LEADING ARTICLE

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Clinical Potential of Sodium-Calcium Exchanger Inhibitors as Antiarrhythmic Agents

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Abstract

The Na^+/Ca^{2+} exchanger (NaCaX) plays an important role in calcium handling in myocytes, but in the setting of calcium overload NaCaX can also contribute to the activation of an arrhythmogenic transient inward current (I_{ti}). Therefore, approaches to inhibit NaCaX could have potential antiarrhythmic effects in pathophysiological states such as heart failure (HF) or myocardial ischaemia and reperfusion. NaCaX typically functions in a forward (Ca^{2+} extrusion) mode but can also function in a reverse (Ca^{2+} influx) mode. The determining factors for the directionality of NaCaX ion movement are the electrochemical gradients of calcium and sodium, and membrane potential (E_m). In HF, upregulated NaCaX plays a dual role: it decreases sarcoplasmic reticulum (SR) calcium load, which leads to contractile dysfunction, and it underlies the I_{ti} responsible for delayed afterdepolarisations (DADs) and ventricular arrhythmias. In myocardial ischaemia and reperfusion, increases in $[Na^+]_i$ (as a result of acidosis and activation of the Na^+/H^+ exchanger [NHE]) lead to calcium overload via the NaCaX and arrhythmogenesis is probably mediated by I_{ti} activation due to NaCaX. As such, inhibition of NaCaX could provide a novel therapeutic approach to the prevention and treatment of arrhythmias. Unfortunately, it is difficult to assess the efficacy of such an approach since there are no specific NaCaX inhibitors. Currently available agents are hampered by their nonspecific effects on other ion channels and carriers.

The potential utility of specific inhibition of forward or reverse mode NaCaX as an antiarrhythmic approach in the settings of HF and ischaemia/ reperfusion is discussed within the context of current knowledge of myocyte calcium and sodium handling. NaCaX is a challenging and complex therapeutic target because of the delicate balance of SR calcium load (too little contributes to contractile dysfunction and too much leads to calcium overload and arrhythmogenesis). Further understanding of NaCaX function, $[Na^+]_i$ and $[Ca^{2+}]_i$ in HF and ischaemia/reperfusion, combined with the development and assessment of specific NaCaX inhibitors, will ultimately define the potential role of NaCaX inhibition in the prevention and treatment of ventricular arrhythmias.

The Na^+/Ca^{2+} exchanger (NaCaX) is a transmembrane carrier that plays a critical role in maintaining calcium balance in cardiac myocytes.[1] However, in the setting of heart failure (HF) and myocardial ischaemia/reperfusion, NaCaX may underlie an arrhythmogenic transient inward current (I_{ti}) responsible for delayed after-depolarisations (DADs) and non-reentrant initiation of ventricular tachycardia (VT).[2-4] The function of the NaCaX and its alteration in pathophysiological states has been extensively reviewed.[1,5-13] In this article, the focus will be on the central role of NaCaX (both forward and reverse mode) in cardiac excitation-contraction coupling, the role of NaCaX in arrhythmogenesis especially in the setting of HF or ischaemia/reperfusion, and the potential role for NaCaX inhibitors as antiarrhythmic agents.

1. Na^+/Ca^{2+} Exchanger (NaCaX) – Role in Excitation Contraction Coupling

Normal cardiac function depends on the balance of calcium fluxes, so that at steady state, the amount of calcium leaving the ventricular myocyte is the same as the amount entering.[1] Following depolarisation of the myocyte by activation of voltage-activated sodium current (I_{Na}), normal excitation-contraction coupling involves calcium influx via the voltage-sensitive L-type calcium channel (I_{Ca}) (figure 1). This entry of calcium activates the calcium release channels (ryanodine receptors, RyR) to release an even greater amount of calcium that is stored in the sarcoplasmic reticulum (SR). As a result of this calcium-induced calcium release there is a large amount of calcium available to bind to troponin C and initiate myocyte contraction. The calcium released from the SR is subsequently taken back up by the SR via the SR Ca^{2+}-ATPase (SERCA). Calcium efflux occurs primarily through the NaCaX, a transmembrane protein that extrudes Ca^{2+} ions in exchange for Na^+ ions. Only a minimal percentage of calcium efflux involves the sarcolemmal (SL) Ca^{2+}-ATPase (SL Ca^{2+}-pump) and the mitochondria (slow components).[1,14] There are substantial differences among species as to the relative contribution of the various calcium efflux mechanisms. For mice and rat heart, SR calcium uptake predominates and the ratio of SR : NaCaX

