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Block of Na^+/Ca^{2+} exchanger by SEA0400 in human right atrial preparations from patients in sinus rhythm and in atrial fibrillation

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ABSTRACT

The Na^+/Ca^{2+} exchanger (NCX) plays a major role in myocardial Ca^{2+} homoeostasis, but is also considered to contribute to the electrical instability and contractile dysfunction in chronic atrial fibrillation (AF). Here we have investigated the effects of the selective NCX blocker SEA0400 in human right atrial cardiomyocytes from patients in sinus rhythm (SR) and AF in order to obtain electrophysiological evidence for putative antiarrhythmic activity of this new class of drugs. Action potentials were measured in right atrial trabeculae using conventional microelectrodes. Human myocytes were enzymatically isolated. Rat atrial and ventricular cardiomyocytes were used for comparison. Using perforated-patch, NCX was measured as Ni^{2+}-sensitive current during ramp pulses. In ruptured-patch experiments, NCX current was activated by changing the extracellular Ca^{2+} concentration from 0 to 1 mM in Na^+-free bath solution (100 mM Na^+ intracellular, "Hilgemann protocol"). Although SEA0400 was effective in rat cardiomyocytes, 10 μM did not influence action potentials and contractility, neither in SR nor AF. SEA0400 (10 μM) also failed to affect human atrial NCX current measured with perforated patch. With the "Hilgemann protocol" SEA0400 concentration-dependently suppressed human atrial NCX current, and its amplitude was larger in AF than in SR cardiomyocytes. Our results confirm higher NCX activity in AF than SR. SEA0400 fails to block Ni^{2+}-sensitive current in human atrial cells unless unphysiological conditions are used. We speculate that block of NCX with SEA0400 depends on intracellular Na^+ concentration.

1. Introduction

The Na^+/Ca^{2+} exchanger (NCX) is the major Ca^{2+} extruding process in mammalian myocardium and therefore plays a dominant role in cardiac Ca^{2+} homoeostasis (Bers, 2002; Sipido, 2000). Because of its electrogenic nature it contributes to transmembrane potential. Negative to the reversal potential, the transmembrane concentration gradient for Na^+ is driving Ca^{2+} out of the cell ("forward mode") resulting in net inward (depolarising) current. When operating in the "reverse" mode, NCX allows Ca^{2+} to enter the cell, producing net outward (repolarizing) current. The antiporter's activity thus modulates both electrical activity and intracellular Ca^{2+} concentration $[Ca^{2+}]_i$ [for review see (Antoons et al., 2012)].

Increased activity of NCX is proarrhythmic, irrespective of upregulation by genetic manipulation (Pott et al., 2012) or due to pathophysiological alterations in hypertrophy and heart failure (Antoons et al., 2012; Hasenfuss et al., 1996; Pogwizd et al., 2001; Sipido, 2000; Bers and Despa, 2006). Remodelling processes associated with chronic atrial fibrillation include upregulation of NCX at mRNA and protein level (El Armouche et al., 2006; Schotten et al., 2002; Voigt et al., 2012). The associated NCX activity increase contributes to the observed electrical instability and contractile dysfunction (Dobrev and Wehrens, 2010). Abnormal Ca^{2+} signalling in AF is recognised as a major pathophysiological cause of triggered activity and maintenance of AF (Dobrev and Nattel, 2008; Lugenbiel et al., 2015; Wakili et al., 2011) with increasing evidence for enhanced diastolic Ca^{2+} leak from

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sarcoplasmic reticular Ca^{2+} stores (Neef et al., 2010; Vest et al., 2005; Voigt et al., 2012), although there is also evidence not supporting such view (Greiser et al., 2014). The upregulated NCX exchanges diastolic leak Ca^{2+} for Na^{+} giving rise to depolarising transient inward currents and increasing the susceptibility to cellular delayed afterdepolarizations and triggered activity (Voigt et al., 2012).

Compounds that selectively block NCX have fuelled great expectations as antiarrhythmic agents by stabilizing the membrane potential and ameliorating contractile dysfunction (Iwamoto et al., 2007; Pogwizd, 2003). The NCX blocker SEA0400 possesses good selectivity for NCX over ion channels especially L-type Ca^{2+} channels (Birinyi et al., 2005; Hobai et al., 1997; Matsuda et al., 2001; Tanaka et al., 2002), however at high concentrations of >3 μM this may no longer be the case (Birinyi et al., 2005). SEA0400 inhibits NCX both in its forward and reverse mode in expression systems and cardiomyocytes of different species (Tanaka et al., 2002) and possesses antiarrhythmic activity in various animal models of arrhythmia (Nagasawa et al., 2005; Nagy et al., 2004, 2014; Szepesi et al., 2015). However, no information is available for SEA0400 in human atria.

In order to evaluate SEA0400 as a putative antiarrhythmic agent for atrial fibrillation (AF), we have investigated its effects in atrial biopsies obtained from patients in sinus rhythm, chronic AF and paroxysmal AF. Cardiomyocytes from atria and ventricles of rat hearts were used for comparative purposes. Although NCX current amplitudes were higher in samples from AF than from SR patients, SEA0400 inhibited human atrial NCX current only under conditions of high intracellular Na^{+}.

2. Material and methods

2.1. Tissue samples and cell isolation

Biopsies from right atrial appendages were obtained from adult patients undergoing open-heart surgery. The study was approved by the ethics committee of the Medical Faculty of Technical University Dresden, Germany (No. EK790799). Each patient gave written, informed consent.

