Pharmacological Inhibition of Na/Ca Exchange Results in Increased Cellular Ca\(^{2+}\) Load Attributable to the Predominance of Forward Mode Block

Semir Ozdemir, Virginie Bito, Patricia Holemans, Laurent Vinet, Jean-Jacques Mercadier, Andras Varro, Karin R. Sipido

Abstract—Block of Na/Ca exchange (NCX) has potential therapeutic applications, in particular, if a mode-selective block could be achieved, but also carries serious risks for disturbing the normal Ca\(^{2+}\) balance maintained by NCX. We have examined the effects of partial inhibition of NCX by SEA-0400 (1 or 0.3 μmol/L) in left ventricular myocytes from healthy pigs or mice and from mice with heart failure (MLP\(^{-/-}\)). During voltage clamp ramps with [Ca\(^{2+}\)], buffering, block of reverse mode was slightly larger than of forward mode (by 25±5%, P<0.05). In the absence of [Ca\(^{2+}\)], buffering and with sarcoplasmic reticulum (SR) fluxes blocked, rate constants for Ca\(^{2+}\) influx and Ca\(^{2+}\) efflux were reduced to the same extent (to 36±6% and 32±4%, respectively). With normal SR function the reduction of inward NCX current (I\(_{\text{NCX}}\)) was 57±10% (n=10); during large caffeine-induced Ca\(^{2+}\) transients, it was larger (82±3%). [Ca\(^{2+}\)], transients evoked during depolarizing steps increased (from 424±27 to 994±127 nmol/L at +10 mV, P<0.05), despite a reduction of I\(_{\text{Ca}}\) by 27%. Resting [Ca\(^{2+}\)] increased; there was a small decrease in the rate of decline of [Ca\(^{2+}\)]. SR Ca\(^{2+}\) content increased more than 2-fold. Contraction amplitude of field-stimulated myocytes increased in healthy myocytes but not in myocytes from MLP\(^{-/-}\) mice, in which SR Ca\(^{2+}\) content remained unchanged. These data provide proof-of-principle that even partial inhibition of NCX results in a net gain of Ca\(^{2+}\). Further development of NCX blockers, in particular, for heart failure, must balance potential benefits of I\(_{\text{NCX}}\) reduction against effects on Ca\(^{2+}\) handling by refining mode dependence and/or including additional targets. (Circ Res. 2008;102:1398-1405.)

Key Words: cardiac myocytes ■ heart failure ■ Na/Ca exchange ■ sarcoplasmic reticulum ■ calcium overload

The Na/Ca exchanger has a key role in the Ca\(^{2+}\) flux balance of cardiac myocytes as it is the major Ca\(^{2+}\) efflux pathway to remove Ca\(^{2+}\) entry occurring during excitation–contraction coupling (reviewed elsewhere\(^1\)-\(^3\)). With each heartbeat, Ca\(^{2+}\) influx across the sarcolemma triggers a release of Ca\(^{2+}\) from the sarcoplasmic reticulum (SR), the major source for Ca\(^{2+}\) for contraction. Ca\(^{2+}\) influx is mainly through the voltage-dependent L-type Ca\(^{2+}\) channels (I\(_{\text{CaL}}\)) and, to a lesser extent, through the Na/Ca exchanger itself. The net transport of Ca\(^{2+}\) through the exchanger during a cycle is thus Ca\(^{2+}\) removal. Changes in flux through the exchanger occur consequent on alterations in [Ca\(^{2+}\)], and [Na\(^{+}\)], (reviewed elsewhere\(^1\)). Such changes can affect the total amount of Ca\(^{2+}\) available in the SR for release.

