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RESEARCH PAPER

Na^+/Ca^{2+} exchanger inhibition exerts a positive inotropic effect in the rat heart, but fails to influence the contractility of the rabbit heart

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Background and purpose: The Na^+/Ca^{2+} exchanger (NCX) may play a key role in myocardial contractility. The operation of the NCX is affected by the action potential (AP) configuration and the intracellular Na^+ concentration. This study examined the effect of selective NCX inhibition by 0.1, 0.3 and 1.0 μM SEA0400 on the myocardial contractility in the setting of different AP configurations and different intracellular Na^+ concentrations in rabbit and rat hearts.
Experimental approach: The concentration-dependent effects of SEA0400 on $I_{Na/Ca}$ were studied in rat and rabbit ventricular cardiomyocytes using a patch clamp technique. Starling curves were constructed for isolated, Langendorff-perfused rat and rabbit hearts. The cardiac sarcolemmal NCX protein densities of both species were compared by immunohistochemistry.
Key results: SEA0400 inhibited $I_{Na/Ca}$ with similar efficacy in the two species; there was no difference between the inhibitions of the forward or reverse mode of the NCX in either species. SEA0400 increased the systolic and the developed pressure in the rat heart in a concentration-dependent manner, for example, 1.0 μM SEA0400 increased the maximum systolic pressures by 12% relative to the control, whereas it failed to alter the contractility in the rabbit heart. No interspecies difference was found in the cardiac sarcolemmal NCX protein densities.
Conclusions and implications: NCX inhibition exerted a positive inotropic effect in the rat heart, but it did not influence the contractility of the rabbit heart. This implies that the AP configuration and the intracellular Na^+ concentration may play an important role in the contractility response to NCX inhibition.
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Keywords: NCX; myocardial contractility; NCX protein density; SEA0400; isolated hearts; EC_{50}

Abbreviations: AP, action potential; ECG, electrocardiogram; E_m, transmembrane potential; I_{CaL}, L-type Ca^{2+} current; I_{Na}, inward Na^+ current; I_{Na}/Ca, Na^+/Ca^{2+} exchanger current; NCX, Na^+/Ca^{2+} exchanger

Introduction

The Na^+/Ca^{2+} exchanger (NCX) is considered to play an important role in the Ca^{2+} handling of cardiac myocytes. In forward mode, the NCX extrudes Ca^{2+} from the cell, whereas in reverse mode it brings Ca^{2+} into the cell from the extracellular space. The direction of operation of the NCX depends mainly on the transmembrane gradients of Ca^{2+} and Na^+ and on the prevailing transmembrane potential (E_m). In the early phase of the action potential, the NCX works in reverse mode and brings Ca^{2+} into the cell, whereas during repolarization, it removes Ca^{2+} from the cell in the forward mode. Thus, the duration of the action potential influences the operation of the NCX (Weber *et al.*, 2002).

The duration and shape of the action potential may vary with the species, due to the uneven distribution of the various transmembrane K^+ channels. For instance, the duration of the action potential and, particularly, that of the plateau phase are much longer in rabbit myocytes as compared with those in rat myocytes, which suggests that the NCX operates differently in rabbit and rat myocytes.

The cardiac contractility and force development depend on the free intracellular Ca^{2+} level and the Ca^{2+} sensitivity

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of the myofilaments. On a beat-to-beat basis, to increase the intracellular Ca^{2+} level, the Ca^{2+} entry should be increased or the Ca^{2+} removal should be decreased. The NCX is regarded as the main Ca^{2+}-extruding mechanism in the heart, and its inhibition may alter the intracellular Ca^{2+} level and the cardiac muscle contractility.

A change in the expression (for example, an overexpression) of the NCX protein can also affect the cardiac muscle contractility. The overexpression of the NCX in genetically manipulated feline myocytes caused a decline in contractility (Weisser-Thomas *et al*., 2005). The contraction and Ca^{2+} transient decreased in NCX-overexpressing rat cardiomyocytes by adenoviral-mediated gene transfer (Bölck *et al*., 2004). A weakened cardiac muscle contractility has likewise been observed in myocardial hypertrophy, heart failure and even myocardial infarction, due to the increased expression and altered activity of the NCX (Quinn *et al*., 2003). However, the levels of NCX expression in different species under physiological conditions have, so far, not been compared. Furthermore, the relationship between the level of NCX expression and the NCX function has not been elucidated.

A number of benzyloxyphenyl analogues, such as KB-R7943, YM244769, SN-6 and SEA0400, are known to act as NCX inhibitors. The IC_{50} value of SEA0400 for both inward and outward directions in guinea pig and canine cardiomyocytes is between 20–300 nM (Tanaka *et al*., 2002; Birinyi *et al*., 2005). SEA0400 is a highly effective inhibitor of the NCX (Matsuda *et al*., 2001), with an inhibitory potency 10- to 100-fold higher than that of KB-R7943 (Takahashi *et al*., 2003). The inhibition rate of the forward and the reverse modes of the NCX with SEA0400 is indeterminate: the drug caused nearly equivalent inhibition for both modes of NCX operation in mouse, guinea pig and canine isolated myocardial cells (Tanaka *et al*., 2002; Nagy *et al*., 2004; Birinyi *et al*., 2005; Namekata *et al*., 2005; Wang *et al*., 2007), whereas it preferentially inhibited the reverse mode in transfected fibroblasts and in *Xenopus laevis* oocyte (Iwamoto *et al*., 2004; Lee *et al*., 2004). SEA0400 is considered to be selective for the NCX up to a concentration of 1.0 μM without influencing any other ion transport mechanism (Matsuda *et al*., 2001; Tanaka *et al*., 2002; Iwamoto and Kita, 2004). SEA0400 at a concentration of 1.0 μM inhibits 70–80% of the NCX function (Matsuda *et al*., 2001; Tanaka *et al*., 2002; Birinyi *et al*., 2005). The concentration-dependent effects of SEA0400 on NCX current (I_{Na}/Ca) have never been studied in rat and rabbit ventricular cardiomyocytes.

Inhibition of the NCX may affect the contractile function of the myocardium under pathological conditions, thus it may have therapeutic implications (Matsuda *et al*., 2001; Takahashi *et al*., 2003). It was recently found that inhibition of the NCX increased the recovery of the contractile force in guinea pigs subjected to ischaemia/reperfusion injury (Namekata *et al*., 2005). Our group has demonstrated that NCX inhibition with SEA0400 increases the contractility and Ca^{2+} transient in rat isolated myocytes (Acsai *et al*., 2007). However, the effects of selective inhibition of the NCX on the contractility in different species with different action potential durations under physiological conditions are currently unknown. Accordingly, our present objective was to examine the effects of NCX inhibition with SEA0400 on the cardiac muscle contractility in isolated Langendorff-perfused hearts in the setting of the long action potential of the rabbit and in the short action potential of the rat. To clarify the inhibitory effect of SEA0400 on the reverse and the forward modes of the NCX in the rabbit and in the rat isolated myocardial cell, $I_{Na/Ca}$ was examined by a patch clamp technique. NCX protein densities of the two species were compared to determine whether interspecies differences in the contractile function can be explained by differences in NCX expression.

Methods

Animals

New Zealand white rabbits and Sprague–Dawley rats, weighing 2.3–2.9 kg and 200–250 g, respectively, were used for the experiments. The animals were handled according to a protocol reviewed and approved by the Ethical Committee for the Protection of Animals in Research at the University of Szeged, Szeged, Hungary.

Isolated, Langendorff-perfused hearts

The anticoagulant sodium heparin was injected into the marginal ear vein of the rabbits (1000 U) or into the intraperitoneal space of the rats (250 U). The rabbits and rats were subsequently stunned by a blow to the neck and cervical dislocation, respectively. The heart was rapidly removed via a thoracotomy and rinsed in ice-cold modified Krebs–Henseleit buffer solution containing (in mM) NaCl 118.5, $CaCl_2$ 2.0, glucose 11.1, $MgSO_4$ 1.0, NaH_2PO_4 1.2, $NaHCO_3$ 25 and KCl 4. The aorta was cannulated and hung on a Langendorff apparatus. The hearts were retrogradely perfused at a constant temperature of 37 °C with the modified Krebs–Henseleit buffer solution described above. A mixture of 95% O_2 and 5% CO_2 was bubbled through the buffer, which was equilibrated to pH 7.4. All solutions were filtered (10 μm pore-size filter) before use. The perfusion pressure was maintained constant at 80 mm Hg. The volume-conducted ECG and left ventricular pressure (described later) were recorded by using National Instruments data acquisition PC card (National Instruments, Austin, TX, USA) and SPEL Advanced Haemosys software (version 2.45, Experimetria Ltd. & Logirex Software Laboratory, Budapest, Hungary). Coronary flow was measured with a glass flowmeter (Cole-Parmer Instrument Company, Vernon Hills, IL, USA) positioned immediately above the retrogradely perfused aorta and was later corrected for the weight of each heart to give values in $ml\,min^{-1}\,g^{-1}$.