All experiments with rat cardiomyocytes were performed in accordance with the local authorities (permission number 24D-9168.24-1/2007-17 of the Regierungspräsidium Dresden) and comply with the European Commission Directive 86/609/EEC regarding the protection and welfare of animals used for experimental as well as scientific purposes. The rats were killed by decapitation under light CO_2 anaesthesia (70% CO_2, 30% O_2). The hearts were excised and mounted on a Langendorff perfusion column by tying the aorta to the outflow cannula. The remaining blood in the hearts was washed out with normal, oxygenated Tyrode solution at 37 °C (5 min). The solution was then changed to nominally Ca^{2+}-free solution for 10 min before the solution was supplemented with enzymes containing collagenase (Type I, Worthington, 233 units/mg; 0.54 mg/ml) and 1% BSA (Sigma). After 10–15 min, the hearts were removed from the Langendorff apparatus. The atria and ventricles were separated, minced into small chunks and further processed like human cardiomyocytes until single cardiomyocytes with clear cross striations appeared in the suspension.

Cardiomyocytes were isolated from right atrial biopsies as described previously (Dobrev et al., 2000). In brief, the tissue was cut into small pieces in Ca^{2+}-free isolation buffer of the following composition (in mM): NaCl 100, KCl 10, KH_2PO_4 1.2, $MgSO_4$ 5, MOPS 5 mM, mM, glucose 20, taurine 50 (pH $=7.0$), and washed 3 times for 3 min. During the complete isolation procedure, the solutions were oxygenated with 100% O_2 at 35 °C. For enzymatic dissociation of the tissue pieces, 246 U/ml collagenase type I (Worthington Biochemical Corp., NJ, USA) and 0.5 mg/ml protease type XXIV (Sigma-Aldrich Co., St Louis, USA) were added to the nominally Ca^{2+}- free buffer. After gentle stirring for 10 min, 0.2 mM Ca^{2+} was added, and the tissue was stirred for another 35 min. Solution was changed and the digestion was continued with 246 U/ml collagenase type I in the presence of 0.2 mM Ca^{2+}. Stirring of the solution containing the tissue fragments was stopped when single rod-shaped, striated myocytes could be detected in test droplets under the microscope. The suspension was centrifuged, myocytes were resuspended and stored until use in Tyrode's solution (supplemented with 0.5 mM Ca^{2+} in three steps) at room temperature.

2.2. Perforated patch-clamp experiments

Perforated patch-clamp experiments were performed on single myocytes within 1–5 h after cell isolation. A gigaohm seal was established using borosilicate glass pipettes (R=2–3 MΩ) in K^{+} free Tyrode's solution [composition in mM/l: Na-methanesulfonic acid 140, $MgCl_2$ 1, $BaCl_2$ 0.2, $CaCl_2$ 1, glucose 10, HEPES 10, (pH adjusted to 7.4 with NaOH)] at 37 °C. The bath was superfused with Tyrode's solution supplemented with 20 μM ouabain, 1 μM nisoldipine and 50 μM lidocaine in order to block Na^{+}/K^{+} pump currents, as well as L-type Ca^{2+} and Na^{+} currents. Amphotericin B (SIGMA-Aldrich Co.) was solved in 98% ethanol and sonicated for 25 min at room temperature (c=40 mg/ml). Stock solution was stored at 4 °C for a maximum of 1 week. 240 μg/ml amphotericin B was added to the pipette solution before the start of the experiments. Pipette solution [composition in mM/l: CsOH 150, NaOH 10, HEPES 10, $MgCl_2$ 1, $CaCl_2$ 10 μM, aspartic acid 75 mM (pH=7.2)] was sonicated for 10 min and was kept on ice. Intracellular Ca^{2+} concentration was calculated to be 165 nM in this pipette solution by WEBMAXCLITE v1.15 software (Chris Patton, Stanford University, CA, USA). Fresh pipette solution was prepared in every 2 h.

I_{NCX} was recorded with the protocol reported by (Hobai et al., 1997). Ramp pulses (100 mV/s) were delivered at a rate of 0.05 Hz. The membrane was depolarised from the holding potential of −40 mV to +60 mV, then hyperpolarized to −100 mV, finally the membrane potential returned to the holding potential. Outward and inward I_{NCX} were determined during the descending limb of the ramp. After the control record was taken, the cell was superfused with the given concentration of SEA0400, finally 10 mM $NiCl_2$ was added in order to fully block I_{NCX}. Total I_{NCX} was determined as a Ni^{2+}-sensitive current.

Extracellular application of agents was performed through a DAD Superfusion System (ALA Scientific Instruments, New York, USA). Standard voltage clamp experiments were conducted with a single electrode patch clamp system using an Axopatch 200B amplifier (MDS Analytical Technologies, Toronto, Canada), a TC-344B dual automatic temperature controller and data storage devices. Current signals were low-pass filtered at 1–3 kHz by the amplifier, digitised, saved and analysed with the patch-clamp software ISO-2 (MFK-Computer, Niedernhausen, Germany).

2.3. Whole-cell patch-clamp experiments (reverse mode NCX activation)

Whole-cell configuration of the patch-clamp method was established in superfusion buffer of the following composition (in mM): LiCl 135, CsCl 10, NaH_2PO_4 0.33, $MgCl_2$ 1, $BaCl_2$ 0.2, TEA-Cl 10, glucose 10, HEPES 10, EGTA 0.5 (pH=7.4). Lidocaine (50 μM), nisoldipine (1 μM) and ouabain (20 μM) were added to block Na^{+} channels, L-type Ca^{2+} channels and the (Na_{+} + K^{+})-ATPase, respectively. The pipette solution contained (in mM): NaOH 100, CsOH 20, $CaCl_2$ 13, $MgCl_2$ 1.1, TEA-Cl 20, EGTA 50, MgATP 4, HEPES

50 (pH=7.0). The intracellular Ca^{2+} concentration was calculated to be 105 nM (WEBMAXCLITE v1.15 software).

