Published in final edited form as:

Curr Drug Targets. 2011 May ; 12(5): 737–747.

Triple Threat: The Na⁺/Ca²⁺ Exchanger in the Pathophysiology of Cardiac Arrhythmia, Ischemia and Heart Failure

Christian Pott^{1,*}, Lars Eckardt¹, and Joshua I. Goldhaber²

¹University Hospital of Muenster, Department of Cardiology and Angiology, Division of Experimental and Clinical Electrophysiology, Albert-Schweitzer-Str. 33, 48149 Muenster

²Cedars-Sinai Heart Institute, 8700 Beverly Blvd, Los Angeles, CA 90077 USA

Abstract

The Na⁺/Ca²⁺ exchanger (NCX) is the main Ca²⁺ extrusion mechanism of the cardiac myocyte and thus is crucial for maintaining Ca²⁺ homeostasis. It is involved in the regulation of several parameters of cardiac excitation contraction coupling, such as cytosolic Ca²⁺ concentration, repolarization and contractility. Increased NCX activity has been identified as a mechanism promoting heart failure, cardiac ischemia and arrhythmia. Transgenic mice as well as pharmacological interventions have been used to support the idea of using NCX inhibition as a future pharmacological strategy to treat cardiovascular disease.

1. INTRODUCTION

The great majority of cardiac disorders are associated with at least one of three main pathophysiologies: arrhythmia, ischemia and heart failure. In clinical settings these entities often occur in combination sometimes mutually reinforcing and/or depending upon one another. As a clinical example, human heart failure renders the heart more susceptible to arrhythmia [1]. Conversely, ischemia can result in heart failure and increased arrhythmia burden [2]. Both extracellular and cellular mechanisms have been identified in the causal chains leading to either heart failure, arrhythmia or ischemia and some of these have been successfully identified as therapeutic targets [3].

In this issue of *Current Drug Targets*, cardiac Ca²⁺ regulating proteins such as the ryanodine receptor [4], TRP channels [5] and mitochondrial Ca²⁺ handling proteins [6] are reviewed regarding their role in cardiac pathophysiology and their potential as future pharmacological targets.

Another Ca²⁺ handling protein, the Na⁺/Ca²⁺ exchanger (NCX), also operates in cardiac myocytes. In the physiological setting, NCX serves as the main Ca²⁺ extrusion mechanism of the cardiac myocyte [7]. However, increased activity of NCX, induced experimentally, can promote arrhythmia; exacerbate ischemic necrosis and cause heart failure. Since

increased exchanger activity has also been observed in several models of these diseases, increased NCX activity could be interpreted as a common denominator in the mediation of these three pathophysiologies. Several groups [8–11], including ours [12–14] have been able to define experimental conditions under which genetic or pharmacological inhibition of NCX activity can indeed protect from arrhythmia and ischemia. Although the above studies have demonstrated a promising therapeutic potential of NCX inhibition, pharmacological NCX inhibition has not, to our knowledge, been tested on a chronic condition. Some information on the chronic effects of altered NCX activity stems from transgenic murine models. We have investigated adaptive mechanisms and pathophysiological aspects of chronically altered NCX activity in transgenic overexpressors of NCX and in cardiac specific NCX knockout (KO) mice (for review see [15–18]). Mice are viable with both ventricular-specific KO and 3-fold overexpression of NCX. Although homozygous cardiac specific KO of NCX eventually leads to hypertrophy and a heart failure phenotype in later development, this does not seem to be the case in heterozygotes when NCX expression is modestly reduced to about 50% [19]. However, these observations do not speak to the safety of chronic pharmacological NCX inhibition since murine cardiac physiology is drastically different than humans. Furthermore, transgenic manipulation in early development may offer unrecognized but beneficial adaptive mechanisms that may not operate during pharmacological suppression started during later development.

Thus, before pharmacological or genetic NCX antagonism can be tested as treatment for human cardiac disease, a considerable amount of data defining bioavailability, safety, and reliability has yet to be collected. Nevertheless, the animal data currently available is encouraging. The conduct of future studies may in the end make available a new therapeutic concept by which we can defend against the “triple threat” of cardiac arrhythmia, ischemia and heart failure by countering one single molecular target.

It is noteworthy that NCX has also been characterized in extracardiac tissue such as arterial smooth muscle [20] and neuronal tissue [21]. Though not directly involved in cardiac disease, arterial and neuronal NCX may play a significant role in arterial hypertension and brain ischemia. These diseases are also part of the burden of cardiovascular disease, however, the role of NCX in these pathophysiologies has been reviewed elsewhere [22, 23].

Here we will give an overview on the role of NCX in cardiac physiology and pathophysiology. We will furthermore review the current data on experimental therapeutic approaches targeting NCX to treat ischemia, arrhythmia and heart failure.

2. NCX IN CARDIAC EXCITATION CONTRACTION COUPLING

In cardiac myocytes, the contractile cycle is initiated by Ca^{2+} entry via voltage activated L-type Ca^{2+} channels (dihydropyridine receptor (DHPR)). This “trigger Ca^{2+} ” binds to the ryanodine receptor (RyR) of the sarcoplasmic reticulum (SR) and thus induces further release of Ca^{2+} into the cytosol, which allows contraction of the myofilaments. During diastole, Ca^{2+} is shifted back into the sarcoplasmic reticulum by the Ca^{2+} ATPase of the sarcoplasmic reticulum (SERCA) (Fig. 1, for review, see [24]).

To maintain Ca^{2+} homeostasis and to ensure diastolic relaxation of the myocyte, the same amount of Ca^{2+} that enters the cell with each contractile cycle has to be extruded. As the main cardiac Ca^{2+} removal mechanism, the $\text{Na}^+/\text{Ca}^{2+}$ exchanger extrudes 1 Ca^{2+} ion in exchange for 3 Na^+ ions [25–28]. As an additional Ca^{2+} removal mechanism, the plasma membrane Ca^{2+} ATPase (PMCA) also extrudes Ca^{2+} from the cell, however in far smaller quantities than NCX [29].

The RyR and the DHPR are colocalized to the cleft at the t-tubular sarcoplasmic-sarcolemmal junctions [30]. The proximity of the RyR and the DHPR is believed to ensure a high fidelity of excitation-contraction coupling. There is also mounting evidence that a sizeable proportion of NCX is also expressed within the dyadic cleft [31–33] and that NCX could thus modulate cleft Ca^{2+} concentration which again may have important physiological implications [34, 35].

Under certain conditions, NCX transport mode can reverse to mediate influx of Ca^{2+} into the myocyte instead of extruding Ca^{2+} [36–38]. Thus, in the presence of increased intracellular Na^+ , the driving force for Ca^{2+} extrusion is reduced or even reversed, so that Na^+ leaves the cell and is exchanged for Ca^{2+} entering the cell. Since NCX is electrogenic, membrane depolarization during early systole also favours NCX reverse mode. It has been a matter of debate whether NCX reverse mode may contribute to systolic Ca^{2+} influx and thus add to force development in cardiac myocytes. Indeed, it was demonstrated that NCX reverse mode induced by long depolarizing voltage clamps could evoke contractions [39, 40]. We have recently produced new evidence from our genetic models that this reverse NCX mechanism is a manifest contributor to trigger Ca^{2+} [41]. As will be discussed below, NCX reverse mode is believed to play a significant role in the pathophysiology of ischemia-reperfusion injury.

In 1990, Nicoll *et al.* [42] first sequenced and published their initial analysis of the transsarcolemmal structure of NCX. It was found that the protein consists of 9 transmembrane segments and a long cytoplasmic loop, which separates the first 5 from the following 4 α helical transmembrane segments. Currently, three isoforms of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger have been characterized, that have about 70% amino acid identity. While NCX1 is the predominant isoform of the heart [43, 44], NCX2 and NCX3 are detected in the central nervous system and in skeletal muscle [45, 46]. An as yet not cloned form of NCX is also thought to be present in mitochondria. Interestingly, this form of NCX does not seem to be electrogenic [47].

NCX activity is regulated by a variety of mechanisms among them Ca^{2+} and Na^+ , that besides being substrates for NCX also exert separate regulatory influences (for review, see [7]). At least one study has found that $\text{Na}^+/\text{Ca}^{2+}$ exchange current (I_{NCX}) may respond to adrenergic stimulation [48] but most studies investigating this effect have been negative [49–51].

3. PRINCIPLES OF NCX INHIBITION

Experimental NCX inhibition has been an important tool in defining the role of NCX in cardiac physiology and pathophysiology. Both pharmacological and genetic inhibition of

NCX have been used experimentally to evaluate the therapeutic potential of NCX suppression. The potential of a future therapy of cardiovascular disease by inhibition of NCX will depend directly on the reliability, specificity and safety of the means available to suppress NCX activity in vivo. We will therefore give a brief review on the tools that are currently available to suppress NCX activity.