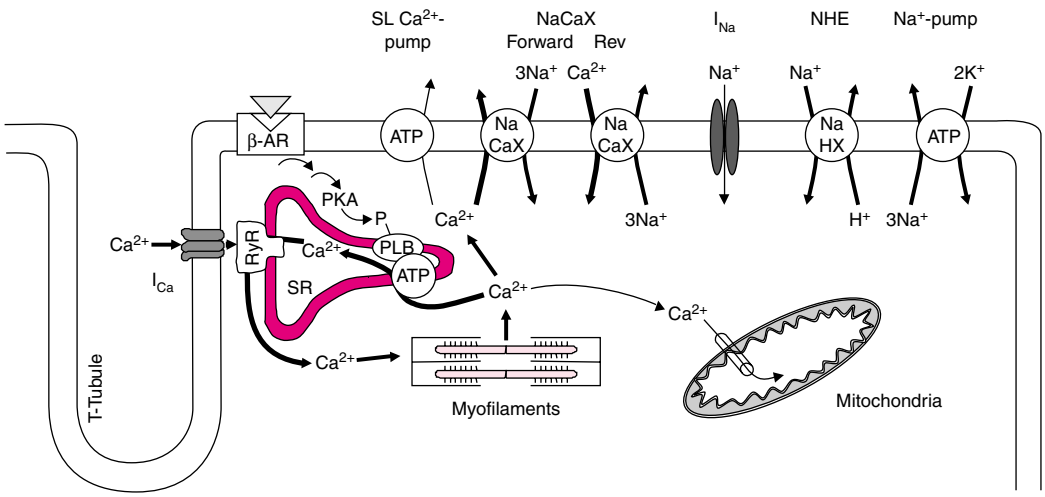

Fig. 1. Schematic of excitation-contraction coupling and the role of forward and reverse Na^+/Ca^{2+} exchanger (NaCaX), as well as other proteins involved in regulating $[Ca^{2+}]_i$ and $[Na^+]_i$ in cardiac ventricular myocytes. **β-AR** = β-adrenergic receptor; **I_{Ca}** = L-type calcium channel; **I_{Na}** = sodium channel; **NHE** = Na^+/H^+ exchanger; **PKA** = protein kinase A; **PLB** = phospholamban; **Rev** = reverse; **RyR** = ryanodine receptor (calcium release channel); **SL** = sarcolemmal; **SR** = sarcoplasmic reticulum.

: slow components is 92 : 7 : 1. In contrast, human heart is similar to rabbit with a greater role of NaCaX and a relative ratio of 70 : 28 : 2.[1,14] This issue becomes important in interpreting the results of agents that have inhibitory effects on NaCaX (see section 3).

1.1 Forward Mode NaCaX

The stoichiometry of the NaCaX has long been accepted as $3Na^+ : 1Ca^{2+}$,[1,15] although some recent data suggest that 4 : 1 stoichiometry may be possible.[16] With three Na^+ ions moving in for every one Ca^{2+} ion moving out, NaCaX is electrogenic – i.e. there is a net charge movement resulting in (inward) current.[5-7] The depolarisation from this inward current contributes to the plateau of the action potential (AP). However, when there is intracellular calcium overload, spontaneous release of calcium from an overloaded SR can be extruded from the cell by NaCaX.[4] The resultant inward current (called a transient inward current – I_{ti}) can underlie the development of delayed afterdepolarisations (DADs) and triggered activity.[4] Thus, NaCaX plays a critical role in calcium efflux, but the extrusion of calcium can activate an arrhythmogenic I_{ti} that can be carried by NaCaX (as well as other currents – see section 2.1).

1.2 Reverse Mode NaCaX

NaCaX is actually a bi-directional carrier protein, capable of calcium efflux as in section 1.1 (forward mode), or of calcium influx (Na^+ out, Ca^{2+} in)[reverse mode](figure 1). There is controversy as to whether calcium influx via NaCaX is sufficient to cause calcium release from the SR.[1,17-22] Calcium influx via NaCaX would not trigger an AP since it is an outward current. However, calcium influx via NaCaX can increase SR calcium loading and contribute to enhanced contractile function under certain conditions.[23-25]

The stoichiometry of reverse mode NaCaX is the same as that of forward mode. However, the directionality of charge movement via NaCaX is dependent on the electrochemical gradients for sodium and calcium (which may be altered in pathophysiological states, such as heart failure or ischaemia and reperfusion). Figure 2 (left side) shows an AP, a calcium transient, and the NaCaX reversal potential (E_{NaCaX}) from a rabbit ventricular myocyte.[1] As for an ion channel, E_{NaCaX} is the level of membrane potential at which the NaCaX current (I_{NaCaX}) reverses direction. $E_{NaCaX} = 3E_{Na} - 2E_{Ca}$ where $E_{Na} = RT/F \ln [Na^+]_o/[Na^+]_i$ and $E_{Ca} = RT/2F \ln [Ca]_o/[Ca]_i$ where R = the universal gas constant, T = temperature (°Kelvin) and F = Faraday's constant (see figure 2 legend). The main point from these equations is that the direction (and duration) of NaCaX is strongly dependent on $[Na^+]_i$ and $[Ca^{2+}]_i$ (which affect E_{NaCaX}) and the AP characteristics, particularly the AP duration (APD) [i.e. E_m].[1] The driving force for ion movement is the difference between E_m and E_{NaCaX} (i.e. the greater the difference between E_{NaCaX} and E_m, the greater the driving force). At rest, E_m is less than E_{NaCaX} (~ –23mV) so I_{NaCaX} is inward (calcium efflux). Despite the large driving force, the amount of calcium efflux is limited by the low level of diastolic $[Ca^{2+}]_i$ (~ 150 nmol/L). During the early part of the AP, when E_m becomes positive but $[Ca^{2+}]_i$ remains low, E_m transiently exceeds E_{NaCaX} and there is a brief interval of outward I_{NaCaX} (calcium influx, shaded area in figure 2 [upper left]). Thus NaCaX transiently reverses direction (reverse mode NaCaX). However, immediately after calcium rises due to I_{Ca}, SR calcium release (and reverse NaCaX), E_{NaCaX} increases and I_{NaCaX} becomes inward again (calcium efflux, forward mode NaCaX) and remains so until the next depolarisation. Weber et al.[26] used inward NaCaX current to predict the time course of the submembrane $[Ca^{2+}]$ ($[Ca^{2+}]_{sm}$, sensed by the NaCaX during the normal AP) and found elevated $[Ca^{2+}]_{sm}$, which would further limit reverse mode NaCaX. Thus, one would expect that reverse NaCaX would contribute very little calcium influx during the AP in control myocytes.[26]