I_{NCX} was recorded at a holding potential of −40 mV. Outward I_{NCX} was activated by applying 1 mM Ca^{2+} extracellularly for 20 s. The bath was perfused again with Ca^{2+}-free solution for 3 min before the second current activation. Myocytes were treated for 3 min with different concentrations of SEA0400 before exposed once more to 1 mM Ca^{2+} under control conditions, in the presence of SEA0400, or 10 mM Ni^{2+}.

2.4. Contractility measurements

Right atrial trabeculae were mounted in organ baths filled with 50 ml of Tyrode solution. The preparations were paced at a frequency of 1 Hz, with 5-ms electrical pulses 10% above threshold intensity and were pre-stretched to 50% of maximum developed force. All preparations were exposed for 90 min to the non-specific, irreversible α-adrenoreceptor blocker phenoxybenzamine (PBA, 6 μM) in order to minimise stimulation of α-adrenoceptors via catecholamines released from endogenous sympathetic nerve endings. PBA was washed by removal of old and addition of fresh bath solution. After an equilibration period of 30 min, the effects of SEA0400 or of an equivalent amount of DMSO (0.1%) as time-matched control were measured on contractile force, recordings of which were saved on a personal computer and analysed off-line by Chart (Version 5.5, AD Instruments Pty Ltd., Castle Hill, NSW, Australia) software. Data was plotted by Prism (Version 4.00, GraphPad Software, Inc., San Diego, USA) software.

2.5. Conventional microelectrode technique

Human right atrial trabeculae muscles were mounted in an organ bath perfused with oxygenated Tyrode's solution. The preparations were stimulated electrically via silver/silver chloride electrodes at a regular frequency of 1 Hz. The muscles were allowed to equilibrate for at least 90 min before intracellular action potentials were recorded with borosilicate glass pipettes, filled with 2.5 M KCl solution (tip resistances were 20–30 MΩ). Drugs were added to the superfusing solution from concentrated stock solutions to yield the final concentration. Action potentials from stable preparations were recorded 20 min after drug addition by HSE-APES software (Department of Pharmacology, Albert Szent-Gyorgyi Medical University, Szeged, Hungary). Action potentials were stored on a personal computer, and the main parameters were analysed off-line, data was plotted by Prism (Version 4.00, GraphPad Software, Inc., San Diego, USA) software.

2.6. Drugs

SEA0400 (2-[4-[(2,5-difluorophenyl)methoxy]phenoxy]−5-ethoxy-aniline) was synthesised by Ferenc Fülöp (Institute of Pharmaceutical Chemistry, University of Szeged, Hungary). The product was isolated as hydrochloride salt. A stock solution of SEA0400 (10 mM) was prepared in 100% DMSO, aliquots of which were stored at –20 °C. All other chemicals and drugs were obtained from Sigma Chemicals.

2.7. Statistical analysis

Statistical significance between groups was analysed by paired or unpaired Student's *t*-test, where appropriate. Data is expressed as mean ± S.E.M., numbers of experiments (n) are given as cells/patients.

3. Results

3.1. Effects of SEA0400 on action potentials and force of contraction

In a first set of experiments the effects of SEA0400 on action potentials and force of contraction were examined in human right atrial trabeculae stimulated at 1 Hz (Fig. 1). Under control conditions, action potentials from patients in SR and AF had the typical "spike-and-dome" and triangular appearance, respectively (Dobrev and Ravens, 2003). However, increasing concentrations between 0.1 and 10 μM of SEA0400 did not produce any significant changes, neither in comparison with pre-drug controls nor with a group of preparations treated with solvent only that served as time-matched controls. In addition, 10 μM of SEA0400 or 0.1% of DMSO had no effect on force of contraction. Since lack of efficacy in superfused multicellular preparations could be due to impaired drug diffusion within the tissue, we tested the efficacy of SEA0400 on NCX current measured in isolated cardiomyocytes.

3.2. Ramp protocol for activation of NCX

At experimental conditions that strongly reduce interfering currents conducted via Na^+, K^+ and Ca^{2+} channels as well as Na^+ pump current, human atrial cardiomyocytes exhibited robust inward and outward currents during the ramp protocol (Fig. 2), irrespective of whether the cells were derived from patients in SR or AF (Fig. 2A and C). SEA0400 (3 μM) had no effect, whereas addition of nickel (10 mM) partially reduced current amplitude. Fig. 2B and D show average SEA0400- and Ni^{2+}-sensitive currents in pA/pF of cells from SR and AF patients. The absolute NCX current densities at membrane potentials relevant for the action potential, i.e. at −60 mV and −40 mV were -0.82 ± 0.15 pA/pF, and -0.51 ± 0.10 pA/pF, respectively, in AF and -0.40 ± 0.12 pA/pF and -0.27 ± 0.08 pA/pF, respectively, in SR. Although current density tended to be larger in AF than in SR, the differences failed to reach the level of statistical significance. SEA0400 had no effect on NCX current measured with this technique, although a robust SEA0400-sensitive current was detected in rat ventricular myocytes (see Supplementary Fig. 1).

