valentin, J.P., Crijns, H.J., Abi-Gerges, N., Volders, P.G., 2010. I(Ks) restricts excessive beat-to-beat variability of repolarization during beta-adrenergic receptor stimulation. Journal of molecular and cellular cardiology 48, 122-130.
Jost, N., Nagy, N., Corici, C., Kohajda, Z., Horvath, A., Acsai, K., Biliczki, P., Levijoki, J., Pollesello, P., Koskelainen, T., Otsomaa, L., Toth, A., Papp, J.G., Varro, A., Virag, L., 2013. ORM-10103, a novel specific inhibitor of the Na+/Ca2+ exchanger, decreases early and delayed afterdepolarizations in the canine heart. British journal of pharmacology 170, 768-778.
Kamimura, D., Ohtani, T., Sakata, Y., Mano, T., Takeda, Y., Tamaki, S., Omori, Y., Tsukamoto, Y., Furutani, K., Komiyama, Y., Yoshika, M., Takahashi, H., Matsuda, T., Baba, A., Umemura, S., Miwa, T., Komuro, I., Yamamoto, K., 2012. Ca2+ entry mode of Na+/Ca2+ exchanger as a new therapeutic target for heart failure with preserved ejection fraction. European heart journal 33, 1408-1416.
Katanosaka, Y., Kim, B., Wakabayashi, S., Matsuoka, S., Shigekawa, M., 2007. Phosphorylation of Na+/Ca2+ exchanger in TAB-induced cardiac hypertrophy. Annals of the New York Academy of Sciences 1099, 373-376.
Kobayashi, S., Yano, M., Suetomi, T., Ono, M., Tateishi, H., Mochizuki, M., Xu, X., Uchinoumi, H., Okuda, S., Yamamoto, T., Koseki, N., Kyushiki, H., Ikemoto, N., Matsuzaki, M., 2009. Dantrolene, a therapeutic agent for malignant hyperthermia, markedly improves the function of failing cardiomyocytes by stabilizing interdomain interactions within the ryanodine receptor. Journal of the American College of Cardiology 53, 1993-2005.
Kobayashi, S., Yano, M., Uchinoumi, H., Suetomi, T., Susa, T., Ono, M., Xu, X., Tateishi, H., Oda, T., Okuda, S., Doi, M., Yamamoto, T., Matsuzaki, M., 2010. Dantrolene, a therapeutic agent for malignant hyperthermia, inhibits catecholaminergic polymorphic ventricular tachycardia in a RyR2(R2474S/+) knock-in mouse model. Circulation journal : official journal of the Japanese Circulation Society 74, 2579-2584.
Kuszczak, I., Kuner, R., Samson, S.E., Grover, A.K., 2010. Proximity of Na+ -Ca2+ -exchanger and sarco/endoplasmic reticulum Ca2+ pump in pig coronary artery smooth muscle: fluorescence microscopy. Molecular and cellular biochemistry 339, 293-300.
Lee, C., Visen, N.S., Dhalla, N.S., Le, H.D., Isaac, M., Choptiany, P., Gross, G., Omelchenko, A., Matsuda, T., Baba, A., Takahashi, K., Hnatowich, M., Hryshko, L.V., 2004. Inhibitory profile of SEA0400 [2-[4-[(2,5-difluorophenyl)methoxy]phenoxy]-5-ethoxyaniline] assessed on the cardiac Na+-Ca2+ exchanger, NCX1.1. The Journal of pharmacology and experimental therapeutics 311, 748-757.

Li, P.C., Yang, Y.C., Hwang, G.Y., Kao, L.S., Lin, C.Y., 2014. Inhibition of reverse-mode sodium-calcium exchanger activity and apoptosis by levosimendan in human cardiomyocyte progenitor cell-derived cardiomyocytes after anoxia and reoxygenation. PloS one 9, e85909.