Pharmacological Inhibition

Synthetic NCX inhibitors have been available since the mid-1990s and have since been used in numerous studies investigating the physiology and pathophysiology of $\text{Na}^+/\text{Ca}^{2+}$ exchange and Ca^{2+} cycling. A common reservation about the use of pharmacologic NCX inhibition vs. genetic ablation is the potential lack of specificity. Indeed, KB-R 7943, the first synthetic NCX inhibitor widely used experimentally shows interactions with several extracardiac [52, 53] and cardiac ion channels and functional proteins. Among the latter are L-type- Ca^{2+} , K^+ and Na^+ channels [54], the RyR [55] and mitochondrial uniporters [56]. SEA0400, a synthetic inhibitor which became available in 2001 [57] appears to offer a higher specificity [54] though there is still evidence that it may also modify cardiac function via a mechanism independent of NCX [58].

Further synthetics with NCX inhibitory potential are under development [59, 60].

Genetic Knockout (KO) of NCX

Global KO of NCX is embryonically lethal in mice [61, 62], while mice with inducible cardiac specific knockout survive into adulthood. Mice with modest (=heterozygous KO) [19, 63] and complete (=homozygous KO) [64] genetic ablation of NCX have been investigated. Cardiac myocytes from NCX KO mice with complete ablation of NCX do not exhibit significant alterations of resting or systolic Ca^{2+} concentration, or sarcoplasmic reticular (SR) Ca^{2+} load when compared to WT littermates. NCX inward current is absent and the decrease of the Ca^{2+} transient is drastically slowed during caffeine exposure, indicating that no alternative Ca^{2+} extrusion mechanism is upregulated to compensate for the absence of NCX. Instead, peak L-type Ca^{2+} current (I_{Ca}) is decreased [32] and AP duration is reduced [65] resulting in a further reduction of net Ca^{2+} entry into the myocyte to 20% [35]. Thus, in the absence of NCX, transsarcolemmal Ca^{2+} traffic is drastically reduced. The plasma membrane Ca^{2+} ATPase (PMCA) has been estimated to provide 10–25% of myocyte Ca^{2+} efflux from the cytosol into the extracellular space depending on species variation [29, 66, 67]. Thus, a Ca^{2+} influx reduced to 20% as observed in NCX KO myocytes is well within the range of capacity of the PMCA (Fig. 2a). Despite these alterations, there is no difference in the cellular Ca^{2+} transients implying an increase in the gain of excitation-contraction coupling in KO cells. The converse has been observed in NCX overexpressor mice [68] (Fig. 2b). Though there are considerable differences between murine cardiac physiology and that of higher mammals, these studies provide information on the cellular adaptations that may be active during future therapeutic NCX manipulation.

Gene-transfection methods to acutely inhibit NCX in isolated cardiac myocytes are currently available [69] and may be investigated as therapeutic tools in future studies.

4. NCX AND CARDIAC ARRHYTHMIA

NCX activity and Action Potential (AP) Kinetics

To understand the involvement of NCX in the generation of cardiac arrhythmia, it is necessary to define the role that NCX plays in the repolarization of the cardiac AP. During systole, when cytosolic Ca^{2+} concentration is high, NCX extrudes Ca^{2+} from the cytosol into the extracellular space. Due to the electrogenic nature of this process, NCX generates an inwardly directed membrane current (I_{NCX}) during SR Ca^{2+} release. It has been assumed that via this mechanism NCX would contribute to maintain the plateau of the cardiac action potential. Indeed, computer modelling revealed that increased I_{NCX} activity significantly delays repolarization of the cardiac AP [70]. Sham and Spencer [71] also demonstrated in guinea pig cardiomyocytes that an inhibition of NCX caused an abbreviation of the AP while a prolongation was observed when NCX activity was maximal. Many groups including ours have used transgenic models with cardiac specific alteration of NCX expression to further define the role of NCX in AP repolarization. A prolonged AP plateau was observed in NCX overexpressor mice, and – conversely – a shortened AP in NCX knockout mice [35] (Fig. 3), although in knockout mice, additional mechanisms contribute to shortening AP duration [65].

In addition to a direct influence of NCX inward current on AP repolarization, an alternative mechanism may explain NCX-mediated repolarization changes: we have proposed a functional interplay between NCX and L-type Ca^{2+} channels. One of the strongest regulators of I_{Ca} is cytosolic Ca^{2+} itself. Thus, at high intracellular Ca^{2+} concentrations, Ca^{2+} binds to calmodulin, thereby inactivating I_{Ca} . At low cytosolic Ca^{2+} concentrations, when no Ca^{2+} is bound, L-type Ca^{2+} current is considered to be maximal [72, 73]. The DHPR is primarily located at sarcolemmal SR junctions in close vicinity to the RyR and NCX [30]. It has been accepted that Ca^{2+} release from the SR is the primary mechanism to influence and terminate L-type Ca^{2+} current [74, 75]. However, due to its close vicinity, NCX activity may also influence I_{Ca} . We have reported that blockade of NCX may increase cleft Ca^{2+} concentration [32–34]. This may have important implications for I_{Ca} regulation via Ca^{2+} induced inactivation: In the absence of NCX activity elevated cleft Ca^{2+} decreases I_{Ca} open probability and accelerates I_{Ca} inactivation. Conversely, when NCX activity is high, this may increase I_{Ca} open probability and delay I_{Ca} inactivation. Indeed, we could demonstrate that in NCX knockout mice, I_{Ca} is reduced via a Ca^{2+} dependent mechanism [32], while – conversely – I_{Ca} in NCX overexpressor mice is increased, a mechanism that also appears Ca^{2+} dependent [68]. Via this functional interplay with I_{Ca} , NCX may – indirectly – influence AP repolarization in the same direction as it does directly via reduced or increased NCX inward current as explained above. Thus, increased I_{NCX} would also increase net I_{Ca} thereby further slowing repolarization. Conversely, reduced I_{NCX} would also reduce I_{Ca} thus reducing AP duration.

NCX and Afterdepolarizations: Pathophysiological Concept

It has been hypothesized that increased NCX activity could induce afterdepolarizations of the ventricular AP that could then trigger tachyarrhythmias [76–78]. Afterdepolarizations are defined as oscillations of the electric membrane potential during (early

afterdepolarization [EAD]) or after (delayed afterdepolarization [DAD]; Fig. 4) the cardiac action potential. Afterdepolarizations can induce reentry and are commonly regarded as a major trigger of cardiac tachyarrhythmias.

EADs have been shown to occur in animal models with slowed AP repolarization [79]. The prolonged AP duration prolongs the period of time the myocytes spend in a depolarized state which again will broaden the “time window” for voltage dependent I_{Na} or I_{Ca} to reactivate and induce an EAD (Fig. 4). This mechanism has been evoked to explain proarrhythmia in animal models of hypertrophy and heart failure where a reduction of K^+ carried outward currents and an increased AP duration have been observed (for review see [80]). Likewise, specific mutations of K^+ channel subunits resulting in loss of function, or Na^+ channel gain-of-function mutations [81], can both slow AP repolarization and cause EADs resulting in ventricular tachyarrhythmia [81–83]. Since NCX also slows repolarization it may be a further candidate for the promotion of EADs.

NCX is also essential for the development of DADs. The initiating event of a DAD consists of a spontaneous release of Ca^{2+} from the SR, as occurs in the setting of SR Ca^{2+} overload. This sudden increase of cytosolic Ca^{2+} then leads to an activation of NCX inward current which depolarizes the sarcolemmal membrane. If this reaches the threshold for Na^+ channel activation, a new and premature AP is triggered [76, 84] (Fig. 4).

Initial studies in isolated cardiac myocytes from several different species have indeed demonstrated that DADs can be triggered via Ca^{2+} overload of the SR [85–88]. An increased occurrence of afterdepolarizations of the cardiac AP has also been observed in animal models of hypertrophy and heart failure [89–94], where NCX is known to be overexpressed [95, 96].

Studies testing the proarrhythmic potential of increased NCX expression have mostly been limited to hypertrophied and failing myocardium. Therefore, their informations on the role of NCX in the generation of cardiac arrhythmia are somewhat limited because a multitude of structural, expressional and functional changes other than the upregulation of NCX have been observed in human and animal failing and hypertrophic myocardium (for review, see [97]) and any one or a combination of these could be potential promoters of cardiac arrhythmia. The NCX overexpressing mouse on the other hand has increased NCX activity in the absence of cellular hypertrophy or reduced K^+ channel activity. Before 14 weeks, these animals have normal whole heart and cellular function and structure and preserved K^+ outward currents. We have found that these animals are prone to whole heart ventricular tachyarrhythmia. In isolated cardiac myocytes, APs are prolonged and there is an increased occurrence of EADs as well as DADs [14]. These observations indicate that NCX may be a promoter for arrhythmia independent of structural or ionic remodelling associated with heart failure.