3.3. NCX activity using the "Hilgemann protocol"

The group of Hilgemann introduced a method for activating NCX, by rapidly changing the ionic compositions of the extracellular bath solution [Fig. 3A; (Hilgemann et al., 1992a, 1992b)]. With the membrane potential clamped to −10 mV (ruptured patch), a large, reversible outward current was recorded when rapidly changing $[Ca^{2+}]_e$ from 0 to 1 mM and back to 0, i.e. by activating NCX in its reverse mode ("Hilgemann protocol"). Such currents could be repeatedly activated also in human atrial cardiomyocytes and were almost completely suppressed by 10 mM Ni^{2+} (Fig. 3B). The inactivation of NCX current during the 20-s period of superfusion with 1 mM Ca^{2+} was not analysed any further. Average peak reverse mode NCX current density was larger in AF than in SR cells, did not change significantly in TMC, but was highly significantly suppressed not only by 10 mM Ni^{2+} but also by 300 nM SEA0400 (Fig. 3C and D). The inhibitory effect of SEA0400 on NCX current measured with the "Hilgemann protocol" was concentration-dependent and complete (Fig. 4A to C). No differences in -logEC_{50} values for SEA0400 were detected between cardiomyocytes from SR and AF patients, i.e. 6.77 and 6.86, respectively. In rat ventricular cardiomyocytes, 10 μM SEA0400 also completely suppressed NCX current (Fig. 4C).

Peak current density measured with the Hilgemann protocol reveal ~40% higher values in atrial cardiomyocytes from patients in AF than in from patients in SR (Fig. 4D). We also had the chance

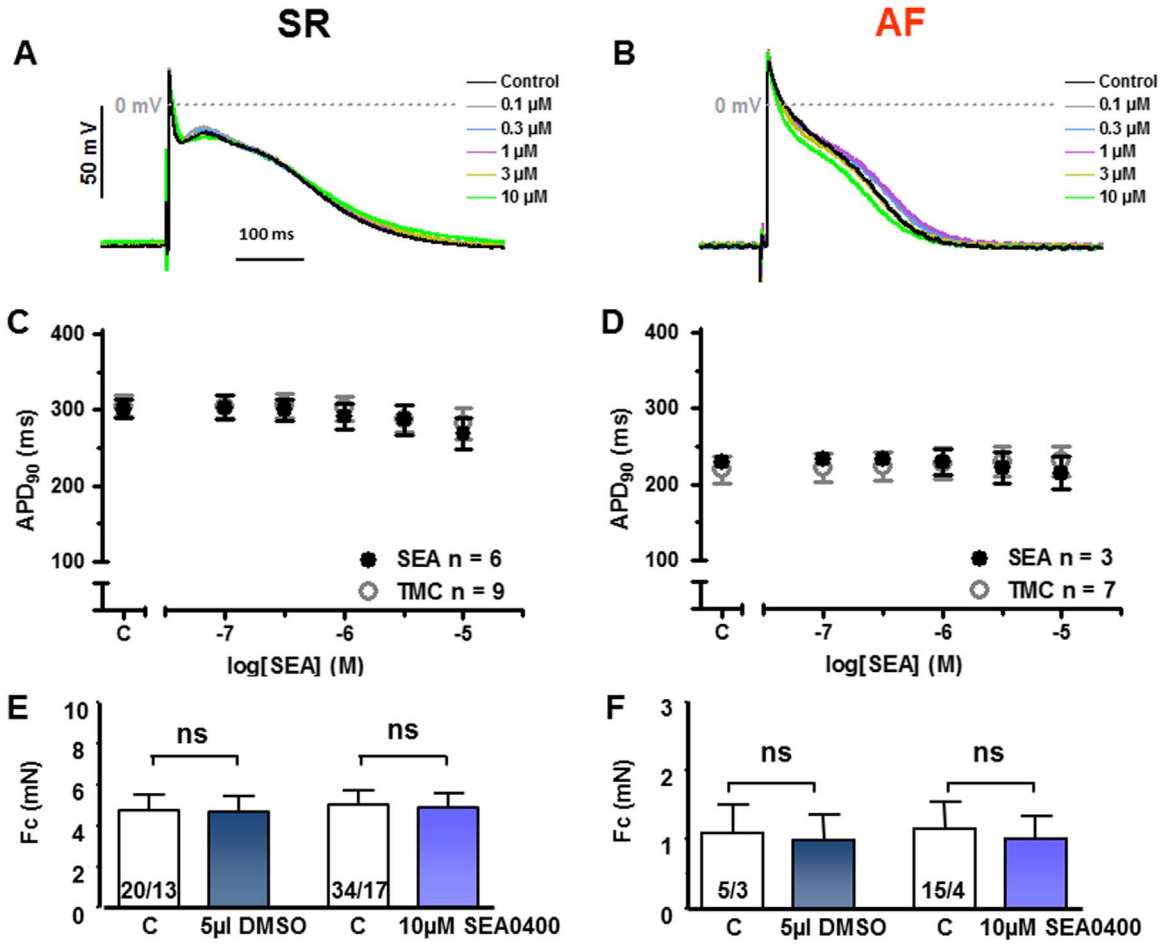

Fig. 1. Effects of SEA0400 on action potentials and force of contraction in human right atrial trabeculae from patients in sinus rhythm (SR) and atrial fibrillation (AF). A and B: action potential tracings at pre-drug control and after 20 min of superfusion with cumulatively increasing concentrations of SEA0400 (0.1–10 µM). C and D: Comparison of concentration-response curves for the effects of SEA0400 on action potential duration at 90% of repolarization (APD90) with time-matched controls (TMCs). E and F: Force of contraction in mN (Fc) before and 30 min after addition of solvent (DMSO 0.1%) or SEA0400 (10 µM). Mean values ± S.E.M. from n trabeculae (action potentials) or number of trabeculae/number of patients (force).

to study some cardiomyocytes from a few patients in paroxysmal AF, in which NCX current density was comparable to the values of SR patients, however, we were not successful investigating SEA0400 effects in paroxysmal AF. Rat atrial cardiomyocytes showed significantly larger amplitudes of NCX current density than rat ventricular cells.