Li, X., Zima, A.V., Sheikh, F., Blatter, L.A., Chen, J., 2005. Endothelin-1-induced arrhythmogenic Ca2+ signaling is abolished in atrial myocytes of inositol-1,4,5-trisphosphate(IP3)-receptor type 2-deficient mice. Circulation research 96, 1274-1281.
Louhelainen, M., Vahtola, E., Kaheinen, P., Leskinen, H., Merasto, S., Kyto, V., Finckenberg, P., Colucci, W.S., Levijoki, J., Pollesello, P., Haikala, H., Mervaala, E.M., 2007. Effects of levosimendan on cardiac remodeling and cardiomyocyte apoptosis in hypertensive Dahl/Rapp rats. British journal of pharmacology 150, 851-861.
Lugenbiel, P., Wenz, F., Govorov, K., Schweizer, P.A., Katus, H.A., Thomas, D., 2015. Atrial fibrillation complicated by heart failure induces distinct remodeling of calcium cycling proteins. PloS one 10, e0116395.
Lynch, R.M., Weber, C.S., Nullmeyer, K.D., Moore, E.D., Paul, R.J., 2008. Clearance of store-released Ca2+ by the Na+-Ca2+ exchanger is diminished in aortic smooth muscle from Na+-K+-ATPase alpha 2-isoform gene-ablated mice. American journal of physiology. Heart and circulatory physiology 294, H1407-1416.
Marx, S.O., Marks, A.R., 2013. Dysfunctional ryanodine receptors in the heart: new insights into complex cardiovascular diseases. Journal of molecular and cellular cardiology 58, 225-231.
Maxwell, J.T., Domeier, T.L., Blatter, L.A., 2012. Dantrolene prevents arrhythmogenic Ca2+ release in heart failure. American journal of physiology. Heart and circulatory physiology 302, H953-963.
Mei, Y., Xu, L., Kramer, H.F., Tomberlin, G.H., Townsend, C., Meissner, G., 2013. Stabilization of the skeletal muscle ryanodine receptor ion channel-FKBP12 complex by the 1,4-benzothiazepine derivative S107. PloS one 8, e54208.
Meissner, A., Min, J.Y., Haake, N., Hirt, S., Simon, R., 1999. Dantrolene sodium improves the force-frequency relationship and beta-adregenic responsiveness in failing human myocardium. European journal of heart failure 1, 177-186.
Meissner, A., Szymanska, G., Morgan, J.P., 1996. Effects of dantrolene sodium on intracellular Ca2(+)-handling in normal and Ca2(+)-overloaded cardiac muscle. European journal of pharmacology 316, 333-342.
Milberg, P., Pott, C., Fink, M., Frommeyer, G., Matsuda, T., Baba, A., Osada, N., Breithardt, G., Noble, D., Eckardt, L., 2008. Inhibition of the Na+/Ca2+ exchanger suppresses torsades de pointes in an intact heart model of long QT syndrome-2 and long QT syndrome-3. Heart rhythm : the official journal of the Heart Rhythm Society 5, 1444-1452.
Milberg, P., Pott, C., Frommeyer, G., Fink, M., Ruhe, M., Matsuda, T., Baba, A., Klocke, R., Quang, T.H., Nikol, S., Stypmann, J., Osada, N., Muller, F.U., Breithardt, G., Noble, D., Eckardt, L., 2012. Acute inhibition of the Na(+)/Ca(2+) exchanger reduces proarrhythmia in an experimental model of chronic heart failure. Heart rhythm : the official journal of the Heart Rhythm Society 9, 570-578.
Min, J.Y., Meissner, A., Feng, X., Wang, J., Malek, S., Wang, J.F., Simon, R., Morgan, J.P., 2003. Dantrolene: effects on abnormal intracellular Ca(2+) handling and inotropy in postinfarcted rat myocardium. European journal of pharmacology 471, 41-47.