E_{NaCaX} is quite sensitive to the level of $[Na^+]_i$, which is regulated by several ion channels and carriers[1] (figure 1). Sodium influx occurs via NaCaX, the voltage-sensitive sodium channel (I_{Na}), a slowly-inactivating sodium channel ($I_{Na(s)}$) (ac-

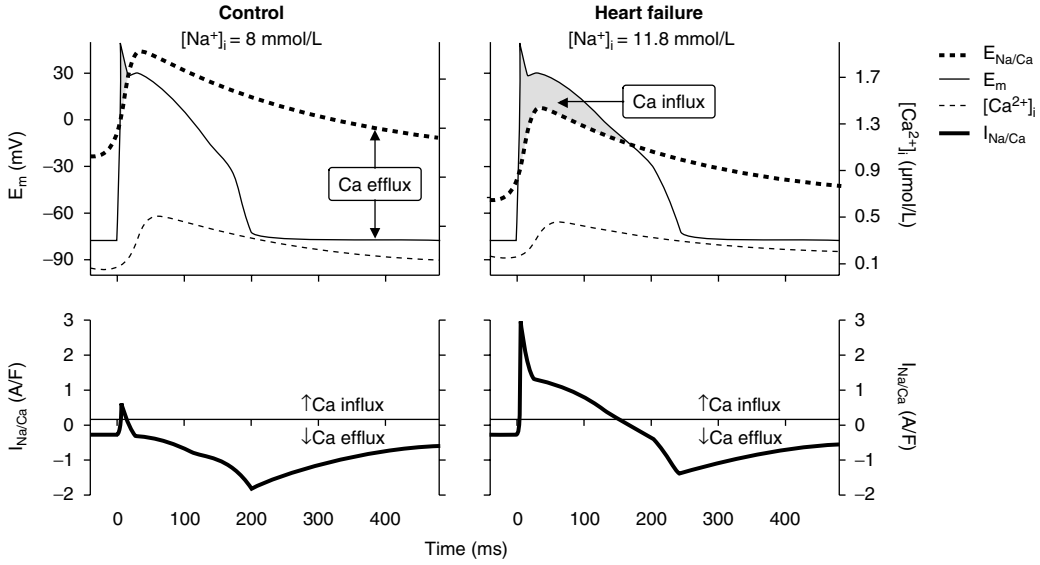

Fig. 2. Changes in $E_{Na/Ca}$ and $I_{Na/Ca}$ during an action potential in control and rabbit ventricular myocytes. When $E_m > E_{Na/Ca}$, Ca^{2+} influx is favoured (shaded regions) and when $E_m < E_{Na/Ca}$, Ca^{2+} efflux is favoured. $E_{NaCaX} = 3E_{Na} - 2E_{Ca}$ where $E_{Na} = RT/F \ln [Na^+]_o/[Na^+]_i$ and $E_{Ca} = RT/2F \ln [Ca^{2+}]_o/[Ca^{2+}]_i$ where A = amps, R = gas constant, T = temperature (°Kelvin) and F = Faraday's constant. Calculation based resting $[Ca^{2+}]_i$ = 152 nmol/L, $[Ca^{2+}]_o$ = 2 mmol/L, $[Na^+]_o$ = 140 mmol/L. Control peak $[Ca^{2+}]_i$ = 0.58 μmol/L (submembrane $[Ca^{2+}]$ = 1.9 μmol/L); HF peak $[Ca^{2+}]_i$ = 0.45 μmol/L (submembrane $[Ca^{2+}]$ = 1.3 μmol/L) based on methods of Weber et al.[26] and Despa et al.[27]

tive in HF[28,29]), and Na^+/H^+ exchanger (NHE) [which extrudes one proton for a Na^+ ion and which is activated during ischaemia and reperfusion].[30,31] The main route of sodium efflux is the Na^+/K^+-ATPase (which can be blocked by digitalis).[32] Alterations in any or all of these channels and carriers (e.g. in HF) could alter $[Na^+]_i$ and shift the balance to a greater or lesser degree of reverse NaCaX. If there is elevation of $[Na^+]_i$ along with decreased peak $[Ca^{2+}]_i$ and prolonged APD (as we have shown occurs in HF[2,27]), it may take longer for NaCaX to switch back to forward mode and there would be greater calcium influx from reverse NaCaX. As shown in figure 2 (right), E_{NaCaX} in HF would be on the order of –50 mV and with depolarisation and a prolonged APD, E_m could exceed E_{NaCaX} for a period of time and reverse NaCaX could contribute considerable calcium influx. Recent experimental data and modelling studies have confirmed this.[27]

Thus, in ventricular myocytes, forward NaCaX plays a critical role in calcium efflux and maintaining intracellular calcium balance by removing the calcium brought in by the L-type calcium channel. In addition, forward mode NaCaX can mediate I_{ti} induced by spontaneous SR calcium release in the setting of calcium overload. The contribution of NaCaX to calcium handling in ventricular myocytes is dependent on the electro-chemical gradients of sodium and calcium. Reverse NaCaX will be more prominent in the setting of increased $[Na^+]$, decreased $[Ca^{2+}]$, and prolonged APD – all of which are characteristic of myocytes from failing hearts. However, the magnitude and directionality of NaCaX can be rather complex, making predictions of the effects of specific NaCaX inhibitors difficult. Recently, much has been learned about the role of NaCaX in underlying arrhythmogenesis, especially in the setting of HF and ischaemia/reperfusion.

