

# SAR340835, a Novel Selective $\text{Na}^+/\text{Ca}^{2+}$ Exchanger Inhibitor, Improves Cardiac Function and Restores Sympathovagal Balance in Heart Failure<sup>[S]</sup>

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## ABSTRACT

In failing hearts,  $\text{Na}^+/\text{Ca}^{2+}$  exchanger (NCX) overactivity contributes to  $\text{Ca}^{2+}$  depletion, leading to contractile dysfunction. Inhibition of NCX is expected to normalize  $\text{Ca}^{2+}$  mishandling, to limit afterdepolarization-related arrhythmias, and to improve cardiac function in heart failure (HF). SAR340835/SAR296968 is a selective NCX inhibitor for all NCX isoforms across species, including human, with no effect on the native voltage-dependent calcium and sodium currents in vitro. Additionally, it showed in vitro and in vivo antiarrhythmic properties in several models of early and delayed afterdepolarization-related arrhythmias. Its effect on cardiac function was studied under intravenous infusion at 250, 750 or 1500  $\mu\text{g}/\text{kg}$  per hour in dogs, which were either normal or submitted to chronic ventricular pacing at 240 bpm (HF dogs). HF dogs were infused with the reference inotrope dobutamine (10  $\mu\text{g}/\text{kg}$  per minute, i.v.). In normal dogs, NCX inhibitor increased cardiac contractility ( $\text{dP}/\text{dt}_{\text{max}}$ ) and stroke volume (SV) and tended to reduce heart rate (HR). In HF dogs, NCX inhibitor significantly and dose-dependently increased SV from the first dose (+28.5%, +48.8%, and +62% at 250, 750, and 1500  $\mu\text{g}/\text{kg}$  per hour, respectively) while significantly increasing  $\text{dP}/\text{dt}_{\text{max}}$  only at 1500 (+33%). Furthermore,

NCX inhibitor significantly restored sympathovagal balance and spontaneous baroreflex sensitivity (BRS) from the first dose and reduced HR at the highest dose. In HF dogs, dobutamine significantly increased  $\text{dP}/\text{dt}_{\text{max}}$  and SV (+68.8%) but did not change HR, sympathovagal balance, or BRS. Overall, SAR340835, a selective potent NCX inhibitor, displayed a unique therapeutic profile, combining antiarrhythmic properties, capacity to restore systolic function, sympathovagal balance, and BRS in HF dogs. NCX inhibitors may offer new therapeutic options for acute HF treatment.

## SIGNIFICANCE STATEMENT

HF is facing growing health and economic burden. Moreover, patients hospitalized for acute heart failure are at high risk of decompensation recurrence, and no current acute decompensated HF therapy definitively improved outcomes. A new potent,  $\text{Na}^+/\text{Ca}^{2+}$  exchanger inhibitor SAR340835 with antiarrhythmic properties improved systolic function of failing hearts without creating hypotension, while reducing heart rate and restoring sympathovagal balance. SAR340835 may offer a unique and attractive pharmacological profile for patients with acute heart failure as compared with current inotrope, such as dobutamine.

## Introduction

A variety of treatments are used to improve depressed left ventricular function in heart failure (HF) during periods of acute decompensation.  $\beta$ -Adrenergic agonists, like dobutamine, are currently the gold standard for inotropic agents in acute decompensated HF (AHF); however, their clinical use