Experimental Inhibition of NCX to Counter Proarrhythmia

Early studies investigating the antiarrhythmic potential of the NCX inhibitor SEA0400 yielded inconsistent results. While Nagy *et al.* [8] observed that the NCX inhibitor SEA0400 inhibited EADs induced by dofetilide and barium in guinea pig cardiac muscle strips, Amran

et al. did not observe a protective effect of SEA0400 in either *in vivo* studies or isolated cardiac myocytes when monitoring aconitine-induced arrhythmia in the same species [98]. Two independent studies investigated the effects of SEA0400 on arrhythmias induced by ouabain. Tanaka *et al.* [9] showed an antiarrhythmic effect in guinea pig right ventricular muscle strips. In an *in vivo* dog model, Nagasawa and coworkers [99] observed that pharmacological inhibition of NCX protected against ouabain-induced arrhythmia but not against arrhythmia induced by acute ischemia. A recent study has specifically investigated the effect of SEA0400 on cardiac myocytes isolated from the pulmonary vein regions of guinea pig left atria [100]. Spontaneous electrical activity generated by this cell population is regarded to be the trigger for atrial fibrillation, one of the most frequent tachyarrhythmias.

We have also attempted to characterize the antiarrhythmic effect of pharmacological NCX inhibition. To produce proarrhythmic experimental conditions in which afterdepolarizations occur, we utilized a recently developed intact heart model of the rabbit heart mimicking the electrophysiological alterations of long QT 2 and 3 syndromes (LQT 2 and 3) [101–103]. Under these experimental conditions, SEA0400 significantly reduced AP duration and suppressed EADs and the Torsade-de-Pointes form of ventricular tachycardia [13].

The discrepancies evident in studies investigating the antiarrhythmic potential of acute NCX inhibition may be due to the following factors: 1. Proarrhythmic interventions of a different nature have been applied (i.e. aconitine-, vs. ouabain-, vs. dofetilide- vs. ischemia-induced arrhythmia), 2. Different species (guinea pig vs. dog vs. rabbit) have been investigated and species differences in the composition of repolarizing ion channels are well known [104]. 3. Finally, different preparations of cardiac tissue ranging from *in vivo* models over trabecular preparations to patch clamp experiments in isolated cardiac myocytes have been used.

5. NCX AND ISCHEMIA

Role of NCX in the Pathophysiology of Ischemia Reperfusion Injury

During cardiac ischemia, a rise in intracellular Na^+ occurs due to a reduction of Na^+/K^+ ATPase activity. This increase in Na^+ concentration is followed by a rise in intracellular Ca^{2+} . This accumulation of cytosolic Ca^{2+} concentration is dependent on the rise in cytosolic Na^+ concentration and it has therefore been suggested that the process of Ca^{2+} accumulation during ischemia is mediated via NCX working in reverse mode [105–110]. As mentioned above, depending on the transsarcolemmal Na^+ and Ca^{2+} gradients and the membrane potential, NCX can switch into reverse mode and transport Ca^{2+} into the cell instead of extruding Ca^{2+} [7]. In reverse mode, NCX extrudes 3 Na^+ in exchange for 1 Ca^{2+} ion entering the cell. NCX reverse mode is thus favored by the high cytosolic Na^+ concentration characteristic for ischemic conditions. Consistent with this hypothesis, mice with heterozygous overexpression of NCX exhibit increased susceptibility to ischemia-reperfusion injury [10, 111].

However, during ischemia, there is also a rise in cytosolic H^+ concentration that contributes to the rise in cytosolic Na^+ concentration via activation of the Na^+/H^+ exchanger (NHE). This increased Na^+ favors NCX reverse mode and cytosolic Ca^{2+} accumulation. Inhibition of NHE blocks the rise in Na^+ and Ca^{2+} and is cardioprotective during ischemia [110, 112].

Some studies have suggested that preventing the rise in cytosolic Na^+ concentration via NHE inhibition may be cardioprotective, independent of the effect on Ca^{2+} accumulation [113, 114]. Both cytosolic accumulation of Ca^{2+} and Na^+ are inhibited by NHE inhibitors, and it is therefore difficult to distinguish between the relative protective effects of preventing the increase in Na^+ vs. Ca^{2+} concentration.

The development of transgenic mice and genetic transfection techniques has provided a unique tool to further investigate this question. We have demonstrated that NCX KO mice are significantly less susceptible to ischemic injury [12]. Cellular studies have demonstrated that there is no detectible Ca^{2+} entry during experimental lowering of the transsarcolemmal Na^+ gradient in NCX KO myocytes while WT myocytes exhibited significant cytosolic Ca^{2+} accumulation under these conditions (Fig. 5). During ischemia-reperfusion, hearts from NCX KO mice exhibited less necrosis, better post-ischemic recovery of cardiac performance, higher levels of high-energy phosphates, and improved recovery of Na^+ homeostasis when compared to WT animals [12]. Maddaford *et al.* [115] have demonstrated that acute targeted genetic suppression of NCX from isolated cardiac myocytes can also protect from ischemic injury. Similarly, it was shown that introduction of specific splice variants of NCX into neonatal cardiac myocytes via transfection prevented ischemia induced cytosolic Ca^{2+} accumulation [116]. These observations add further support to the hypothesis that NCX is a direct mediator of ischemic injury. Moreover, these data indicate that inhibition of NCX seems to be protective independent of a direct inhibition of H^+ accumulation via NHE.

NCX Inhibition in Models of Ischemia-Reperfusion Injury

Pharmacological studies in animal models have also been conducted to investigate the therapeutic potential of NCX inhibitors as a means to protect against ischemia-reperfusion injury.

The most frequently used agents to test the effect of NCX inhibition on ischemia reperfusion injury are KB-R7943 and more recently SEA0400. KB-R7943 has been demonstrated to reduce ischemic injury under a variety of experimental conditions ranging from cellular to *in vivo* models. Thus, it has been shown to inhibit ischemia-reperfusion induced cytosolic Ca^{2+} accumulation in isolated cardiac myocytes [117, 118] and to improve post-ischemic contractility in trabecular preparations [119, 120] and whole heart models [121–123].

In a similar range of experimental settings, SEA0400 has as well been demonstrated to reduce cellular Ca^{2+} entry upon ischemia-reperfusion [124], limit myocardial infarction size [125] and improve post-ischemic contractility [124, 125] even several days after the ischemia-reperfusion event [126]. A new NCX inhibitor, NCC-135, has also been shown to inhibit Ca^{2+} overload and reduce ischemia-reperfusion injury [127]. One comparative study using a whole heart rabbit model to investigate the protective effect of SEA0400 and KB-R7943 under identical experimental conditions has observed a similar efficiency of both drugs in reducing infarct size. However in this model only SEA0400 improved post-ischemic cardiac contractility while KB-R7943 significantly reduced heart rate and cardiac output [125]. This may be a consequence of the previously reported lack of KB-R7943's specificity.

6. NCX AND CHRONIC HEART FAILURE

NCX and Chronic Heart Failure: Pathophysiological Concept

In the failing human heart, contractile force is reduced and relaxation is retarded. These alterations are caused in part by defective intracellular Ca^{2+} -handling (for review see [128]). In failing myocytes, there is an increase in diastolic and a decrease of systolic Ca^{2+} [129] resulting in a reduced Ca^{2+} transient and contractile force, especially at increased cardiac frequencies [130]. These alterations have been attributed to decreased sarcoplasmic Ca^{2+} -load, which could be caused by insufficient Ca^{2+} -reuptake in diastole due to decreased activity of the SERCA [131, 132] or to excessive Ca^{2+} leak through hyperphosphorylated ryanodine receptors [133]. NCX is upregulated in the failing and hypertrophic heart in both activity and expression [93, 96]. It is unknown whether increased NCX activity is a cause or a consequence of chronic heart failure. Evidence suggests that increased NCX activity may provide an adaptive mechanism which protects against diastolic Ca^{2+} overload [95]. Yet, it may also be possible that the increased NCX current in the failing heart competes with the reduced SERCA of failing myocardium for cytosolic Ca^{2+} , a maladaptive mechanism contributing to sarcoplasmic reticular Ca^{2+} loss and reduced contractility.

Cardiac glycosides such as digoxin have been used for over a 100 years to manipulate this relationship between SERCA and NCX in order to restore contractility in failing myocardium. Digoxin inhibits the Na^+/K^+ ATPase, resulting in increased cytoplasmic Na^+ . This increased Na^+ concentration alters the transmembrane gradient for Na^+ , which reduces Ca^{2+} efflux via NCX and allows SERCA to accumulate Ca^{2+} . In heart failure, the restored SR Ca^{2+} load provided by this indirect manipulation of NCX improves contractility. As noted above, this can unfortunately result in SR Ca^{2+} overload, DADs and triggered arrhythmias, which likely explains why digoxin does not provide a mortality benefit [128]. If intracellular Na^+ could be more carefully regulated, it might reduce the arrhythmias associated with digoxin. The approach of reducing digoxin target levels may help in this regard.

