Review

Altered Na/Ca exchange activity in cardiac hypertrophy and heart failure: a new target for therapy?

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Received 16 May 2001; accepted 10 September 2001

Abstract

Increased Na/Ca exchange (NCX) expression may be part of the genetic reprogramming in cardiac remodeling. In this review we address the following questions: (1) Is increased NCX activity a general feature of cardiac remodeling in hypertrophy and heart failure? (2) How does this contribute to the contractile and electrical phenotype of hypertrophy and heart failure? (3) Should we consider NCX a potential therapeutic target? From a review of the literature we found that NCX activity can be increased, unchanged, or even downregulated during cardiac remodeling. When NCX activity is increased, it can be considered compensatory for contractile function, but with negative side-effects, including an increased risk of arrhythmias. Changes in activity do not necessarily reflect changes in gene expression. Altered NCX activity can also be consequent to changes in other Ca fluxes or in [Na+] homeostasis. The role of NCX in contractile alterations and arrhythmogenesis varies with the different stimuli or stages of cardiac remodeling. Pharmacological block of NCX in heart failure or hypertrophy may thus be useful, but most likely only in specific conditions, perhaps as part of a combined approach. Development of drugs that target only a specific mode of the exchanger may offer a further advantage.

Keywords: Arrhythmia (mechanisms); Hypertrophy; Heart failure; Na/Ca-exchanger

1. Background and aims

Several recent studies have reported increased expression and/or activity of the Na/Ca exchanger in animal models of hypertrophy and heart failure (e.g. [1]), as well as in the human heart with end-stage failure (e.g. [2]). The importance of changes of Na/Ca exchange (NCX) activity for the pathophysiology of hypertrophy and heart failure stems from the involvement of the exchanger in regulation of both contractile behavior and electrical events. Alterations in NCX activity could therefore contribute to contractile (dys)function, but could also alter the action potential configuration and could potentially contribute to arrhythmogenesis. As drugs aimed at the exchanger have been developed and may become clinically available, they are certainly going to be evaluated in the area of hypertrophy and failure. So it is timely to evaluate potential benefits and hazards of such interventions.

This review aims to answer the following questions: (1) Is increased NCX activity a general feature of cardiac remodeling in hypertrophy and heart failure? (2) How does this contribute to the contractile and electrical phenotype of hypertrophy and heart failure? (3) Should we consider NCX a potential therapeutic target?

The introductory sections on the properties of the exchanger have been kept brief, the reader is referred to a number of excellent reviews recently published [3–5].

2. Molecular properties of the Na/Ca exchanger

The Na/Ca exchanger is a Ca²⁺ and Na⁺ transport protein, found in the membranes of most cell types. It is...
particularly abundant in heart cells, where it plays a crucial role in maintaining Ca\(^{2+}\) balance (see below). The molecular structure/function relations have been explored in detail, following the cloning of the cardiac exchanger [15], and reviewed in Ref. [3]. During Western blot analysis of exchanger expression usually three bands are found, 170, 120 and 70 kDa. These different bands result from incomplete reduction and probable fragmentation during the preparation. Most reports refer to the 120 kDa band, or include both the 120 and the 70 kDa band, which may be important to keep in mind for the comparison of literature data.

Immunohistochemistry studies have found the exchanger on both external peripheral and T-tubular sarcolemma membranes, with reports in favor of even distribution [7], or in favor of a higher density in the T-tubules [8]. This controversy is reviewed in Ref. [3], but remains currently unresolved due to technical limitations. Binding of the exchanger to cytoskeletal proteins can contribute to specific localization [9], which may be affected during cardiac cellular remodeling.

Ca\(^{2+}\) efflux through the exchanger is rather slow, with an estimated turnover rate of 2500 ions s\(^{-1}\) in the intact cardiac cell [10]. These values are in the same order of magnitude as for the Ca\(^{2+}\)-ATPases, but the capacity of the sarcoplasmic reticulum (SR) Ca\(^{2+}\)-ATPase, SERCA, is much larger. Therefore SERCA constitutes the most powerful pathway for Ca\(^{2+}\) removal from the cytoplasm during twitch relaxation (reviewed in Ref. [11]). However, to extrude Ca\(^{2+}\) from the cell, NCX is the major pathway. Turnover rate of NCX for Ca\(^{2+}\) influx is as high as 5000 ions s\(^{-1}\) in excised patches [12] or sarcolemmal vesicle suspension [13]. Yet, the ‘single protein current’ obtained from noise-analysis in giant excised patches is still some two to three orders of magnitude smaller than for an L-type Ca\(^{2+}\) channel [12,14]. This implies that Ca\(^{2+}\) influx via NCX is less likely to result in high near-membrane Ca\(^{2+}\) gradients as compared to Ca\(^{2+}\) influx via L-type Ca\(^{2+}\) channels [15].

The Na/Ca exchanger is generally thought to move three Na\(^{+}\) ions for one Ca\(^{2+}\) (reviewed in Ref. [3]), although recently evidence for a scheme of four Na\(^{+}\) ions for one Ca\(^{2+}\) was given [16]. The transport is therefore electrogenic and can be measured as an ionic current. Net Ca\(^{2+}\) movement across the exchanger is dependent on the electrochemical gradients for Na\(^{+}\) and Ca\(^{2+}\) which determine the reversal potential \(E_{\text{NCX}}\) [17,18]. During a cardiac cycle important rightward shifts in \(E_{\text{NCX}}\) will occur in response to the transient rise in intracellular Ca\(^{2+}\) thus promoting Ca\(^{2+}\) efflux; a rise in intracellular Na\(^{+}\) will shift \(E_{\text{NCX}}\) to the left and decrease Ca\(^{2+}\) efflux and enhance Ca\(^{2+}\) influx, resulting in a net gain in cellular Ca\(^{2+}\) content. The current–voltage relation is not linear, but increases exponentially with depolarization while the current decreases at strong hyperpolarization (e.g. Fig. 3 in Ref. [19], with 20 mM [Na\(^{+}\)], 0.067 mM [Ca\(^{2+}\)], and 1 mM [Ca\(^{2+}\)]\(_0\)). This behavior reflects the kinetics of ion transfer (reviewed in detail in Ref. [3]). In addition, the Na/Ca exchanger has a number of regulatory sites. Intracellular Ca\(^{2+}\) is required for activation, independent of the transport mode. From giant-patch experiments the \(K_D\) is estimated at 0.3 \(\mu\)M [20], but in intact cells it is estimated that the regulatory site is fully occupied at [Ca\(^{2+}\)] of 50 nM [21]. Using a physiological approach to vary [Ca\(^{2+}\)] in intact cells, Weber et al. [22] recently calculated a \(K_D\) of 125 nM. They estimated that allosteric regulation is fast enough to account for a beat to beat variation in NCX activity. These observations also imply that in hypertrophy exchanger activity could be altered due to this allosteric regulation. It has to be kept in mind that the presence of allosteric regulation is apparently species-dependent [22]. It has so far not been reported for human cells.

In the excised patch, cytoplasmic MgATP stimulates the exchanger current, but not via classic phosphorylation [23]. There is however evidence that in the intact cardiac cell the exchanger can be phosphorylated. In guinea-pig cardiac myocytes, phosphorylation through \(\beta\) receptors increases exchanger currents [24]. In the rat heart, adrenergic stimulation via \(\alpha\), but not \(\beta\), receptors increases the current through a PKC-dependent pathway [25,26]. In frog [27] and shark [28] cardiac myocytes, \(\beta\)-adrenergic stimulation predominantly reduces exchanger activity.

3. Na/Ca exchange in cardiac excitation–contraction coupling

3.1. NCX and Ca\(^{2+}\) removal: contribution to relaxation and inward currents during the action potential

The exchanger is the major Ca\(^{2+}\) efflux pathway to maintain the cellular Ca\(^{2+}\) balance during steady state contractions, and compensates for the Ca\(^{2+}\) influx via the L-type Ca\(^{2+}\) current [29,30]. Because of this the exchanger also contributes to the rate of decline of [Ca\(^{2+}\)], and this process is voltage-dependent [18,31]. The relative importance is species-dependent [32], and species differences also exist in density and kinetics of the exchanger [33]. Because the exchanger is electrogenic, an inward current flows during the [Ca\(^{2+}\)], transient, as can most easily be appreciated during a caffeine-induced Ca\(^{2+}\) release [34,35] or during flash photolysis of caged Ca\(^{2+}\) [36]. With SR Ca\(^{2+}\) release, the current is inward even at positive membrane potentials [36–38]. The exact time course of the exchanger during the cardiac action potential is likely to be complex. During the initial depolarization, before [Ca\(^{2+}\)], has increased substantially, the membrane potential is more positive than \(E_{\text{NCX}}\) and the current will be outward. As [Ca\(^{2+}\)] increases, \(E_{\text{NCX}}\) becomes more positive, and the current becomes inward. Experimental evidence indicates that this inward component predominates [39] and is
an important determinant of the action potential duration (reviewed in Ref. [40], see Fig. 1, right panels). Earlier mathematical models were consistent with these data [41], but more recent models of the action potential include a larger outward NCX current during the early phase of the action potential which lasts throughout most of the plateau phase [42-44] (Fig. 1, left panels). Consequently in these models the contribution of inward exchanger current to the action potential duration is small. One of the major difficulties in modeling the time course of the current is its dependence on the subsarcolemmal \([Ca^{2+}]_s\), which will differ substantially from the bulk \([Ca^{2+}]_b\) during SR Ca\(^{2+}\) release [45]. For the experimentalist it seems unlikely that the outward current would predominate during the action potential plateau, as Ca\(^{2+}\) release even at positive potentials induces an inward current (see e.g. Fig. 8 in Ref. [46]). Preliminary reports of recent action potential clamp studies confirm the earlier data that indeed in normal conditions the current is inward for most of the duration of the plateau [47,48]. This information on the time course of the NCX current during the action potential is critical for understanding the effects of altered NCX activity in cardiac hypertrophy and failure.