The Na/Ca exchange (NCX) current (I\(_{\text{NCX}}\)) is inward for Ca\(^{2+}\) removal (forward mode) and outward for Ca\(^{2+}\) influx (reverse mode). I\(_{\text{NCX}}\) can be arrhythmogenic because the inward current during Ca\(^{2+}\) release from the SR can give rise to delayed afterdepolarizations when SR Ca\(^{2+}\) release occurs outside the normal excitation–contraction coupling cycle (reviewed elsewhere\(^4\)). This can be attributable to Ca\(^{2+}\) overload with or without a lowering of the threshold for release because of altered properties of the ryanodine receptor.\(^5\)-\(^7\) Outward I\(_{\text{NCX}}\) can contribute to Ca\(^{2+}\) loading when [Na\(^{+}\)] is increased, as, for example, during Na/K pump block but also in heart failure.\(^8\)

From the purely electrophysiological viewpoint, reducing inward I\(_{\text{NCX}}\) could theoretically be antiarrhythmic, whereas selective block of the outward I\(_{\text{NCX}}\) would reduce Ca\(^{2+}\) loading.\(^9\)-\(^11\) Several recent developments have rekindled the interest in potential benefits of blocking, or at least reducing, NCX. KBR-7943 and the more potent and selective SEA-0400 were reported to have a higher potency in blocking reverse mode than forward mode, which could be related to Na-dependent binding.\(^12\)-\(^14\) Such drugs could thus reduce Ca\(^{2+}\) overload related to reverse mode NCX. Indeed, in conditions of increased Ca\(^{2+}\) influx via reverse mode, such as block of the Na/K pump, KBR-7943 and SEA-0400 reduced
arrhythmias.\textsuperscript{15,16} Mice with cardiac-specific knockout of the Na/Ca exchanger had a higher resistance to ischemia/reperfusion damage.\textsuperscript{17} Despite absence of NCX, they had normal overall [Ca\textsuperscript{2+}] transients, maintaining Ca\textsuperscript{2+} balance most likely because of a reduced Ca\textsuperscript{2+} influx.\textsuperscript{18,19} However, caveats against NCX block have also been raised. Theoretical modeling and experiments with XIP predict an increase in Ca\textsuperscript{2+} load from a block of NCX because of its role in Ca\textsuperscript{2+} efflux.\textsuperscript{20} Under conditions in which the exchanger would thus not be blocked by SEA-0400, it could have different effects. Second, the selective block of reverse mode more than forward mode by these agents is less pronounced in more physiological conditions,\textsuperscript{21,22} although subsequent changes in Ca\textsuperscript{2+} handling were so far not studied.

In the present study, we tested the hypothesis that in normal myocytes and physiological conditions SEA-0400 would not exhibit mode dependence and that even partial inhibition of NCX would lead to a net gain of Ca\textsuperscript{2+}. Our findings support this hypothesis. We also confirm the potency of this drug to reduce potentially arrhythmogenic inward NCX currents. Further development of NCX inhibitors should thus weigh the potential benefits of current block against the effects on Ca handling.

Materials and Methods

Cell Isolation

Single left ventricular (LV) myocytes were enzymatically isolated from the hearts of domestic pigs of either sex (body weight, 40 to 45 kg; N = 18) as previously described.\textsuperscript{23} We used only cells isolated from the midmyocardial layer of the left ventricle. For mice, we used LV myocytes without distinguishing layers within the left ventricle; some specific and complementary experiments were done in pig myocytes only.

Solutions and Drugs

Experiments were done in normal Tyrode solution (in mmol/L): NaCl 137, KCl 5.4, MgCl\textsubscript{2} 0.5, CaCl\textsubscript{2} 1.8, Na-Hepes 11.8, glucose 10; pH 7.40. The pipette solution for whole-cell patch clamp contained (in mmol/L): K-aspartate 120, NaCl 10, KCl 20, K-Hepes 10, MgATP 5, K\textsubscript{2}HPO\textsubscript{4} 3 0.05; pH 7.2. To measure Ca\textsuperscript{2+} and NCX currents, K\textsuperscript{+} was replaced with Cs\textsuperscript{+} in pipette and extracellular solutions.