4. Discussion

The major findings of our present study were that (i) I_{NCX} measured with 2 independent protocols is of larger amplitude in cells from AF than from SR patients; (ii) SEA0400 did not modulate action potentials of atrial trabeculae from patients, neither in SR nor with AF; (iii) NCX current was detected as Ni^{2+}-sensitive current in patch-clamped atrial myocytes during ramp pulses, but SEA0400 had no effect neither on forward nor reverse mode NCX; (iv) SEA0400 was able to suppress NCX current activated by reverse mode NCX activity at −10 mV under unphysiological intra- and extracellular Na^+ and Ca^{2+} concentrations.

4.1. Lack of effect of SEA0400 on action potentials of human atrial trabeculae

Since the antiporter is controlled by membrane potential and the concentration gradients for Ca^{2+} and Na^+, the time course of NCX current during an action potential is complicated, and simulation models of the exchanger help to predict it (Blaustein and Lederer, 1999; Hilgemann, 2004; Pogwizd et al., 2003). When considering only potential-dependence, NCX current is expected to affect the cardiac action potential in a different way (Fig. 5A) than when considering only changes in cardiac concentration gradient (Fig. 5B), depending on the membrane potential. In its reverse mode during the early plateau phase, net outward NCX current will shorten action potential duration, whereas in the forward mode during late repolarisation, inward NCX current will delay final repolarization. For NCX blockers, the opposite changes are anticipated with early prolongation and late shortening. To our surprise we did not observe any effects at all on atrial action potentials even with SEA0400 concentrations as high as 10 µM, which completely blocked NCX current in expression systems. It should be noted, however, that at such high concentrations SEA0400 may no longer be selective for inhibition of NCX but also block L-type Ca^{2+} channels (Birinyi et al., 2005), which is expected to shorten the action potential. At the time of our study, the more selective NCX blockers were not available to us (Jost et al., 2013).

Amongst the many possible causes for this lack of effect we could exclude chemical inactivation of the compound because action potentials were shortened in rat myocardium (unpublished result). Another reason could have been that SEA0400 cannot penetrate multicellular tissue because of diffusion barriers.