Mishra, S., Sabbah, H.N., Rastogi, S., Imai, M., Gupta, R.C., 2005. Reduced sarcoplasmic reticulum Ca2+ uptake and increased Na+-Ca2+ exchanger expression in left ventricle myocardium of dogs with progression of heart failure. Heart and vessels 20, 23-32.

Mollova, M.Y., Katus, H.A., Backs, J., 2015. Regulation of CaMKII signaling in cardiovascular disease. Frontiers in pharmacology 6, 178.
Morita, N., Mandel, W.J., Kobayashi, Y., Karagueuzian, H.S., 2014. Cardiac fibrosis as a determinant of ventricular tachyarrhythmias. Journal of arrhythmia 30, 389-394.
Nagasawa, Y., Zhu, B.M., Chen, J., Kamiya, K., Miyamoto, S., Hashimoto, K., 2005. Effects of SEA0400, a Na+/Ca2+ exchange inhibitor, on ventricular arrhythmias in the in vivo dogs. European journal of pharmacology 506, 249-255.
Nagy, N., Kormos, A., Kohajda, Z., Szebeni, A., Szepesi, J., Pollesello, P., Levijoki, J., Acsai, K., Virag, L., Nanasi, P.P., Papp, J.G., Varro, A., Toth, A., 2014. Selective Na(+) /Ca(2+) exchanger inhibition prevents Ca(2+) overload-induced triggered arrhythmias. British journal of pharmacology 171, 5665-5681.
Nagy, Z.A., Virag, L., Toth, A., Biliczki, P., Acsai, K., Banyasz, T., Nanasi, P., Papp, J.G., Varro, A., 2004. Selective inhibition of sodium-calcium exchanger by SEA-0400 decreases early and delayed after depolarization in canine heart. British journal of pharmacology 143, 827-831.
Nakatani, Y., Sakamoto, T., Nishida, K., Kataoka, N., Yamaguchi, Y., Sakabe, M., Fujiki, A., Mizumaki, K., Inoue, H., 2015. Bepridil enhances aprindine-induced prolongation of atrial effective refractory period in a canine atrial rapid pacing model. Journal of cardiology.
Namekata, I., Nakamura, H., Shimada, H., Tanaka, H., Shigenobu, K., 2005. Cardioprotection without cardiosuppression by SEA0400, a novel inhibitor of Na+-Ca2+ exchanger, during ischemia and reperfusion in guinea-pig myocardium. Life sciences 77, 312-324.
Ohkusa, T., Hisamatsu, Y., Ueyama, T., Kobayashi, S., Yano, M., Maekawa, T., Matsuzaki, M., 1998. Effects of dantrolene sodium on progression of left ventricular hypertrophy induced by pressure overload in rats. Journal of cardiovascular pharmacology 31, 520-524.
Oros, A., Houtman, M.J., Neco, P., Gomez, A.M., Rajamani, S., Oosterhoff, P., Attevelt, N.J., Beekman, J.D., van der Heyden, M.A., Ver Donck, L., Belardinelli, L., Richard, S., Antoons, G., Vos, M.A., 2010. Robust anti-arrhythmic efficacy of verapamil and flunarizine against dofetilide-induced TdP arrhythmias is based upon a shared and a different mode of action. British journal of pharmacology 161, 162-175.
Ottolia, M., Torres, N., Bridge, J.H., Philipson, K.D., Goldhaber, J.I., 2013. Na/Ca exchange and contraction of the heart. Journal of molecular and cellular cardiology 61, 28-33.
Ozdemir, S., Bito, V., Holemans, P., Vinet, L., Mercadier, J.J., Varro, A., Sipido, K.R., 2008. Pharmacological inhibition of na/ca exchange results in increased cellular Ca2+ load attributable to the predominance of forward mode block. Circulation research 102, 1398-1405.
Pelleg, A., Roth, A., Shargordsky, B., Belhassen, B., Chagnac, A., Laniado, S., 1985. Effects of dantrolene sodium on occlusion and reperfusion arrhythmias in the canine heart. Methods and findings in experimental and clinical pharmacology 7, 239-243.