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recording the baseline current, SEA-0400 was applied. A steady state was typically achieved after 5 minutes and the calculated difference current represented the SEA-sensitive current. Then, we added NiCl₂ to have full block of NCX and measured total Iₐ. From these data, the fractional block of Iₐ by SEA-0400 was calculated. At 1 μmol/L, SEA-0400 significantly decreased both the forward and reverse mode NCX. When measured at similar driving force of 60 mV, the extent of reverse mode block was larger than of forward mode (Figure 1B). This was more pronounced in mouse than in pig myocytes, and, in both species, was more pronounced when pipette [Na⁺] was higher. When studying Ca²⁺ handling in the mouse, we used a lower dose of SEA-0400, 0.3 μmol/L, resulting in a similar block of forward mode Iₐ as in the pig (see below).

**Relative Block of Ca²⁺ Influx via Reverse Mode and Efflux via Forward Mode**

We next analyzed mode selectivity without Ca²⁺ buffering but with Ca²⁺ fluxes other than NCX inhibited (Ca²⁺ channels blocked with nifedipine, SR fluxes blocked with ryanodine and thapsigargin). In these conditions, a strong depolarizing pulse evokes reverse mode NCX and Ca²⁺ influx, and on repolarization, Ca²⁺ efflux occurs through forward mode NCX, illustrated in Figure 2A. The initial rates of Ca²⁺ increase on depolarization and of Ca²⁺ removal on repolarization were fit by a single exponential function (Figure 2B). The rate constants were decreased to a similar extent for forward and reverse mode (Figure 2C).

We also quantified the decrease of Ca²⁺ influx via reverse mode NCX in more physiological conditions, during short steps to +60 mV of 300 ms. The increase of [Ca²⁺], was reduced by 45±5% (n=10, data not shown).

**Selectivity of NCX Block: Presence of Ca²⁺ Channel Block**

Despite its higher selectivity for NCX than KBR-7943, SEA-0400 has also been reported to have some Ca²⁺ channel blocking effect. Current–voltage curves obtained before and after application of SEA-0400 (Figure 3A) suggested a decrease of Iₐ at +10 mV of 27% to 35%. Because of potentially confounding effects of rundown of Iₐ, we recorded simultaneously the time course of block of Iₐ and Iₐ during wash in of SEA-0400 (data obtained in pig myocytes). We used a double pulse protocol, repeated every 10 seconds, with a step to +10 mV to measure Iₐ and a second depolarizing step to +60 mV to measure outward Iₐ (Figure 3Ba). These data confirmed that SEA-0400 at 1 μmol/L indeed reduced Iₐ. As described in dog myocytes, the block of Iₐ had distinct properties. It occurred faster than the block of Iₐ, recovered partially, and was reversible in all cells that were tested (n=6), whereas the block of Iₐ by SEA-0400 on the same time scale was not. The difference in time course of the inhibition between Iₐ and Iₐ is highly significant (Figure 3Bb).

The block of Iₐ was also frequency-dependent and, in pig myocytes, increased from 35% at 0.1 Hz to 60% at 1 Hz (P<0.05, n=8). However, when measured in Na⁺-free solutions and with intracellular Ca²⁺ buffering, current block and frequency dependence were blunted (29±5% at 0.1 Hz and 37±10% at 1 Hz; P=NS; n=5). This suggests that the block...
of $I_{CaL}$ is at least partially consequent on the effects of NCX block, most likely through the increase of resting $[Ca^{2+}]_{i}$.

**SEA Increases $[Ca^{2+}]_{i}$**

During wash in of SEA-0400 we observed an increase in both resting $[Ca^{2+}]_{i}$ and $[Ca^{2+}]_{t}$, transient amplitude. The effect on resting $[Ca^{2+}]_{i}$ was analyzed in a time course experiment designed to distinguish potentially confounding effects of alterations in dye loading from changes in resting $[Ca^{2+}]_{i}$ during long experiments (Figure 4A). After optimal dye loading, $Ca^{2+}$ signals were recorded at 1 and 0.1 Hz for 1- and 10-minute periods in the absence of SEA-0400. The drug was then washed in during low frequency stimulation. The small increase in basal fluorescence during the first 12 minutes most likely reflects further dye loading and a small $Ca^{2+}$ increase. However, after wash in of SEA-0400, there was a faster increase in the baseline values which must reflect an increase in resting $[Ca^{2+}]_{i}$. The slopes calculated by linear fit to the data points were significantly steeper after SEA-0400 application, implying a net increase in resting $Ca^{2+}$ levels (Figure 4A, bar graph). This was also supported by analysis of resting cell length. Resting cell length, on average, decreased by $1.2\pm0.4\%$ ($P<0.05$) in pig myocytes and by $1.3\pm0.4\%$ ($P<0.05$) in mice.