2. Role of Altered NaCaX in Arrhythmogenesis

2.1 Arrhythmias in Heart Failure

Chronic HF affects over 2 million Americans and when severe is associated with a 50% 2-year mortality.[33] While many patients die from pump failure, nearly one-half of deaths are sudden, primarily from VT and ventricular fibrillation (VF).[33] In three-dimensional cardiac mapping studies both in experimental animal models of HF and in the failing human heart, we have shown that VT can initiate by a non-reentrant mechanism such as delayed or early after-depolarisations.[34-36] We characterised altered calcium and sodium handling in a novel arrhythmogenic rabbit model of HF (combined pressure and volume overload).[2,27,34,37] Aside from severe left ventricular (LV) dysfunction, 90% of HF rabbits exhibit nonsustained VT and 10% die suddenly.[2,34,37] Isolated myocytes and LV tissue from failing hearts exhibit DADs and after-contractions (from activation of I_{ti}) in response to catecholamine infusion.[2,38]

Others and we have demonstrated that HF is associated with upregulation of the NaCaX,[37,39-41] and we found that upregulated NaCaX in HF plays a dual role.[2] Firstly, it unloads the SR which leads to contractile dysfunction. Secondly, NaCaX current (I_{NaCaX}) underlies the I_{ti} that is activated by catecholamine infusion. We found no significant contribution in HF from two other currents that have been proposed to underlie I_{ti} – a calcium-activated chloride current ($I_{Cl(Ca)}$) and a nonspecific cationic current (I_{NS}).[2] It seemed paradoxical that HF myocytes exhibited contractile dysfunction secondary to low SR calcium load, yet could exhibit activation of I_{ti} with SR calcium overload. The resolution of this paradox was the finding of preserved β-adrenergic responsiveness, which enables SR calcium load to increase to levels at which spontaneous SR calcium release and activation of I_{ti} occur.[2] We also found that decreased inward rectifying potassium currents (I_{K1}) in HF enhanced the arrhythmogenic effects of I_{ti}. We have proposed the paradigm shown in figure 3 in which upregulated NaCaX, preserved β-adrenergic responsiveness and decreased I_{K1} all conspire to enhance arrhythmogenicity in the failing heart.[2] These findings suggest that NaCaX could be a potential therapeutic target for antiarrhythmic approaches for the arrhythmias in HF. However, as discussed in sections 4.1–4.3, the critical role of NaCaX in maintaining intracellular calcium homeostasis complicates this issue.

2.2 Ischaemia-Induced Arrhythmias

VT and VF are extremely common in the setting of both myocardial ischaemia and subsequent reperfusion, and much has been learned about the underlying electrophysiological and biochemical mechanisms.[42] VT during ischaemia can initiate both by reentry and a non-reentrant mechanism. In 3-dimensional cardiac mapping studies in the feline heart, we found that 75% of VTs during ischaemia were initiated by intramural reentry.[43,44] This reentrant activity arises from depression of transmembrane APs (secondary to acidosis, hyperkalaemia and hypoxia),[42] AP shortening (as a result of activation of ATP-sensitive potassium channels [$I_{K\text{-}ATP}$]),[45] and reduced conduction velocity (from long chain acylcarnitines and lysophospholipids released during ischaemia[46] and decreased gap junctional conductance[47] associated with dephosphorylation of the gap junctional protein connexin 43).[48]

We found that 25% of ischaemic VTs were initiated by a non-reentrant mechanism[43] such as DADs (which have been demonstrated in ischaemic tissue),[49,50] and there are alterations in calcium and sodium handling that probably contribute. As shown in figure 4, myocardial ischaemia is associated with a rapid and progressive rise in $[Na^+]_I$, which is primarily mediated by NHE (although sodium entry via tetrodotoxin-sensitive sodium channels may occur).[31,51] This may explain the protective benefits of NHE inhibitors during both ischaemia and reperfusion,[30] and make more complicated the interpretation of results of agents possessing both NaCaX and NHE inhibitory activ-

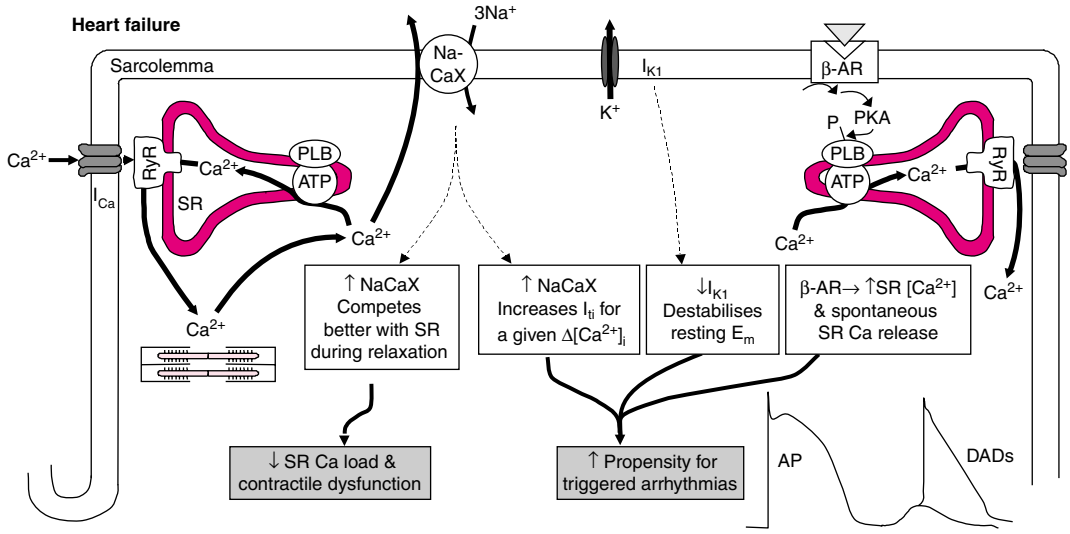

Fig. 3. Schematic of the role of altered Na^+/Ca^{2+} exchanger (NaCaX) in arrhythmogenesis in heart failure (HF). **AP** = action potential; **β-AR** = β-adrenergic receptor; **DADs** = delayed after-depolarisations; $\mathbf{E_m}$ = membrane potential; $\mathbf{I_{Ca}}$ = L-type Ca^{2+} channel; $\mathbf{I_{K1}}$ = inward rectifying K^+ channel; $\mathbf{I_{ti}}$ = transient inward current; **PKA** = protein kinase A; **PLB** = phospholamban; **RyR** = ryanodine receptor (calcium release channel); **SR** = sarcoplasmic reticulum; ↓ indicates decreased; ↑ indicates increased; → indicates causes. Reproduced from Pogwizd et al.[2] with permission.