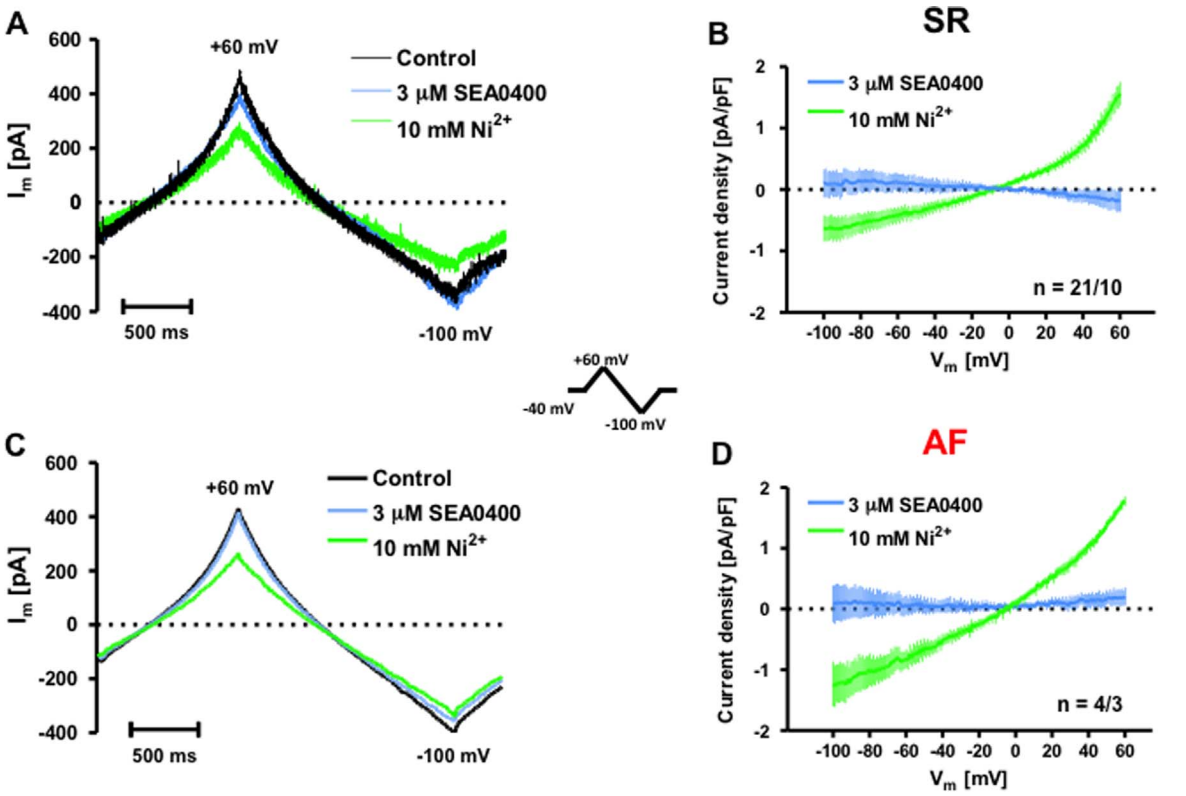

Fig. 2. NCX currents in human right atrial cardiomyocytes from patients in sinus rhythm (SR) and atrial fibrillation (AF). A and C: Current traces elicited by a 3-s double ramp pulse from a holding potential of −40 mV to +60 mV, to −100 mV and back to −40 mV (see inset) at pre-drug control (black traces), and after 2 min of superfusion of an SR (A) and an AF (C) cardiomyocyte in the presence of SEA0400 (3 μM, blue traces) and after further 2 min of superfusion with Ni^{2+} (10 mM, green traces). B and D: Mean values ± S.E.M of SEA0400- (blue) and Ni^{2+}-sensitive (green) current density expressed in pA/pF in 21 cardiomyocytes from 10 SR and 4 cardiomyocytes from 3 AF-patients.

Although most drugs require higher concentrations in multicellular tissue than in single cardiomyocytes SEA0400 was reported to be active in ventricular tissue of various species (Nagy et al., 2014). A chamber specific effect, i.e. atria versus ventricle, could also be excluded because SEA0400 shortened action potentials both in atrial and ventricular strips of rat hearts. Last not least, we considered that SEA0400 might be ineffective because the NCX contributes very little current to the human atrial action potential, despite of several reports suggesting a pathophysiological role of NCX in triggering and maintaining atrial fibrillation (Schotten et al., 2011; Voigt et al., 2012). Therefore NCX currents were measured directly in human atrial cardiomyocytes.

4.2. Lack of SEA0400 effect in NCX recorded with a ramp protocol

To the best of our knowledge, I_{NCX} currents have not previously been reported for native human atrial cardiomyocytes neither from patients in AF nor in SR. This may not hold true for human ventricular cardiomyocytes. Voigt et al. (2012) reported differences between AF and SR cells by comparing the decay rate of transient outward current after caffeine-induced Ca^{2+} release from the sarcoplasmic reticulum, and did not report any drug effects. The standard ramp protocol for measuring NCX current (Hobai et al., 1997) yielded Ni^{2+}-sensitive current in human atrial myocytes, indicating that a robust NCX current is also present in human atria. Moreover, current density was larger in cells from patients in AF than in SR. Yet again, SEA0400 did not affect Ni^{2+}-sensitive current neither in myocytes from SR nor AF patients. This was again a surprising finding, since SEA0400 inhibited NCX current in rat atrial and ventricular myocytes also in our hands [Supplementary Fig. 1].

4.3. Block of NCX current by SEA0400 in human atrial myocytes in its reverse mode ("Hilgemann protocol")

In our final set of experiments with high $[Na^+]_i$ and zero $[Na^+]_e$ and zero $[Ca^{2+}]_e$ we measured large amplitude, Ni^{2+}-sensitive outward currents upon switching to 1 mM $[Ca^{2+}]_e$, which slowly inactivated partially during superfusion with 1 mM $[Ca^{2+}]_e$. The time course of the current was not considered any further, and only peak current amplitude was analysed. In analogy to the work by Hilgemann and coworkers we define peak current as "reverse mode" NCX current amplitude (Hilgemann et al., 1992a, 1992b). Our results indicate larger NCX current amplitude in AF than SR myocytes, which is consistent with the increased expression of NCX in remodelled atria both at mRNA and protein level (El Armouche et al., 2006; Schotten et al., 2002; Voigt et al., 2012). Interestingly, myocytes from patients in paroxysmal AF had NCX amplitudes similar to SR supporting the general notion that little electrical remodelling takes place in myocytes from these patients (Loose et al., 2014). Reverse-mode NCX currents activated with the "Hilgemann protocol" were inhibited by SEA0400 in a concentration-dependent manner indicating that SEA0400 can in fact block NCX current also in human atrial myocytes, albeit under special conditions. Only when the reverse mode was enforced by reversing the Na^+ concentration gradient, but not by membrane potential (see Fig. 5), could we detect efficacy of SEA0400, suggesting that high $[Na^+]_i$ is required for efficacy. We therefore speculate that the NCX-blocking effect of SEA0400 strongly depends on the $[Na^+]_i$, which is substantially higher in rat [~17 mM, for references, see (Lewalle et al., 2014)] than in human myocardium [~8 mM (Hoey et al., 1994)]. This idea is supported by a recent report on the potency of SEA0400 block of NCX1.1 expressed in Xenopus oocytes. The concentration-response curve for