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**ABBREVIATIONS:** AHF, acute decompensated HF; AVB, atrioventricular block; BRS, baroreflex sensitivity; CO, cardiac output; DAD, delayed afterdepolarization; DBP, diastolic blood pressure;  $\text{dP}/\text{dt}_{\text{max}}$ , maximal rate of rise of left ventricular pressure;  $\text{dP}/\text{dt}_{\text{min}}$ , minimal rate of rise of (usually) left ventricular pressure; DT, deceleration time; E/A, the ratio of peak velocity blood flow from left ventricular relaxation in early diastole (the E wave) to peak velocity flow in late diastole caused by atrial contraction (the A wave); EAD, early afterdepolarization; E-C, excitation-contraction; HF, heart failure; HFpEF, HF with preserved ejection fraction; HR, heart rate; LAV, left atria vulnerability; LF, low frequency; LV, left ventricle; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; MAD, median absolute deviation; MVO<sub>2</sub>, Myocardial oxygen consumption; NCX,  $\text{Na}^+/\text{Ca}^{2+}$  exchanger; nu, normalized unit; PR, progesterone; SBP, systolic blood pressure; SV, stroke volume; TdP, Torsades de Pointe.

is limited by major drawbacks. Firstly,  $\beta$ -adrenergic receptor desensitization requires continuous augmentation of the dose to maintain inotropic efficacy. Secondly, stimulation of adrenergic receptors induces tachycardia and increases myocardial oxygen consumption out of proportion to their positive inotropic action, potentially reducing cardiac efficiency at midterm. Finally, they exert deleterious effects on membrane electrical stability, favoring the occurrence of arrhythmias and cardiovascular death (Van Bilsen et al., 2017).

It is well known that hemodynamic alterations accompanying HF are associated with abnormal regulation of intracellular  $\text{Ca}^{2+}$ , leading to electrophysiological and excitation-contraction (E-C) alterations at the cellular level. Reduction of the amplitude of intracellular  $\text{Ca}^{2+}$  transient and of its rate of decay have been reported in isolated myocytes from failing human and dog hearts (O'Rourke et al., 1999; Menick et al., 2007; Bögeholz et al., 2017). The cardiac plasma membrane  $\text{Na}^+/\text{Ca}^{2+}$  exchanger (NCX) is the main  $\text{Ca}^{2+}$  extrusion mechanism of the cardiac myocyte and is crucial for maintaining  $\text{Ca}^{2+}$  homeostasis. NCX is a key player of cardiac E-C coupling that regulates cytosolic  $\text{Ca}^{2+}$  and  $\text{Na}^+$  concentration, repolarization process, and contractility (Wei et al., 2007; Ottolia et al., 2013). Moreover, NCX expression and activity are consistently upregulated in both patients with HF (Sipido et al., 2002; Pott et al., 2011) and animal models of HF (O'Rourke et al., 1999; Goldhaber and Philipson, 2013; (Bögeholz et al., 2017)). This overactivity, which is associated with a reduced efficacy of sarcoplasmic reticulum  $\text{Ca}^{2+}$ -ATPase to pump cytosolic  $\text{Ca}^{2+}$  back into the sarcoplasmic reticulum during diastole, favors excessive  $\text{Ca}^{2+}$  extrusion from the cytosol, which leads to a reduction in sarcoplasmic reticulum  $\text{Ca}^{2+}$  content and thereby contributes to cardiac contractility impairment.

In addition, increased NCX current favors generation of early afterdepolarization (EAD)-related arrhythmias and delayed afterdepolarization (DAD)-related arrhythmias. Enhanced expression of NCX is recognized as one of the molecular mechanisms that increases the risk of arrhythmias during the development of HF with reduced ejection fraction (Hobai et al., 2004; Peana and Domeier, 2017). Therefore, NCX blockers have been proposed as positive inotropes and antiarrhythmic agents in the treatment of HF. The therapeutic objective in patients with HF is therefore to limit NCX overactivity to maintain adequate sarcoplasmic reticulum  $\text{Ca}^{2+}$  refilling, to improve cardiac dysfunction, and additionally to reduce the risk of arrhythmias through restoration of proper calcium-induced calcium release. Indeed, partial inhibition of NCX increases intracellular  $\text{Ca}^{2+}$  available for sarcoplasmic reticulum  $\text{Ca}^{2+}$ -ATPase and improves systolic and diastolic function in both normal and failing canine cardiomyocytes (O'Rourke et al., 1999; Hobai et al., 2004) and prevents the occurrence of EAD- and DAD-related arrhythmias, which are commonly observed in patients with HF (Kohajda et al., 2016). Only one NCX inhibitor, caldaret, has been clinically developed that aims at normalizing disturbed calcium handling in patients with either myocardial infarction or HF. Although caldaret was shown to be safe in patients, the compound was stopped due to its limited efficacy on myocardial infarction size or LV dysfunction (Bär et al., 2006). However, the limited literature about caldaret did not allow a conclusion as to whether its potency and specificity to