With cellular Ca\(^{2+}\) overload, spontaneous Ca\(^{2+}\) release occurs and Ca\(^{2+}\)-activated transient inward currents induce potentially arrhythmogenic delayed afterdepolarizations (DADs). There is no doubt that in all species and conditions, inward exchanger currents are a major factor in these DADs as shown in a large number of studies (reviewed in Ref. [49]). What is still open for debate is to what extent currents such as the Ca\(^{2+}\)-activated Cl\(^-\) current and/or non-specific cation current may add to the

Fig. 1. Time course of NCX current during the ventricular action potential. **Left panel**, top, calculated time course of the action potential in a LV midmyocardial cell from a normal dog (solid lines) and from a dog with tachycardia-induced heart failure (dashed lines). The altered time course of the action potential is the result of combined changes in ionic currents and \([Ca^{2+}]_s\) homeostasis. The bottom panel shows the corresponding calculated amplitude, direction and time course of the NCX current (reproduced with permission from Ref. [43]). **Right panel**, top, action potential of a ferret LV myocyte before and after buffering of \([Ca^{2+}]_s\) by application of BAPTA-AM. Lower panel, the BAPTA-sensitive current during an action potential clamp with the action potential configuration as above. This current is predominantly NCX inward current, but may be contaminated by outward current components such as the Ca\(^{2+}\)-activated Cl\(^-\) current and the enhanced inactivation of \(I_{calc}\) during SR Ca\(^{2+}\) release in control (reproduced with permission from Ref. [177]).
arrhythmogenic current. This is likely to depend on species and conditions [49]. In human ventricular cells, the inward exchanger current is apparently the only Ca\(^{2+}\)-activated current, whereas in human atrial cells a non-selective cation current is also present [50].

Inward NCX currents may also contribute to the diastolic depolarization of pacemaker cells. This is supported by observations that the pacemaker frequency of sinoatrial node cells is slowed after blocking SR Ca\(^{2+}\) release [51,52]. Huser et al. [53] recently observed the localized Ca\(^{2+}\) release events during the diastolic depolarization in cat pacemaker cells. They proposed that these events were triggered by T-type Ca\(^{2+}\) channels. Block of the T-type channels, and consequently the release events and associated NCX current, significantly slowed the rate of the pacemaker cells.

3.2. NCX and Ca\(^{2+}\) entry: modulation of Ca\(^{2+}\) release

Emphasis on Ca\(^{2+}\) removal by the exchanger has led to the terminology of forward mode for Ca\(^{2+}\) efflux and reverse mode for Ca\(^{2+}\) influx. Although in experimental practice both modes can be studied separately, in terms of cell function there is no such separation. During perturbations of the steady-state, a net increase/decrease in Ca\(^{2+}\) efflux via the exchanger (or decrease/increase in Ca\(^{2+}\) influx), can lead to a net loss/gain of the cellular Ca\(^{2+}\) load, affecting the SR Ca\(^{2+}\) content [54]. In this way the exchanger is an important pathway to modulate the amount of Ca\(^{2+}\) available in the SR and of the amount actually released. Cellular Ca\(^{2+}\) loss or gain via the exchanger will also depend on the SERCA pump competing with NCX for cytosolic Ca\(^{2+}\) removal [55]. In (patho)physiological conditions cellular Ca\(^{2+}\) gain via the exchanger is often the consequence of changes in internal Na\(^{+}\), and the gain of Ca\(^{2+}\) may occur during diastole [56].

The exchanger could also modulate Ca\(^{2+}\) release by providing trigger Ca\(^{2+}\) during depolarization and in the presence of a (locally) increased Na\(^{+}\). The first reports on triggering of Ca\(^{2+}\) release by NCX proposed that the local accumulation of Na\(^{+}\) following the Na\(^{+}\) current induced reverse mode exchange providing sufficient trigger Ca\(^{2+}\) [57,58]. The issue has remained controversial with reports in favor [59,60], and reports that the \(I_{Na}\)-related release was related to spurious activation of unblocked Ca\(^{2+}\) channels [61–63]. Ca\(^{2+}\) entry through the exchanger in the absence of \(I_{Ca}\) can trigger release [64,65]. Experiments with rapid solution changes have suggested that the exchanger was capable of triggering Ca\(^{2+}\) release as fast as \(I_{Ca}\) [66,67]. However, with full block of \(I_{Ca}\) NCX by itself appears to be a poor trigger compared to \(I_{Ca}\) [46,69]. \(I_{Ca}\) is thus the major trigger for SR Ca\(^{2+}\) release, but NCX can modulate this signal [70–72].

From the above it follows that altered exchanger activity in disease states could reflect on the rate of [Ca\(^{2+}\)]\(_i\) decline, on the SR Ca\(^{2+}\) content and on the amplitude of the [Ca\(^{2+}\)]\(_i\) transient, on action potential duration, and on inward currents during arrhythmogenic afterdepolarizations. Altered activity can occur in the absence of changes in expression levels, as a consequence of changes in [Ca\(^{2+}\)]\(_i\), [Na\(^{+}\)]\(_i\), homeostasis, or of altered regulation.

4. Cardiomyocyte function with overexpression of NCX

Overexpression of NCX has been studied independently from the remodeling during hypertrophy or heart failure. Using the canine cardiac Na/Ca exchanger cDNA, under control of the αMHC promoter, transgenic mice over-expressing NCX were generated [73]. The first studies were carried out on heterozygous mice. These animals had no signs of cardiac hypertrophy or heart failure. Myocyte size was normal, and global [Ca\(^{2+}\)]\(_i\) transients were not different from control. Exchanger activity in SL vesicles was increased by 148%. [Ca\(^{2+}\)]\(_i\) transients triggered by caffeine application in voltage-clamped myocytes showed a much faster relaxation rate; the accompanying peak inward exchanger current was about three times larger. The SR Ca\(^{2+}\) content tended to be slightly larger, but only if tested in the presence of Ni\(^{2+}\) to block Ca\(^{2+}\) efflux via the exchanger. When directly measured during strong depolarizing steps, Ca\(^{2+}\) influx via the exchanger was likewise increased. This Ca\(^{2+}\) influx still was unable to trigger a fast and early Ca\(^{2+}\) release. It was concluded that the overexpression did not affect triggering of SR Ca\(^{2+}\) release directly, but Ca\(^{2+}\) removal near the release sites could alter Ca\(^{2+}\) release indirectly. Yao et al. [74] studied the same transgenic mice. Decline of [Ca\(^{2+}\)]\(_i\) in field-stimulated transgenic cells was significantly faster, and so was the decline of [Ca\(^{2+}\)]\(_i\) transients in the presence of caffeine. Decline of [Ca\(^{2+}\)]\(_i\), transients after block of the exchanger was not different. These authors observed Ca\(^{2+}\) release triggered by reverse mode NCX in the transgenic line, but not in control. This release was however slow and delayed. Action potentials showed a more prominent early repolarization, and delayed late repolarization. These observations could be consistent with early outward current and later inward current, although direct support for this interpretation is currently lacking. Data from Terracciano et al. [75] partly confirmed the studies above, but found additional evidence for increased Ca\(^{2+}\) influx via the exchanger. Cellular Ca\(^{2+}\) gain occurs during rest, as evidenced by rest potentiation. Ca\(^{2+}\) influx may also occur during the latter part of decay of the [Ca\(^{2+}\)]\(_i\) transient. This was suggested by the observation that the decay of [Ca\(^{2+}\)]\(_i\) (with caffeine and thus in the absence of functional SR uptake) was faster with block of the exchanger. In addition they found that the SR Ca\(^{2+}\) content was increased. None of the above studies found evidence for concurrent changes in other Ca\(^{2+}\) fluxes (\(I_{Ca}\) [73,74], SERCA [74,75], calsequestrin [75]), or in [Na\(^{+}\)]\(_i\) [74].
Although these heterozygous mice had no apparent cardiac dysfunction in vivo, it was recently reported that the incidence of sudden death was increased to 20 vs. 6% in wild type [76]. This mortality occurred in the post-partum female mice. This was even more pronounced in the homozygous mice where post-partum mortality was 65% at 8 months [76]. These mice had clear signs of heart failure, and cardiac hypertrophy and dilatation. It still needs to be investigated whether this phenotype is truly related to the NCX overexpression or whether concomitant genetic changes are involved.

Further work with the heterozygous mice has shown that the overexpression of the exchanger may be protective during ischemia [77] and interacts with expression of SERCA in the calsequestrin transgenic mouse [78]. In cells from the heterozygous mice, SERCA function can be moderately reduced while keeping the duration of the [Ca\textsuperscript{2+}] transient comparable to that of control cells [79,80].

Taken together, these data indicate that at least in the heterozygous mouse moderate overexpression of NCX doesn’t depress contractility, and may on the contrary even enhance SR Ca\textsuperscript{2+} load. A very different picture emerged from a study of acute overexpression of NCX in adult rabbit ventricular myocytes [81]. These adult cells were put into culture and transfected with adenovirus-linked NCX gene. After 48 h, protein levels of NCX increased by about 200%, and the protein levels of SERCA, phospholamban and calsequestrin remained unchanged. In the transfected cells increased frequency of stimulation failed to increase the extent of shortening, and decay of the contraction with rest was more pronounced. The amplitude of caffeine contractures was also smaller, but the decay was much faster. These data are consistent with the concept that overexpression leads to net loss of Ca\textsuperscript{2+} from the cell. The contrast with the findings in the transgenic mouse must be ascribed to the different background of Ca\textsuperscript{2+} handling, with perhaps a more active SERCA and/or higher [Na\textsuperscript{+}] in the mouse, and perhaps adaptations in the transgenic animals. These studies are a nice example of the importance of the concomitant Ca\textsuperscript{2+} handling in determining the functional consequences of overexpression of NCX.

### 5. Is increased NCX activity a general feature of cardiac remodeling in hypertrophy and heart failure?

This section is summarized in Tables 1–7, with numbering corresponding to the subsections below. The tables also contain data on the degree of hypertrophy and cardiac function, if available. Comments on the particulars of the experiments, comparison between data, and potential criticisms are given in the text. Within each section, the studies performed on a given animal model are grouped, and within these groups, studies are presented chronologically.