Therapeutic Approaches and Lessons from Transgenic Mice

As mentioned above, there is evidence, that in *acute* heart failure induced by ischemia NCX inhibition can be beneficial (for review see [134]). However, experimental testing of the therapeutic potential of NCX inhibition in *chronic* heart failure is difficult. In an ideal experimental setup, the development of chronic heart failure would be monitored in the absence and presence of pharmacological NCX inhibition. However, depending on the model, the development from a non-failing phenotype to overt heart failure may take up to several weeks. Alternatively, one could choose an approach in which NCX inhibitors are administered in a phenotype that already exhibits heart failure to test whether a reduction of NCX activity may cause reverse remodelling. However, to detect macro- or microstructural remodelling in chronic heart failure, one would require an observation period of at least weeks. To our knowledge there is no study that has administered pharmacological NCX inhibitors over such a long period of time. This may in part be due to the fact that there is no published information available on the bioavailability or plasma removal rates of pharmacological NCX inhibitors.

Some information on the effect of altered NCX activity on the development of heart failure has been gathered in transgenic mice. In accordance with the hypothesis that increased NCX activity can promote chronic heart failure, transgenic mice with 3-fold overexpression develop clinically overt heart failure under experimental conditions under which their WT littermates do not [135].

In mice with complete ablation of NCX using cre/lox technology, cellular excitation-contraction coupling and Ca^{2+} handling can adapt so that cellular contractility is preserved [16, 35, 64]. Nevertheless, with progressing age, these animals also exhibit overt clinical heart failure and premature death [19]. This does not necessarily contradict the hypothesis that reduction of NCX activity may protect from heart failure since a beneficial effect may only be evident with modest NCX inhibition. Accordingly, heterozygous NCX KO mice show normal cardiac function and structure with normal lifespan [19]. Thus the heterozygous model may be better suited to further investigate the potential of reduced NCX activity in the therapy of chronic heart failure.

7. FUTURE DIRECTIONS: NCX INHIBITION AS A NEW THERAPEUTIC TOOL?

A wide variety of therapeutic tools exist to treat cardiovascular disease. These consist of catheter-based, surgical and pharmacological approaches. Nevertheless, cardiovascular disease remains the leading cause of death in western industrial nations. Since the 1980s, several innovations have been made in the field of interventional therapies such as catheter based coronary revascularization and arrhythmia ablation, implantable cardioverter/defibrillators and cardiac resynchronization therapy to name only a few. Innovations have also been made in the pharmacological field. Since the advent of angiotensin converting enzyme blockers (ACE inhibitors), novel promising drugs such as ranolazine or ivabradine have emerged. The overwhelming experimental data reviewed here point towards a benefit of using NCX inhibition as a novel therapy as well. However, research on NCX inhibition – may it be pharmacological or genetical – has to be taken to the next level. Long term animal feeding studies will have to be conducted to give some information on the safety of pharmacological NCX inhibitors. This will be a precondition of eventually testing NCX inhibitors on their safety in humans.

Contemplating the broad spectrum of pathophysiologies that depend on NCX activity (arrhythmia, ischemia, heart failure, arterial hypertension) the authors imagine, that once established in terms of safety and benefit, inhibition of NCX for the treatment of cardiovascular disease may have a similar impact on the field as the advent of ACE inhibitors in the 1980s.

Acknowledgments

This work was supported by grants Interdisziplinäre Medizinische Forschung (IMF Po 12 06 07), a stipend by the Deutsche Forschungsgemeinschaft (DFG Po 1004-1/2) (both to C.P.), NIH (R01 HL070828) to J.I.G. L.E. holds the Peter-Osypka Professorship for Clinical and Experimental electrophysiology.

References

1. Eckardt L, Haverkamp W, Breithardt G. Antiarrhythmic therapy in heart failure. *Heart Fail Monit.* 2002; 2:110–9. [PubMed: 12634885]
2. Barth AS, Tomaselli GF. Cardiac metabolism and arrhythmias. *Circ Arrhythm Electrophysiol.* 2009; 2:327–35. [PubMed: 19808483]
3. Scott J. Pathophysiology and biochemistry of cardiovascular disease. *Curr Opin Genet Dev.* 2004; 14:271–9. [PubMed: 15172670]
4. Dulhunty AF, Casarotto MG, Beard NA. The ryanodine receptor: a pivotal Ca^{2+} regulatory protein and potential therapeutic drug target. *Curr Drug Targets.* :XXX.
5. Jiang LH, Gamper N, Beech DJ. Properties and therapeutic potential of TRP channels with putative roles in adversity. *Curr Drug Targets.* :XXX.
6. Viola HM, Hool LC. Targeting calcium and the mitochondria in prevention of pathology in the heart. *Curr Drug Targets.* :XXX.
7. Philipson KD, Nicoll DA. Sodium-calcium exchange: a molecular perspective. *Annu Rev Physiol.* 2000; 62:111–33. [PubMed: 10845086]
8. Nagy ZA, Virag L, Toth A, Biliczki P, Acsai K, Banyasz T, et al. Selective inhibition of sodium-calcium exchanger by SEA-0400 decreases early and delayed after depolarization in canine heart. *Br J Pharmacol.* 2004; 143:827–31. [PubMed: 15504749]
9. Tanaka H, Shimada H, Namekata I, Kawanishi T, Iida-Tanaka N, Shigenobu K. Involvement of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger in ouabain-induced inotropy and arrhythmogenesis in guinea-pig myocardium as revealed by SEA0400. *J Pharmacol Sci.* 2007; 103:241–6. [PubMed: 17310075]
10. Cross HR, Lu L, Steenbergen C, Philipson KD, Murphy E. Overexpression of the cardiac $\text{Na}^+/\text{Ca}^{2+}$ exchanger increases susceptibility to ischemia/reperfusion injury in male, but not female, transgenic mice. *Circ Res.* 1998; 83:1215–23. [PubMed: 9851938]
11. Takahashi T, Takahashi K, Onishi M, Suzuki T, Tanaka Y, Ota T, et al. Effects of SEA0400, a novel inhibitor of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger, on myocardial stunning in anesthetized dogs. *Eur J Pharmacol.* 2004; 505:163–8. [PubMed: 15556149]
12. Imahashi K, Pott C, Goldhaber JJ, Steenbergen C, Philipson KD, Murphy E. Cardiac-specific ablation of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger confers protection against ischemia/reperfusion injury. *Circ Res.* 2005; 97:916–21. [PubMed: 16179590]
13. Milberg P, Pott C, Fink M, Frommeyer G, Matsuda T, Baba A, et al. Inhibition of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger suppresses torsades de pointes in an intact heart model of long QT syndrome-2 and long QT syndrome-3. *Heart Rhythm.* 2008; 5:1444–52. [PubMed: 18929333]
14. Pott, C.; Muszynski, A.; Ruhe, M.; Schulte, JS.; Milberg, P.; Fabritz, L.; Goldhaber, JJ.; Breithardt, G.; Philipson, KD.; Kirchhof, P.; Müller, FU. Proarrhythmia in a non-failing murine model of $\text{Na}^+/\text{Ca}^{2+}$ exchanger overexpression: Cellular and molecular mechanisms; abstract. *Heart Rhythm Meeting*; Denver. 2010;
15. Pott C, Goldhaber JJ, Philipson KD. Genetic manipulation of cardiac $\text{Na}^+/\text{Ca}^{2+}$ exchange expression. *Biochem Biophys Res Commun.* 2004; 322:1336–40. [PubMed: 15336980]
16. Pott C, Henderson SA, Goldhaber JJ, Philipson KD. $\text{Na}^+/\text{Ca}^{2+}$ exchanger knockout mice: plasticity of cardiac excitation-contraction coupling. *Ann N Y Acad Sci.* 2007; 1099:270–5. [PubMed: 17446467]
17. Goldhaber JJ, Henderson SA, Reuter H, Pott C, Philipson KD. Effects of $\text{Na}^+/\text{Ca}^{2+}$ exchange expression on excitation-contraction coupling in genetically modified mice. *Ann N Y Acad Sci.* 2005; 1047:122–6. [PubMed: 16093490]
18. Reuter H, Pott C, Goldhaber JJ, Henderson SA, Philipson KD, Schwinger RH. $\text{Na}^+/\text{Ca}^{2+}$ exchange in the regulation of cardiac excitation-contraction coupling. *Cardiovasc Res.* 2005; 67:198–207. [PubMed: 15935336]
19. Jordan MC, Henderson SA, Han T, Fishbein MC, Philipson KD, Roos KP. Myocardial Function With Reduced Expression of the Sodium-Calcium Exchanger. *J Card Fail.* 2010; 16:786–796. [PubMed: 20797603]
20. Reuter H, Blaustein MP, Haeusler G. Na-Ca exchange and tension development in arterial smooth muscle. *Philos Trans R Soc Lond B Biol Sci.* 1973; 265:87–94. [PubMed: 4144702]