The contractile function of the left ventricle was measured by using a non-elastic balloon filled with water, connected to a pressure transducer. The balloon was inserted into the left ventricle via an incision in the left atrium and the mitral valve. The ventricular systolic, end-diastolic pressures and the developed pressure (that is, the systolic pressure minus the diastolic pressure) were recorded.

SEA0400 concentrations and Langendorff contractility protocol

SEA0400 was chosen for study because of its high specificity and potency for the NCX. At concentrations up to 3 μM,

SEA0400 has been shown to have no effect on ion currents other than the NCX (Tanaka *et al*., 2002). With regard to the literature and our own measurement of $I_{Na/Ca}$, SEA0400 concentrations of 0.1, 0.3 and 1.0 μM were chosen for the experiments, as 0.1 and 0.3 μM are around the measured IC_{50} and SEA0400 is considered selective for the NCX up to a concentration of 1.0 μM (Figures 1c and d).

A control group (which received the solvent of SEA0400) and the three treated groups (which received the different concentrations of SEA0400: 0.1, 0.3 and 1.0 μM) were compared in both the rabbit heart and the rat heart. Each of the groups in the rabbit study contained 10 hearts, and each of the four groups in the rat study contained 12 hearts. The protocol involved a randomized design with a time-matched control group, and blind experimentation, data collation and analysis. The choice of drug solution was made by reference to a randomization table. Randomization was achieved by coding each group with a letter whose meaning was unknown to the operator. Blind analysis was achieved by using stock solutions prepared by a second operator, who did not participate in the heart perfusion or data analysis. For the assessment of the vehicle control or each individual concentration of the drug, each heart was used only once.

Each heart was set up under Krebs perfusion and the balloon was inserted into the left ventricle. The 'zero volume' was determined (where the first pressure wave appeared). The experiment started with a 10 min isovolumetric (constant) period, when the balloon was inflated to an added volume of 0.5 ml in the rabbit heart and of 0.08 ml in the rat heart, which induced a left ventricular pressure that was approximately 60% of the maximum developed pressure. Next, commencing from 'zero volume', 0.1 ml increments in the rabbit heart and 0.02 ml increments in the rat heart were added to the balloon volume every minute to reach the maximum developed pressure (a Starling curve was constructed), the final diastolic pressure not being allowed to exceed 10 mm Hg. This was followed by another 5 min isovolumetric period and the whole procedure was repeated in the presence of the test drug.

Individual measurements of coronary flow, left ventricle pressure and ECG variables were made 1 min before, 4 min after and every minute during the Starling curves. This made

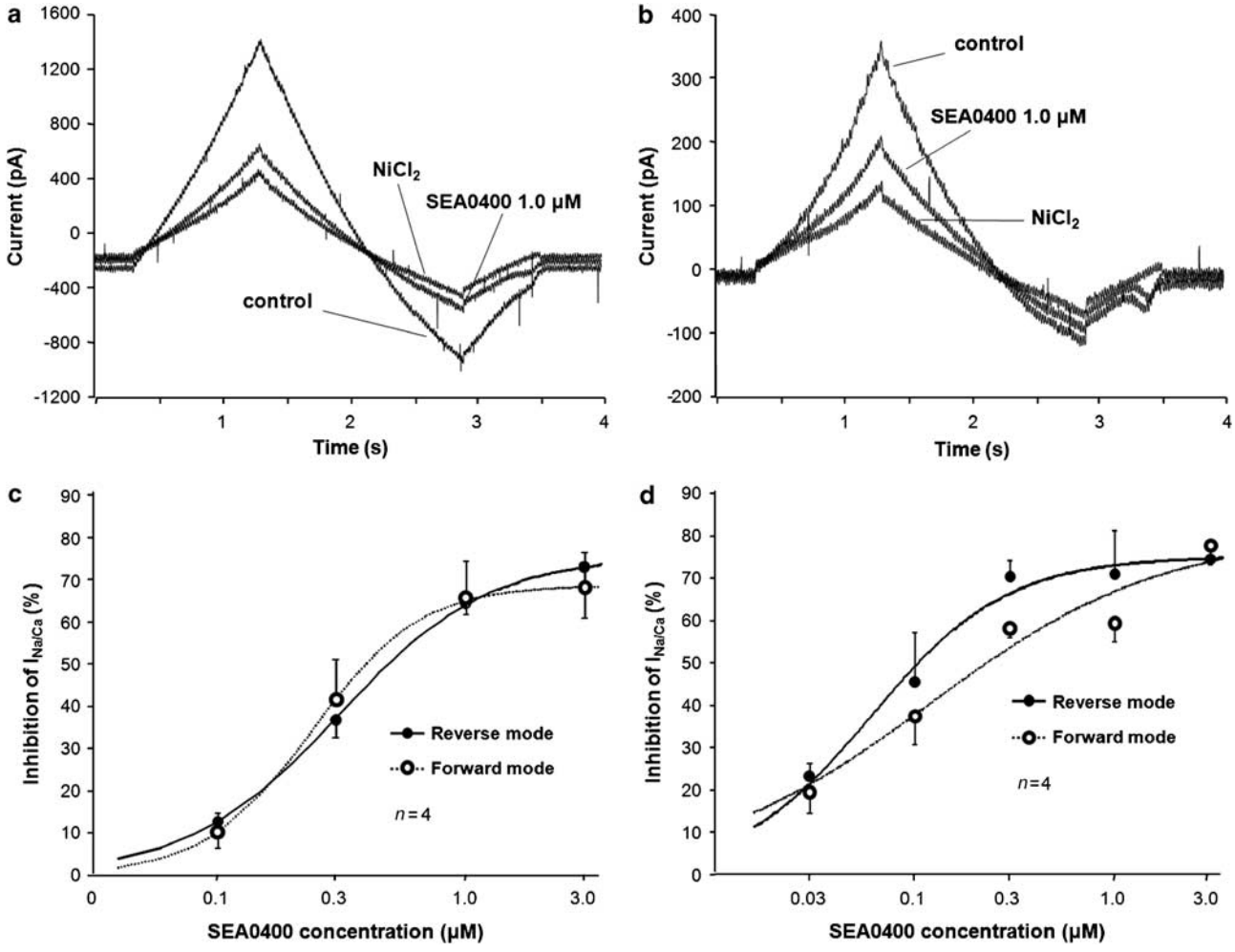

Figure 1 Concentration-dependent effects of SEA0400. Superimposed current traces obtained in K^+-free bath solution (control), in the presence of 1.0 μM SEA0400 and after addition of 10 mM $NiCl_2$ to block completely $I_{Na/Ca}$ in the rabbit (**a**) and the rat (**b**) myocyte. Outward and inward $I_{Na/Ca}$ were determined during the descending limb of the ramp at +40 and −80 mV. Concentration-dependent blocking effect of SEA0400 on forward $I_{Na/Ca}$ and reverse $I_{Na/Ca}$ in rabbit (**c**) and in rat (**d**) myocytes. The solid line was obtained by fitting data to the Hill equation. Four cells were challenged in every examined concentrations of SEA0400. Symbols and bars represent mean ± s.e.mean.

it possible to evaluate the immediate effects of the drug on the heart function under both isovolumetric and non-isovolumetric conditions. The constant balloon volume inflation applied (0.5 and 0.08 ml in the rabbit heart and the rat heart, respectively), which had been shown to give rise to approximately 60% maximum pressure development, was appropriate for the assessment of either positive or negative effects of the test drug on the ventricular function. The first Starling curve was constructed to test the health of each heart and to provide drug-free baseline data. The two Starling curves (before and during drug perfusion) were used to evaluate the inotropic and lusitropic effects completely. At the end of each experiment, the atria were removed from the heart and the ventricles were weighed. The protocol is outlined in Figure 2.