Poburko, D., Fameli, N., Kuo, K.H., van Breemen, C., 2008. Ca2+ signaling in smooth muscle: TRPC6, NCX and LNats in nanodomains. Channels (Austin, Tex.) 2, 10-12.
Preckel, B., Schlack, W., Comfere, T., Thamer, V., 2000. Effect of dantrolene in an in vivo and in vitro model of myocardial reperfusion injury. Acta anaesthesiologica Scandinavica 44, 194-201.
Pulina, M.V., Zulian, A., Baryshnikov, S.G., Linde, C.I., Karashima, E., Hamlyn, J.M., Ferrari, P., Blaustein, M.P., Golovina, V.A., 2013. Cross talk between plasma membrane Na(+)/Ca (2+) exchanger-1 and TRPC/Orai-containing channels: key players in arterial hypertension. Advances in experimental medicine and biology 961, 365-374.

Radwanski, P.B., Brunello, L., Veeraraghavan, R., Ho, H.T., Lou, Q., Makara, M.A., Belevych, A.E., Anghelescu, M., Priori, S.G., Volpe, P., Hund, T.J., Janssen, P.M., Mohler, P.J., Bridge, J.H., Poelzing, S., Gyorke, S., 2015. Neuronal Na+ channel blockade suppresses arrhythmogenic diastolic Ca2+ release. Cardiovascular research 106, 143-152.
Radwanski, P.B., Poelzing, S., 2011. NCX is an important determinant for premature ventricular activity in a drug-induced model of Andersen-Tawil syndrome. Cardiovascular research 92, 57-66.
Rainer, P.P., Primessnig, U., Harenkamp, S., Doleschal, B., Wallner, M., Fauler, G., Stojakovic, T., Wachter, R., Yates, A., Groschner, K., Trauner, M., Pieske, B.M., von Lewinski, D., 2013. Bile acids induce arrhythmias in human atrial myocardium--implications for altered serum bile acid composition in patients with atrial fibrillation. Heart (British Cardiac Society) 99, 1685-1692.
Rodriguez, J.S., Velez Rueda, J.O., Salas, M., Becerra, R., Di Carlo, M.N., Said, M., Vittone, L., Rinaldi, G., Portiansky, E.L., Mundina-Weilenmann, C., Palomeque, J., Mattiazzi, A., 2014. Increased Na(+)/Ca(2)(+) exchanger expression/activity constitutes a point of inflection in the progression to heart failure of hypertensive rats. PloS one 9, e96400.
Satoh, H., Ginsburg, K.S., Qing, K., Terada, H., Hayashi, H., Bers, D.M., 2000. KB-R7943 block of Ca(2+) influx via Na(+)/Ca(2+) exchange does not alter twitches or glycoside inotropy but prevents Ca(2+) overload in rat ventricular myocytes. Circulation 101, 1441-1446.
Satoh, M., Ishide, N., Shinozaki, T., Kagaya, Y., Shirato, K., 1997. Effect of dantrolene sodium on calcium-overloaded heart. Japanese circulation journal 61, 855-863.
Slodzinski, M.K., Blaustein, M.P., 1998. Physiological effects of Na+/Ca2+ exchanger knockdown by antisense oligodeoxynucleotides in arterial myocytes. The American journal of physiology 275, C251-259.
Suetomi, T., Yano, M., Uchinoumi, H., Fukuda, M., Hino, A., Ono, M., Xu, X., Tateishi, H., Okuda, S., Doi, M., Kobayashi, S., Ikeda, Y., Yamamoto, T., Ikemoto, N., Matsuzaki, M., 2011. Mutation-linked defective interdomain interactions within ryanodine receptor cause aberrant Ca(2)(+)release leading to catecholaminergic polymorphic ventricular tachycardia. Circulation 124, 682-694.