21. Blaustein MP, Oborn CJ. The influence of sodium on calcium fluxes in pinched-off nerve terminals in vitro. *J Physiol.* 1975; 247:657–86. [PubMed: 238034]
22. Blaustein MP, Zhang J, Chen L, Song H, Raina H, Kinsey SP, et al. The pump, the exchanger, and endogenous ouabain: signaling mechanisms that link salt retention to hypertension. *Hypertension.* 2009; 53:291–8. [PubMed: 19104005]
23. Jeffs GJ, Meloni BP, Bakker AJ, Knuckey NW. The role of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX) in neurons following ischaemia. *J Clin Neurosci.* 2007; 14:507–14. [PubMed: 17430774]
24. Bers DM. Cardiac excitation-contraction coupling. *Nature.* 2002; 415:198–205. [PubMed: 11805843]
25. Bers DM, Bridge JH, Spitzer KW. Intracellular Ca^{2+} transients during rapid cooling contractures in guinea-pig ventricular myocytes. *J Physiol.* 1989; 417:537–53. [PubMed: 2621609]
26. Bridge JH, Smolley JR, Spitzer KW. The relationship between charge movements associated with I_{Ca} and $\text{I}_{\text{Na-Ca}}$ in cardiac myocytes. *Science.* 1990; 248:376–8. [PubMed: 2158147]
27. Crespo LM, Grantham CJ, Cannell MB. Kinetics, stoichiometry and role of the Na-Ca exchange mechanism in isolated cardiac myocytes. *Nature.* 1990; 345:618–21. [PubMed: 2348872]
28. Reuter H, Seitz N. The dependence of calcium efflux from cardiac muscle on temperature and external ion composition. *J Physiol.* 1968; 195:451–70. [PubMed: 5647333]
29. Bassani JW, Bassani RA, Bers DM. Relaxation in rabbit and rat cardiac cells: species-dependent differences in cellular mechanisms. *J Physiol.* 1994; 476:279–93. [PubMed: 8046643]
30. Scriven DR, Dan P, Moore ED. Distribution of proteins implicated in excitation-contraction coupling in rat ventricular myocytes. *Biophys J.* 2000; 79:2682–91. [PubMed: 11053140]
31. Thomas MJ, Sjaastad I, Andersen K, Helm PJ, Wasserstrom JA, Sejersted OM, et al. Localization and function of the $\text{Na}^+/\text{Ca}^{2+}$ -exchanger in normal and detubulated rat cardiomyocytes. *J Mol Cell Cardiol.* 2003; 35:1325–37. [PubMed: 14596789]
32. Pott C, Yip M, Goldhaber JI, Philipson KD. Regulation of cardiac L-type Ca^{2+} current in $\text{Na}^+/\text{Ca}^{2+}$ exchanger knockout mice: functional coupling of the Ca^{2+} channel and the $\text{Na}^+/\text{Ca}^{2+}$ exchanger. *Biophys J.* 2007; 92:1431–7. [PubMed: 17114214]
33. Neco P, Rose B, Huynh N, Zhang R, Bridge JH, Philipson KD, Goldhaber JI. Sodium-Calcium Exchange is Essential for Effective Triggering of Calcium Release in Mouse Heart. *Biophys J.* 2010; 99:755–64. [PubMed: 20682252]
34. Goldhaber JI, Lamp ST, Walter DO, Garfinkel A, Fukumoto GH, Weiss JN. Local regulation of the threshold for calcium sparks in rat ventricular myocytes: role of sodium-calcium exchange. *J Physiol.* 1999; 520(Pt 2):431–8. [PubMed: 10523412]
35. Pott C, Philipson KD, Goldhaber JI. Excitation-contraction coupling in $\text{Na}^+/\text{Ca}^{2+}$ exchanger knockout mice: reduced transsarcolemmal Ca^{2+} flux. *Circ Res.* 2005; 97:1288–95. [PubMed: 16293789]
36. Leblanc N, Hume JR. Sodium current-induced release of calcium from cardiac sarcoplasmic reticulum. *Science.* 1990; 248:372–6. [PubMed: 2158146]
37. Levesque PC, Leblanc N, Hume JR. Role of reverse-mode $\text{Na}^+/\text{Ca}^{2+}$ exchange in excitation-contraction coupling in the heart. *Ann N Y Acad Sci.* 1991; 639:386–97. [PubMed: 1785865]
38. Lipp P, Niggli E. Sodium current-induced calcium signals in isolated guinea-pig ventricular myocytes. *J Physiol.* 1994; 474:439–46. [PubMed: 8014905]
39. Barcenas-Ruiz L, Beuckelmann DJ, Wier WG. Sodium-calcium exchange in heart: membrane currents and changes in $[\text{Ca}^{2+}]_i$. *Science.* 1987; 238:1720–2. [PubMed: 3686010]
40. Hume JR, Uehara A. Properties of “creep currents” in single frog atrial cells. *J Gen Physiol.* 1986; 87:833–55. [PubMed: 2425041]
41. Larbig R, Torres N, Bridge JH, Goldhaber JI, Philipson KD. Activation of Reverse $\text{Na}^+/\text{Ca}^{2+}$ Exchange by the Na^+ Current during an Action Potential Augments the Calcium Transient: Evidence from NCX Knockout Mice. *J Physiol.* 2010 Jul 19. Epub ahead of print.
42. Nicoll DA, Longoni S, Philipson KD. Molecular cloning and functional expression of the cardiac sarcolemmal $\text{Na}^+/\text{Ca}^{2+}$ exchanger. *Science.* 1990; 250:562–5. [PubMed: 1700476]