Exclusion criteria

Any heart with a coronary flow less than 5 ml min^{-1} g^{-1}, or those with an impaired contractility function observed in the first Starling curve or those not in a constant sinus rhythm were excluded. Excluded hearts were replaced to maintain equal group sizes.

Coronary flow measurement and ECG analysis

Coronary flow and ECG intervals were measured at predetermined time points. Coronary flow values were read directly from the flowmeter during the experiment. After completion of the experiments, the data were replayed and the PQ, QRS, QT and RR intervals were measured by manual positioning of on-screen markers. The QT interval was defined as the time between the first deviation from the isoelectric line during the PQ interval until the end of the TU wave. In the rabbit hearts, where the T or U wave overlapped the following P wave or the QRS complex of the subsequent sinus beat, the extrapolation method was used to measure the length of the QT (or QU) interval (Farkas *et al*., 2004).

Isolation of cardiac myocytes

Rabbits and rats (see above) were used for the isolation of myocytes, and the hearts were prepared as described above. Briefly, the hearts were excised and Langendorff-perfused. A modified Krebs–Henseleit buffer solution containing (in mM) NaCl 118.5, KCl 4, $MgSO_4$ 1.0, NaH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 11.1 at pH 7.4 was used as the perfusate for the rat hearts, and a modified Tyrode buffer solution containing (in mM) NaCl 130, HEPES 23, taurin 20, creatine 5, $MgCl_2$ 5, Na-pyruvate 5, KCl 4.5, NaH_2PO_4 1 and glucose 21 at pH 7.3 was used as the perfusate for the rabbit hearts. In the first 5 min, additional $CaCl_2$ (2 mM for the rat heart and 1 mM for the rabbit heart) was added to the buffer, and this was followed by a 5 min Ca^{2+}-free period. In the next step, collagenase, hyaluronidase and $CaCl_2$ (200 μM) were added to the buffer for the rats, whereas collagenase, protease and $CaCl_2$ (100 μM) were added to the buffer for the rabbits, and the hearts were perfused for an additional 10 min. The left ventricular myocardium was then minced and gently agitated. Isolated cells were stored in KB solution, containing (in mM) KOH 89, glutamic acid 70, taurin 15, KCl 30, KH_2PO_4 10, HEPES 10, $MgCl_2$ 0.5, glucose 11 and EGTA 0.5, the pH being titrated to 7.3 with KOH, at room temperature.

Measurement of $I_{Na/Ca}$

To examine the effects of SEA0400 on the NCX current, the whole-cell configuration of the patch clamp technique was applied (Birinyi *et al*., 2005). After establishment of the whole-cell configuration in Tyrode solution, the cell was superfused with a special K^+-free bath solution containing (in mM) NaCl 135, CsCl 10, $CaCl_2$ 1, $MgCl_2$ 1, $BaCl_2$ 0.2, NaH_2PO_4 0.33, TEACl 10, HEPES 10, glucose 10, pH 7.4, supplemented with 20 μM ouabain, 1 μM nisoldipine and 50 μM lidocaine to block Na^+, K^+, Ca^{2+} and Na/K pump currents. The pipette solution contained (in mM) CsOH 140, aspartic acid 75, TEACl 20, Mg-ATP 5, HEPES 10, NaCl 20, EGTA 20, $CaCl_2$ 10, pH 7.2. The Ca^{2+} concentration was estimated to be approximately 160 nM in this pipette solution. $I_{Na/Ca}$ was recorded by using ramp pulses (having a velocity of 100 mV s^{-1}) delivered at a rate of 0.05 Hz. The membrane was initially depolarized from the holding potential of −40 mV to +60 mV, then hyperpolarized to −100 mV, and finally the membrane potential returned to the holding potential. The outward and the inward $I_{Na/Ca}$ were determined during the descending limb of the ramp, at

a

	Krebs buffer			SEA0400		
Exp. time (min)	1-10	11-25	26-30	31-40	41-55	56-60
Intervals (min)	10	15	5	10	15	5
Balloon V (ml)	0.5	Starling curve	0.5	0.5	Starling curve	0.5

b

	Krebs buffer			SEA0400		
Exp. time (min)	1-10	11-19	20-24	25-34	35-43	44-48
Intervals (min)	10	9	5	10	9	5
Balloon V (ml)	0.08	Starling curve	0.08	0.08	Starling curve	0.08

Figure 2 The protocol applied in isolated, Langendorff-perfused hearts: in the first half of the experiment, all the hearts were perfused with Krebs buffer; the perfusion solution was then switched to the test drug in the second half of the experiment. (**a**) Protocol for rabbit hearts. (**b**) Protocol for rat hearts. Exp. time, experimental time; balloon V, volume of the left ventricular balloon; Starling curve, starting from the 'zero volume' 0.1 ml increments for the rabbit heart and 0.02 ml increments for the rat heart were added to the balloon volume every minute.

+40 and –80 mV, respectively. After the control recording in K^+-free solution, the cell was superfused with the given concentration of SEA0400 and finally 10 mM $NiCl_2$ was added to block $I_{Na/Ca}$ completely. Thus, total $I_{Na/Ca}$ was determined at both membrane potentials as a Ni^{2+}-sensitive current by subtracting the third record from the first. The fraction of the blockade induced by SEA0400 was expressed as a percentage of the total $I_{Na/Ca}$.

Immunohistochemistry

Rabbit and rat isolated cardiomyocytes from the left ventricle were fixed with acetone. Before staining, the samples were rehydrated in Ca^{2+}-free phosphate-buffered saline and blocked for 120 min at room temperature with phosphate-buffered saline with 0.01% Tween containing 1% bovine serum albumin. Indirect immunofluorescence staining was carried out by using mouse anti-NCX primary antibody in a dilution of 1:1000 and with a fluorescent secondary antibody conjugated goat anti-mouse IgG in a dilution of 1:1000. An incubation period of 60 min with the primary antibody at room temperature was followed by a 60 min incubation with the secondary antibody. Between and after the incubations, the samples were washed thoroughly with phosphate-buffered saline with 0.01% Tween. Control samples were incubated only with the secondary antibody. For microscopic examination, the cells were mounted in Aqua PolyMount.

Confocal laser scanning microscopy and quantification of fluorescence intensities

Confocal laser scanning microscopy was applied for the quantification of the NCX on the sarcolemmal surface of cardiac myocyte (Seprényi *et al*., 2006). This method of semiquantitative confocal image analysis can reliably distinguish 10–15% difference.

Serial images of the immunostained samples were captured by means of an Olympus FV1000 confocal laser scanning microscope (Olympus Co., Tokyo, Japan) with standard parameter settings. For quantitative analysis, the pictures were converted to greyscale TIFF file format with Adobe Photoshop software (Adobe Systems Inc., San Jose, CA, USA). The immunofluorescence of the cardiac muscle cells was quantitatively analysed with ImageQuant software (Molecular Dynamics, Sunnyvale, CA, USA) as follows: first the background was eliminated with the threshold set-up. The fluorescent images of 25 randomly selected rat and 25 rabbit cardiac myocytes, in each case derived from four animals, were subjected to immunofluorescent profile analysis: in each image, 2–5 random bands were selected and their fluorescent profiles were plotted in the profile plots, and the baseline pixels were identified and subtracted from the total profile area. In this way, the actual fluorescent pixel intensities were obtained from the specific staining. The average values and the standard error of the fluorescent intensity per pixel were calculated.

Drugs and materials

Perfusion solutions were prepared fresh each day. The 'vehicle stock' was a 7 ml solution of DMSO (Sigma-Aldrich Inc., St Louis, MO, USA). The '1.0 μM SEA0400 stock' was prepared from 30.58 mg of SEA0400 dissolved in 3.0 ml of DMSO. The '0.3 μM SEA0400' solution was prepared by mixing 0.6 ml of '1.0 μM SEA0400 stock' and 1.4 ml of 'vehicle stock'. The '0.1 μM SEA0400 stock' was prepared by mixing 0.2 ml of '1.0 μM SEA0400 stock' and 1.8 ml of 'vehicle stock'. A quantity of 0.1 ml of the chosen stock dissolved in 2.5 l of modified Krebs–Henseleit buffer yielded the appropriate solution.