The changes in $[Ca^{2+}]_{i}$ transient amplitude and the voltage dependence were evaluated using depolarizing steps to different voltages (Figure 4B). The peak $Ca^{2+}$ values increased significantly with all voltage steps, both in pig and in mouse myocytes; resting $[Ca^{2+}]_{i}$, increased from $115\pm12$ to $350\pm38$ nmol/L in pig myocytes and from $67\pm11$ to $124\pm20$ nmol/L in mouse myocytes. For the step to +10 mV, we further analyzed the kinetics of the $[Ca^{2+}]_{i}$ transient. The time to peak and half-relaxation of the transients prolonged. The kinetics of $I_{CaL}$ decline were not significantly altered (data not shown).

**Effects of SEA-040 on the Sarcoplasmic Reticulum $Ca^{2+}$ Load and Rate of $Ca^{2+}$ Removal by NCX**

We examined the $Ca^{2+}$ load of the SR during caffeine-induced $Ca^{2+}$ release (Figure 5A). $Ca^{2+}$ load was increased as...
measured from the peak of the [Ca\textsuperscript{2+}] transient as well as from the integrated $I_{NCX}$ (Figure 5Ba and 5Bb), yet maximal peak inward $I_{NCX}$ was reduced (Figure 5Bc).

From the caffeine experiments, we also quantified the rate of Ca\textsuperscript{2+} removal by NCX. The rate constant of Ca\textsuperscript{2+} decay was significantly increased (Figure 5Bd).

**Extent of Block of the Inward $I_{NCX}$**

We further measured the effectiveness of SEA-0400 on suppressing the inward current during SR Ca\textsuperscript{2+} release by quantifying $I_{NCX}$ density as a function of [Ca\textsuperscript{2+}], during the caffeine pulses (Figure 6A). In a phase plane analysis for the entire duration of the caffeine application, the loop shifted upward and to the right, and the slope became less steep after SEA-0400. The slope, a measure of current density as a function of [Ca\textsuperscript{2+}] and calculated during the decline of the Ca\textsuperscript{2+} transient was reduced 3-fold for the pig and 2-fold for the mouse.

These data during caffeine release are consistent with measurements of inward $I_{NCX}$ on repolarization from the step to +60 mV during the current–voltage protocol. Inward $I_{NCX}$ density, normalized to [Ca\textsuperscript{2+}], at that time, was likewise reduced 2- to 3-fold by SEA-0400 (Figure 6B).

The data of Figure 6B during 10s caffeine applications suggest that reduction of peak inward $I_{NCX}$ may actually prolong $I_{NCX}$. We therefore also measured block of inward $I_{NCX}$ during brief (300-ms) applications of caffeine in pig myocytes. This protocol mimics more closely spontaneous Ca\textsuperscript{2+} release as it allows normal SR reuptake (Figure 6C).29 Under those conditions peak inward $I_{NCX}$ was similarly reduced (from $1.85 \pm 0.61$ pA/pF per $\mu$mol/L Ca; $n=5$) but without prolongation ($\tau$ from 235 to 279 ms).

**SEA-0400 As Inotropic Agent**

We measured the effects of SEA-0400 on the amplitude and kinetics of contraction during field stimulation at 1 Hz (Figure 7). In pig myocytes, the amplitude of shortening increased; the time to peak shortening also increased with a trend to slower relaxation, but this was not significant. SEA-0400 never induced spontaneous activity (18 cells tested). In mouse myocytes, contraction amplitude similarly
increased ($P=0.07$) and the kinetics were slower, both for rate of contraction and relaxation.