#### 5.1 In vitro models

Since hypertrophy mostly develops in response to

<table>
<thead>
<tr>
<th>Table 1 In vitro models, ventricular myocytes in culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell type</td>
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<tr>
<td>-----------</td>
</tr>
<tr>
<td>Kent and Mc Dermott (1996) [84]</td>
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<tr>
<td>Cadre et al. (1998) [86]</td>
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<tr>
<td>Bassani et al. (1998) [87]</td>
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<tr>
<td>Ju et al. (1996) [88]</td>
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<tr>
<td>Reinecke et al. (1997) [89]</td>
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<tr>
<td>Venuri et al. (1989) [91]</td>
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<tr>
<td>Kent et al. (1993) [92]</td>
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<tr>
<td>Kent and Mc Dermott (1996) [84]</td>
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</tbody>
</table>

Abbreviations used in the tables: Ao, Aorta; ang II, angiotensin II; AP, action potential; APD, action potential duration; DCM, dilated cardiomyopathy; FS, fractional shortening in echocardiography; HW, heart weight, HW/BW, heart weight normalized to body weight; ICM, ischemic cardiomyopathy; K, kidney; LAD, left anterior descending branch coronary artery; LCA, left coronary artery; LV, left ventricle; LVEDP, LV end-diastolic pressure; PA, pulmonary artery; PMCA, plasma membrane Ca\textsuperscript{2+} pump; PLB, phospholamban; RV, right ventricle; SERCA, SR Ca\textsuperscript{2+} pump; SL, sarcolemma; VHD, valvular heart disease.
<table>
<thead>
<tr>
<th>Genetic models</th>
<th>Hamster strain</th>
<th>Cardiac function</th>
<th>NCX mRNA</th>
<th>NCX current</th>
<th>SL vesicles Ca(^{2+}) uptake</th>
<th>Remarks</th>
<th>Electrical changes?</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) The cardiomyopathic hamster</td>
<td>Wagner et al. (1989) [97]</td>
<td>BIO14.6</td>
<td>Postnatal (10-15 days)</td>
<td>Compensated hypertrophy (30 days)</td>
<td>Failure (360 days)</td>
<td>No change</td>
<td>5 fold increase</td>
</tr>
<tr>
<td></td>
<td>Hatem et al. (1994) [98]</td>
<td>BIO14.6</td>
<td>Hypertrophy–failure late stage (250–300 days)</td>
<td>Unchanged for a given [Ca(^{2+})]</td>
<td>NCX current is larger because [Ca(^{2+})], decline is compromised (SERCA?)</td>
<td>AP prolongation</td>
<td></td>
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<tr>
<td></td>
<td>Deroubaix et al. (1996) [99]</td>
<td>MS200</td>
<td>Dilution, no hypertrophy</td>
<td>Larger, but not normalized for [Ca(^{2+})]</td>
<td>NCX current important in later stage</td>
<td>AP prolonged and plateau voltage increased</td>
<td></td>
</tr>
<tr>
<td>(b) The spontaneously hypertensive rat</td>
<td>David-Du®lho et al. (1986) [100]</td>
<td>3–4 weeks (no hypertension)</td>
<td>Normal</td>
<td>Normal</td>
<td>SL vesicles Ca(^{2+}) uptake +23%</td>
<td>Secondary to increased CaM stimulation?</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nakamshi et al. (1989) [101]</td>
<td>22 weeks</td>
<td>LV +32%</td>
<td>Non-failing</td>
<td>SL vesicles uptake increased by 53%</td>
<td>Might be partially due to less Na/K pump activity and higher [Na(^{+})]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Brooks et al. (2000) [102]</td>
<td>24 months</td>
<td>LV +15%</td>
<td>Heart failure</td>
<td>(\gamma2)→(\gamma6)</td>
<td>Captopril treatment started at 12 months, but the functional status at 24 months is not described</td>
<td>AP prolongation known and reversible by treatment e.g. [179]</td>
</tr>
</tbody>
</table>
Table 3
LV pressure overload

<table>
<thead>
<tr>
<th>Model</th>
<th>Cardiac mass</th>
<th>Cardiac function</th>
<th>NCX mRNA</th>
<th>NCX protein</th>
<th>NCX current</th>
<th>NCX function</th>
<th>Remarks</th>
<th>Electrical changes?</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Renovascular hypertension in the rat</td>
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<tr>
<td>Andrawis et al. (1988) [103]</td>
<td>2K-1C</td>
<td>Goldblatt model</td>
<td>4–12 weeks</td>
<td></td>
<td></td>
<td></td>
<td>50% decrease of SL vesicle uptake rate</td>
<td>Wide range of duration of hypertension</td>
</tr>
<tr>
<td>Naqvi and Macleod (1994) [104]</td>
<td>2K-1C</td>
<td>Goldblatt model</td>
<td>11 weeks</td>
<td></td>
<td></td>
<td></td>
<td>Slower decline of [Ca^{2+}], in the presence of caffeine</td>
<td></td>
</tr>
<tr>
<td>Montaz et al. (1996) [105]</td>
<td>1K+DOCA</td>
<td></td>
<td>9–10 weeks</td>
<td>HW+50%</td>
<td></td>
<td></td>
<td>Non-failing</td>
<td>NCX changes do not contribute to APD increase</td>
</tr>
<tr>
<td>(b) Aortic banding</td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Heyliger et al. (1985) [106]</td>
<td>Rabbit</td>
<td>Supraaortic Ao. banding</td>
<td>18–20 weeks</td>
<td>HW/BW +38%</td>
<td></td>
<td></td>
<td>No signs of failure</td>
<td>SL vesicles, uptake=PMCA binding increased</td>
</tr>
<tr>
<td>Haf et al. (1988) [107]</td>
<td>Rat</td>
<td>Supraaortic Ao. clip</td>
<td>4 weeks</td>
<td>HW/BW +30–70%</td>
<td></td>
<td></td>
<td>Non-failing?</td>
<td>SL vesicles, uptake ~40%, efflux ~50%</td>
</tr>
<tr>
<td>Nakashiki et al. (1989) [101]</td>
<td>Rat</td>
<td>Supraaortic Ao banding</td>
<td>4 weeks</td>
<td>HW/BW +41%</td>
<td></td>
<td></td>
<td>No signs of failure</td>
<td>Vesicles, ( V_{\text{max}} )=54%, ( K_{i} )=No increase at 4 days, small increase at 7 days</td>
</tr>
<tr>
<td>Ryder et al. (1993) [108]</td>
<td>Guinea pig</td>
<td>Infrarenal Ao. banding</td>
<td>20 weeks</td>
<td>HW/BW +10%</td>
<td></td>
<td></td>
<td>No signs of failure</td>
<td>AP prolongation combined effect of increased ( I_{\text{CAL}} )–( I_{\text{NCX}} )</td>
</tr>
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\( K_{i} \): initial \( K_{i} \), \( K_{i} \): equilibrium \( K_{i} \), \( V_{\text{max}} \): maximum velocity.
Table 3. Continued

<table>
<thead>
<tr>
<th>Model</th>
<th>Cardiac mass</th>
<th>Cardiac function</th>
<th>NCX mRNA</th>
<th>NCX protein</th>
<th>NCX current</th>
<th>Remarks</th>
<th>Electrical changes?</th>
</tr>
</thead>
<tbody>
<tr>
<td>McCall et al. (1998) [110]</td>
<td>Rat</td>
<td>Suprarenal aortic clip</td>
<td>HW/BW +25%</td>
<td>No signs of failure</td>
<td>Reduction of 35%</td>
<td>[Ca$^{2+}$], decline in the presence of caffeine [110,111]</td>
<td>Discrepancy between mRNA and NCX function</td>
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<td>Maier et al. (1998) [111]</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Vesi uptake= [110]</td>
<td></td>
</tr>
<tr>
<td>Weinberg et al. (1999) [114]</td>
<td>Rat</td>
<td>Ascending Ao. banding</td>
<td>LV female +68%</td>
<td>No signs of failure</td>
<td>×2</td>
<td>No significant difference between female and male</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Slower decline of [Ca$^{2+}$], SERCA and PLB are up at 4 weeks, with SERCA/PLB−, while SERCA/PLB is decreased at 7 weeks</td>
<td></td>
</tr>
<tr>
<td>Ito et al. (2000) [112]</td>
<td>Mouse</td>
<td>Ascending Ao. banding</td>
<td>LV/BW +68%</td>
<td>Preserved</td>
<td>×2</td>
<td>No signs of failure</td>
<td></td>
</tr>
<tr>
<td>Ahmmed et al. (2000) [115]</td>
<td>Guinea-pig</td>
<td>Thoracic Ao. banding</td>
<td>HW/BW +25%</td>
<td>Normal</td>
<td>Density ×2.5</td>
<td>No signs of failure</td>
<td></td>
</tr>
<tr>
<td>Wang et al. (2001) [116]</td>
<td>Mouse</td>
<td>Thoracic Ao. banding</td>
<td>HW/BW +50%</td>
<td>Normal +26%</td>
<td>+71% l_{ sarc } − 68% l_{ intr } − 32%</td>
<td>No change in SR Ca$^{2+}$ load, hypertrophy and changes in NCX prevented by calcineurin</td>
<td></td>
</tr>
<tr>
<td>Bouteng et al. (2001) [117]</td>
<td>Rat</td>
<td>Abdominal Ao. banding</td>
<td>HW +36%</td>
<td>No data</td>
<td>=</td>
<td>=</td>
<td>Decline of [Ca$^{2+}$], transients with caffeine=</td>
</tr>
</tbody>
</table>
hemodynamic loading, stretch of the myocardial cell may be one of the initial trigger events [82]. The cellular signaling cascade leading to hypertrophy has been studied in vitro using cardiac myocytes in primary culture subjected to stretch. This intervention indeed induced expression of the early gene program and protein synthesis [83]. Such a system has in analogy been used to look at expression of NCX. Kent and McDermott [84] used adult cat myocytes and found that passive stretch of unstimulated cells induced protein synthesis and increased the mRNA levels of NCX. This could not be reproduced by treatment of the cells with angiotensin II (ang II), and the stretch effect was not blocked by AT1 receptor antagonists. These results suggest that the pathway is independent of the previously reported induction of immediate early genes c-fos and c-jun during stretch of neonatal cells which was related to autocrine stimulation by ang II [85]. Neonatal cells may however be quite different from adult cells, since mechanical loading by stretching or during spontaneous contractions in neonatal rat myocytes had no direct effect on NCX expression [86,87]. This is also supported by the different effects of ang II on NCX expression: the expression level was actually decreased in cultured neonatal myocytes, but was unaffected in adult rat myocytes in culture [88].