inhibit NCX were appropriate to increase cardiac function in these patients. Recently, highly specific and potent NCX inhibitors were reported (Primessnig et al., 2019; Otsomaa et al., 2020). They clearly improved cardiac function in both the normal and HF condition in rat and rabbit and showed antiarrhythmic properties (Kohajda et al., 2016).

SAR340835 is a water-soluble prodrug of SAR296968, a potent, selective, short-acting NCX inhibitor (Czechizky et al., 2013). At 30 minutes after intravenous injection, SAR340835 is totally transformed into SAR296968, its active moiety, which is responsible for its NCX inhibitory activity.

The purpose of this study was to evaluate the antiarrhythmic, hemodynamic, and cardiac effects of SAR340835 in conscious normal dogs and in dogs with rapid right ventricular pacing-induced heart failure. The antiarrhythmic properties were investigated in experimental models using mammalian species with electrophysiological properties close to human (guinea pig, rabbit, pig). To examine E-C coupling in more detail, ex vivo contractility studies in response to SAR296968 were also performed on normal and failing canine cardiomyocytes. SAR340835 profiling was performed in comparison with dobutamine, which was used as a calibrator in the rapid right ventricular pacing-induced HF dogs.

## Materials and Methods

The purpose of this study was to evaluate the therapeutic potential for AHF of SAR340835, a new potent NCX inhibitor, by successively profiling its in vitro potency and specificity, evaluating its antiarrhythmic properties, and determining the cardiodynamic profile in normal and HF dogs. As we were hypothesizing that NCX inhibition could be beneficial in patients with acute heart failure, the canine heart failure studies were performed versus dobutamine, a well known therapeutic reference for AHF. Electrophysiological investigations were performed in mammalian species with electrophysiological features close to humans, i.e., showing a marked plateau of action potential (guinea pig, rabbit, dog, pig) and in which NCX is known to behave as in human heart (Bers et al., 2006).

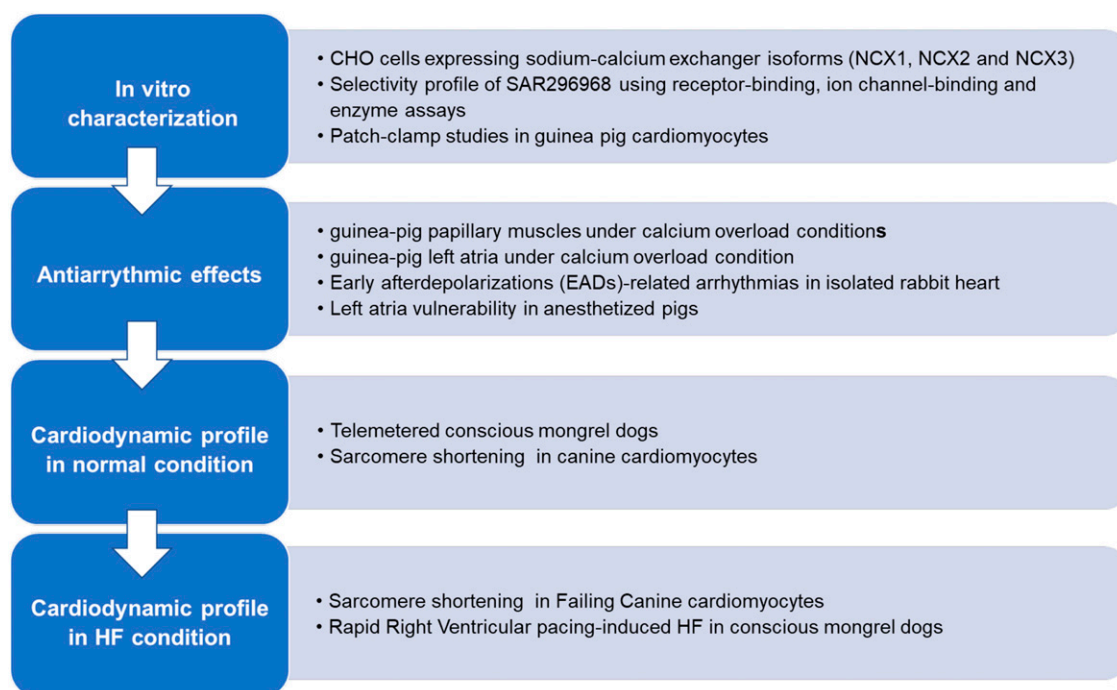
The successive steps of our methodological approach to fully characterize SAR340835, the prodrug of SAR296968, are illustrated by Fig. 1. A brief summary of methods is provided here. The full experimental conditions of those experiments are detailed in Supplemental Material.