To see whether the positive inotropic effect could be advantageous in heart failure, we tested the effects of SEA-0400 in MLP$^{-}$ mice, a known model for heart failure (Figure 7B, left). Amplitude of contraction was less than in WT at baseline, but, surprisingly, SEA-0400 even at 1 $\mu$mol/L failed to increase the amplitude, though relaxation was slowed (half-time to relaxation, from 121±5 to 140±13 ms) and resting length decreased by 2.3±0.9%. We tested the same concentration in WT myocytes, in which a significant increase was seen (not shown), with occurrence of spontaneous activity in 7 of 8 cells. These data suggested that, in the MLP$^{-}$ myocytes, the SR content failed to increase. Indeed, the amplitude of $[\text{Ca}^{2+}]_i$ transients did not increase and neither did the amplitude of the caffeine-induced Ca$^{2+}$ release or the integrated $I_{\text{SCX}}$ (data not shown). There was however an increase in resting $[\text{Ca}^{2+}]_i$. Presence of block of $I_{\text{SCX}}$ was evidenced by the decrease of peak inward current decreased (to $-0.50\pm0.07$ versus $-1.42\pm0.19$ pA/pF at baseline, $P<0.01$) and the rate of decay of the caffeine-induced Ca$^{2+}$ transient (r, 1.89±0.30 versus 0.96±0.12 seconds at baseline, $P<0.01$). We also tested the effect of SEA-0400 in myocytes from mice with 8 weeks of transverse aortic constriction (TAC), another well-established model of cardiac hypertrophy and failure. Heart weight/body weight ratio was, on average, increased by 44%, and lung weight by 48% (n=5). Contraction of isolated myocytes was less impaired than in the MLP$^{-}$ mice, and SEA-0400 had a significant inotropic effect both at 0.3 and 1 $\mu$mol/L (Figure 7B, middle and right).

**Discussion**

**Selectivity and Mode Dependence of NCX Block by SEA-0400**

Earlier studies in guinea pig had reported a very high selectivity of SEA-0400 with only a 10% block of $I_{\text{CaL}}$ at the EC50 of 50 $\mu$mol/L. In the dog, a concentration of 0.3 $\mu$mol/L, close to the EC50 for the inward $I_{\text{SCX}}$, reduced $I_{\text{CaL}}$ by 10%, and at 1 $\mu$mol/L, by 20%. SEA-0400 has been used at 1 and 10 $\mu$mol/L in the mouse, but its selectivity was not previously determined. In the present study, we chose to work with a concentration of 1 $\mu$mol/L, a concentration that has been used in most functional studies so far. In the pig, this resulted in a block of $I_{\text{CaL}}$ of some 25%, whereas it was even higher in the mouse (data not shown). We also measured $I_{\text{CaL}}$ in the mouse with a lower dose of 0.3 $\mu$mol/L, but block was still $\approx 25\%$. These data suggest that if the goal is a block of $I_{\text{SCX}}$ of at least 50%, concomitant block of $I_{\text{CaL}}$ is unavoidable. They also indicate that beneficial effects of SEA-0400 cannot be equivocally ascribed to NCX block, but could be related to the concomitant $I_{\text{CaL}}$ block.

Possible mode dependence of NCX block, ie, different degree of block in reverse versus forward mode, remains an issue of debate. It was first described for KB-R7943, followed by studies that found a much less pronounced, or even absent, mode dependence. In dog myocytes, KB-R7943 showed mode dependence even during symmetrical ramp protocols. For SEA-0400, giant-patch experiments showed higher potency of the drug when the reverse mode was favored by very high cytosolic Na$^+$ concentration. These observations led to the idea that drug-binding could be mode-dependent and favored by the Na-dependent inactivated state. In our experiments, we recorded simultaneously forward and reverse mode and measured currents at equal distance from the reversal potential. We observed only a small but significant difference, with reverse mode blocked to a larger extent, in particular in mouse myocytes. On the other hand, increasing the pipette Na concentration did not greatly affect the degree of block. These results are not easily reconciled with the Na-dependent hypothesis, unless Na binding would be voltage-dependent. We also measured rates of Ca$^{2+}$ influx and removal by NCX and could not demonstrate a mode dependence of block (Figure 2).