Exploring other pathways of hypertrophy signaling, Reinecke et al. treated adult rat myocytes in culture with phenylephrine. They found an increase in both mRNA and protein levels [89]. As Ca\(^2\+)
 may be involved in the signal transduction pathway [90], cellular Ca\(^2\+)
 loading has been used as well. It was shown to increase NCX expression and activity [84,91,92].

In summary, in vitro studies suggest that stretch of adult cells appears to increase NCX expression, and this may occur independent of ang II stimulation. In addition to stretch, other stimuli may affect NCX expression independently, such as \(\alpha\)-adrenergic stimulation.

These studies also illustrate that neonatal cells behave differently from adult cells, and should be used with caution for extrapolation to growth of adult myocytes.

### 5.2. Genetic models of cardiac hypertrophy and failure

Recently several transgenic mice with cardiac hypertrophy and heart failure [93–96] have been described. Since this review is focusing on hypertrophy and heart failure as a consequence of increased hemodynamic loading of the normally developed heart, these models are not included here. Some animal strains which develop hypertrophy, and eventually heart failure, but not as a result of genetic engineering, are nevertheless mentioned as they are thought to be useful models.

The cardiomyopathic (CMP) hamster may be a model for idiopathic dilated cardiomyopathy. Hamsters of the BIO 14.6 strain develop hypertrophy following necrotic cell loss, which evolves at a later stage into decompensated heart failure [97]. Wagner et al. found an increase in NCX activity of membrane vesicles during the compensated hypertrophy stage with a decrease at the end-stage failure stage [97]. In myocytes from animals at the stage just prior to decompensation Hatem et al. [98] found increased inward exchanger currents which contributed to the action potential prolongation. This enhanced activity was most likely secondary to decreased Ca\(^2\+)
 sequestration into the SR, and not to an increased density of the exchanger proper. The hamsters of the MS200 strain, derived from BIO 53.58, develop dilated cardiomyopathy without clear compensated hypertrophy stage. In myocytes from this strain, Deroubaix et al. found that block of the inward NCX current shortened the action potential to a larger extent than in controls, consistent with a higher NCX activity [99]. In this study no distinction could be made between a secondary functional increase in activity or an increased expression level.

The spontaneously hypertensive rat, SHR, has been studied extensively as a model for hypertensive disease with evolution to cardiac hypertrophy and heart failure. Increased NCX activity was found in sarcolemmal (SL) vesicles of 3- to 4-week-old SHR, before hypertension developed [100]. Measurements on SL vesicles from 22-week-old SHR also found an increase in Na\(^+\)-dependent Ca\(^{2+}\) uptake [101]. A recent comprehensive study looked at contractile function, [Ca\(^{2+}\)], and gene expression in 24-month-old SHR with heart failure [102]. The authors found an upregulation of NCX at the mRNA level which correlated with depressed systolic function. Both could be corrected by treatment with ACE inhibitors.

In summary, in these genetic models, increased NCX

### Table 4

| Model       | Cardiac mass | Cardiac function | NCX mRNA | NCX protein | NCX current | NCX function | Remarks | Electrical changes?
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Kent et al. (1993) [92]</td>
<td>Cat PA banding</td>
<td>4 h</td>
<td>×2</td>
<td>×3</td>
<td>×1.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Espinosa et al. (2000) [119]</td>
<td>Rat Hypoxia</td>
<td>3 weeks</td>
<td>+33%</td>
<td>No signs of failure</td>
<td>Density</td>
<td>I(_{\text{max}}) ×2, unchanged with ([\text{Ca}^{2+}]_i) buffering</td>
<td></td>
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</tbody>
</table>

In summary, in these genetic models, increased NCX activity and contractile function is evident, with a variety of mechanisms influencing NCX expression and activity. Further studies are needed to elucidate the role of NCX in cardiac hypertrophy and failure.
### Table 5
Hypertrophy and failure related to volume overload

<table>
<thead>
<tr>
<th>Model</th>
<th>Cardiac mass</th>
<th>Cardiac function</th>
<th>NCX mRNA</th>
<th>NCX protein</th>
<th>NCX current</th>
<th>NCX Remarks</th>
<th>Electrical function changes?</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>(1) Exercise</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Palmer et al. (1999) [121]</td>
<td>Rat</td>
<td>LV/BW +12%</td>
<td>Preserved</td>
<td>NCX-dependent decline of [Ca(^{2+})], following caffeine is slower</td>
<td></td>
<td></td>
<td>No data</td>
</tr>
<tr>
<td>Treadmill 20–22 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Laughlin et al. (1991) [122]</td>
<td>Mini-pig</td>
<td>HW +19%</td>
<td>Improved</td>
<td></td>
<td>SL vesicles Ca(^{2+}) uptake, no change</td>
<td>No data</td>
<td></td>
</tr>
<tr>
<td>Treadmill 16–22 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td><strong>(2) Bradycardia</strong></td>
<td></td>
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</tr>
<tr>
<td>Sepido et al. (2000) [124]</td>
<td>Dog</td>
<td>HW/BW +40%</td>
<td>Good to improved</td>
<td>3-fold increase of (I_{\text{Na}}) at +40 mV, (I_{\text{Na}}) increased for [Ca(^{2+})], 1 µM</td>
<td>Increased SR Ca(^{2+}) content, no data on SERCA but relaxation not significantly slowed, (I_{\text{Ca}}) = APD prolongation and EAD in vitro [126], EAD- and DAD-dependent arrhythmias in vivo [123,164]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic A-V block 6–9 weeks</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td><strong>(3) Tachycardia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O'Rourke et al. (1999,2000) [128,129]</td>
<td>Dog</td>
<td>Dilatation, no increased wall thickness cell capacitance =</td>
<td>Clinical signs of heart failure, depressed in vivo function</td>
<td>×2</td>
<td>SR content decreased, SERCA downregulation, (I_{\text{Ca}}) = APD prolongation [180], EAD in vitro [167], malignant arrhythmias in vivo [166]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tachycardia pacing 3–4 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yao et al. (1998) [130]</td>
<td>Rabbit</td>
<td>LV/BW +21%</td>
<td>Clinical signs of failure, decreased FS with echo</td>
<td>(I_{\text{Na}}) at +40 mV, −36%</td>
<td>([\text{Na}^{+}]) = ([\text{Ca}^{2+}]), transient decreased, SERCA function decreased, (I_{\text{Ca}}) downregulated</td>
<td>APD prolongation reported in [131]</td>
<td></td>
</tr>
<tr>
<td>Tachycardia pacing 3–5 weeks</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><strong>(4) Combined pressure-volume overload</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pogwizd et al. (2000) [1,165]</td>
<td>Rabbit</td>
<td>HW +77%</td>
<td>Signs of failure, decreased FS with echo ×2</td>
<td>(I_{\text{Na}}) at +60 mV, (I_{\text{Na}}) also increased</td>
<td>Relaxation of caffeine twitch is faster, SR content is decreased</td>
<td>Poor LV function correlates with high NCX mRNA</td>
<td></td>
</tr>
<tr>
<td>Ao. banding and Ao. valvular regurgitation &gt;6 months</td>
<td></td>
<td>cell length +31% width +45%</td>
<td>×2.5 (from [1] Fig. 5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
expression can be observed during certain stages. Increased activity can also be observed independent of increased expression, as a consequence of decreased SR uptake. It has to be kept in mind though that in these models, the alterations are not necessarily directly related to the hemodynamic stress, but may also be genetically determined, independent of the hypertrophy.

5.3. LV pressure-overload hypertrophy and failure

Hypertrophy related to renovascular hypertension has been studied in various models and NCX activity has been studied in a number of these. In the 2-kidney 1-clip Goldblatt rat, a decrease of NCX activity was found in the SL vesicles from animals studied between 4 and 12 weeks [103]. Similar results were obtained by Naqvi and MacLeod [104]. Momtaz et al. [105] measured the current to the hemodynamic stress, but may also be genetically determined, independent of the hypertrophy.

![Graph showing density of the Ni²⁺-sensitive outward current vs. voltage](image1)