Sun, G.B., Sun, H., Meng, X.B., Hu, J., Zhang, Q., Liu, B., Wang, M., Xu, H.B., Sun, X.B., 2014. Aconitine-induced Ca2+ overload causes arrhythmia and triggers apoptosis through p38 MAPK signaling pathway in rats. Toxicology and applied pharmacology 279, 8-22.
Syyong, H.T., Poburko, D., Fameli, N., van Breemen, C., 2007. ATP promotes NCX-reversal in aortic smooth muscle cells by DAG-activated Na+ entry. Biochemical and biophysical research communications 357, 1177-1182.
Szentandrassy, N., Birinyi, P., Szigeti, G., Farkas, A., Magyar, J., Toth, A., Csernoch, L., Varro, A., Nanasi, P.P., 2008. SEA0400 fails to alter the magnitude of intracellular Ca2+ transients and contractions in Langendorff-perfused guinea pig heart. Naunyn-Schmiedeberg's archives of pharmacology 378, 65-71.
Szepesi, J., Acsai, K., Sebok, Z., Prorok, J., Pollesello, P., Levijoki, J., Papp, J.G., Varro, A., Toth, A., 2015. Comparison of the efficiency of Na+/Ca2+ exchanger or Na+/H+ exchanger inhibition and their combination in reducing coronary reperfusion-induced arrhythmias. Journal of physiology and pharmacology : an official journal of the Polish Physiological Society 66, 215-226.
Takahashi, K., Takahashi, T., Suzuki, T., Onishi, M., Tanaka, Y., Hamano-Takahashi, A., Ota, T., Kameo, K., Matsuda, T., Baba, A., 2003. Protective effects of SEA0400, a novel and

selective inhibitor of the Na+/Ca2+ exchanger, on myocardial ischemia-reperfusion injuries. European journal of pharmacology 458, 155-162.
Tanaka, H., Shimada, H., Namekata, I., Kawanishi, T., Iida-Tanaka, N., Shigenobu, K., 2007. Involvement of the Na+/Ca2+ exchanger in ouabain-induced inotropy and arrhythmogenesis in guinea-pig myocardium as revealed by SEA0400. Journal of pharmacological sciences 103, 241-246.
Taniguchi, S., Furukawa, K., Sasamura, S., Ohizumi, Y., Seya, K., Motomura, S., 2004. Gene expression and functional activity of sodium/calcium exchanger enhanced in vascular smooth muscle cells of spontaneously hypertensive rats. Journal of cardiovascular pharmacology 43, 629-637.
Tsai, C.T., Chiang, F.T., Chen, W.P., Hwang, J.J., Tseng, C.D., Wu, C.K., Yu, C.C., Wang, Y.C., Lai, L.P., Lin, J.L., 2011. Angiotensin II induces complex fractionated electrogram in a cultured atrial myocyte monolayer mediated by calcium and sodium-calcium exchanger. Cell calcium 49, 1-11.
Tzimas, C., Terrovitis, J., Lehnart, S.E., Kranias, E.G., Sanoudou, D., 2015. Calcium/calmodulin-dependent protein kinase II (CaMKII) inhibition ameliorates arrhythmias elicited by junctin ablation under stress conditions. Heart rhythm : the official journal of the Heart Rhythm Society 12, 1599-1610.
Valdivia, H.H., Lugenbiel, P., Wenz, F., Govorov, K., Schweizer, P.A., Katus, H.A., Thomas, D., 2015. Atrial fibrillation complicated by heart failure induces distinct remodeling of calcium cycling proteins. Proceedings of the National Academy of Sciences of the United States of America 10, e0116395.
Voigt, N., Li, N., Wang, Q., Wang, W., Trafford, A.W., Abu-Taha, I., Sun, Q., Wieland, T., Ravens, U., Nattel, S., Wehrens, X.H., Dobrev, D., 2012. Enhanced sarcoplasmic reticulum Ca2+ leak and increased Na+-Ca2+ exchanger function underlie delayed afterdepolarizations in patients with chronic atrial fibrillation. Circulation 125, 2059-2070.