Drugs were purchased from the following sources: collagenase (0.05%, type 1A) from Worthington Biochemical Corporation (Lakewood, NJ, USA); NCX antibody from Novus Biologicals Inc. (Littleton, CO, USA); fluorescent secondary antibody conjugated goat antimouse IgG from Molecular Probe, Invitrogen Co. (Carlsbad, California, USA); Aqua PolyMount from Polysciences Inc. (Warrington, PA, USA). Other drugs for the isolation of myocytes were from Sigma-Aldrich Inc. (St Louis, MO, USA).

All other salts were purchased from Molar Chemical Ltd. (Budapest, Hungary). Water for the preparation of perfusion solutions was obtained from a reverse osmosis system (Milli-Q RG, Millipore Ltd., Billerca, MA, USA) fed by distilled water and had a specific resistivity of $>18\,M\Omega$.

The synthesis of 2-(4-((2,5-difluorophenyl)methoxy)phenoxy)-5-ethoxy-aniline (SEA0400) was performed by Ferenc Fülöp (Department of Pharmaceutical Chemistry, University of Szeged, Szeged, Hungary) according to a method described in the literature (Aibe *et al*., 2000). The product was isolated as hydrochloride salt. Melting point = 172–174 °C (recrystallized from ethanol-ether). 1H-NMR (400 MHz, DMSO) $\delta = 1.3$ (t, 3H, CH3), 3.93–3.98 (m, 2H, OCH2), 5.09 (s, 2H, OCH2), 6.49–6.51 (m, 1H, Ar-H), 6.73–6.76 (m, 2H, Ar-H), 6.92–6.94 (d, 2H, Ar-H), 7.02–7.04 (d, 2H, Ar-H), 7.25–7.4 (m, 3H, Ar-H).

Statistical evaluation

Continuous data were expressed as means ± s.e.mean. When drug effects were analysed, continuous data from independent samples were subjected to repeated measures analysis of variance, whereas NCX protein density data from independent samples were compared through one-way analysis of variance. $P<0.05$ was taken as indicative of a statistically significant difference between values.

Results

Effects of SEA0400 on $I_{Na/Ca}$ *in isolated myocytes*

$I_{Na/Ca}$ was recorded as a Ni^{2+}-sensitive current using the descending limb of a ramp pulse changing slowly from +60 to −100 mV during 1.6 s. The outward and the inward $I_{Na/Ca}$ were determined at +40 and –80 mV, respectively (Figures 1a and b).

Rabbit. SEA0400 suppressed both the inward and the outward $I_{Na/Ca}$, that is, the Ni^{2+}-sensitive current was significantly decreased by these compounds in a concentration-dependent manner (Figure 1c). IC_{50} values of the inward (forward) and the outward (reverse) currents were 243 and 309 nM, respectively. Data were fitted to the Hill

equation. As full inhibition of $I_{Na/Ca}$ was not achieved under our experimental conditions, drug concentrations causing 50% reduction of the currents were higher than the respective EC_{50} values. No significant difference was seen in the SEA0400-induced suppression of the inward and the outward $I_{Na/Ca}$ (Figure 1c).

Rat. IC_{50} values of the inward (forward) and the outward (reverse) currents were 120 and 61 nM in rat myocytes, respectively (Figure 1d). SEA0400 blocked $I_{Na/Ca}$ by 70% at a concentration of 1 μM. No significant difference was found between the inhibitions of the two modes of NCX.

Systolic, end-diastolic and developed pressures

Rabbit. During the first Starling curve and at a constant balloon volume (Krebs solution), the applied balloon stretch increased the systolic and developed pressures very similarly in all four groups, whereas the diastolic pressures did not exceed 10 mm Hg even at maximum balloon inflation (1.5 ml), proving that the hearts were not overstretched. The SEA0400 concentrations applied (0.1, 0.3 and 1.0 μM) failed to alter the systolic and developed pressures at the constant balloon volume and in the second Starling curve as compared with the control group (Table 1a). When the systolic pressures measured at the maximum balloon volume of the second Starling curve were divided by the corresponding values of the drug-free first Starling curve, a concentration-dependent, but slight and insignificant, positive inotropic effect of SEA0400 was revealed (Figures 3a and 4a). A similar tendency was observed in the corresponding values of the developed pressure (Table 1a). The diastolic pressure was not changed significantly during the second Starling curve and the developed pressure did not differ between the groups during perfusion with SEA0400 (Table 1a).

Rat. Following the striking resemblance between the four groups in the drug-free first Starling curve, the applied SEA0400 concentrations increased the systolic pressure in a concentration-dependent manner both at a constant balloon volume and during the second Starling curve. The 1.0 μM SEA0400 group differed significantly from the control (Table 1b, Figure 4b). A significant and concentration-dependent positive effect of SEA0400 was also revealed when the systolic pressures measured at the maximum balloon volume of the second Starling curve were divided by the corresponding values of the drug-free first Starling curve (Figure 3b). The developed pressure also increased in a concentration-dependent manner during perfusion with SEA0400, as the systolic pressure increased, whereas the diastolic pressure remained unchanged (Table 1b).

ECG intervals and heart rate

The ECG intervals were almost constant during both Starling curves in both species. Perfusion with SEA0400 did not influence the PQ, QRS and QT intervals in either species (Table 2). The increased left ventricle stretch did not affect the ECG intervals; no differences were found between the constant and alternating balloon volume periods. SEA0400 perfusion did not exert any effect on the heart rate in either species. The corresponding ECG intervals of the SEA0400-perfused groups of hearts did not differ from those for the control group in either the rabbit or the rat study (Table 2).

Coronary flow

The coronary artery flow was measured during both isovolumetric and increasing stretch periods in the absence and presence of SEA0400. The increasing left ventricular stretch induced by the balloon inflation caused a slight flow elevation in every group of both species. The mean coronary flow ranged from 6.8 ± 0.7 to 9 ± 0.9 ml min^{-1} g^{-1} and from 7.9 ± 0.3 to 12.5 ± 1.2 ml min^{-1} g^{-1} in the rabbit heart and in the rat heart, respectively. There was no significant difference between the flow values of the various groups at any time point of the experiments in either species.

NCX protein density

Immunohistochemistry was performed to characterize the NCX protein density of the plasma membrane in the rabbit and rat isolated myocytes. Immunohistochemistry with confocal microscopy imaging revealed similar scattered distributions of the NCX protein on the surface of the rabbit and the rat myocytes. The physiological cardiac sarcolemmal NCX protein density did not differ in the rabbit and the rat (Figure 5).

Discussion

Our results demonstrate that selective inhibition of the NCX by SEA0400, which is considered to be a selective inhibitor of the NCX (Matsuda *et al*., 2001; Tanaka *et al*., 2002; Iwamoto and Kita, 2004), did not considerably influence the contractility in the rabbit isolated heart, whereas it increased the systolic and developed pressures in a concentration-dependent manner in the rat isolated heart. The NCX inhibition with the selective inhibitor SEA0400 was almost identical in the rabbit and rat myocytes, when the highest concentration of the drug was used, which significantly increased the contractile force in rats. The immunohistochemistry did not indicate a significant difference between the sarcolemmal NCX protein densities of the rabbit and the rat. Furthermore, heart perfusion with the NCX inhibitor SEA0400 did not influence the ECG intervals (PQ, QRS, QT and RR) or the coronary flow, the latter revealing that the drug did not affect the cardiac vessels either.