**Density of the Ni²⁺-sensitive outward current**

**Acute complete AV block**

**Chronic complete AV block**

Fig. 2. Enhanced NCX currents and arrhythmias in the CAVB dog with compensated hypertrophy. **Left panel**, top, NCX outward current density, measured as the Ni²⁺-sensitive current at the onset of 2-s depolarizing steps in the presence of nifedipine to block \( I_{Ca} \), and ryanodine to suppress SR release (solid symbols, CAVB, open symbols, control). Bottom panel, the amplitude of the transient inward current on repolarization plotted as a function of the \([Ca^{2+}]_i\), at that time (reproduced with permission from [124]). **Right panel**, simultaneous in vivo recording of ECG, lead AVR, monophasic action potentials (MAP) from the LV and RV, and LV pressure (LVP) tracings. Immediately after atroventricular block, a train of conditioning stimuli does not elicit a spontaneous beat. After 8 weeks of chronic AVB, the developed LVP is larger and the conditioning train induces DADs, marked by the arrows, and a number of premature spontaneous beats (reproduced with permission from Ref. [164]).
<table>
<thead>
<tr>
<th>Model</th>
<th>Cardiac mass</th>
<th>Cardiac function</th>
<th>NCX mRNA</th>
<th>NCX protein</th>
<th>NCX current</th>
<th>NCX function</th>
<th>Remarks</th>
<th>Electrical changes?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dixon et al. (1992) [135]</td>
<td>Rat - LCA ligation</td>
<td>4 weeks</td>
<td>LV weight</td>
<td>In vivo function</td>
<td>SL vesicles, uptake decreased</td>
<td>by 23%</td>
<td>This model is known to have APD prolongation from other studies, e.g. [181,182]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>8 weeks</td>
<td>+33% down, heart</td>
<td>by 50%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>16 weeks</td>
<td>+100%</td>
<td>by 50%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sethi et al. (1999) [136]</td>
<td>Rat - LCA ligation</td>
<td>7 weeks</td>
<td>HW+40%</td>
<td>Decreased dP/dt</td>
<td>SL vesicles, uptake decreased</td>
<td>by 50%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Makino et al. (1996) [137]</td>
<td>Rat - LCA ligation</td>
<td>8–12 weeks</td>
<td>HW+40%</td>
<td>Decreased dP/dt, increased LVEDP</td>
<td>SL vesicles, uptake decreased</td>
<td>by 40%</td>
<td>Changes reversed with AT1 antagonist</td>
<td></td>
</tr>
<tr>
<td>Zhang et al. (1996, 1998, 1999) [138–140]</td>
<td>Rat - LCA ligation</td>
<td>3 weeks</td>
<td>Cell capacity</td>
<td>Reduced cell shortening</td>
<td>= Decreased outward current</td>
<td>Slowing of decay of [Ca(^{2+})] transient in the presence of caffeine</td>
<td>Decreased SR Ca(^{2+}) content. All changes were partially reversed by sprint training</td>
<td></td>
</tr>
<tr>
<td>Wasserstrom et al. (2000) [141]</td>
<td>Rat - LCA ligation</td>
<td>6 weeks</td>
<td>HW+74%</td>
<td>Reduced cell shortening</td>
<td>+30–40% +37% Increased outward current</td>
<td>Increased contribution of NCX current to triggering SR Ca(^{2+}) release</td>
<td>I(_{Ca,L}) unchanged, [Na(^{+})] unchanged, SR Ca(^{2+}) content unknown [163]</td>
<td></td>
</tr>
<tr>
<td>Yoshiyama et al. (1997) [142]</td>
<td>Rat - LAD ligation</td>
<td>LV/BW</td>
<td>Signs of failure at 3 months, increased LVEDP</td>
<td>REM, ADJ</td>
<td>RIM=remote area, ADJ=adjacent area</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 week</td>
<td>−10%</td>
<td>×1.6</td>
<td>×2.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 weeks</td>
<td>=</td>
<td>×1.6</td>
<td>×1.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 month</td>
<td>+15%</td>
<td>×1.6</td>
<td>×0.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Litwin and Bridge (1997) [143]</td>
<td>Rabbit ligation of the RCX</td>
<td>8 weeks</td>
<td>LV weight</td>
<td>Increased LVEDP, in vivo, reduced cell shortening</td>
<td>Increased outward current</td>
<td>Increased contribution of NCX current to SR Ca(^{2+}) loading</td>
<td>SR content not significantly altered, reduced I(_{Ca,L}), APD increased at lower stimulation frequencies</td>
<td></td>
</tr>
<tr>
<td>Pu et al. (2000) [145]</td>
<td>Dog</td>
<td>Cell capacity</td>
<td>Reduced cell shortening at higher pacing frequency</td>
<td>No change</td>
<td>Reduced [Ca(^{2+})] transients and SR Ca(^{2+}) content, reduced I(_{Ca,L})</td>
<td>Loss of AP plateau and altered repolarization [144]</td>
<td></td>
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</tr>
</tbody>
</table>
[101] reported an increase in NCX activity. These last authors do not refer to the earlier work by Hanf et al., and the discrepancies can not easily be resolved.

Ryder et al. [108] have used a guinea-pig model with infra-renal aortic banding, which produces a milder degree of hypertrophy than suprarenal banding. In single myocytes from hypertrophied hearts the inward NCX current measured during the plateau of the action potential was increased more than 2-fold. Since Ca$^{2+}$ current was also increased, it is possible that the increased NCX currents were the result of larger Ca$^{2+}$ transients.

Delbridge et al. [109], McCall et al. [110], and Maier et al. [111] studied rats with suprarenal aortic banding. They found no evidence for altered Ca$^{2+}$ removal by NCX in single cells, or in LV trabeculae. However, at the mRNA level a moderate reduction was observed [110]. The SERCA activity of vesicles and the SERCA mRNA levels were smaller in hypertrophied hearts.

Weinberg et al. applied banding of the ascending aorta in rats and looked at gender differences [114]. For the Na/Ca exchanger they found an increased expression at the mRNA level, but without gender difference for a similar degree of hypertrophy.

In an elegant study Ito et al. [112] investigated the changes associated with the transition from compensated hypertrophy to decompensation in mice with banding of the ascending aorta. In the hypertrophy stage NCX was already upregulated, but the SERCA and PLB protein levels were also increased, with a preserved SERCA/PLB ratio. In vivo function at this time was also normal (or improved) [113] and contractile reserve of isolated myocytes was likewise preserved. At 7 weeks when both in vivo and myocyte function were deteriorating, NCX protein levels were still up, but now the SERCA/PLB ratio was decreased. It was proposed that the combination of these changes could account for the failure of maintaining and regulating SR Ca$^{2+}$ load.

In a recent study Ahmmed et al. [115] investigated the link between action potential prolongation and changes in Ca$^{2+}$-dependent membrane currents in guinea-pigs following thoracic aortic banding. In the compensated hypertrophy stage (4 weeks) as well as in the failure stage (8 weeks) increased protein levels and larger inward exchanger currents were observed. Currents seemed to get smaller at 8 weeks, but no statistic evaluation between 4 and 8 weeks was provided. The action potential prolongation was predominantly ascribed to a smaller degree of inactivation of I_{Ca,L}. Although the authors did not consider this in their discussion, larger inward exchanger currents are likely to contribute as well.

Wang et al. [116] used a mouse model of aortic banding and found an upregulation of NCX at the transcript and protein level, as described by Ito et al. [112], but examining Ni$^{2+}$-sensitive currents, they found a decrease in current density.

Boateng et al. [117] did not measure current density in their rat model, but evaluated NCX function from the decline of caffeine contractures. Interestingly, they found no change in function in hypertrophy, but an improved Ca$^{2+}$ removal by NCX when hypertrophied animals were treated with ramipril. The authors suggested that the changes in NCX function might be secondary to changes in [Na$^+$], rather than in NCX proper.

In summary, in a total of 10 studies on LV pressure overload by aortic banding, 6 report an increase in NCX expression and/or function. Importantly, changes in mRNA and/or protein levels are not necessarily concordant with changes in NCX function [110,116], and NCX current densities were measured in 2 studies only. The degree of hypertrophy varied from moderate to large, but function tended to be preserved. An increase in NCX was seen only after at least 4 weeks after the intervention, and possibly a decrease at a later stage.

5.4. RV pressure-overload hypertrophy and failure

In the cat with pulmonary banding, Duthinh and Houser [118] reported that a lower activity of the Na/K pump led to altered NCX activity in the hypertrophied RV. Kent et al. [92] focused on early expression of the Na/Ca exchanger and found an increase in the transcription at 1 and 4 h, as well as after 48 h when protein levels were also up. In a rat model of chronic hypoxia, Espinosa et al. [119] provided evidence for increased NCX inward current related to changes in [Ca$^{2+}$], but most likely with unchanged expression levels. The increased current contributed to the action potential prolongation in this rat model.

5.5. Hypertrophy and failure due to volume-overload or combined pressure–volume overload

5.5.1. Exercise

Endurance training induces an eccentric hypertrophy that mostly results from volume loading of the heart. This type of hypertrophy, at least in the less extreme forms, does not evolve into heart failure, and can be considered compensatory with improved intrinsic cardiac performance. In animal models the means of coercing animals into exercise training may however add an element of stress with potential catecholamine-related effects.

In rats, treadmill training leads to improved cardiac function and myocyte shortening at low pacing frequencies [120]. In this model, rate of decline of [Ca$^{2+}$], transients was less and Na$^+$-dependent Ca$^{2+}$ removal following caffeine exposure of single cells was decreased, suggesting downregulation of the exchanger [121]. In a model of treadmill training in mini-pigs [122] no changes in NCX were observed.

5.5.2. Bradycardia-related volume overload

Dogs with chronic atrioventricular block (CAVB) de-
velop a biventricular eccentric hypertrophy related to bradycardia and consequent volume overload. At 6–9 weeks of CAVB, systolic and diastolic function are maintained, indicating that at this stage the dog has a compensated hypertrophy [123]. At this time CAVB dogs also have an enhanced susceptibility to arrhythmias (see Section 6.2). Isolated myocytes have a larger extent of shortening and a larger amplitude of Ca\(^{2+}\) release at low frequencies of stimulation [124]. We found that in this model the density of the NCX outward currents is increased and Ca\(^{2+}\) influx via reverse mode NCX enhanced. Inward NCX currents are also larger, but the increase is less pronounced (Fig. 2, left panel). These changes are more pronounced in LV than in RV myocytes. SR Ca\(^{2+}\) content is larger at low frequencies of stimulation, contributing to the enhanced contractility. This is probably the combined result of the increased NCX activity and of an increase in subsarcolemmal Na\(^{+}\) [125]. The action potentials in this hypertrophy model are prolonged [126]. This can be ascribed to the decrease in delayed rectifier K\(^{+}\) currents [127] as well as to the increased inward NCX currents.

5.5.3. Tachycardia-related cardiomyopathy

Ventricular tachycardia pacing in the dog leads to heart failure without clear phase of functionally compensated hypertrophy. O’Rourke et al. [128] reported that in this model Ca\(^{2+}\) removal by the Na/Ca exchanger was increased, partly due to increased expression at the protein level, partly as a consequence of decreased SERCA activity. More recently the authors reported a large increase in NCX current density proper, observed only in the absence of [Ca\(^{2+}\)], buffering [129]. In their mathematical model of the action potential in this heart failure dog [43], the NCX current is predominantly outward during the larger part of the action potential plateau (Fig. 1, left panels), and therefore increased inward NCX current has a minor role in the action potential prolongation. Such increased currents could however be important for after-depolarizations.

In the rabbit with pacing-induced heart failure NCX outward currents were reduced, and mRNA levels decreased [130]. Ca\(^{2+}\) currents and SERCA activity were also decreased. A similar reduction in I\(_{\text{Ca,L}}\) was also reported in [131]. These authors observed a significant prolongation of the action potential duration, which could be ascribed to downregulation of K\(^{+}\) currents.