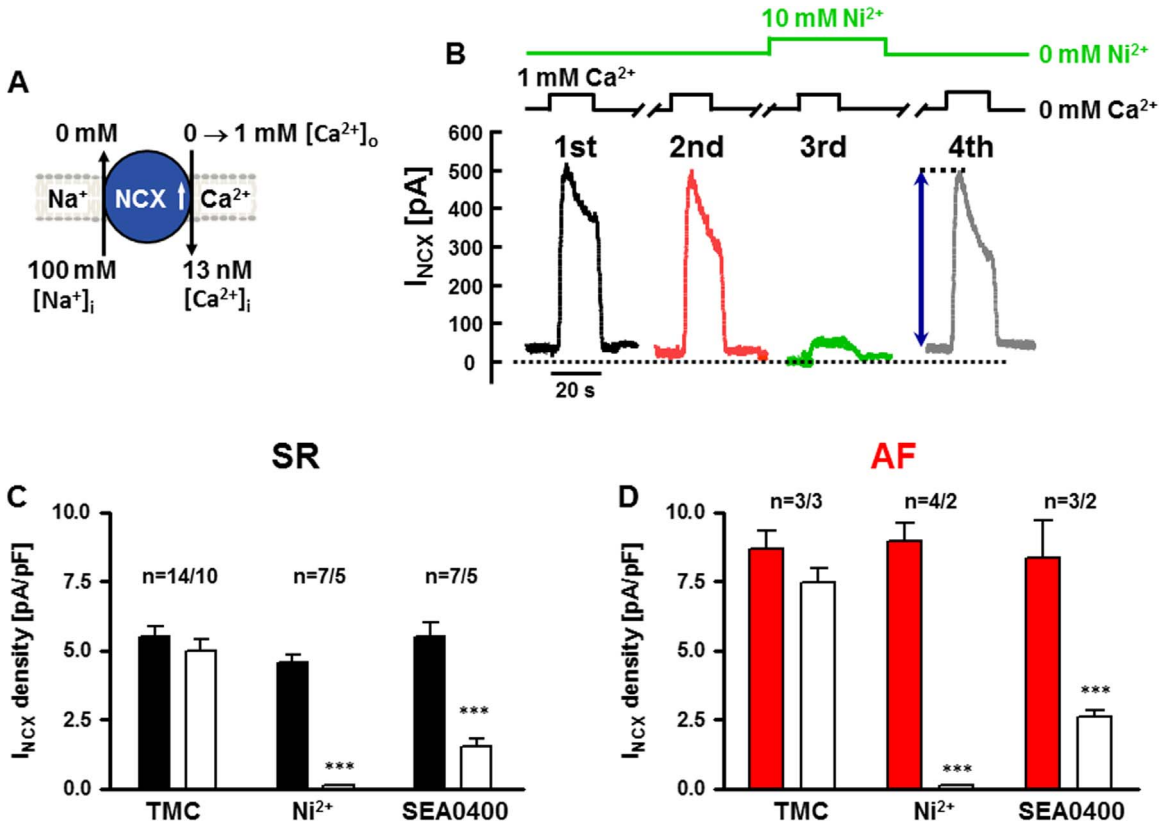

Fig. 3. Effects of Ni^{2+} and SEA0400 on NCX currents measured with the "Hilgemann protocol". A: Scheme of Na^+ and Ca^{2+} concentrations in the extracellular superfusion and intracellular (pipette) solutions. B: NCX current was activated by changing the extracellular Ca^{2+} from 0 to 1 mM Ca^{2+} for 20 s. The third change of extracellular Ca^{2+} was carried out in the presence of 10 mM Ni^{2+}. NCX amplitude was measured as peak current (arrow). C and D: NCX current density in time-matched controls (TMCs), in the presence of Ni^{2+} (10 mM) or SEA0400 (10 μM) in cardiomyocytes from SR or AF patients. Mean values ± S.E.M. from number of cells/number of patients as indicated.