Pharmacodynamic effects of SEA0400

SEA0400 up to a concentration of 3.0 μM is considered to be selective for the NCX without influencing any other ion transport mechanism (Tanaka *et al*., 2002), although Birinyi *et al*. (2005) found that SEA0400 at a concentration of 1.0 μM inhibited the L-type Ca^{2+} current (I_{CaL}) in canine myocytes, which may explain the lack of positive contractility response in the rabbit heart. Recently, we found

Table 1 Systolic, diastolic and developed pressures in the presence of SEA0400 in the rabbit heart (a) and the rat heart (b)

Balloon V (ml)	*0.5*	*0.1*	*0.3*	*0.5*	*0.7*	*0.9*	*1.1*	*1.3*	*1.5*	*0.5*	
SEA0400											
(a) Rabbit											
Systolic P (mm Hg)											
Control	100 ± 9	78 ± 7	89 ± 7	98 ± 9	106 ± 10	114 ± 10	122 ± 10	128 ± 10	128 ± 12	84 ± 10	
0.1 (μm)	98 ± 7	77 ± 10	88 ± 9	95 ± 7	102 ± 7	109 ± 6	116 ± 7	124 ± 7	134 ± 7	94 ± 6	
0.3 (μm)	92 ± 9	70 ± 11	81 ± 10	90 ± 10	101 ± 10	109 ± 9	120 ± 11	126 ± 9	129 ± 8	78 ± 15	
1.0 (μm)	111 ± 7	91 ± 7	97 ± 7	106 ± 7	114 ± 7	120 ± 4	129 ± 8	136 ± 8	141 ± 7	100 ± 6	
Diastolic P (mm Hg)											
Control	1.3 ± 0.6	−3.2 ± 1.3	0.6 ± 0.7	1.6 ± 0.7	2.8 ± 0.7	3.7 ± 0.7	4.5 ± 0.9	5.5 ± 0.9	6.0 ± 1.4	0.9 ± 0.6	
0.1 (μm)	0.6 ± 0.4	−2.8 ± 0.8	1.2 ± 0.5	1.2 ± 0.4	1.9 ± 0.4	2.4 ± 0.5	3.1 ± 0.6	4.1 ± 0.9	5.5 ± 1.2	0.9 ± 0.5	
0.3 (μm)	0.9 ± 0.6	−2.9 ± 0.6	0.1 ± 0.6	1.1 ± 0.6	2.6 ± 0.5	3.8 ± 0.8	4.1 ± 0.7	4.0 ± 0.8	4.9 ± 1.0	1.7 ± 0.7	
1.0 (μm)	1.7 ± 0.4	−2.5 ± 0.4	0.9 ± 0.4	1.7 ± 0.4	2.4 ± 0.4	3.5 ± 0.5	4.7 ± 0.6	5.8 ± 0.9	6.9 ± 1.3	1.4 ± 0.4	
Developed P (mm Hg)											
Control	99 ± 9	81 ± 7	89 ± 7	96 ± 9	103 ± 10	110 ± 10	117 ± 10	122 ± 9	122 ± 11	83 ± 10	
0.1 (μm)	98 ± 6	79 ± 9	89 ± 8	94 ± 6	100 ± 6	106 ± 6	113 ± 6	120 ± 6	129 ± 7	94 ± 6	
0.3 (μm)	91 ± 9	73 ± 11	91 ± 10	89 ± 9	98 ± 9	105 ± 9	115 ± 11	122 ± 8	108 ± 17	76 ± 14	
1.0 (μm)	109 ± 6	93 ± 7	96 ± 7	104 ± 6	111 ± 7	117 ± 7	125 ± 7	131 ± 8	134 ± 7	99 ± 6	
	0.08	*0.02*	*0.04*	*0.06*	*0.08*	*0.10*	*0.12*	*0.14*	*0.16*	*0.18*	*0.08*
Balloon V (ml)	*0.5*	*0.1*	*0.3*	*0.5*	*0.7*	*0.9*	*1.1*	*1.3*	*1.5*	*0.5*	
(b) Rat											
Systolic P (mm Hg)											
Control	77 ± 3	35 ± 3	53 ± 2	68 ± 3	83 ± 3	97 ± 3	109 ± 3	118 ± 3	127 ± 3	135 ± 4	70 ± 3
0.1 (μm)	85 ± 3	33 ± 3	58 ± 3	74 ± 3	91 ± 3	103 ± 3	112 ± 4	124 ± 4	127 ± 6	138 ± 5	80 ± 4
0.3 (μm)	84 ± 4	38 ± 4	60 ± 4	77 ± 4	91 ± 4	104 ± 4	115 ± 3	125 ± 4	134 ± 3	146 ± 4	84 ± 5
1.0 (μm)	90 ± 4*	38 ± 2*	64 ± 3*	84 ± 4*	100 ± 5*	115 ± 6*	126 ± 6*	136 ± 7*	146 ± 7*	153 ± 8*	99 ± 6*
Diastolic P (mm Hg)											
Control	−0.1 ± 0.6	−8.3 ± 0.9	−2.4 ± 0.7	−0.8 ± 0.6	0.4 ± 0.6	1.3 ± 0.6	2.1 ± 0.6	3.4 ± 0.6	4.8 ± 0.5	6.7 ± 0.5	−0.5 ± 0.6
0.1 (μm)	−0.1 ± 0.6	−10.9 ± 1.2	−2.2 ± 0.7	−0.6 ± 0.6	0.8 ± 0.7	1.4 ± 0.7	2.5 ± 0.8	4.1 ± 0.9	3.9 ± 0.7	6.0 ± 1.1	−0.3 ± 0.6
0.3 (μm)	0.1 ± 0.3	−8.7 ± 0.6	−2.3 ± 0.2	−0.5 ± 0.2	0.8 ± 0.3	1.8 ± 0.3	2.4 ± 0.4	3.0 ± 0.3	4.0 ± 0.3	5.4 ± 0.4	−0.1 ± 0.3
1.0 (μm)	0.6 ± 0.3	−11.2 ± 0.6	−2.3 ± 0.3	0.0 ± 0.4	1.2 ± 0.4	2.4 ± 0.5	3.2 ± 0.6	4.3 ± 0.9	6.0 ± 1.0	5.8 ± 0.7	0.6 ± 0.4
Developed P (mm Hg)											
Control	77 ± 3	43 ± 3	55 ± 2	59 ± 3	82 ± 4	96 ± 4	107 ± 4	115 ± 4	122 ± 3	129 ± 4	70 ± 3
0.1 (μm)	85 ± 3	43 ± 2	61 ± 3	74 ± 3	91 ± 3	102 ± 3	110 ± 3	120 ± 4	123 ± 6	132 ± 5	80 ± 4
0.3 (μm)	84 ± 4	47 ± 4	62 ± 4	77 ± 4	90 ± 4	102 ± 4	113 ± 3	122 ± 4	130 ± 3	141 ± 3	84 ± 5
1.0 (μm)	89 ± 4*	50 ± 2*	66 ± 3*	84 ± 4*	99 ± 4*	112 ± 6*	123 ± 6*	132 ± 6*	140 ± 6*	147 ± 8*	99 ± 6*

Abbreviations: Balloon V, volume of the left ventricular balloon; developed P, developed pressure (systolic minus end-diastolic pressure); diastolic P, left ventricular diastolic pressure; systolic P, left ventricular systolic pressure.
*$P<0.05$ vs control.

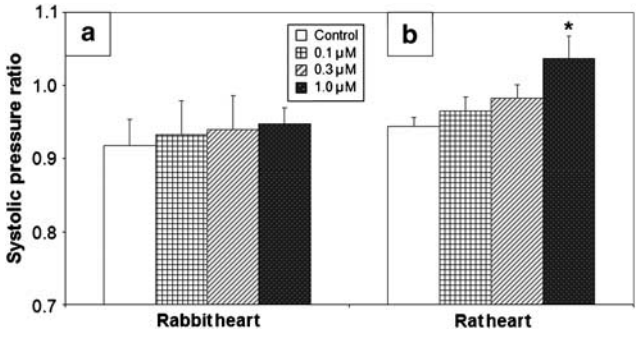

Figure 3 Systolic pressure ratios of the maximum balloon volume of the second (drug-perfused) and the first (drug-free) Starling curves in rabbit heart (**a**) and in the rat heart (**b**). Values are means and vertical lines show s.e.mean. *$P<0.05$ vs control.

in patch clamped rat myocytes that SEA0400-induced increase in the Ca^{2+} transient and cell shortening was accompanied by significant reduction of I_{CaL} (Acsai *et al.*, 2007). These effects can be explained by the autoregulatory nature of cardiac Ca^{2+} handling, as the reduced Ca^{2+} efflux from the cell results in an increased Ca^{2+} load to the sarcoplasmic reticulum leading to increased Ca^{2+} release, which in turn may decrease the I_{CaL} by acceleration of Ca^{2+}-dependent inactivation of I_{CaL} (Acsai *et al.*, 2007). Nagy *et al.* (2004) reported that SEA0400 at a concentration of 1.0 μM failed to change I_{CaL} significantly in canine myocytes. Similarly, our data did not reveal any effect of SEA0400 on the ECG PQ intervals, which would have been widened if I_{CaL} had been blocked by the drug (Farkas *et al.*, 1999). Nevertheless, SEA0400 inhibited Ca^{2+} transients in heart tubes from NCX1 knockout mice, arguing against strict selectivity of the drug for NCX1 (Reuter *et al.*, 2002). In the current study, the lack of any effect of SEA0400 on the coronary flow, the heart rate and the ECG intervals implies that the observed difference in contractility of the rat and rabbit heart caused by NCX inhibition with SEA0400 was not related to any effect of the drug on the coronary flow or the