5.5.4. Combined pressure–volume overload

The rabbit with combined aortic banding and valvular insufficiency develops severe heart failure and has an increased incidence of arrhythmias [132,133]. Pogwizd et al. studied [Ca\(^{2+}\)], homeostasis in single cells from these animals at the time when they had developed overt heart failure [1]. [Ca\(^{2+}\)] transients were smaller. NCX current density was increased, and decay of [Ca\(^{2+}\)] in the presence of caffeine was faster. This functional increase in NCX apparently leads to unloading of the SR, as the SR Ca\(^{2+}\) content tended to be smaller. This occurred despite absence of clear evidence for a downregulation of SERCA. The authors conclude that upregulation of NCX leads to larger inward exchanger currents, which tends to unload the SR. These larger inward currents may also contribute to the triggered arrhythmias in this model (see Section 6.2).

5.6. Post-myocardial infarction hypertrophy and failure

When a large myocardial infarction (MI) occurs in the LV, the remaining tissue will hypertrophy. Changes in non-muscular tissue and infarct expansion also contribute to the slow structural remodeling with LV cavity dilatation and eventually congestive heart failure (reviewed in Ref. [134]). ACE inhibitors have been shown to prevent or at least slow-down this evolution. Animal models rarely mimic the preceding stages of coronary atherosclerotic disease and intermittent ischemia in humans, but ligation of the left coronary artery (or its anterior descending branch) usually reproduces the compensatory hypertrophy, remodeling and evolution to heart failure. Exchanger activity has been studied in a number of these models.

In the rat LV post-MI, Dixon et al. [135] reported a decrease in SL vesicle NCX activity which was most pronounced after 16 weeks, when the animals had clear signs of heart failure. From the same lab it was later reported that treatment with L-carnitine partially reversed the decrease in exchanger activity, and also improved LV function [136]. Makino et al. also found decreased NCX activity in the same model, at 8 and 12 weeks post-MI [137]. Hypertrophy and remodeling, as well as downregulation of exchanger activity, could be partially prevented by AT1 receptor blockade. Zhang et al. [138–140] also studied the same model at 3 weeks post-MI. They found a decrease in outward exchanger current density. The Ca\(^{2+}\) content of the SR was also reduced. High intensity sprint training post-MI partially reversed these changes [139]. While these three different labs consistently report a decrease of NCX, Wasserstrom et al. [141] reported recently that NCX currents were increased in the rat with LCA ligation at 6 weeks post-MI, and that reverse mode NCX could contribute substantially to triggering of SR Ca\(^{2+}\) release. The discrepancies between this and the above studies are not easily explained, since in all the same animal model was used. Although Zhang et al. studied myocytes in an earlier stage post-MI, the other studies did not. One intriguing possibility is that sampling of myocytes may have been different, and that the different outcome reflects regional variation in the post-MI heart. Indeed, Yoshiiyama et al. [142] reported significant differences for NCX protein levels between the areas adjacent to and remote from the MI. Whereas mRNA levels were increased at all times post-MI in the remote area, they were initially increased and later on decreased in the adjacent
area. Wasserstrom et al. [141] also report a very high mRNA level in the scar itself. However, in all studies above homogenates or cells were prepared from the whole of the LV non-infarcted tissue, leaving this possibility open to speculation.

Litwin and Bridge [143] have studied NCX function in the rabbit heart post-MI, focusing on the myocytes from the infarct border zone. They found an upregulation of outward NCX currents. Increased Ca\(^{2+}\) influx could contribute to SR Ca\(^{2+}\) loading and provide extra Ca\(^{2+}\) for triggering of SR release, in the presence of downregulation of the Ca\(^{2+}\) channel. The authors speculated that reverse mode NCX during the long action potential plateau could contribute to the prolongation of contractions seen during field stimulation. A potential role for increased inward exchanger currents in the action potential changes was not investigated directly, but is not compatible with the hypothesis that net Ca\(^{2+}\) influx occurs during the action potential plateau.

Boyden and co-workers have characterized in extenso the electrical changes occurring in myocytes from the epicardial infarct borderzone in the dog at 5 days post-MI [144] and recently reported on NCX currents in this model [145]. Cells in this area were significantly larger than the controls. [Ca\(^{2+}\)]\(_i\) transients in myocytes from the borderzone were significantly depressed in the voltage range where \(I_{\text{Ca,L}}\) is the predominant trigger, but less so in the positive voltage range. Yet, NCX currents were not significantly different. Since \(I_{\text{Ca,L}}\) is downregulated in this model, it is possible that NCX may functionally be more prominent even without intrinsic upregulation. This may then reflect primarily on [Ca\(^{2+}\)]\(_i\), transients at more positive potentials.

In summary, reports on alterations of NCX in the surviving LV myocytes post-MI are not consistent. More than in other hypertrophy/failure conditions, regional and temporal heterogeneity is likely to occur post-MI. Regional variations in hemodynamic loading will interact with differences in perfusion with probable ischemia in the border zone. This may superimpose on underlying basal regional differences [146].

5.7. End-stage heart failure: studies on human tissues

Human ventricular tissues studied for exchanger activity have so far mostly been obtained from hearts explanted from patients with end-stage heart failure at the time of transplantation. Therefore little or no information is available concerning earlier stages of (compensated) hypertrophy. In the majority of these studies NCX expression is increased, although mRNA and protein levels do not always match (Table 7). However, mRNA and protein levels do not always match and results are not always consistent, even in reports from the same lab. Flesch et al. [147] reported an increase in NCX protein levels which could account for the enhanced sensitivity of the contractile response to BDF-9148, an agent which increases intracellular Na\(^+\) by slowing inactivation of the Na\(^+\)-K\(^+\)-ATPase channel. Recently however, the same lab reexamined the response to a substance from the same class, namely BDF-9198 [148]. Although a similar increased sensitivity of failing hearts was observed, NCX protein levels were

Table 7

<table>
<thead>
<tr>
<th>Pathology</th>
<th>NCX mRNA</th>
<th>NCX protein</th>
<th>NCX current</th>
<th>NCX function</th>
<th>Remarks</th>
<th>Electrical changes?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Komuro et al.</td>
<td>DCM (13)</td>
<td></td>
<td></td>
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<td></td>
<td>APD prolongation described, e.g.</td>
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<td>(1992) [183]</td>
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<td>[152]</td>
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<tr>
<td>Studer et al.</td>
<td>DCM (13)</td>
<td></td>
<td></td>
<td>&gt; ×2</td>
<td></td>
<td>SL vesicles</td>
</tr>
<tr>
<td>(1994) [149]</td>
<td>ICM (11)</td>
<td></td>
<td>&gt; ×1.8</td>
<td></td>
<td></td>
<td>+160% +87%</td>
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<tr>
<td>Reinecke et al.</td>
<td>DCM (5),</td>
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<tr>
<td>(1996) [184]</td>
<td>ICM (6), results pooled</td>
<td></td>
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<tr>
<td>Flesch et al.</td>
<td>DCM (8)</td>
<td></td>
<td>+79%</td>
<td>+36%</td>
<td>contractions more sensitive to increasing Na(^+) influx</td>
<td></td>
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<tr>
<td>(1996) [147]</td>
<td>ICM (6)</td>
<td></td>
<td>+58%</td>
<td>+20%</td>
<td>contractions more sensitive to increasing Na(^+) influx</td>
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<tr>
<td>Schwerin et al.</td>
<td>DCM (21)</td>
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<tr>
<td>Hasenfuss et al.</td>
<td>DCM, non-end-stage (22)</td>
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<tr>
<td>(2000) [150]</td>
<td>DCM, non-end-stage (18)</td>
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<tr>
<td>Piper et al.</td>
<td>DCM/ICM, end-stage (13)</td>
<td></td>
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<td>×4</td>
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</table>

APD, action potential duration; DCM, dilated cardiomyopathy; ICM, ischemic cardiomyopathy; NCX, Na\(^+\)Ca\(^{2+}\) exchanger; SERCA, sarco(end)plasmic reticulum Ca\(^{2+}\)-ATPase; SERCA, sarco(end)plasmic reticulum Ca\(^{2+}\)-ATPase.
not significantly enhanced in these samples, when tested by immunoblotting. The authors thus ascribed the increased sensitivity to BDF-9198 to the observed decrease in Na/K-ATPase density. The authors compare their data with the report of Studer et al. [149] who included a 40 kDa band in their measurements, possibly allowing for higher readings, but do not explain why their own earlier results [147] were different. One potential explanation comes from the report by Hasenfuss et al. [2]. These authors divided the tissue samples from 29 hearts into three groups according to the relaxation properties of the papillary muscles during high frequency stimulation. In group III, with poor relaxation and increased diastolic tension at high frequency, NCX protein levels were not increased, whereas in group I with maintained relaxation and no increase of diastolic tension, NCX protein levels were increased. As Fig. 3 illustrates, a large scatter is present in these data, further supporting the idea that different phenotypes may exist. Besides etiology, concurrent medication, age, and stage of hypertrophy/failure may all be involved. The recent report of Piper et al. [150] is consistent with this notion. These authors didn’t find increased NCX transcript levels in endocardial biopsies of heart failure patients, until the end-stage.

Only few studies have reported on the amplitude of NCX currents in human cardiac tissues. In atrial tissue Sham et al. [33] reported that the density of the exchanger was lower than in hamster, but more or less equal to guinea-pig ventricular cells. Benardeau et al. [151] also measured currents in atrial tissue and clearly demonstrated that inward currents contribute to the plateau of the action potential. Beuckelmann et al. measured [Ca\(^{2+}\)], transients during voltage clamp [152], but current density of the transient inward current was not reported. We have measured Ca\(^{2+}\) influx through the exchanger in ventricular cells from end-stage failure and found very modest values, both for increase in [Ca\(^{2+}\)], and for current densities [153]. Reverse mode NCX was by itself not capable of triggering SR Ca\(^{2+}\) release [154].