43. Kofuji P, Hadley RW, Kieval RS, Lederer WJ, Schulze DH. Expression of the Na-Ca exchanger in diverse tissues: a study using the cloned human cardiac Na-Ca exchanger. *Am J Physiol.* 1992; 263:C1241–9. [PubMed: 1476165]
44. Komuro I, Wenninger KE, Philipson KD, Izumo S. Molecular cloning and characterization of the human cardiac Na⁺/Ca²⁺ exchanger cDNA. *Proc Natl Acad Sci U S A.* 1992; 89:4769–73. [PubMed: 1374913]
45. Li Z, Matsuoka S, Hryshko LV, Nicoll DA, Bersohn MM, Burke EP, et al. Cloning of the NCX2 isoform of the plasma membrane Na⁺-Ca²⁺ exchanger. *J Biol Chem.* 1994; 269:17434–9. [PubMed: 8021246]
46. Nicoll DA, Quednau BD, Qui Z, Xia YR, Lusis AJ, Philipson KD. Cloning of a third mammalian Na⁺-Ca²⁺ exchanger, NCX3. *J Biol Chem.* 1996; 271:24914–21. [PubMed: 8798769]
47. Palty R, Silverman WF, Hershfinkel M, Caporale T, Sensi SL, Parnis J, et al. NCLX is an essential component of mitochondrial Na⁺/Ca²⁺ exchange. *Proc Natl Acad Sci U S A.* 2009; 107:436–41. [PubMed: 20018762]
48. Zhang YH, Hinde AK, Hancox JC. Anti-adrenergic effect of adenosine on Na⁺-Ca²⁺ exchange current recorded from guinea-pig ventricular myocytes. *Cell Calcium.* 2001; 29:347–58. [PubMed: 11292391]
49. Ballard C, Schaffer S. Stimulation of the Na⁺/Ca²⁺ exchanger by phenylephrine, angiotensin II and endothelin 1. *J Mol Cell Cardiol.* 1996; 28:11–7. [PubMed: 8745210]
50. Main MJ, Grantham CJ, Cannell MB. Changes in subsarcolemmal sodium concentration measured by Na-Ca exchanger activity during Na-pump inhibition and beta-adrenergic stimulation in guinea-pig ventricular myocytes. *Pflugers Arch.* 1997; 435:112–8. [PubMed: 9359910]
51. Ginsburg KS, Bers DM. Isoproterenol does not enhance Ca-dependent Na/Ca exchange current in intact rabbit ventricular myocytes. *J Mol Cell Cardiol.* 2005; 39:972–81. [PubMed: 16242149]
52. Liang GH, Kim JA, Seol GH, Choi S, Suh SH. The Na⁺/Ca²⁺ exchanger inhibitor KB-R7943 activates large-conductance Ca²⁺-activated K⁺ channels in endothelial and vascular smooth muscle cells. *Eur J Pharmacol.* 2008; 582:35–41. [PubMed: 18237728]
53. Pezier A, Bobkov YV, Ache BW. The Na⁺/Ca²⁺ exchanger inhibitor, KB-R7943, blocks a nonselective cation channel implicated in chemosensory transduction. *J Neurophysiol.* 2009; 101:1151–9. [PubMed: 19118110]
54. Tanaka H, Nishimaru K, Aikawa T, Hirayama W, Tanaka Y, Shigenobu K. Effect of SEA0400, a novel inhibitor of sodium-calcium exchanger, on myocardial ionic currents. *Br J Pharmacol.* 2002; 135:1096–100. [PubMed: 11877314]
55. Barrientos G, Bose DD, Feng W, Padilla I, Pessah IN. The Na⁺/Ca²⁺ exchange inhibitor 2-(2-(4-(4-nitrobenzyloxy)phenyl)ethyl)isothiourea methanesulfonate (KB-R7943) also blocks ryanodine receptors type 1 (RyR1) and type 2 (RyR2) channels. *Mol Pharmacol.* 2009; 76:560–8. [PubMed: 19509218]
56. Santo-Domingo J, Vay L, Hernandez-Sanmiguel E, Lobaton CD, Moreno A, Montero M, et al. The plasma membrane Na⁺/Ca²⁺ exchange inhibitor KB-R7943 is also a potent inhibitor of the mitochondrial Ca²⁺ uniporter. *Br J Pharmacol.* 2007; 151:647–54. [PubMed: 17471180]
57. Matsuda T, Arakawa N, Takuma K, Kishida Y, Kawasaki Y, Sakaue M, et al. SEA0400, a novel and selective inhibitor of the Na⁺-Ca²⁺ exchanger, attenuates reperfusion injury in the in vitro and in vivo cerebral ischemic models. *J Pharmacol Exp Ther.* 2001; 298:249–56. [PubMed: 11408549]
58. Reuter H, Henderson SA, Han T, Matsuda T, Baba A, Ross RS, et al. Knockout mice for pharmacological screening: testing the specificity of Na⁺-Ca²⁺ exchange inhibitors. *Circ Res.* 2002; 91:90–2. [PubMed: 12142340]
59. Niu CF, Watanabe Y, Ono K, Iwamoto T, Yamashita K, Satoh H, et al. Characterization of SN-6, a novel Na⁺/Ca²⁺ exchange inhibitor in guinea pig cardiac ventricular myocytes. *Eur J Pharmacol.* 2007; 573:161–9. [PubMed: 17644086]
60. Secondo A, Pannaccione A, Molinaro P, Ambrosino P, Lippiello P, Esposito A, et al. Molecular pharmacology of the amiloride analog 3-amino-6-chloro-5-[(4-chloro-benzyl)amino]-n-[(2,4-dimethylbenzyl)-amino]iminomethyl]-pyrazinecarboxamide (CB-DMB) as a pan inhibitor of the Na⁺-Ca²⁺ exchanger isoforms NCX1, NCX2, and NCX3 in stably transfected cells. *J Pharmacol Exp Ther.* 2009; 331:212–21. [PubMed: 19602550]

61. Koushik SV, Wang J, Rogers R, Moskophidis D, Lambert NA, Creazzo TL, et al. Targeted inactivation of the sodium-calcium exchanger (Ncx1) results in the lack of a heartbeat and abnormal myofibrillar organization. *Faseb J*. 2001; 15:1209–11. [PubMed: 11344090]
62. Reuter H, Henderson SA, Han T, Mottino GA, Frank JS, Ross RS, et al. Cardiac excitation-contraction coupling in the absence of Na^+ - Ca^{2+} exchange. *Cell Calcium*. 2003; 34:19–26. [PubMed: 12767889]
63. Komuro I, Ohtsuka M. Forefront of $\text{Na}^+/\text{Ca}^{2+}$ exchanger studies: role of $\text{Na}^+/\text{Ca}^{2+}$ exchanger-lessons from knockout mice. *J Pharmacol Sci*. 2004; 96:23–6. [PubMed: 15359083]
64. Henderson SA, Goldhaber JJ, So JM, Han T, Motter C, Ngo A, et al. Functional adult myocardium in the absence of $\text{Na}^+/\text{Ca}^{2+}$ exchange: cardiac-specific knockout of NCX1. *Circ Res*. 2004; 95:604–11. [PubMed: 15308581]
65. Pott C, Ren X, Tran DX, Yang MJ, Henderson S, Jordan MC, et al. Mechanism of shortened action potential duration in $\text{Na}^+/\text{Ca}^{2+}$ exchanger knockout mice. *Am J Physiol Cell Physiol*. 2007; 292:C968–73. [PubMed: 16943244]
66. Bassani JW, Yuan W, Bers DM. Fractional SR Ca release is regulated by trigger Ca and SR Ca content in cardiac myocytes. *Am J Physiol*. 1995; 268:C1313–9. [PubMed: 7762626]
67. Lamont C, Eisner DA. The sarcolemmal mechanisms involved in the control of diastolic intracellular calcium in isolated rat cardiac trabeculae. *Pflügers Arch*. 1996; 432:961–9. [PubMed: 8781189]
68. Pott C, Goldhaber JJ, Philipson KD. Homozygous overexpression of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger in mice: evidence for increased transsarcolemmal Ca^{2+} fluxes. *Ann N Y Acad Sci*. 2007; 1099:310–4. [PubMed: 17446472]
69. Hurtado C, Wible JT, Dibrov E, Maddaford TG, Pierce GN. A comparison of adenovirally delivered molecular methods to inhibit $\text{Na}^+/\text{Ca}^{2+}$ exchange. *J Mol Cell Cardiol*. 2007; 43:49–53. [PubMed: 17540404]
70. Noble D. Influence of Na/Ca exchange stoichiometry on model cardiac action potentials. *Ann N Y Acad Sci*. 2002; 976:133–6. [PubMed: 12502551]
71. Spencer CI, Sham JS. Effects of $\text{Na}^+/\text{Ca}^{2+}$ exchange induced by SR Ca^{2+} release on action potentials and afterdepolarizations in guinea pig ventricular myocytes. *Am J Physiol Heart Circ Physiol*. 2003; 285:H2552–62. [PubMed: 12933341]
72. Peterson BZ, DeMaria CD, Adelman JP, Yue DT. Calmodulin is the Ca^{2+} sensor for Ca^{2+} -dependent inactivation of L-type calcium channels. *Neuron*. 1999; 22:549–58. [PubMed: 10197534]
73. Zuhlke RD, Pitt GS, Deisseroth K, Tsien RW, Reuter H. Calmodulin supports both inactivation and facilitation of L-type calcium channels. *Nature*. 1999; 399:159–62. [PubMed: 10335846]
74. Sham JS, Cleemann L, Morad M. Functional coupling of Ca^{2+} channels and ryanodine receptors in cardiac myocytes. *Proc Natl Acad Sci U S A*. 1995; 92:121–5. [PubMed: 7816800]
75. Sham JS, Song LS, Chen Y, Deng LH, Stern MD, Lakatta EG, et al. Termination of Ca^{2+} release by a local inactivation of ryanodine receptors in cardiac myocytes. *Proc Natl Acad Sci U S A*. 1998; 95:15096–101. [PubMed: 9844021]
76. Pogwizd SM, Bers DM. Cellular basis of triggered arrhythmias in heart failure. *Trends Cardiovasc Med*. 2004; 14:61–6. [PubMed: 15030791]
77. Sipido KR, Varro A, Eisner D. Sodium calcium exchange as a target for antiarrhythmic therapy. *Handb Exp Pharmacol*. 2006:159–99. [PubMed: 16610344]
78. Goldhaber JJ. Sodium-calcium exchange: the phantom menace. *Circ Res*. 1999; 85:982–4. [PubMed: 10571527]
79. Eckardt L, Haverkamp W, Borggrefe M, Breithardt G. Experimental models of torsade de pointes. *Cardiovasc Res*. 1998; 39:178–93. [PubMed: 9764199]
80. Shah M, Akar FG, Tomaselli GF. Molecular basis of arrhythmias. *Circulation*. 2005; 112:2517–29. [PubMed: 16230503]
81. Nuyens D, Stengl M, Dugarmaa S, Rossenbacker T, Compennolle V, Rudy Y, et al. Abrupt rate accelerations or premature beats cause life-threatening arrhythmias in mice with long-QT3 syndrome. *Nat Med*. 2001; 7:1021–7. [PubMed: 11533705]