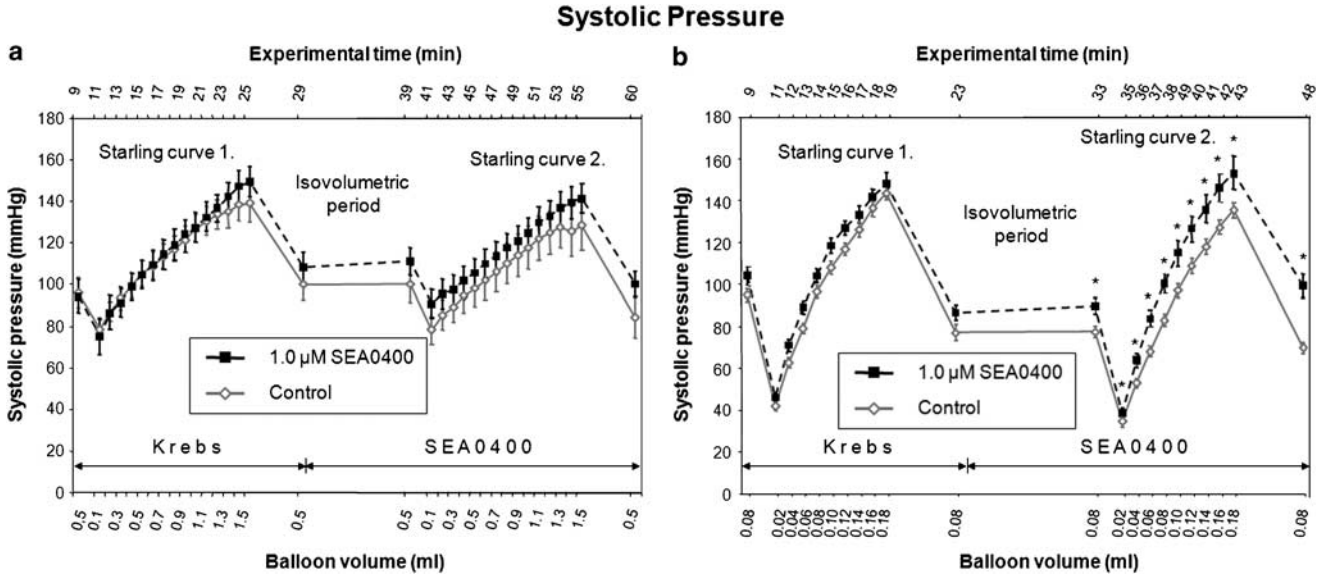

Figure 4 Starling curves, that is, systolic pressures plotted vs balloon volume, in the rabbit heart (**a**) and the rat heart (**b**). In the first half of the experiment, all the hearts were perfused with Krebs buffer (first Starling curve); the perfusion solution was then switched to the test drug in the second half of the experiment (second Starling curve). Between the two Starling curves, an isovolumetric period was applied in the presence of the drug (see Figure 1 for the protocol). Control and 1.0 μM SEA0400 concentration values are presented as means with vertical lines showing s.e.mean. $*P<0.05$ vs control.

sarcolemmal ion channel functions reflected by the ECG intervals.

In a recent study, Tanaka *et al*. (2002) measured slightly lower EC_{50} (IC_{50}) values (40 and 32 nM for the inward and the outward NCX current, respectively) of the SEA0400 for the NCX in guinea pig myocytes as compared with those we measured in rat and rabbit isolated myocytes. Either the different species or the different experimental circumstances may explain this difference between the results of this and earlier studies. In this study, we found that SEA0400 had a slightly but non-significantly greater affinity to NCX in the rat than in the rabbit, although the maximum inhibition (specific activity) was almost equivalent (~70%) in both species. Nevertheless, a significant contractile elevating effect of SEA0400 was only found at a concentration of 1.0 μM when the NCX block was almost maximal in both species. This indicates that the slight difference in the affinity of the drug for the rat or rabbit NCX is not the main reason for the species-dependent contractile force difference in this study.

There are several reports where SEA0400 exerted a greater degree of inhibition for reverse currents than for forward currents in transfected fibroblasts and in mutant *X. laevis* oocytes (Bouchard *et al*., 2004; Iwamoto and Kita, 2004; Lee *et al*., 2004), similar to other NCX benzyloxyphenyl derivative inhibitors, such as KB-R7943 and SN-6 (Iwamoto and Kita, 2004). However, in this study, SEA0400 (0.03–3.0 μM) inhibited both the forward and the reverse modes of the NCX operation, with similar efficacy, either in rabbit or rat isolated myocytes. These results corroborate the previous findings on mouse, guinea pig and canine isolated myocardial cells and neuronal cells (Matsuda *et al*., 2001; Tanaka *et al*., 2002; Birinyi *et al*., 2005; Namekata *et al*., 2005; Wang *et al*., 2007). Accordingly, the potency of SEA0400 for the two modes of NCX varies depending upon experimental conditions. These data suggest that different experimental models reveal different SEA0400 effects on NCX, and in non-manipulated isolated myocytes, SEA0400 similarly inhibits both inward and outward $I_{Na/Ca}$ in these mammalian species.

The effect of NCX on contractility is determined by species-dependent and pathological factors

SEA0400 1.0 μM was found to increase the contractile force, the cell shortening and the Ca^{2+} transient amplitude in an isolated ventricular tissue preparation of the mouse, which possesses a very short action potential duration (Tanaka *et al*., 2005). In a recent study with rat isolated myocytes, the amplitude of the intracellular Ca^{2+} transient and cell shortening was significantly increased by SEA0400 in field-stimulated and voltage-clamped myocytes (Acsai *et al*., 2007). These results are consistent with our whole rat heart contractile force results. In contrast, in the guinea pig myocardium, which exhibits a longer action potential duration, 1.0 μM SEA0400 increased the contractile force by only 5% (Tanaka *et al*., 2007). SEA0400 at a concentration of 1.0 μM significantly decreased the ouabain-induced inotropy when the reverse mode of NCX operation was favoured due to the high intracellular Na^+ (Na_i) level in the guinea pig myocardium (Tanaka *et al*., 2007). SEA0400 1.0 μM did not affect the contractile force or the contractile force decay under normal conditions in guinea pig myocardium, but it significantly enhanced the recovery of the contractile force in ischaemia/reperfusion (Namekata *et al*., 2005). This is supported by our findings in the healthy rabbit heart, which has a longer action potential duration, as 1.0 μM SEA0400 did not elevate the contractile force significantly. In myocardial stunning in anaesthetized dogs, SEA0400 had no direct effect

Table 2 The electrocardiographic PQ, QRS, QT intervals and the heart rate in the presence of SEA0400 in rabbit heart (a) and in rat heart (b)