We further studied NCX block under more physiological conditions. When normalized to $[\text{Ca}^{2+}]_i$, inward $I_{\text{SCX}}$ density was reduced more than 3-fold during SR Ca$^{2+}$ release. The suppression of outward current during the double pulse protocol amounted to approximately 50% (data not shown), consistent with the reduction of Ca$^{2+}$ influx via reverse mode by $\approx 50\%$.

These data suggest that mode dependence is absent or even reversed during normal excitation–contraction coupling. Possibly, block of inward $I_{\text{SCX}}$ is potentiated by high $[\text{Ca}^{2+}]_i$, because the highest degree of block was seen at the peak of caffeine-induced Ca$^{2+}$ release (82% reduction of $I_{\text{SCX}}$; Figure 6A).

**Ca$^{2+}$ Handling With SEA-0400**

In both mouse and pig myocytes, SEA-0400 increased the amplitude of the Ca$^{2+}$ transients, increased resting Ca$^{2+}$, and increased the Ca$^{2+}$ content of the SR. The rate of decline of the Ca$^{2+}$ transient was slowed down. Although a number of studies have used SEA-0400 as a tool to block NCX, reports on the consequences for Ca$^{2+}$ handling are limited. In the mouse, the increase of $[\text{Ca}^{2+}]_i$ transient amplitude was reported before but with 10 $\mu$mol/L SEA-0400. These authors observed, but did not further comment on, the increase in resting Ca$^{2+}$. They also did not report an increase of spontaneous activity, but it is possible that the higher dose of SEA-0400 had a higher concomitant degree of $I_{\text{CaL}}$ block; this was not tested. In the dog, only a minor effect on $[\text{Ca}^{2+}]_i$, transients was seen, possibly because of the block of $I_{\text{CaL}}$.

The effects on Ca$^{2+}$ handling are consistent with a net decrease in Ca$^{2+}$ removal and gain of Ca$^{2+}$ load of the myocytes in mice and pig, despite moderate block of $I_{\text{CaL}}$. In the NCX knockout mouse, there was no such net gain of Ca$^{2+}$ and this was ascribed to the reduction of $I_{\text{CaL}}$ by 60%, which exceeds the reduction with SEA-0400. It is therefore conceivable that drugs that would have a higher degree of $I_{\text{CaL}}$ block would not result in cellular Ca$^{2+}$ gain.

Our data indicate clearly that during normal excitation–contraction coupling, the physiology of NCX with net Ca$^{2+}$ removal during a cardiac cycle outweighs an intrinsic mode dependence of block. Beneficial effects of NCX block or knockout on ischemia-reperfusion injury could result from a predominance of Ca$^{2+}$ influx via NCX under these conditions but at the same time suggest there are alternate ways for Ca$^{2+}$ removal. Another possible interpretation is that the mode
dependence is much more pronounced under those conditions because of very high [Na⁺]. Our current data with 20 versus 10 mmol/L pipette Na⁺ do not reveal such big differences but may underestimate the increase in subsarcolemmal Na⁺ during ischemia/reperfusion.

Species Differences in NCX Inhibition
When we set up the study, we expected to find significant differences between mouse and pig myocytes. In the mouse, Ca²⁺ reuptake into the SR is the major Ca²⁺ removal system, and, based on the data from the NCX knockout mouse, we expected less effect on Ca²⁺ and, based on the data from the NCX knockout mouse, we expected less effect on Ca²⁺ handling. We also hypothesized that mouse myocytes would have higher [Na⁺], and more preferential reverse mode inhibition, which would result in a reduction of contraction rather than increase.