Volders, P.G., Vos, M.A., Szabo, B., Sipido, K.R., de Groot, S.H., Gorgels, A.P., Wellens, H.J., Lazzara, R., 2000. Progress in the understanding of cardiac early afterdepolarizations and torsades de pointes: time to revise current concepts. Cardiovascular research 46, 376-392.
Wagner, S., Maier, L.S., Bers, D.M., 2015. Role of sodium and calcium dysregulation in tachyarrhythmias in sudden cardiac death. Gut 116, 1956-1970.
Wang, Y., Chen, L., Li, M., Cha, H., Iwamoto, T., Zhang, J., 2015. Conditional knockout of smooth muscle sodium calcium exchanger type-1 lowers blood pressure and attenuates Angiotensin II-salt hypertension. Physiological reports 3.
Watanabe, Y., Iwamoto, T., Matsuoka, I., Ono, T., Shigekawa, M., Kimura, J., 2004. Effects of amiodarone on mutant Na+/Ca2+ exchangers expressed in CCL 39 cells. European journal of pharmacology 496, 49-54.
Watanabe, Y., Iwamoto, T., Shigekawa, M., Kimura, J., 2002. Inhibitory effect of aprindine on Na+/Ca2+ exchange current in guinea-pig cardiac ventricular myocytes. British journal of pharmacology 136, 361-366.
Watanabe, Y., Kimura, J., 2000. Inhibitory effect of amiodarone on Na(+)/Ca(2+) exchange current in guinea-pig cardiac myocytes. British journal of pharmacology 131, 80-84.
Watanabe, Y., Kimura, J., 2001. Blocking effect of bepridil on Na+/Ca2+ exchange current in guinea pig cardiac ventricular myocytes. Japanese journal of pharmacology 85, 370-375.
Watanabe, Y., Kimura, J., 2010. Inhibitory effect of azimilide on Na+/Ca2+ exchange current in guinea-pig cardiac myocytes. Journal of pharmacological sciences 114, 111-114.

Westenbrink, B.D., Edwards, A.G., McCulloch, A.D., Brown, J.H., 2013. The promise of CaMKII inhibition for heart disease: preventing heart failure and arrhythmias. Expert opinion on therapeutic targets 17, 889-903.
Wongcharoen, W., Chen, Y.C., Chen, Y.J., Chang, C.M., Yeh, H.I., Lin, C.I., Chen, S.A., 2006. Effects of a Na+/Ca2+ exchanger inhibitor on pulmonary vein electrical activity and ouabain-induced arrhythmogenicity. Cardiovascular research 70, 497-508.
Xu, X., Yano, M., Uchinoumi, H., Hino, A., Suetomi, T., Ono, M., Tateishi, H., Oda, T., Okuda, S., Doi, M., Kobayashi, S., Yamamoto, T., Ikeda, Y., Ikemoto, N., Matsuzaki, M., 2010. Defective calmodulin binding to the cardiac ryanodine receptor plays a key role in CPVT-associated channel dysfunction. Biochemical and biophysical research communications 394, 660-666.
Yano, M., Kobayashi, S., Kohno, M., Doi, M., Tokuhisa, T., Okuda, S., Suetsugu, M., Hisaoka, T., Obayashi, M., Ohkusa, T., Kohno, M., Matsuzaki, M., 2003. FKBP12.6-mediated stabilization of calcium-release channel (ryanodine receptor) as a novel therapeutic strategy against heart failure. Circulation 107, 477-484.
Zamiri, N., Masse, S., Ramadeen, A., Kusha, M., Hu, X., Azam, M.A., Liu, J., Lai, P.F., Vigmond, E.J., Boyle, P.M., Behradfar, E., Al-Hesayen, A., Waxman, M.B., Backx, P., Dorian, P., Nanthakumar, K., 2014. Dantrolene Improves Survival Following Ventricular Fibrillation by Mitigating Impaired Calcium Handling in Animal Models. Circulation.