ity. The increase in $[Na^+]_i$ via NHE is limited by the low extracellular pH_o during ischaemia. Moreover, pH_o declines more than intracellular pH (pH_i) [presumably because the myocyte is trying to maintain normal pH].[52,53] A rise in $[Na^+]_i$ reduces calcium efflux via forward NaCaX and/or induces calcium influx via reverse NaCaX (depending on the sodium gradient and E_m). Diastolic levels of calcium thus rise[54] and calcium overload activates I_{ti} that is probably carried by NaCaX.[55] NaCaX is inhibited by acidosis but increased production of fatty acids (e.g. arachidonic acid) would increase NaCaX activity,[56] so the net effect of ischaemia on NaCaX remains unclear. Yet overall, NaCaX appears to play an important role in mediating arrhythmogenesis due to calcium overload brought on by increasing levels of $[Na^+]_i$.

2.3 Arrhythmogenesis During Myocardial Reperfusion

Our 3-D mapping studies showed that 75% of reperfusion VTs are initiated by a non-reentrant mechanism, and that the transition from VT to VF was as a result of acceleration by a non-reentrant mechanism that ultimately led to the multiple simultaneous reentrant circuits characteristic of VF.[57] DADs have been demonstrated in this setting and have been implicated as the mechanism for reperfusion VT.[50] With reperfusion, pH_o rapidly normalises and creates a large outward $[H^+]$ gradient. This further increases $[Na^+]_i$ via NHE (figure 4), and leads to a marked $[Ca^{2+}]_i$ rise via reverse NaCaX[58] (NaCaX also becomes more active as pH_i normalises). The resultant calcium overload activates I_{ti} (probably carried by NaCaX)[55] leading to non-reentrant VT. Enhanced oxygen free radical production with reperfusion probably worsens arrhythmogenesis, as they have been shown to activate NaCaX, increase $[Ca^{2+}]_i$, activate I_{ti} and lead to triggered APs.[59-63] Thus, NaCaX plays a central role in mediating arrhythmogenesis during both myocardial ischaemia and reperfusion and, as such, represents a potential therapeutic target for treating ventricular arrhythmias in this setting.

3. NaCaX Inhibitors

NaCaX is inhibited by a number of agents.[5-7, 11-13,64] However, most of these have low potency and all of them have effects on other ion channels and carriers that limit their specificity and utility, precluding us from making conclusions regarding the effects of selective NaCaX inhibition. Nonetheless, some of these agents have been studied for antiarrhythmic efficacy or functional effects *in vivo* or *in vitro*, particularly in the setting of myocardial ischaemia and/or reperfusion. The results of some of these studies are briefly summarised.

3.1 Amiloride and Derivatives

Amiloride, an acyclguanidine diuretic, inhibits NaCaX but at low potency, with an IC_{50} (half maximum concentration of inhibition) in the mmol/L range.[65] However, it has significant inhibitory effects on NHE, so that many of the biochemical and antiarrhythmic effects of amiloride, including: (i) a decrease in $[Ca^{2+}]_i$ and $[Na^+]_i$ during ischaemia;[66] (ii) a decrease in reperfusion VT and VF[67,68] and reperfusion injury;[67] (iii) elimination of post-infarction inducible sustained VT;[69] and (iv) prevention of ouabain-induced VF[70] are most likely to be as a result of NHE inhibition.[1,30] Amiloride also blocks delayed rectifying potassium channels and Na^+/K^+-ATPase.[71-73]

Derivatives of amiloride such as 3′,4′-dichlorobenzamil (DCB) and 2′,4′-dimethylbenzamil (DMB) exhibit greater potency (IC_{50} in the 10–20 μmol/L range)[74] and DCB has a 50-fold greater inhibition on forward NaCaX than on reverse NaCaX.[75] DCB inhibits I_{ti} in atrial myocytes,[76] suppresses digitalis-induced ventricular arrhythmias (but not ischaemic and reperfusion VT or VF) *in vivo*[77] and attenuates reoxygenation-induced calcium