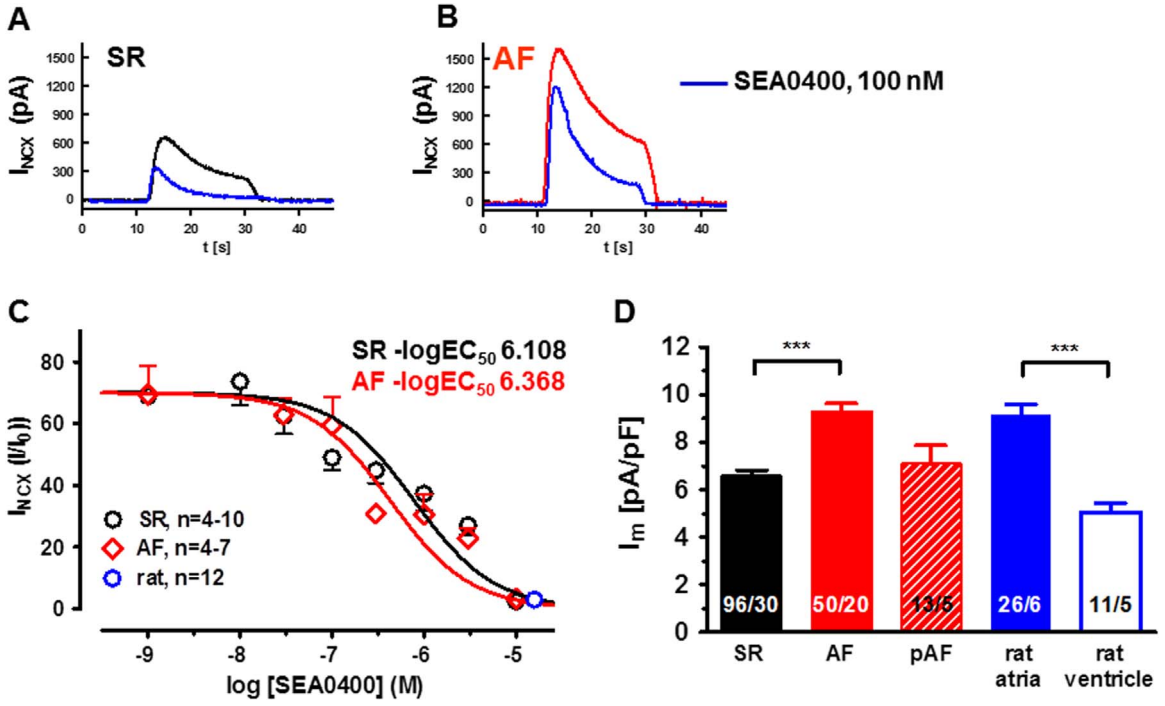

Fig. 4. Concentration-dependent block of NCX by SEA0400. A and B: Outward current during a 20 s of stimulation of NCX in backward mode ("Hilgemann protocol", see Fig. 3) under control conditions (black, red) and in the presence of SEA0400 (100 nM, blue) in a SR (A) and an AF cardiomyocyte (B). C: Concentration-dependent effect of SEA0400 on NCX current in SR and AF cells, and effect of 10 μM SEA0400 on NCX in rat atrial cardiomyocytes (note that the latter point was shifted by 0.2 log units to the right on the graph for better visibility). D: Amplitudes of NCX measured as Ni^{2+}-sensitive current with the "Hilgemann protocol" in human atrial cardiomyocytes from patients in SR, AF and paroxysmal AF (pAF), and in atrial and ventricular cardiomyocytes from rat hearts. Mean values ± S.E.M. from number of cells /number of patients or animals as indicated by the numbers at the base of the columns. *** $P < 0.001$, Student's *t*-test between columns.

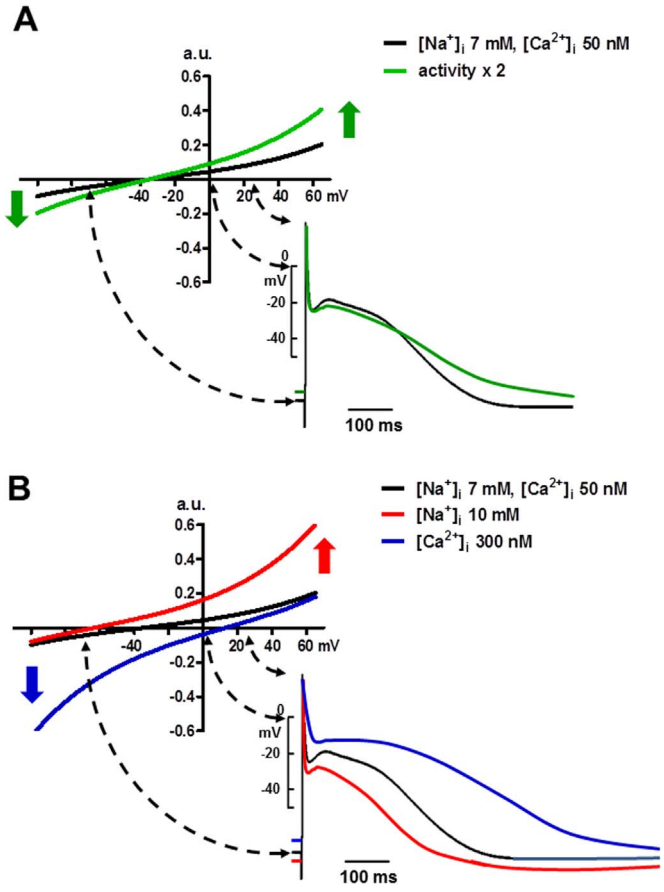

Fig. 5. Scheme of changes in NCX activity (i.e. current amplitude) by enhanced expression (A) or by changes in ion concentrations (B) and their expected influence on the shape of human atrial action potentials. The I/V curves (arbitrary current units) were calculated according to the formula given by (Sipido et al., 2006). The lay-out was adapted from (Ravens and Wettwer, 1989). The coloured downward and upward arrows indicate increases in inward current (forward mode) or outward current (reverse mode) according to the depicted changes in NCX activity.

SEA0400 was shifted by half a log unit to higher sensitivity upon $[Na^+]_i$ increase from 25 mM to 100 mM (Lee et al., 2004). Thus, although NCX current amplitudes were higher in samples from AF than from SR patients, SEA0400 inhibited human atrial NCX current only under conditions of high intracellular Na^+.

In our view, modulators of NCX activity are extremely interesting as potential antiarrhythmic drugs. However, unless block of NCX can be demonstrated directly in human cardiomyocytes under physiological conditions, any antiarrhythmic efficacy has most likely some other mechanism than NCX block. Hence, extrapolation from animal models requires great caution.

In conclusion, we have shown that human atrial cardiomyocytes exhibit larger NCX current amplitudes in AF than SR. Although SEA0400 blocks Ni^{2+}-sensitive current in rat cardiomyocytes, it fails to do so in human atrial cardiomyocytes unless the intracellular internal Na^+ concentration is very high. We speculate that block of NCX with SEA0400 depends on intracellular Na^+ concentration. Further experiments in human atrial tissue are required to investigate the efficacy of NCX blockers under physiological conditions in order to estimate their antiarrhythmic potential in atrial fibrillation.

Authors contributions

The study was conceived by E. W., A. V. and U. R., and was designed by T. C., E. W. and U. R.; T. C. performed all organ bath experiments; P. K., T. C. and E. W. performed the patch clamp experiments; M. K. obtained informed consent from all patients and provided atrial biopsies and clinical data; P. K., E. W., and U. R. compiled the data and made all draft figures; N. J., K. A. and U. R. wrote a draft manuscript which was revised to its final form and approved by all authors. All authors also agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ejphar.2016.06.050.

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