82. Keating MT, Sanguinetti MC. Molecular and cellular mechanisms of cardiac arrhythmias. *Cell*. 2001; 104:569–80. [PubMed: 11239413]
83. Salama G, London B. Mouse models of long QT syndrome. *J Physiol*. 2007; 578:43–53. [PubMed: 17038432]
84. Fujiwara K, Tanaka H, Mani H, Nakagami T, Takamatsu T. Burst emergence of intracellular Ca^{2+} waves evokes arrhythmogenic oscillatory depolarization via the Na^+ - Ca^{2+} exchanger: simultaneous confocal recording of membrane potential and intracellular Ca^{2+} in the heart. *Circ Res*. 2008; 103:509–18. [PubMed: 18635824]
85. De Ferrari GM, Viola MC, D'Amato E, Antolini R, Forti S. Distinct patterns of calcium transients during early and delayed afterdepolarizations induced by isoproterenol in ventricular myocytes. *Circulation*. 1995; 91:2510–5. [PubMed: 7743611]
86. Kimura S, Cameron JS, Kozlovskis PL, Bassett AL, Myerburg RJ. Delayed afterdepolarizations and triggered activity induced in feline Purkinje fibers by alpha-adrenergic stimulation in the presence of elevated calcium levels. *Circulation*. 1984; 70:1074–82. [PubMed: 6149823]
87. Sicouri S, Antzelevitch C. Afterdepolarizations and triggered activity develop in a select population of cells (M cells) in canine ventricular myocardium: the effects of acetylcholine and Bay K 8644. *Pacing Clin Electrophysiol*. 1991; 14:1714–20. [PubMed: 1721163]
88. Tseng GN, Wit AL. Effects of reducing $[\text{Na}^+]_o$ on catecholamine-induced delayed afterdepolarizations in atrial cells. *Am J Physiol*. 1987; 253:H115–25. [PubMed: 3605357]
89. de Groot SH, Schoenmakers M, Molenschot MM, Leunissen JD, Wellens HJ, Vos MA. Contractile adaptations preserving cardiac output predispose the hypertrophied canine heart to delayed afterdepolarization-dependent ventricular arrhythmias. *Circulation*. 2000; 102:2145–51. [PubMed: 11044434]
90. Meszaros J, Khananshvil D, Hart G. Mechanisms underlying delayed afterdepolarizations in hypertrophied left ventricular myocytes of rats. *Am J Physiol Heart Circ Physiol*. 2001; 281:H903–14. [PubMed: 11454597]
91. Pogwizd SM, Qi M, Yuan W, Samarel AM, Bers DM. Upregulation of Na^+ / Ca^{2+} exchanger expression and function in an arrhythmogenic rabbit model of heart failure. *Circ Res*. 1999; 85:1009–19. [PubMed: 10571531]
92. Pogwizd SM, Schlotthauer K, Li L, Yuan W, Bers DM. Arrhythmogenesis and contractile dysfunction in heart failure: Roles of sodium-calcium exchange, inward rectifier potassium current, and residual beta-adrenergic responsiveness. *Circ Res*. 2001; 88:1159–67. [PubMed: 11397782]
93. Sipido KR, Volders PG, de Groot SH, Verdonck F, Van de Werf F, Wellens HJ, et al. Enhanced Ca^{2+} release and Na/Ca exchange activity in hypertrophied canine ventricular myocytes: potential link between contractile adaptation and arrhythmogenesis. *Circulation*. 2000; 102:2137–44. [PubMed: 11044433]
94. Volders PG, Kulcsar A, Vos MA, Sipido KR, Wellens HJ, Lazzara R, et al. Similarities between early and delayed afterdepolarizations induced by isoproterenol in canine ventricular myocytes. *Cardiovasc Res*. 1997; 34:348–59. [PubMed: 9205549]
95. Hasenfuss G, Schillinger W, Lehnart SE, Preuss M, Pieske B, Maier LS, et al. Relationship between Na^+ - Ca^{2+} -exchanger protein levels and diastolic function of failing human myocardium. *Circulation*. 1999; 99:641–8. [PubMed: 9950661]
96. Reinecke H, Studer R, Vetter R, Holtz J, Drexler H. Cardiac Na^+ / Ca^{2+} exchange activity in patients with end-stage heart failure. *Cardiovasc Res*. 1996; 31:48–54. [PubMed: 8849588]
97. Liew CC, Dzau VJ. Molecular genetics and genomics of heart failure. *Nat Rev Genet*. 2004; 5:811–25. [PubMed: 15520791]
98. Amran MS, Hashimoto K, Homma N. 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. *J Pharmacol Exp Ther*. 2004; 310:83–9. [PubMed: 15028781]
99. Nagasawa Y, Zhu BM, Chen J, Kamiya K, Miyamoto S, Hashimoto K. Effects of SEA0400, a Na^+ / Ca^{2+} exchange inhibitor, on ventricular arrhythmias in the in vivo dogs. *Eur J Pharmacol*. 2005; 506:249–55. [PubMed: 15627435]

100. Namekata I, Tsuneoka Y, Takahara A, Shimada H, Sugimoto T, Takeda K, et al. Involvement of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger in the automaticity of guinea-pig pulmonary vein myocardium as revealed by SEA0400. *J Pharmacol Sci.* 2009; 110:111–6. [PubMed: 19423954]
101. Milberg P, Ramtin S, Monnig G, Osada N, Wasmer K, Breithardt G, et al. Comparison of the in vitro electrophysiologic and proarrhythmic effects of amiodarone and sotalol in a rabbit model of acute atrioventricular block. *J Cardiovasc Pharmacol.* 2004; 44:278–86. [PubMed: 15475823]
102. Milberg P, Reinsch N, Wasmer K, Monnig G, Stypmann J, Osada N, et al. Transmural dispersion of repolarization as a key factor of arrhythmogenicity in a novel intact heart model of LQT3. *Cardiovasc Res.* 2005; 65:397–404. [PubMed: 15639478]
103. Eckardt L, Haverkamp W, Mertens H, Johna R, Clague JR, Borggreffe M, et al. Drug-related torsades de pointes in the isolated rabbit heart: comparison of clofilium, d,l-sotalol, and erythromycin. *J Cardiovasc Pharmacol.* 1998; 32:425–34. [PubMed: 9733356]
104. Nerbonne JM, Kass RS. Molecular physiology of cardiac repolarization. *Physiol Rev.* 2005; 85:1205–53. [PubMed: 16183911]
105. Imahashi K, Kusuoka H, Hashimoto K, Yoshioka J, Yamaguchi H, Nishimura T. Intracellular sodium accumulation during ischemia as the substrate for reperfusion injury. *Circ Res.* 1999; 84:1401–6. [PubMed: 10381892]
106. Kusuoka H, Camilion de Hurtado MC, Marban E. Role of sodium/calcium exchange in the mechanism of myocardial stunning: protective effect of reperfusion with high sodium solution. *J Am Coll Cardiol.* 1993; 21:240–8. [PubMed: 8417067]
107. Kusuoka H, Porterfield JK, Weisman HF, Weisfeldt ML, Marban E. Pathophysiology and pathogenesis of stunned myocardium. Depressed Ca^{2+} activation of contraction as a consequence of reperfusion-induced cellular calcium overload in ferret hearts. *J Clin Invest.* 1987; 79:950–61. [PubMed: 3818956]
108. Ladilov Y, Haffner S, Balser-Schafer C, Maxeiner H, Piper HM. Cardioprotective effects of KB-R7943: a novel inhibitor of the reverse mode of $\text{Na}^+/\text{Ca}^{2+}$ exchanger. *Am J Physiol.* 1999; 276:H1868–76. [PubMed: 10362665]
109. Murphy E, Cross H, Steenbergen C. Sodium regulation during ischemia versus reperfusion and its role in injury. *Circ Res.* 1999; 84:1469–70. [PubMed: 10381900]
110. Murphy E, Perlman M, London RE, Steenbergen C. Amiloride delays the ischemia-induced rise in cytosolic free calcium. *Circ Res.* 1991; 68:1250–8. [PubMed: 1902148]
111. Sugishita K, Su Z, Li F, Philipson KD, Barry WH. Gender influences $[\text{Ca}^{2+}]_i$ during metabolic inhibition in myocytes overexpressing the $\text{Na}^+/\text{Ca}^{2+}$ exchanger. *Circulation.* 2001; 104:2101–6. [PubMed: 11673353]
112. Hartmann M, Decking UK. Blocking Na^+/H^+ exchange by cariporide reduces Na^+ -overload in ischemia and is cardioprotective. *J Mol Cell Cardiol.* 1999; 31:1985–95. [PubMed: 10591025]
113. Iwai T, Tanonaka K, Inoue R, Kasahara S, Kamo N, Takeo S. Mitochondrial damage during ischemia determines post-ischemic contractile dysfunction in perfused rat heart. *J Mol Cell Cardiol.* 2002; 34:725–38. [PubMed: 12099713]
114. Sawyer DB, Suter TM, Apstein CS. The sting of salt on an old, but open, wound--is Na^+ the cause of mitochondrial and myocardial injury during ischemia/reperfusion? *J Mol Cell Cardiol.* 2002; 34:699–702. [PubMed: 12099708]
115. Maddaford TG, Dibrov E, Hurtado C, Pierce GN. Reduced expression of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger in adult cardiomyocytes via adenovirally delivered shRNA results in resistance to simulated ischemic injury. *Am J Physiol Heart Circ Physiol.* 2010; 298:H360–6. [PubMed: 19966047]
116. Hurtado C, Prociuk M, Maddaford TG, Dibrov E, Mesaali N, Hryshko LV, et al. Cells expressing unique $\text{Na}^+/\text{Ca}^{2+}$ exchange (NCX1) splice variants exhibit different susceptibilities to Ca^{2+} overload. *Am J Physiol Heart Circ Physiol.* 2006; 290:H2155–62. [PubMed: 16399865]
117. Wang J, Zhang Z, Hu Y, Hou X, Cui Q, Zang Y, et al. SEA0400, a novel $\text{Na}^+/\text{Ca}^{2+}$ exchanger inhibitor, reduces calcium overload induced by ischemia and reperfusion in mouse ventricular myocytes. *Physiol Res.* 2007; 56:17–23. [PubMed: 16497099]
118. Wei GZ, Zhou JJ, Wang B, Wu F, Bi H, Wang YM, et al. Diastolic Ca^{2+} overload caused by $\text{Na}^+/\text{Ca}^{2+}$ exchanger during the first minutes of reperfusion results in continued myocardial stunning. *Eur J Pharmacol.* 2007; 572:1–11. [PubMed: 17822695]