### Ethical Approvals

All the procedures described in the present study were performed in agreement with the European regulation (2010/63/UE) and under the approval and control of SANOFI's ethics committee. All procedures were performed in Association for Assessment and Accreditation of Laboratory Animal Care International-accredited facilities in full compliance with the standards for the care and use of laboratory animals and in accordance either with the French Ministry for Research or with the German animal protection law.

### In Vitro Characterization of SAR296968, the Active Principle of SAR340835

An extended method for these in vitro studies is available in Supplemental Material. Briefly, in vitro potency on the NCX isoforms was assessed by a cell-based calcium mobilization assay on CHO cell lines expressing either NCX1, NCX2, or NCX3 with a fluorescent imaging plate reader and using the  $\text{Ca}^{2+}$ -sensitive dye Fluo4-AM for measurement of the intracellular  $\text{Ca}^{2+}$  concentration. The specificity of action toward NCX was investigated in guinea pig cardiomyocytes by performing patch-clamp studies to evaluate the activity of SAR296968 on the endogenous NCX, calcium, and sodium currents.



**Fig. 1.** Brief summary of our methodological approach to fully characterize SAR340835, the prodrug of SAR296968.

Moreover, an extended profiling of SAR296968 was carried out using receptor binding, ion channel binding, and enzyme assays (Supplemental Table 6).

### Effects of SAR296968 on Atrial and Ventricular Arrhythmias

**Confirmatory Studies.** The antiarrhythmic properties of the NCX inhibitor were assessed in a battery of in vitro and in vivo models using the active principle SAR296968. NCX inhibition was extensively described in literature to reduce the occurrence of early and delayed afterdepolarization. We performed confirmatory studies with our compound in guinea pig papillary muscles and left atria under calcium overload condition to determine its ability to reduce DAD-related arrhythmias. Furthermore, the efficacy of SAR296968 against EADs was tested in isolated rabbit heart perfused according to the Langendorff method. The extended methods for those experiments are described in Supplemental Material.