The baseline characteristics in kinetics of [Ca²⁺], transients and contractions are striking, with the mouse being faster and having larger contractions (Figures 4 and 7). Differences in the effect of NCX inhibition, however, are small. Even though the mode dependence in the ramp protocol at 1 μmol/L SEA-0400 was slightly more pronounced, the predominant effect in mice of NCX inhibition was still a gain of Ca²⁺. During field stimulation, mouse myocytes had a tendency to spontaneous activity after several minutes of exposure to 1 μmol/L SEA-0400, which was never seen in pig myocytes. This could correspond to a higher potential for SR accumulation and there was indeed a trend toward higher increase of SR content (Figure 5).

Overall, our data suggest that quantitative differences are present but do not indicate differences in the principal actions of NCX inhibition between mouse or pig myocytes.

NCX Block As an Positive Inotropic Intervention
In healthy pig and mouse myocytes, SEA-0400 increased amplitude of contractions, consistent with the increase in [Ca²⁺]. In myocytes from dogs with heart failure, NCX block with intracellular XIP not only increased the amplitude of the Ca²⁺ transient but also led to a faster relaxation.20 This was ascribed to a shift from excessive NCX removal to enhanced SR uptake. In the MLP+/− mouse with heart failure, we could not achieve such beneficial effects. We have previously reported that, in these mice, the SR loading is not diminished at baseline but the SR has a reduced capacity for increasing its load.24 The ryanodine receptor also has a higher degree of phosphorylation, and myocytes may have an increased cycling across the sarcolemma through NCX.24 For these reasons, NCX block may be unable to enhance SR Ca²⁺ uptake and raise SR Ca²⁺ load in this heart failure model. In contrast, in myocytes from mice with TAC, the inotropic effect was preserved. These observations indicate that the effects in heart failure depend on the associated changes in other transports or myofilament function. The value of SEA-0400 in heart failure clearly needs to be studied further and cannot be extrapolated simply from data obtained in healthy myocytes. Large animal models with a greater reliance on NCX in heart failure should be included because their response may be more pronounced.

Perspectives for NCX Inhibitors
SEA-0400 was very effective in reducing the maximal peak current associated with Ca²⁺ release at resting membrane potentials, as measured during caffeine-induced Ca²⁺ release. In the presence of a functional SR, this could provide specific antiarrhythmic protection, currently not achieved with any other class of drugs. Several groups have reported reduction of delayed afterdepolarizations and also early afterdepolarizations using either KBR-7943 or SEA-0400. Given that none of these studies used concentrations that were selective for NCX, concomitant Ca²⁺ channel block may have contributed to the beneficial effects. To further tease out the role of NCX inhibition proper, a more selective blocker will be useful. Yet, in the clinical perspective, a concomitant block of I_{Ca,L} may be an advantage and part of the therapeutic profile of antiarrhythmic drugs.11,16,32

Our data strongly suggest that NCX block can have adverse effects in heart failure, because it raises resting Ca²⁺ and slows down relaxation. Yet, there is also a potential benefit from increasing SR Ca²⁺ load. This requires that SR uptake is preserved, also to prevent an excessive raise in resting Ca²⁺.

In conclusion, further development of NCX inhibitors should weigh the potential benefits of current block against the effects on Ca²⁺ handling. Inhibitors of NCX without effects on other Ca²⁺ transport are highly desirable for research purposes but may not necessarily be the first choice for clinical use. If mode selectivity of NCX inhibition could be developed, it would have clear advantages, but this goal has not yet been achieved.

Acknowledgments
SEA-0400 was generously supplied by Dr Fülöp (University of Szeged). We thank E. Detre, C. Huysmans, and K. Vermeulen for assistance.

Sources of Funding
This study was supported by grants to K.R.S from the Fund for Scientific Research (Flanders) (grant G.0384.07), the European Union (grant LSHM-CT-2005-018833, EUGeneHeart), and the Belgian Science Program (grant IAP6/31).

Disclosures
None.

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Circ Res. 2008;102:1398-1405; originally published online May 1, 2008; doi: 10.1161/CIRCRESAHA.108.173922

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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