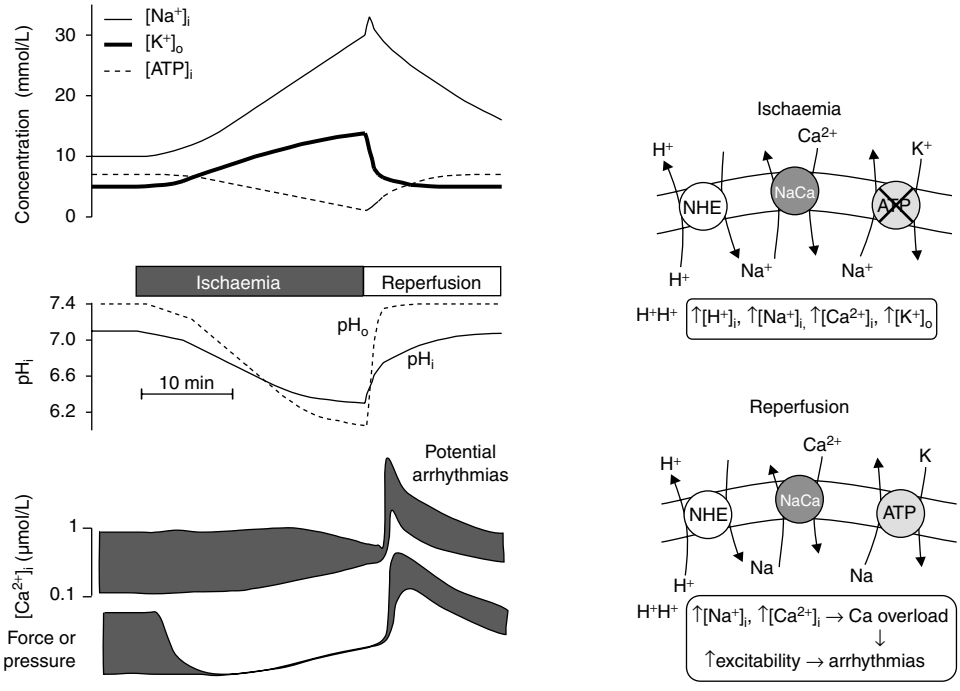

Fig. 4. Schematic diagram of overall changes during myocardial ischaemia and reperfusion based on data from numerous sources (not intended to be an accurate quantitative description). **NaCaX** = sodium/calcium exchanger; **NHE** = sodium/hydrogen exchanger; **pH_i** = intracellular pH; **pH_o** = extracellular pH. Reproduced from Bers[1] with permission.

overload.[78] However, amiloride analogues (as well as amiloride) inhibit Na^+/K^+-ATPase, SERCA, cAMP-dependent phosphodiesterase, voltage-gated sodium and calcium channels, and I_{K-ATP}.[64,71,72,74,79,80] Of note, several amiloride derivatives including DCB have been shown to decrease contractile force[65,81] and to inhibit the positive inotropic response of ouabain.[65] Thus, while amiloride and its derivatives had inhibitory effects on forward and reverse NaCaX (to varying degrees) and some evidence of antiarrhythmic efficacy against ventricular arrhythmias in various settings, the many nonspecific effects of these agents preclude drawing any conclusions on the beneficial effects of NaCaX inhibition.

3.2 Antiarrhythmic Agents

The antiarrhythmic agents mepacrine (quinacrine) and bepridil inhibit NaCaX.[64] Mepacrine inhibits both forward and reverse mode NaCaX,[82] and blocks both inward and outward I_{ti} induced by isoproterenol.[83] But mepacrine can stimulate as well as inhibit NaCaX.[82] depending on the experimental conditions. Bepridil completely blocks forward NaCaX but only partially inhibits reverse NaCaX.[84] However, mepacrine and bepridil affect other ion transporters and are not specific for NaCaX.[64]

3.3 Exchanger Inhibitory Peptide

Exchanger Inhibitory Peptide (XIP) is a peptide that was synthesised based on the amino acid sequence of the cardiac NaCaX.[85,86] XIP inhibits NaCaX with high potency (IC_{50} = 0.1–1 μmol/L)[85] but interacts with calmodulin (modulating calmodulin-activated enzymes such as sarcolemmal Ca^{2+}-ATPase).[85] Its major shortcoming is its lack of membrane permeability. Since it acts from the cytoplasmic surface,[86] XIP is only effective if delivered intracellularly, limiting its applicability for therapeutic use.

3.4 KB-R7943

KB-R7943, an isothiourea derivative, has been found to have a 50-fold greater effect on reverse NaCaX (IC_{50} = 0.3 μmol/L) than forward NaCaX (IC_{50} = 17 μmol/L),[75,87] and has been used to assess the role of reverse NaCaX and the effects of reverse NaCaX inhibition in various pathophysiological states. The mechanism by which KB-R7943 inhibits NaCaX remains unknown.

KB-R7943 has been used as a tool to assess the role of reverse NaCaX in physiological and pathophysiological states. Satoh et al.[88] performed studies in isolated rat ventricular myocytes and found that KB-R7943 did not alter steady-state twitches, calcium transients or SR calcium load. They concluded that reverse NaCaX does not appear to contribute significantly to excitation-contraction coupling in the rat. KB-R7943 has been used to study the role of reverse NaCaX in failing human ventricular myocytes[24,25] and the findings to date suggest reverse NaCaX does contribute to calcium influx during the AP in human HF myocytes. KB-R7943 at 0.3-1 μmol/L inhibits the positive inotropic response to angiotensin II[89] and endothelin-1,[90] suggesting a role of reverse NaCaX in mediating these effects.

Additional studies have focused on whether inhibition of reverse NaCaX has cardioprotective and antiarrhythmic effects during myocardial ischaemia and reperfusion. KB-R7943 (10 μmol/L) has been shown to protect against reoxygenation injury in guinea pig papillary muscle[91] and isolated rat myocytes,[92] and against reperfusion injury in perfused rat hearts.[93] KB-R7943 also decreased the rate of recovery of $[Na^+]_i$ after reperfusion (assessed by ^{23}Na nuclear magnetic resonance spectroscopy),[94] suggesting a key role for reverse NaCaX in this setting. KB-R7943 suppressed ischaemia-reperfusion arrhythmias both in the intact rat heart *in vivo*[93] and in isolated perfused rabbit hearts *in vitro*,[95] although Lu et al. reported no effects on ischaemia and reperfusion-induced arrhythmias with KB-R7943 in anaesthetised Wistar rats.[96] Satoh et al.[88] found that KB-R7943, at a dose (10 μmol/L) that they showed inhibits reverse

but not forward NaCaX in the rat, abolished the spontaneous oscillations and arrhythmias induced by strophanthidin, but not the inotropic effects. They concluded that net calcium influx via reverse NaCaX (not merely reduced calcium efflux via forward NaCaX) was involved in the arrhythmogenic calcium overload of digitalis toxicity.