**Left Atria Vulnerability in Anesthetized Pigs.** The antiarrhythmic property of SAR296968 was further investigated by measuring the left atria vulnerability (LAV) in pentobarbital-anesthetized pigs. The purpose of this investigation was to determine the effect of NCX inhibition on atrial refractoriness and electrically induced atrial arrhythmias. Pigs were premedicated with 2 ml Rompun 2%, i.m. (xylazine HCl, 23.3 mg/ml) and 1 ml of Zoletil 100 (100 mg/ml; 50 mg/ml tiletamine and 50 mg/ml zolazepam) and anesthetized with an intravenous bolus of 3 ml Narcoren (pentobarbital, 160 mg/ml), followed by a continuous intravenous infusion of 12–17 mg/kg per hour pentobarbital. Pigs were ventilated with room air and oxygen by a respirator. Blood gas analysis (partial pressure of oxygen or  $pO_2$ , partial pressure of carbon dioxide or  $pCO_2$ ) was performed at regular time intervals to control the oxygen supply via the respirator to maintain  $pO_2 > 100$  mm Hg and  $pCO_2 < 35$ –40 mm Hg. A left thoracotomy was performed at the fifth intercostal space, the lung was retracted, the pericardium was incised, and the heart was suspended in a pericardial cradle. The atrial effective refractory period, determined by the S1-S2 method, and cardiac contractility ( $dp/dt_{max}$ ) were monitored at baseline and under treatment with SAR296968 (1.5 mg/kg over 20 minutes) dissolved in a mixture of DMSO (1 ml) and

PEG400 (9 ml). LAV was determined as described previously (Wirth et al., 2007). Briefly, the S1-S2 stimulation procedure induced short self-terminating episodes of atrial tachycardia (fibrillation or flutter). The number of atrial repetitive action potentials after the premature beat S2 had to exceed 4 for a full score (1). Three or four repetitive action potentials were counted as a half score (0.5). The procedure was applied while increasing the coupling S1-S2 interval by 5 milliseconds and was repeated at three basic cycle lengths (150, 200, and 250 bpm). A total of 45 S1-S2 stimulation procedures were repeated before and after infusion of SAR296968 in eight pigs. The same procedure was performed on a separate control group of seven pigs according to the same protocol with infusion of the vehicle.

### Effect of SAR340835 on Cardiac Hemodynamics in Normal and HF Dogs

**Animal and Surgical Procedure.** In total, 12 adult mongrel dogs (body weight 27–31 kg) were implanted with telemetry devices (L21-F2; Datasciences International). Six of them were additionally equipped with a pacemaker (Adapta model; Medtronic, MI) with bipolar epicardial Pacing Lead (CapSure Epi; Medtronic) for induction of heart failure by tachypacing: 240 beats per minute for 4 weeks.

For drug infusion, dogs were implanted under anesthesia with a vascular access port.

**Rapid Right Ventricular Pacing-Induced HF.** Heart failure was induced by chronic rapid right ventricle pacing at 240 beats per minute for 4 weeks with the programmable pacemaker. Baseline echocardiogram and hemodynamic recordings were performed before and at the end of the 4-week pacing period to assess the development of heart failure.

**Study Design in Normal and Pacing-Induced HF Dogs.** The same study design was applied to normal and HF dogs. All the experiments were performed in conscious animals 4 weeks after the induction of HF by rapid pacing. Dogs were trained daily to remain quiet during the hemodynamic and echocardiography procedures before and after surgery. Before each echocardiography and telemetry monitoring session, pacing was turned off and maintained off during the whole recording period.

Each animal was subjected to four treatment sessions over the following 2 weeks with vehicle or SAR340835 infused at 250, 750, or 1500  $\mu\text{g/kg}$  per hour. Pacing-induced heart failure dogs received dobutamine infused at 10  $\mu\text{g/kg}$  per minute during an additional session for comparison purposes. SAR340835 was intravenously administered with a loading dose over 2 minutes (0.29, 0.86, or 1.73  $\text{mg/kg}$  for 250, 750, and 1500  $\mu\text{g/kg}$  per hour, respectively), followed by an intravenous infusion maintained for 3 hours in HF dogs and 6 hours in normal dogs (250, 750, or 1500  $\mu\text{g/kg}$  per hour, respectively). For simplification, doses are designated by the maintenance infusion rate in the tables and figures. A minimum washout period of 2 days in accordance with the short half-life of compounds was allowed between two sessions.

During each session, after a 15-minute stabilization period, telemetry signals were continuously recorded throughout the treatment infusion. Echocardiography was performed before starting the treatment infusion and over the last minutes of the 3- or 6-hour treatment infusion.