Balloon V (ml)	*0.5*	*0.1*	*0.3*	*0.5*	*0.7*	*0.9*	*1.1*	*1.3*	*1.5*	*0.5*	
SEA0400											
(a) Rabbit											
PQ (ms)											
Control	61 ± 1	58 ± 2	59 ± 2	60 ± 2	62 ± 3	60 ± 2	59 ± 2	59 ± 3	60 ± 3	57 ± 2	
0.1 (μm)	68 ± 3	67 ± 3	67 ± 4	66 ± 3	68 ± 3	67 ± 4	67 ± 4	67 ± 4	69 ± 4	68 ± 4	
0.3 (μm)	63 ± 3	61 ± 3	60 ± 3	62 ± 3	61 ± 3	62 ± 3	61 ± 3	60 ± 4	55 ± 2	60 ± 3	
1.0 (μm)	64 ± 4	67 ± 5	66 ± 4	67 ± 4	65 ± 4	64 ± 3	61 ± 4	64 ± 3	63 ± 3	64 ± 3	
QRS (ms)											
Control	60 ± 2	60 ± 2	59 ± 2	59 ± 3	60 ± 3	62 ± 3	63 ± 3	64 ± 3	67 ± 3	61 ± 5	
0.1 (μm)	60 ± 3	60 ± 3	59 ± 3	57 ± 3	61 ± 3	62 ± 2	60 ± 3	61 ± 3	62 ± 3	59 ± 4	
0.3 (μm)	67 ± 4	66 ± 4	60 ± 4	64 ± 6	63 ± 5	64 ± 4	67 ± 5	65 ± 4	67 ± 5	66 ± 3	
1.0 (μm)	67 ± 3	66 ± 3	66 ± 4	65 ± 4	65 ± 2	64 ± 2	67 ± 2	66 ± 3	68 ± 3	65 ± 2	
QT (ms)											
Control	201 ± 7	199 ± 6	200 ± 6	201 ± 7	200 ± 7	200 ± 6	198 ± 6	199 ± 8	194 ± 9	205 ± 8	
0.1 (μm)	214 ± 9	213 ± 8	212 ± 8	214 ± 8	210 ± 9	207 ± 8	206 ± 9	207 ± 5	208 ± 8	209 ± 10	
0.3 (μm)	211 ± 9	205 ± 10	206 ± 9	216 ± 15	109 ± 15	204 ± 6	204 ± 7	199 ± 6	211 ± 11	202 ± 9	
1.0 (μm)	209 ± 4	210 ± 4	207 ± 7	206 ± 7	204 ± 7	199 ± 7	199 ± 8	198 ± 8	201 ± 7	207 ± 6	
HR (min^{-1})											
Control	186 ± 8	187 ± 9	187 ± 8	187 ± 8	186 ± 8	187 ± 8	185 ± 8	187 ± 9	188 ± 10	175 ± 8	
0.1 (μm)	169 ± 12	171 ± 11	171 ± 12	169 ± 11	191 ± 12	180 ± 12	179 ± 11	173 ± 12	173 ± 12	173 ± 12	
0.3 (μm)	184 ± 13	186 ± 12	187 ± 12	185 ± 13	186 ± 12	186 ± 12	186 ± 11	183 ± 11	175 ± 13	190 ± 14	
1.0 (μm)	173 ± 7	173 ± 7	174 ± 7	174 ± 7	175 ± 7	1175 ± 7	175 ± 7	175 ± 8	174 ± 8	174 ± 8	
	0.08	*0.02*	*0.04*	*0.06*	*0.08*	*0.10*	*0.12*	*0.14*	*0.16*	*0.18*	*0.08*
Balloon V (ml)	*0.5*	*0.1*	*0.3*	*0.5*	*0.7*	*0.9*	*1.1*	*1.3*	*1.5*	*0.5*	
(b) rat											
PQ (ms)											
Control	46 ± 2	46 ± 2	46 ± 3	45 ± 3	46 ± 3	46 ± 2	43 ± 3	45 ± 3	45 ± 3	48 ± 2	43 ± 3
0.1 (μm)	45 ± 2	45 ± 2	47 ± 2	44 ± 2	44 ± 2	44 ± 2	46 ± 2	45 ± 2	44 ± 2	47 ± 2	43 ± 2
0.3 (μm)	49 ± 2	49 ± 3	45 ± 3	47 ± 3	47 ± 3	48 ± 3	49 ± 3	48 ± 3	48 ± 3	50 ± 3	49 ± 3
1.0 (μm)	47 ± 3	49 ± 3	47 ± 3	48 ± 3	48 ± 3	49 ± 3	48 ± 3	47 ± 2	46 ± 2	47 ± 3	48 ± 3
QT (ms)											
Control	98 ± 3	95 ± 3	97 ± 3	96 ± 3	96 ± 4	96 ± 3	95 ± 4	96 ± 4	95 ± 4	96 ± 4	98 ± 3
0.1 (μm)	99 ± 4	95 ± 5	97 ± 4	96 ± 4	96 ± 4	98 ± 4	99 ± 4	100 ± 4	98 ± 4	100 ± 4	97 ± 3
0.3 (μm)	84 ± 3	88 ± 3	90 ± 2	92 ± 4	89 ± 3	92 ± 4	91 ± 4	92 ± 3	94 ± 4	90 ± 2	88 ± 3
1.0 (μm)	89 ± 4	89 ± 4	92 ± 3	91 ± 4	91 ± 3	91 ± 4	90 ± 2	91 ± 3	92 ± 3	92 ± 4	92 ± 3
HR (min^{-1})											
Control	290 ± 7	294 ± 8	292 ± 8	290 ± 8	289 ± 6	290 ± 7	291 ± 6	288 ± 6	287 ± 7	285 ± 9	287 ± 7
0.1 (μm)	290 ± 10	290 ± 10	290 ± 10	287 ± 10	286 ± 9	285 ± 9	286 ± 9	283 ± 8	274 ± 10	275 ± 13	282 ± 10
0.3 (μm)	287 ± 13	289 ± 12	289 ± 12	288 ± 12	283 ± 12	285 ± 12	285 ± 12	287 ± 12	285 ± 11	283 ± 11	284 ± 12
1.0 (μm)	289 ± 6	289 ± 6	287 ± 6	289 ± 7	288 ± 7	289 ± 7	292 ± 7	285 ± 8	286 ± 8	286 ± 10	291 ± 7

Abbreviations: Balloon V, volume of the left ventricular balloon; HR, heart rate.
QRS interval was not measured in rat hearts, as the QRS and the QT interval is merged in the rat ECG.

on contractility (Takahashi *et al*., 2004). However, 1.0 μM SEA0400 significantly improved the recovery of the post-ischaemic left ventricle pressure in the rabbit heart (Magee *et al*., 2003) and in the rat heart (Takahashi *et al*., 2003). This SEA0400 concentration likewise increased the contraction in rat hearts with a short action potential duration in our study, although under physiological conditions.

The above-mentioned findings emphasize the importance of different species-dependent and pathological factors of NCX functioning. The results imply that, under physiological conditions, NCX inhibition increases the contractility only in species that possess a short action potential duration; under pathological conditions, NCX inhibition may increase the contractile force even in species with a longer action potential duration.

Species-dependent relationship between the NCX function and contractility: possible mechanisms

The direction of operation of the NCX depends on (i) the intracellular and the extracellular Ca^{2+} and Na^{+} concentrations, (ii) the prevailing phase and the shape of the action potential, and (iii) the pH (Blaustein and Lederer, 1999). Physiologically, the intracellular pH does not differ considerably in the rat heart and the rabbit heart. In contrast, the rabbit and the rat differ in some functional properties (for example, action potential and intracellular Na^{+}) as concerns the operation of the NCX (Bers, 2001). The action potential duration of the rat (30–60 ms) is much shorter than that of the rabbit (150–200 ms) (Szigligeti *et al*., 1996). The actual membrane potential (E_m) and the reversal potential of the NCX ($E_{Na/Ca}$) define the direction of operation of the