Houser and colleagues have examined the contribution of the exchanger to Ca\(^{2+}\) handling in myocytes from failing human hearts [155–157]. During field stimulation, when contractions are triggered by action potentials, [Ca\(^{2+}\)], transients had a phasic and a tonic component. This tonic component was suppressed by KB-R7943, an inhibitor of NCX [158,159], and was not increased by isoproterenol, whereas the phasic component was. This led the authors to propose that the tonic component results from Ca\(^{2+}\) influx via reverse mode NCX. Experimental data clearly indicate that this component does not result from protracted release, making this a plausible hypothesis. However alternative explanations may still be explored. For one, KB-R7943 may shorten the action potential and the tonic component may result from poor Ca\(^{2+}\) removal by forward mode NCX at depolarized potentials, or Ca\(^{2+}\) influx through non-inactivated Ca\(^{2+}\) channels. In human myocytes the small amplitude of SR Ca\(^{2+}\) release is indeed more likely to reduce Ca\(^{2+}\)-dependent inactivation of Ca\(^{2+}\) channels. In their studies Houser and colleagues also looked at the Ca\(^{2+}\) removal by the Na/Ca exchanger. At 37°C onset of fast relaxation was dependent on repolarization, indicating the contribution of voltage-dependent processes. The decay of the [Ca\(^{2+}\)], transient following a brief (100 ms) application of caffeine was nearly as fast as in control, suggesting NCX is capable of substituting for SERCA activity.

**In summary**, data on NCX function in single human ventricular myocytes show the importance of the exchanger for Ca\(^{2+}\) removal in the failing heart. The role of reverse mode NCX is not fully established, but Ca\(^{2+}\) entry during the latter part of the action potential plateau could contribute to Ca\(^{2+}\) loading and to the diastolic dysfunction of the failing heart. Many studies report increased NCX expression in end-stage heart failure, but a large phenotypic variability is likely to be present. In addition, evidence for re-remodeling of the failing human heart [160] indicates that there is a high degree of plasticity. The contribution of NCX is thus likely to vary not only between patients, but also with time within the same patient.

5.8. Conclusions

From the above literature review it is clear that hypertrophy/heart failure is not unequivocally associated with increased NCX function. In the total of 29 studies imposing cardiac overload in adult animals (Tables 3–6),
14 report an increase in NCX expression and/or function, 10 a decrease, and 5 no change. It is important to consider whether this heterogeneity is due to animal models not faithfully reproducing (human) pathology. Many of the studies have been performed in rats. Of these 16 rat studies, only 5 report an increase. In contrast in the 13 studies on other animal species, nine studies show an increase of NCX. This suggests that rats are less likely to upregulate NCX. Yet, if we compare within the same species, or across time, it is clear that heterogeneity is likely to be a genuine property of cardiac remodeling. The importance of the nature of the stimuli leading to hypertrophy/failure can be appreciated from the comparison of heart failure in the rabbit induced by tachycardia-pacing [130] vs. combined LV pressure and volume overload [1], where NCX is down-regulated in the first, but up-regulated in the second model. Secondly, the time course of the contractile remodeling, i.e. evolution from compensated to failure state is likely to be important as well. Though only few studies have addressed this issue, they clearly show evolution of expression levels with time [115,142]. Human studies further support the notion that important heterogeneity may exist. Lastly, the literature review underscores that, in the absence of changes in expression level, function can still be up, e.g. due to altered regulation (changes in [Na\(^{+}\]), perhaps phosphorylation), or due to changes in other [Ca\(^{2+}\)] fluxes, e.g. increased Ca\(^{2+}\) release, or slower Ca\(^{2+}\) uptake by the SR Ca\(^{2+}\) pump.

6. How does increased NCX activity contribute to the contractile and electrical phenotype of hypertrophy and heart failure?

6.1. Contractile phenotype

A few studies report a decrease in NCX activity, but the functional consequences are unclear and no definite hypothesis has been put forward. Transgenic mice with knockout of NCX are lethal when homozygous [161,162], but lack a clear phenotype at the cellular level when heterozygous [162].

For an increased activity, the hypotheses which have been formulated fall into two broad categories related to the concomitant changes in [Ca\(^{2+}\)] homeostasis.

In the first view, increased NCX improves relaxation, but leads to unloading of the SR [81]. This may be true in general for all conditions where the exchanger is operating predominantly in the Ca\(^{2+}\) efflux mode, and could explain the findings in cultured rabbit cells [81]. Enhanced NCX activity therefore contributes to a decrease in systolic function, even if diastolic function is improved. This phenotype is seen in heart failure in humans, and in the heart failure model of tachycardia-induced cardiomyopathy in the dog [128]. In the rabbit with combined aortic insufficiency and constriction, higher NCX function was correlated with a decrease in SR content [1]. This phenotype is not always associated with a decrease in SERCA function, but if present, SR depletion will be more pronounced. In Ref. [130] [Na\(^{+}\)] was unchanged, but further data on [Na\(^{+}\)] is currently lacking.

In the second view increased NCX enhances cellular Ca\(^{2+}\) gain. This could lead to an increased SR Ca\(^{2+}\) content as we observed in the dog with CAVB, where it may be related to a concomitant increase of [Na\(^{+}\)]. The gain in cellular Ca\(^{2+}\) would then occur primarily during diastole. In this dog model we found no direct evidence for a larger contribution of NCX to trigger Ca\(^{2+}\). In contrast in the rabbit post-MI [143], increased Ca\(^{2+}\) influx may contribute to the trigger for SR Ca\(^{2+}\) release, and the increase in SR content is less clear. In this rabbit model \(I_{\text{Ca, L}}\) is down-regulated, probably allowing for a relative increase in the dependence on Ca\(^{2+}\) influx via the NCX. The concomitant decrease in amplitude of the [Ca\(^{2+}\)], transient will also allow for a larger influx via the NCX. This may apply to other models where \(I_{\text{Ca, L}}\) is decreased, as in Ref. [145], but should be less prominent if \(I_{\text{Ca, L}}\) is unchanged, as in Ref. [163]. In the rabbit post-MI, [Na\(^{+}\)], was not altered, but in the dog with CAVB it may be increased [125].

6.2. Electrical phenotype: is there evidence for a link between altered NCX expression and increased risk of arrhythmias?

As there are no hypotheses on the effect of a decrease in NCX activity on electrical phenotype, we will focus here on the consequences of an increase in NCX activity. These could reflect on action potential configuration, on membrane currents during spontaneous Ca\(^{2+}\) release, and on the incidence of arrhythmogenic spontaneous release.

For the action potential configuration, different views have been expressed. In the modeling of the action potential of the dog with heart failure [43], the NCX is predominantly outward, and increased expression leads to enhanced repolarizing current during the plateau; inward current is present only during the rapid repolarization phase and at diastolic potentials (Fig. 1, left panel). In this view the increased NCX current can be only a minor factor in the prolongation of the action potential plateau so commonly observed in heart failure, but it could contribute to late repolarization delays as shown by Pribe et al. [44]. If NCX current is however predominantly inward during the plateau (see Section 3, and Fig. 1, right panel), then an increase in NCX might lead to prolongation of the action potential. In this way NCX may facilitate the occurrence of early afterdepolarizations (EADs) [49]. We support this view in particular for our dog model of hypertrophy, as in compensated hypertrophy the amplitude of the [Ca\(^{2+}\)], transient is preserved or even enhanced [124]. In heart failure the small amplitude of the [Ca\(^{2+}\)], transients will decrease the amplitude of the inward currents, perhaps
even inducing Ca\(^{2+}\) influx at the end of the action potential, but on the other hand loss of SERCA function will favor (slow) extrusion via NCX. Clearly the issue of the direction and amplitude of the current during the action potential is not yet settled. In this discussion it is also important to keep in mind that the amount of charge extruded by the exchanger in steady state is determined by the amount of Ca\(^{2+}\) entry, not by the expression/activity levels of NCX [29,30]. The latter will however determine the kinetics of the current and time course of Ca\(^{2+}\) removal. Another element is that heterogeneity in exchanger current density, already present at baseline [146], and potentially enhanced during remodeling [124,142], may contribute to dispersion of repolarization.

During spontaneous Ca\(^{2+}\) release at diastolic potentials, increased activity will lead to larger depolarizing currents and facilitate arrhythmogenic delayed afterdepolarizations (DADs). Whether increased NCX expression enhances the likelihood of spontaneous release depends on the concomitant changes in SERCA, \(I_{\text{CaL}}\), and [Na\(^{+}\)], which will co-determine potential changes in SR content (cfr. supra).

Among the studies described in Section 5, a number have emphasized the potential link between increased activity of NCX and increased incidence of arrhythmias. In the dog with CAVB both EAD and DAD related triggered arrhythmias occur [123,164]. In the case of the DAD-related arrhythmias, the increased SR content and larger NCX current are likely to be a major factor. This is supported by the observations that in vivo the occurrence of DADs is related to enhanced contractile function. This is illustrated in Fig. 2, right panel.

In the failing rabbit heart, Vermeulen et al. [132] demonstrated the higher incidence of afterdepolarizations in vitro. In the same model, Pogwizd et al. [133] demonstrated that arrhythmias in vivo are mostly triggered, and can be easily provoked by adrenergic stimulation. In this rabbit model increased exchanger currents during spontaneous release are likely to be an important factor. Pogwizd et al. have shown that spontaneous Ca\(^{2+}\) release in myocytes from these rabbits will more easily induce spontaneous activity because the NCX current is larger and because the (stabilizing) \(I_{K1}\) current is decreased [165].

Pak et al. [166] have reported the increased sudden death and polymorphic ventricular tachycardia in the dog with pacing-induced heart failure. In vitro experiments [167] show an increased incidence of EADs at the cellular level and spontaneous depolarizations that are not related to spontaneous Ca\(^{2+}\) release. The role of NCX in these events in unclear, as in the modeling of the [Ca\(^{2+}\)]\(_{\text{i}}\), transient and action potential of the heart failure dog [43] NCX is predominantly an outward current, except at the very end of the plateau, and is a large inward current during early diastole. These inward currents could be important in late EADs, but this remains to be determined.