However, the effects of KB-R7943 are more complex. As noted in the previous paragraph, Satoh et al.[88] showed no effects on twitch contractions in rat ventricular myocytes with 10 μmol/L KB-R7943, and there were no reported effects on LV function in isolated Sprague-Dawley and Fischer 344 rat hearts with 10 μmol/L KB-R7943.[97] However, KB-R7943 has been shown to depress contractile function in several studies. Isolated adult rabbit ventricular myocytes exhibited attenuated cell shortening and calcium transients in a concentration-dependent manner with KB-R7943 at 0.3 μmol/L and higher, and 3 μmol/L KB-R7943 completely abolished cell shortening.[90] Elias et al.[95] found a negative inotropic effect (28% decrease in LV developed pressure) in isolated perfused rabbit hearts with 3 μmol/L KB-R7943. KB-R7943 has also been shown to significantly decrease LV contractile force in isolated blood-perfused canine heart preparations.[98] The basis for this contractile dysfunction is species other than rat is unknown. It may relate to different baseline levels of $[Na^+]_i$ (rat has much higher levels than rabbit, guinea pig, dog[1,99]) or to the smaller contribution of NaCaX to overall calcium flux in mouse and rats.[100] Another possible explanation is differing nonspecific inhibitory actions that are more prominent in certain species. Nonetheless, these data suggest that direct comparison of experimental studies among different animal species should be done with caution. Along these lines, Yamamura et al.[97] recently found that 10 μmol/L KB-R7943 improved post reperfusion recovery of LV function in Sprague-Dawley rats but the same dose was toxic in Fischer 344 rats. They found that much lower doses of KB-R7943 (1 nmol/L) improved post-reperfusion recovery, decreased reperfusion VT and VF, and inhibited reverse NaCaX in Fischer rats. These findings suggest that there may be substantial differences in response to KB-R7943 not only between species, but also between different strains in the same species.

A major limitation of many of these studies was the lack of control experiments demonstrating selective block of reverse NaCaX under the conditions of those studies. In addition, KB-R7943 also inhibits voltage-gated sodium current, calcium current and inward rectifier potassium current (I_{K1}) with IC_{50} values in the 7–14 μmol/L range.[75] Lastly, while KB-R7943 has been used as a selective blocker of reverse NaCaX, there is more recent evidence suggesting that KB-R7943 may not be as directionally-specific as previously thought.[101] Elias et al.[95] propose that differences in ionic conditions and, as a result, differences in distinct exchange transport states, may determine the mode selectivity of the NaCaX to KB-R7943 (even if KB-R7943 can inhibit both forward and reverse mode NaCaX equally). These recent developments emphasise the need to better understand the mechanisms by which KB-R7943 and related agents inhibit NaCaX (for subsequent design of more specific inhibitors).

Overall, these studies are intriguing in that they suggest a potential antiarrhythmic effect from inhibition of reverse NaCaX. However, these data should be interpreted with caution because none of the agents studied to date, including KB-R7943, is a truly specific blocker of NaCaX and that differences in $[Na^+]_i$, $[Ca^{2+}]_i$, APD and response to agents may vary among different strains of animals (let alone different species) and in different pathophysiological conditions.

3.5 SEA-0400

SEA-0400 is a newly synthesised compound that has been reported to be the most potent and selective NaCaX inhibitor available. SEA-0400 inhibited NaCaX current with a 10-fold higher potency than KB-R7943 and, unlike KB-R7943, it had no effects on sodium, calcium and potassium (I_K, I_{K1}) currents.[102] It attenuated reperfusion injury in cerebral ischaemic models both *in vitro* and

in vivo.[103] However, recent work by Reuter et al.[104] in heart tubes from NaCaX knockout mice shows that SEA-0400 (as well as KB-R7943) depresses calcium transients even in the absence of NaCaX. Thus, SEA-0400, as well as KB-R7943, are not specific inhibitors of NaCaX.

4. Potential of Specific NaCaX Inhibitors as Antiarrhythmics

In the absence of specific inhibitors of NaCaX, a discussion of the potential benefits or shortcomings of NaCaX inhibition on arrhythmogenesis must be made on theoretical grounds based on our understanding of the role of NaCaX in mediating arrhythmias. In this section, the potential role of a pure forward NaCaX inhibitor, a pure reverse NaCaX inhibitor, and an agent that inhibits both forward and reverse NaCaX (figure 5) is discussed.

4.1 Inhibition of Forward Mode NaCaX

Since I_{ti} in HF appears to be primarily due to NaCaX current (forward mode), forward NaCaX inhibition could directly inhibit I_{ti} and have an antiarrhythmic effect. The concurrent enhancement of SR calcium load as a result of forward NaCaX inhibition of calcium decline would have a positive inotropic effect. However, if excessive, the increased SR calcium load could result in greater spontaneous SR calcium release with more frequent activation of I_{ti} (albeit an I_{ti} of decreased magnitude) and potentially offset or even counteract an apparent antiarrhythmic effect. An unfortunate corollary of the fact that I_{ti} in HF is carried almost exclusively by NaCaX is that one is limited in the extent to which one can inhibit I_{ti} since NaCaX is so critical in calcium handling. Were I_{ti} to be carried by I_{NS} or $I_{Cl(Ca)}$, targeting these channels and specifically suppressing their activity might be easier and with (perhaps) fewer consequences on cellular ionic flux. While systolic function could be enhanced by increased SR calcium load from inhibition of forward NaCaX, diastolic dysfunction might result from impaired relaxation associated with slowing of forward NaCaX-mediated calcium decline.