Zarain-Herzberg, A., Fragoso-Medina, J., Estrada-Aviles, R., 2011. Calcium-regulated transcriptional pathways in the normal and pathologic heart. IUBMB life 63, 847-855.
Zaugg, C.E., Wu, S.T., Barbosa, V., Buser, P.T., Wikman-Coffelt, J., Parmley, W.W., Lee, R.J., 1998. Ventricular fibrillation-induced intracellular Ca2+ overload causes failed electrical defibrillation and post-shock reinitiation of fibrillation. Journal of molecular and cellular cardiology 30, 2183-2192.
Zhang, J., Lee, M.Y., Cavalli, M., Chen, L., Berra-Romani, R., Balke, C.W., Bianchi, G., Ferrari, P., Hamlyn, J.M., Iwamoto, T., Lingrel, J.B., Matteson, D.R., Wier, W.G., Blaustein, M.P., 2005. Sodium pump alpha2 subunits control myogenic tone and blood pressure in mice. The Journal of physiology 569, 243-256.
Zhou, Y.H., Piao, X.M., Liu, X., Liang, H.H., Wang, L.M., Xiong, X.H., Wang, L., Lu, Y.J., Shan, H.L., 2013. Arrhythmogenesis toxicity of aconitine is related to intracellular ca(2+) signals. International journal of medical sciences 10, 1242-1249.
Zou, Y., Liang, Y., Gong, H., Zhou, N., Ma, H., Guan, A., Sun, A., Wang, P., Niu, Y., Jiang, H., Takano, H., Toko, H., Yao, A., Takeshima, H., Akazawa, H., Shiojima, I., Wang, Y., Komuro, I., Ge, J., 2011. Ryanodine receptor type 2 is required for the development of pressure overload-induced cardiac hypertrophy. Hypertension 58, 1099-1110.
Zulian, A., Baryshnikov, S.G., Linde, C.I., Hamlyn, J.M., Ferrari, P., Golovina, V.A., 2010. Upregulation of Na+/Ca2+ exchanger and TRPC6 contributes to abnormal Ca2+ homeostasis in arterial smooth muscle cells from Milan hypertensive rats. American journal of physiology. Heart and circulatory physiology 299, H624-633.

Figure legends

Figure 1: Bimodal operation of NCX. NCX is the main Ca^{2+} extrusion mechanism when operating in its forward mode. However, this operation generates an inward current, which can lead to aberrant membrane depolarization and arrhythmia generation. When operating in reverse mode, NCX controls the cellular Ca^{2+} load, which can result in cellular Ca^{2+} overlaod and pathological Ca^{2+} signaling.

Figure 2: Central role of NCX in vascular smooth muscle cells. Due to co-localization and functional coupling with ion-channels and transporters, NCX integrates the vasomotor signals and controls the intracellular Ca^{2+} level. Increased reverse NCX activity due to intracellular Na^{+} load leads to increased vascular responsiveness and hypertension.

Figure 3: Targeting Ca^{2+} handling in heart failure. NCX inhibition can normalize the increased Ca^{2+} extrusion from the cell, resulting in elevated Ca^{2+} load of the SR. However, parallel block of the reverse NCX may deleteriously affect the Ca^{2+}-induced Ca^{2+} release. Inhibition of the pathological Ca leak via stabilizing the RyR activity may be useful to increase the SR Ca^{2+} content, which strengthens the systolic Ca^{2+} release and force of contraction.

Figure 4: Antiarrhythmic action of RyR and NCX inhibition. RyR stabilization inhibits the aberrant Ca^{2+} release events, thereby preventing the activation of the forward NCX and the development of abnormal membrane depolarizations such as early and delayed afterdepolarizations. Parallel inhibition of NCX may have synergistic effects, reducing at the same time the cellular Ca^{2+} load via inhibition of the revers NCX activity.