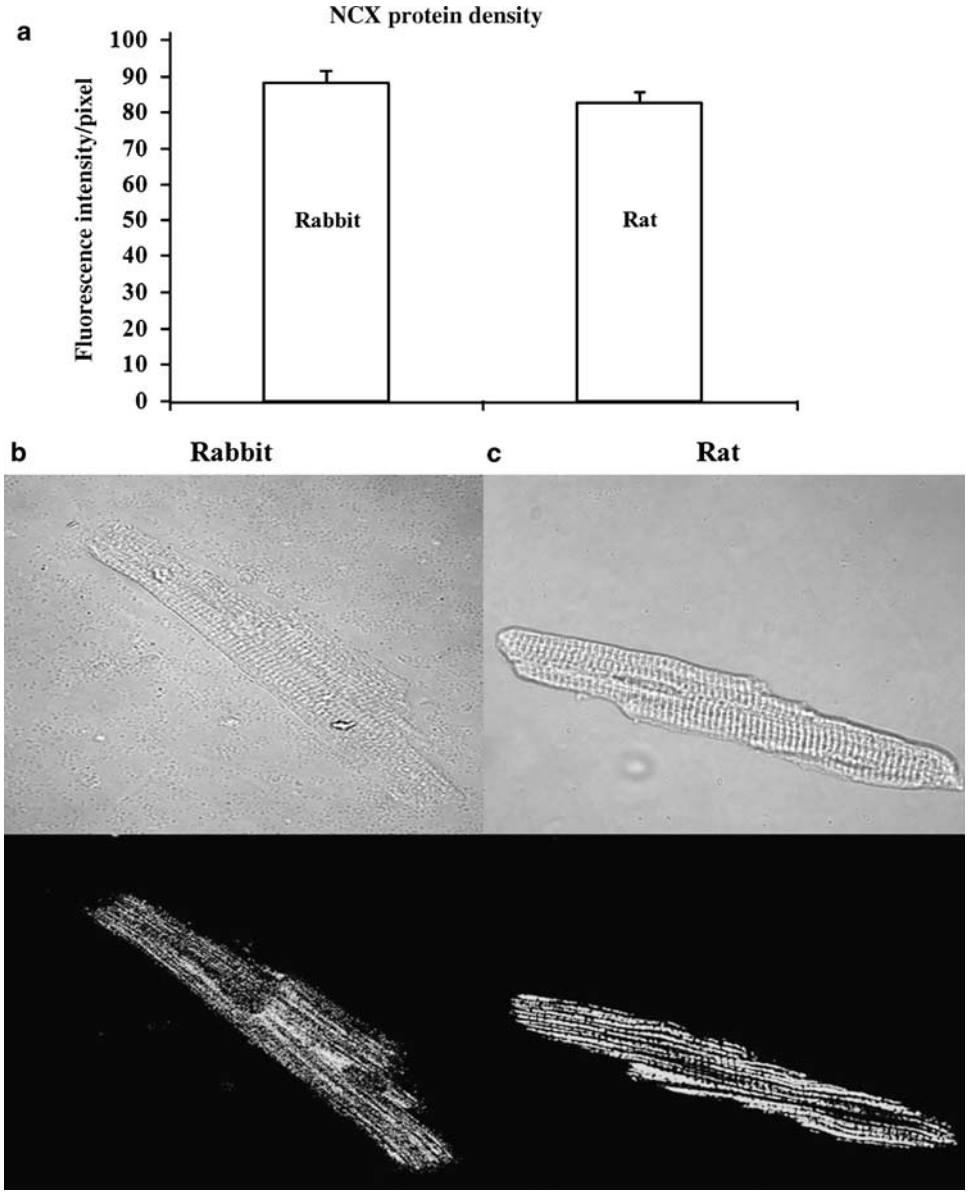

Figure 5 Cardiac sarcolemmal NCX protein density in rabbit and rat myocytes (**a**). Native and NCX immunofluorescent confocal laser scanning microscope images of the rabbit myocytes (**b**) and the rat myocytes (**c**).

NCX: the reversal potential depends on the Na_i which is higher in the rat (12.7–16.0 mM) than in the rabbit (7.2–9.0 mM) (Bers, 2001). During depolarization, E_m is more positive than $E_{Na/Ca}$, which favours the reverse mode (Ca^{2+} influx). When E_m becomes more negative, the NCX operates in the forward mode (Ca^{2+} efflux). Because of the shorter action potential and higher Na_i in rats, the NCX operates mainly in forward mode, even in systole (Bers, 2001), and the functional role of Ca^{2+} extrusion through the forward mode of the NCX is larger in species with a shorter action potential duration (Tanaka *et al*., 2007). The longer action potential duration of the rabbit allows the NCX to operate in the reverse mode for a longer duration as compared with that in the rat. Accordingly, inhibition of the NCX operation in the settings of a short action potential in the mouse or the rat mainly affects the Ca^{2+} efflux (forward mode), which may result in intracellular Ca^{2+} accumulation on a beat-to-beat basis in the cardiac cycle under physiological conditions, thereby leading to an increased contractile force. In contrast, contractility fails to increase significantly in species with the settings of a longer action potential (for example, guinea pig, rabbit and dog). Our rat and rabbit heart contractility results support this hypothesis. Despa and Bers found that NCX blocking Ni^{2+} reduced resting Na^+ influx by almost 40% in rat (Despa *et al*., 2002), which may underline the important role of the NCX in the Ca^{2+} extrusion in rats.

However, there are some experimental data that do not support this explanation. Weber *et al*. observed that during the depolarization phase of the action potential, the reverse mode of the NCX may bring Ca^{2+} into the cell only up to 15 ms in the rabbit (Weber *et al*., 2002). It was found that the NCX had a more important role in Ca^{2+} removal in rabbits than in rats, and the ability of the NCX to extrude Ca^{2+}

(forward mode) from the ventricular myocytes varied in different species according to the sequence hamster > guinea pig > rabbit > ferret > cat > dog > mouse > > rat (Bers, 2001). This suggests that NCX inhibition should cause a greater increase in the contractile force in the rabbit than in the rat. However, compensating Ca^{2+} transport mechanisms may also have role in the NCX inhibition. The sarcolemmal Ca^{2+}-ATPase may adequately compensate for the absent NCX (Choi and Eisner, 1999a, b). Thus, it is possible that the greater effect of SEA0400 on the contractility in the rat reflects the fact that NCX plays a larger role than does the sarcolemmal Ca^{2+}-ATPase in Ca^{2+} extrusion in the rat as compared with the rabbit. However, previous studies do not support this hypothesis. The functional sarcolemmal Ca^{2+}-ATPase was required beside the NCX to maintain Ca^{2+} at the normal low resting level in rat myocytes, and during the inhibition of NCX more than 70% of the Ca^{2+} was removed from the cytoplasm by the sarcolemmal Ca^{2+}-ATPase (Choi and Eisner, 1999b). A previous study on rabbit and ferret myocytes found no effect of the Ca^{2+}-ATPase inhibitor carboxyeosin on resting Ca^{2+} (Bassani *et al*., 1995). Indirect I_{CaL} inhibition due to the NCX inhibition is also possible (Acsai *et al*., 2007), but no signs of I_{CaL} block was detected.

The inhibitory effect of SEA0400 can be affected by the level of Na_i. The inhibitory potency of SEA0400 was found to decrease tenfold in response to a fourfold decrease in Na_i (Lee *et al*., 2004). This indicates that the interspecies difference in the contractility response to the NCX inhibition by SEA0400 was due to the different Na_i levels. However, NCX inhibition were almost identical in the rabbit and rat myocytes when the highest concentration of the SEA0400 was used, but this concentration only increased the contractile force significantly in rats. Thus, clarification of the exact mechanism of the observed interspecies difference in the contractility response to NCX inhibition needs further investigation.

Contractility and NCX protein expression

The NCX protein expression may increase under certain pathological conditions, such as heart failure or myocardial hypertrophy (Quinn *et al*., 2003). The function and protein expression of the NCX decreases during the development of the mouse (Reppel *et al*., 2007). The overexpression of the NCX in genetically manipulated feline myocytes causes a decline in contractility (Weisser-Thomas *et al*., 2005). Similarly, overexpression of the NCX in rabbit ventricular myocytes gave rise to a more pronounced decay of the contraction and a failure to increase the extent of shortening for the increased frequency of stimulation, which was in contrast to the findings obtained in the NCX-overexpressing transgenic mouse (Sipido *et al*., 2002). The overexpression of NCX protein by somatic gene transfer in rat myocytes enhanced both intracellular systolic Ca^{2+} and contraction amplitude at low stimulation rates (0.25 Hz), whereas it reduced cell shortening at higher stimulation frequencies (>2 Hz) (Bölck *et al*., 2004). The NCX protein expression levels have not been compared between different species under physiological conditions. In this study, the NCX protein densities in the rat and the rabbit were compared to ascertain whether the different contractility results were attributable to different levels of NCX protein expression. As the NCX is widely distributed over the cell surface (Kieval *et al*., 1992), immunohistochemistry was performed in isolated myocytes from rat and rabbit hearts. The fluorescence density did not differ between the two species, though this does not exclude the existence of different NCX activities despite the similar protein expressions. A decreased level of phosphorylation of the NCX could lead to a net reduction of NCX activity in spite of an increased expression (Quinn *et al*., 2003). Phosphorylation and the activity of the NCX were not examined in this study. However, the different effects of NCX inhibition on contractility in rat and rabbit hearts cannot be attributed to different levels of NCX expression in these experiments.

Conclusions

These results indicate that the selective inhibition of the NCX with SEA0400 increases the contractility in a concentration-dependent manner in the rat isolated heart with a short action potential duration and a higher intracellular Na^+ concentration, but it does not exert an appreciable influence on the contractile force in the rabbit isolated heart with a longer action potential duration and a lower intracellular Na^+ concentration. These data reveal important functional interspecies differences in contractility as a result of NCX inhibition. However, the role of the NCX in contractility and modulation of the NCX function necessitate further investigations to reveal the exact mechanism of the observed interspecies difference in the contractility response to NCX inhibition in rat and rabbit isolated hearts.

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Conflict of interest

The authors state no conflict of interest.

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