Priebe and Beuckelmann have modeled the action potential of human cells from end-stage failure, and incorporated in their model an increase of NCX currents by 65%. Although the NCX current is also predominantly outward during the plateau phase, it nevertheless contributes to the delayed repolarization. During simulated spontaneous Ca\(^{2+}\) release, NCX currents are smaller than in non-failing myocytes, and the enhanced likelihood of DADs is due to the concomitant loss of repolarizing K\(^{+}\) current. It is of interest to note that spontaneous release is not easy to ‘induce’ because of the low SERCA activity, and spontaneous [Ca\(^{2+}\)]\(_{\text{i}}\) transients are of low amplitude. Experimental data indicate however that DADs will occur with rather high likelihood in human preparations under adrenergic stimulation [132,168].

### 6.3. Conclusions

Several contractile phenotypes may result from altered NCX activity depending on concomitant changes. A common feature however is that NCX upregulation appears to have a compensatory function. When upregulation of the exchanger is associated with a decrease in SERCA activity, the exchanger may improve twitch relaxation and diastolic function. With higher [Na\(^{+}\)]\(_{\text{i}}\), and preserved SERCA function, reverse mode exchanger may actually increase cellular Ca\(^{2+}\) load, and improve systolic function. These adaptive changes have however negative side-effects. With low SERCA activity and/or [Na\(^{+}\)]\(_{\text{i}}\), increased Ca\(^{2+}\) efflux through the exchanger is likely to contribute to loss of Ca\(^{2+}\) from the cell, and to decreased systolic performance [102]. In conditions where [Na\(^{+}\)]\(_{\text{i}}\) is elevated and SERCA function preserved, the increased Ca\(^{2+}\) influx is likely to increase the risk of Ca\(^{2+}\) overload and spontaneous Ca\(^{2+}\) release. There is little doubt that during spontaneous Ca\(^{2+}\) release the Na/Ca exchanger will generate an inward current that may be involved in afterdepolarizations. Although direct evidence linking increased risk of arrhythmias and increased NCX activity in hypertrophy and failure is missing, current data favors the idea that increased NCX currents might contribute to increased susceptibility to arrhythmias in hypertrophy and failure. Concomitant changes in ionic currents, e.g. down-regulation of K\(^{+}\) currents, may amplify the effect of increased NCX.

### 7. Can we consider NCX a potential therapeutic target in hypertrophy and heart failure?

#### 7.1. What goals are to be achieved?

Throughout the spectrum of compensated hypertrophy to end-stage heart failure, there are clearly multiple phenotypes associated with increased NCX activity. Consequently, we can not simply consider block of exchanger function as a strategy in hypertrophy or heart failure in general. We may however be able to identify particular
conditions where this may be desirable. In heart failure it is conceivable that reducing Ca$^{2+}$ efflux through NCX would improve systolic function and reduce arrhythmogenic risk. The risk of worsening diastolic function should be considered carefully. In most species, SERCA is quantitatively the most important factor for twitch relaxation. If SERCA is downregulated, reducing NCX may be realistic only with a simultaneous increase in SERCA function (see Section 7.2). An alternative approach to reduce Ca$^{2+}$ efflux via NCX is to alter [Na$^+$], and shift the reversal potential (see Section 7.2). Each of these interventions should increase SR Ca$^{2+}$ content, but only increasing SERCA activity will improve diastolic function.

In conditions where enhanced NCX activity contributes to cellular Ca$^{2+}$ influx, reducing NCX may still be beneficial to reduce arrhythmic risk. It remains to be established whether this requires a selective block of reverse mode NCX. With a global reduction of NCX activity the reduced Ca$^{2+}$ influx might be expected to worsen systolic function, but this may be offset by a concomitant reduction of Ca$^{2+}$ efflux. Such predictions remain however speculative, and should be tested experimentally.

7.2. What tools do we have and/or need?

Block of NCX in the experimental settings is very often achieved by removing Na$^+$, or blocking exchanger by Ni$^{2+}$ [19]. These are however non-specific interventions, and can only be used in well-defined conditions. The most specific blocker so-far has been the inhibitory peptide, XIP [169,170], but as this peptide must be applied intracellularly, its use is restricted. Amiloride and its derivatives, e.g. 3'-4'-dichlorobenzamil (DCB) [171] have been used as blockers, but their use is limited, due to non-specific side effects. The most recent derivative, KB-R7943, has a higher potency, and at the lower concentrations has only minimal effects on other ion transports [158,172]. Although at first it was proposed to be a more potent blocker of the reverse mode than of the forward mode, this may be true only in well-defined conditions, when the exchanger operates nearly exclusively in either of the two modes [159]. Nevertheless, KB-R7943 was shown to be useful in suppressing Ca$^{2+}$ overload and associated arrhythmias induced by block of the Na/K pump in the guinea pig, in vivo and in atrial strips, without affecting inotropy [173]. This effect was different from the effect of DCB, which is a more potent blocker of inward mode. Similar results were obtained by Satoh et al. [174] who used KB-R7943 in single rat ventricular myocytes (Fig. 4). In this study it was shown that at the dose used, KB-R7943 suppressed reverse mode, but not forward mode. KB-R7943 may therefore prove to be a useful tool in conditions where increased Ca$^{2+}$ influx through the exchanger may be predominant, perhaps in the presence of an increased [Na$^+$].

Indirect approaches to alter NCX activity should also be considered. When upregulation of the exchanger is associated with a decrease in SERCA activity, loss of Ca$^{2+}$ from the cell, and decreased systolic performance, it may be better to aim at improving Ca$^{2+}$ uptake into the SR. Several experimental data support the usefulness of enhancing SERCA in heart failure [175], but a clinically applicable method is not yet available.

Increasing [Na$^+$] through inhibition of the Na/K-ATPase thereby shifting $E_{NCX}$ and reducing Ca$^{2+}$ efflux, has been a long-standing practice in heart failure. However, the large multicenter trial on the use of digoxin in heart failure [176] clearly illustrates the trade-off to be considered. Total mortality was unchanged, but the reduction in death due to worsening heart failure was offset by an increase in presumed arrhythmic death.

Altering action potential duration will also affect the NCX Ca$^{2+}$ fluxes. For Ca$^{2+}$ overload, one could consider promoting Ca$^{2+}$ efflux by shortening the action potential.

8. Conclusions

Upregulation of NCX is not a general feature of cardiac hypertrophy and failure. When NCX activity is increased, it can be considered compensatory for contractile function, but with negative side-effects, including an increased risk of arrhythmias. Because of these negative consequences, therefore the exchanger may be a useful therapeutic target, but only in specific conditions. Several caveats remain, warranting further studies.

First, we have to identify more clearly in which conditions NCX is altered and when its role in contractile dysfunction and/or arrhythmogenesis is critical. For this...
purpose we need longitudinal studies, and at each time point a clear evaluation of the changes in NCX activity. Studies of mRNA and protein may help to establish whether altered activity is due to changes in expression, but cannot substitute for functional tests in the intact cell and/or tissue. Altered activity may be indeed be secondary to changes of other Ca\(^{2+}\) transport systems, or of Na\(^{+}\) homeostasis. Functional consequences must be viewed in relation to these accompanying changes. Although ideally this information should come from human studies, we currently have to explore these issues in animal studies. Despite the cost and problems involved, larger animals may offer clear advantages. Studies of function and arrhythmogenesis in vivo are easier and the data can be more easily related to humans, because of the lower heart rate and similarities in anatomy. Smaller rodents may also respond differently as far as NCX is concerned.

Second, altering NCX activity will always be a trade-off, since it may interfere with compensatory mechanisms. Yet some studies with KB-R7943 suggest that it may be possible to suppress selective effects of increased NCX activity. It is conceivable that drugs that suppress more potently Ca\(^{2+}\) influx or Ca\(^{2+}\) efflux have a larger potential.

Thirdly, to investigate these issues we need better drugs. The current information cannot guarantee that such drugs will eventually have a wide applicability. Yet there are enough data indicating that it is worthwhile to develop specific blockers to be tested experimentally.

Lastly, hypertrophy and heart failure are multifactorial syndromes. Therefore multifactorial approaches tailored to particular phenotypes may ultimately be needed. NCX may be one of the targets of such a ‘cocktail’ approach.

Acknowledgements

We thank Professor D. Eisner for critical reading of the manuscript. R.L.H.M.G Spätjens assisted with figure layout. This study was supported by the Fund for Scientific Research – Flanders (F.V. and K.R.S.).

References


[63] Su Z, Sugishita K, Ritter M, Li F, Spitzer KW, Barry WH. The sodium pump modulates the influence of I(Na) on [Ca$^{2+}$], transients in mouse ventricular myocytes. Biophys J 2001;80:1230–1237.


Terracciano CM, Phipps J, MacLeod KT. Overexpression of the Na\(^+\)–Ca\(^{2+}\) exchanger and inhibition of the sarcoplasmic reticular Ca\(^{2+}\)–ATPase in ventricular myocytes from transgenic mice. Cardiovasc Res 2001;49:38–47.


Naqvi RU, MacLeod KT. Effect of hypertrophy on the mechanical activity in isolated cardiac myocytes from guinea pig. Am J Physiol 1994;267:H1851–H1861.


proteins underlie cardiac action potential prolongation in a pressure-overloaded guinea pig model with cardiac hypertrophy and failure, Circ Res 2000;86:558–570.


[125] Volders PGA, Vos MA, Sipido KR. Cardiac hypertrophy is associated with an increase in subsarcolemmal Na⁺ (abstract). Biophys J 2001;80:589A.


[141] Pu J, Robinson RB, Boyden PA. Abnormalities in Ca²⁺ handling in myocytes that survive in the infarcted heart are not just due to alterations in repolarization. J Mol Cell Cardiol 2000;32;1509–1523.


[149] Sipido KR, Stankovicova T, Mubagwa K et al. Despite increased expression of Na/Ca exchange (NCX) in heart failure, Ca²⁺ influx...
via reverse mode NCX is a poor trigger for Ca\(^{2+}\) release from the sarcoplasmic reticulum in human ventricular myocytes (abstract).

Circulation 1997;96:1138.


177] JANVIER NC, HARRISON SM, BOYETT MR. The role of inward Na\(^+\)/Ca\(^{2+}\) exchange current in the ferret ventricular action potential. J Physiol (Lond) 1997;498:611±625.


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