119. Mukai M, Terada H, Sugiyama S, Satoh H, Hayashi H. Effects of a selective inhibitor of $\text{Na}^+/\text{Ca}^{2+}$ exchange, KB-R7943, on reoxygenation-induced injuries in guinea pig papillary muscles. *J Cardiovasc Pharmacol.* 2000; 35:121–8. [PubMed: 10630742]
120. Satoh H, Mukai M, Urushida T, Katoh H, Terada H, Hayashi H. Importance of Ca^{2+} influx by $\text{Na}^+/\text{Ca}^{2+}$ exchange under normal and sodium-loaded conditions in mammalian ventricles. *Mol Cell Biochem.* 2003; 242:11–7. [PubMed: 12619860]
121. Seki S, Taniguchi M, Takeda H, Nagai M, Taniguchi I, Mochizuki S. Inhibition by KB-r7943 of the reverse mode of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger reduces Ca^{2+} overload in ischemic-reperfused rat hearts. *Circ J.* 2002; 66:390–6. [PubMed: 11954956]
122. Schafer C, Ladilov Y, Inserte J, Schafer M, Haffner S, Garcia-Dorado D, et al. Role of the reverse mode of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger in reoxygenation-induced cardiomyocyte injury. *Cardiovasc Res.* 2001; 51:241–50. [PubMed: 11470463]
123. Nakamura A, Harada K, Sugimoto H, Nakajima F, Nishimura N. Effects of KB-R7943, a novel $\text{Na}^+/\text{Ca}^{2+}$ exchange inhibitor, on myocardial ischemia/reperfusion injury. *Nippon Yakurigaku Zasshi.* 1998; 111:105–15. [PubMed: 9558649]
124. Takahashi K, Takahashi T, Suzuki T, Onishi M, Tanaka Y, Hamano-Takahashi A, et al. Protective effects of SEA0400, a novel and selective inhibitor of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger, on myocardial ischemia-reperfusion injuries. *Eur J Pharmacol.* 2003; 458:155–62. [PubMed: 12498920]
125. Magee WP, Deshmukh G, Deninno MP, Sutt JC, Chapman JG, Tracey WR. Differing cardioprotective efficacy of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger inhibitors SEA0400 and KB-R7943. *Am J Physiol Heart Circ Physiol.* 2003; 284:H903–10. [PubMed: 12446284]
126. Yoshiyama M, Nakamura Y, Omura T, Hayashi T, Takagi Y, Hasegawa T, et al. Cardioprotective effect of SEA0400, a selective inhibitor of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger, on myocardial ischemia-reperfusion injury in rats. *J Pharmacol Sci.* 2004; 95:196–202. [PubMed: 15215644]
127. Satoh N, Kitada Y. Cardioprotective effect of MCC-135 is associated with inhibition of Ca^{2+} overload in ischemic/reperfused hearts. *Eur J Pharmacol.* 2004; 499:179–87. [PubMed: 15363965]
128. Goldhaber JJ, Hamilton MA. Role of inotropic agents in the treatment of heart failure. *Circulation.* 2010; 121:1655–60. [PubMed: 20385962]
129. Beuckelmann DJ, Nabauer M, Erdmann E. Intracellular calcium handling in isolated ventricular myocytes from patients with terminal heart failure. *Circulation.* 1992; 85:1046–55. [PubMed: 1311223]
130. Mulieri LA, Hasenfuss G, Leavitt B, Allen PD, Alpert NR. Altered myocardial force-frequency relation in human heart failure. *Circulation.* 1992; 85:1743–50. [PubMed: 1572031]
131. Schwinger RH, Bohm M, Schmidt U, Karczewski P, Bavendiek U, Flesch M, et al. Unchanged protein levels of SERCA II and phospholamban but reduced Ca^{2+} uptake and Ca^{2+} -ATPase activity of cardiac sarcoplasmic reticulum from dilated cardiomyopathy patients compared with patients with nonfailing hearts. *Circulation.* 1995; 92:3220–8. [PubMed: 7586307]
132. Mercadier JJ, Lompre AM, Duc P, Boheler KR, Fraysse JB, Wisnewsky C, et al. Altered sarcoplasmic reticulum Ca^{2+} -ATPase gene expression in the human ventricle during end-stage heart failure. *J Clin Invest.* 1990; 85:305–9. [PubMed: 2136864]
133. Wehrens LS, Reiken S, Vest JA, Wronska A, Marks AR, et al.
134. Ryanodine receptor/calcium release channel PKA phosphorylation: a critical mediator of heart failure progression. *Proc Natl Acad Sci U S A.* 2006; 103(3):511–8.
135. Lee C, Dhalla NS, Hryshko LV. Therapeutic potential of novel $\text{Na}^+/\text{Ca}^{2+}$ exchange inhibitors in attenuating ischemia-reperfusion injury. *Can J Cardiol.* 2005; 21:509–16. [PubMed: 15917880]
136. Roos KP, Jordan MC, Fishbein MC, Ritter MR, Friedlander M, Chang HC, et al. Hypertrophy and heart failure in mice overexpressing the cardiac sodium-calcium exchanger. *J Card Fail.* 2007; 13:318–29. [PubMed: 17517353]

Fig. 1.

Role of NCX in cellular Ca²⁺ cycling during excitation-contraction coupling in cardiac myocytes. During the action potential Ca²⁺ enters the myocyte via the voltage dependent sarcolemmal L-type Ca²⁺ channel, and thus triggers further Ca²⁺ release from the sarcoplasmic reticulum via sarcoplasmic Ca²⁺ release channels (ryanodine receptors). The released Ca²⁺ diffuses towards the myofilaments and induces the contraction. During diastole Ca²⁺ is shifted back into sarcoplasmic reticulum by the Ca²⁺ ATPase of the sarcoplasmic reticulum (SERCA). The Na⁺/Ca²⁺ exchanger extrudes Ca²⁺ from the cytosol into the extracellular space.

Fig. 2.
Concept of cellular mechanisms of adaptation to reduced and increased NCX activity as modelled from studies in NCX knockout (a, [32, 35]) and overexpressor mice (b, [68]).

Fig. 3.

NCX activity regulates AP repolarization in adult cardiac myocytes. Middle column: typical “pyramid”-like mouse AP recorded in a myocyte isolated from a WT animal. Left column: A lack of the plateau phase due to a faster repolarization of the AP is observed in myocytes isolated from NCX KO mice. Right column: In myocytes isolated from mice that homozygously overexpress NCX (HOM), a slowed repolarization with a pronounced plateau phase can be observed (modified from [35, 68]).

Fig. 4.
Pathophysiological concept on the role of NCX in the mediation of cardiac arrhythmia
(modified from [76]).

Fig. 5. Ca^{2+} entry induced by Na^+ depletion is absent in NCX KO myocytes: Recording of cytosolic Ca^{2+} concentration in patch clamped cardiac myocytes isolated from NCX KO and WT mice. Ca^{2+} entry via NCX reverse mode was induced in WT myocytes by hyperpolarization and simultaneous rapid removal of external Na^+ . Under these conditions, no increase in cytosolic Ca^{2+} concentration was observed in NCX KO cells. (Modified from [12]).