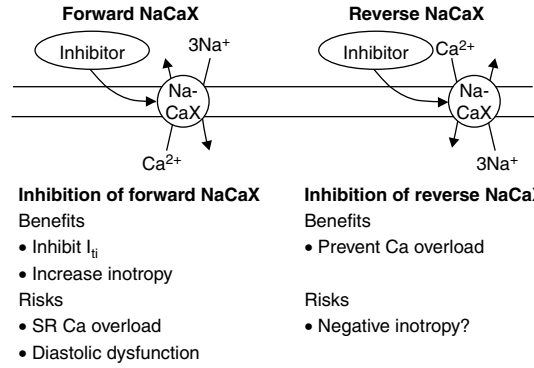

Fig. 5. Summary of potential risks and benefits of inhibiting forward or reverse Na^+/Ca^{2+} exchange (NaCaX) [as an antiarrhythmic in setting of heart failure or ischaemia/reperfusion].

The issue in HF is thus one of balance: too little SR calcium load can contribute to contractile dysfunction but too much SR calcium load can lead to spontaneous SR calcium release and ultimately arrhythmogenesis. It must be kept in mind that this balance issue is more than a theoretical construct. There are definite lessons to be learned from previous attempts to improve contractile function in the failing heart by modulating intracellular calcium (whether by catecholamines or phosphodiesterase inhibition) that resulted in enhanced arrhythmogenesis.[105] The issue in ischaemia and reperfusion is similar in that inhibition of forward NaCaX alone would be expected to impair calcium efflux and increase high diastolic levels of $[Ca^{2+}]_i$ (figure 4) even further. Although I_{ti} carried by NaCaX would be inhibited, there would be greater spontaneous SR calcium release that is likely to be arrhythmogenic even with a reduction of the magnitude of I_{ti}.

4.2 Inhibition of Reverse Mode NaCaX

Inhibition of reverse NaCaX may have a greater potential for antiarrhythmic efficacy by preventing calcium overload mediated by NaCaX. For normal hearts exposed to ischaemia and reperfusion, the prevention of calcium overload would be expected to be beneficial, and the data with KB-R7943 suggest this (despite the fact that KB-R7943 does not

appear to be a pure reverse NaCaX inhibitor). The antiarrhythmic effects could potentially be additive to those observed with inhibition of NHE.[30] For failing hearts, reverse NaCaX inhibition may or may not be a useful antiarrhythmic. Reverse NaCaX appears to play a greater role in calcium flux, and its inhibition could have antiarrhythmic effects, especially if reverse NaCaX helps mediate the SR calcium overload induced by β-adrenergic stimulation.

The potential shortcoming would be an adverse effect on LV function. If upregulation of NaCaX and an increase contribution of reverse NaCaX to calcium loading occurs as a compensatory mechanism to altered SERCA function in HF,[41,106] reverse NaCaX inhibition could have a negative inotropic effect. The negative inotropic effects of KB-R7943 in isolated rabbit cardiac myocytes[90] and even in isolated perfused rabbit hearts[95] suggest, albeit indirectly, that this may be the case. An alternative approach to get around the negative inotropy would be to combine a reverse NaCaX inhibitor with another inotropic agent to offset any contractile dysfunction but maintain antiarrhythmic effect, but SR calcium overload must be avoided.

4.3 Inhibition of Both Forward and Reverse Mode NaCaX

Lastly, a combined approach with an agent that inhibits both forward and reverse NaCaX may have the advantage of two offsetting effects on SR load and contractile function (see sections 4.1 and 4.2), while still having direct effects on inhibiting I_{ti} mediated by NaCaX. However, the effects on calcium decline and relaxation may still be an issue with higher degrees of forward NaCaX inhibition. Overall, the data discussed above all point to the complexity of modulating NaCaX and the difficulty with predicting drug efficacy in the absence of specific NaCaX inhibitors. Aside from the development of such agents, a greater understanding of the role of reverse NaCaX in HF and ischaemia/reperfusion using experimental animal models that demonstrate arrhythmias, as well as mathematical models of excitation-contraction coupling in cardiac myocytes,[26,107-110] will be critical to developing novel approaches to antiarrhythmic therapy.

5. Conclusions

Our understanding of the mechanisms of arrhythmogenesis in HF and ischaemia/reperfusion have rapidly evolved over the last few years and it is clear that NaCaX plays a very important role – both directly by mediating I_{ti} and indirectly by modulating $[Ca^{2+}]_i$ and SR calcium load. While NaCaX is an obvious potential pharmacological target for preventing and treating arrhythmias in these settings, the complex interplay of various membrane transporters and ion channels, and their even more complex alterations in pathophysiological states, makes the task more challenging. The central role of NaCaX will undoubtedly require careful titration to achieve benefits while preventing toxic effects. The good news is: (i) we have learned much about the structure and function of the NaCaX and its role in arrhythmogenesis; (ii) we have found agents that can inhibit NaCaX (albeit without the specificity needed to serve as a prototype antiarrhythmic); and (iii) we have arrhythmogenic models of HF and ischaemia/reperfusion that will allow careful testing. However, the ultimate resolution of whether and how modulation of NaCaX can prevent and treat arrhythmias will require the development of specific NaCaX inhibitors and carefully controlled studies of their effects.

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