**Echocardiography Measurements.** Cardiac function was assessed by echocardiography using a Philips CX 50 (Philips, Amsterdam, The Netherlands) with a 5-MHz phased-array transducer. Additional methodological details are provided online in Supplemental Methods.

**Telemetry Recordings and Analysis.** Telemetry signals (LVP, ECG, aortic blood pressure) were continuously recorded throughout the experiment starting 15 minutes before and until the end of vehicle or treatment infusion at a sampling rate of 500 Hz. Measurements were averaged over at least 20-second periods using HEM software (Notocord System, Croissy, France). Several derived parameters were calculated: diastolic (DBP) and systolic (SBP) aortic blood pressure, left ventricular end-diastolic pressure (LVEDP),  $dP/dt_{\text{max}}$ , and  $dP/dt_{\text{min}}$ .

Myocardial oxygen consumption (MVO2 in milliliters of  $\text{O}_2/\text{min}$  per 100 g) was calculated using the following equation developed by Rooke and Feigl (1982):

$$\text{MVO2} = 0.000408 \cdot (\text{SBP} \cdot \text{HR}) + 0.000325 \cdot [(0.8 \cdot \text{SBP} + 0.2 \cdot \text{DBP}) \cdot \text{HR} \cdot \text{SV}] / \text{BW} + 1.43.$$

**Evaluation of Autonomic Tone and Baroreflex.** The effects of SAR340835 on the autonomic nervous system (ANS) were explored. Spectral analysis of heart rate variability was performed for the evaluation of autonomic tone in all telemetered dogs before and at the end of the 3 hours of dosing. This spectral analysis using a fast-Fourier transform algorithm on sequences of 512 points (5 minutes) was performed with the HEM CsA10 software (Notocord Systems, Croissy, France).

Specific frequency bands of heart rate variability permitted the simultaneous assessment of sympathetic [Low Frequency] and parasympathetic [High Frequency] modulation, with the Low Frequency/High Frequency ratio illustrating the sympathovagal balance. Spectral powers were determined as the area under the curve calculated for the very low frequency (0.04–0.05 Hz), low frequency (0.05–0.15 Hz), and high frequency (0.15–0.5 Hz) bands. The results are expressed in normalized units (nu) for spectral indices calculated as follows:

$$\begin{aligned} \text{Low Frequency (nu, \%)} &= (\text{Low Frequency} / (\text{Low Frequency} + \text{High Frequency})) \cdot 100; \\ \text{High Frequency (nu, \%)} &= (\text{High Frequency} / (\text{Low Frequency} + \text{High Frequency})) \cdot 100. \end{aligned}$$

To investigate the ability of heart rate changes to counteract arterial blood pressure variations, spontaneous baroreflex efficiency was evaluated using the sequence method (Verwaerde et al., 1999; Gronda et al., 2014). Additional methodological details are provided online in Supplemental Methods.

**ECG Analysis.** The ECG signals of all animals were examined for any test article-related abnormality in wave form morphology. The progesterone (PR) interval was evaluated on each dosing day, at least at each selected time point, over a 60-second period. Examination of second-degree AVB was performed on the totality of the 24-hour recording of ECG.

## Dog Cardiomyocyte Studies

Detailed methods of dog cardiomyocyte studies are provided in the Supplemental Material.

## Statistical Analysis

Detailed statistical analysis is described in Supplemental Methods.

## Results

### In Vitro Profile of SAR296968

**NCX Inhibition Potency of SAR296968.** In CHO cell lines expressing sodium-calcium exchanger isoforms, SAR296968 potentially inhibited the human NCX1, with an  $\text{IC}_{50}$  of 74 nM (Fig. 2). Inhibition of human NCX2 and NCX3 occurred within similar ranges. Testing of SAR296968 on NCX1 orthologs from dog, guinea pig, pig, rabbit, and rat also showed the same range of inhibitory potency (Fig. 2).

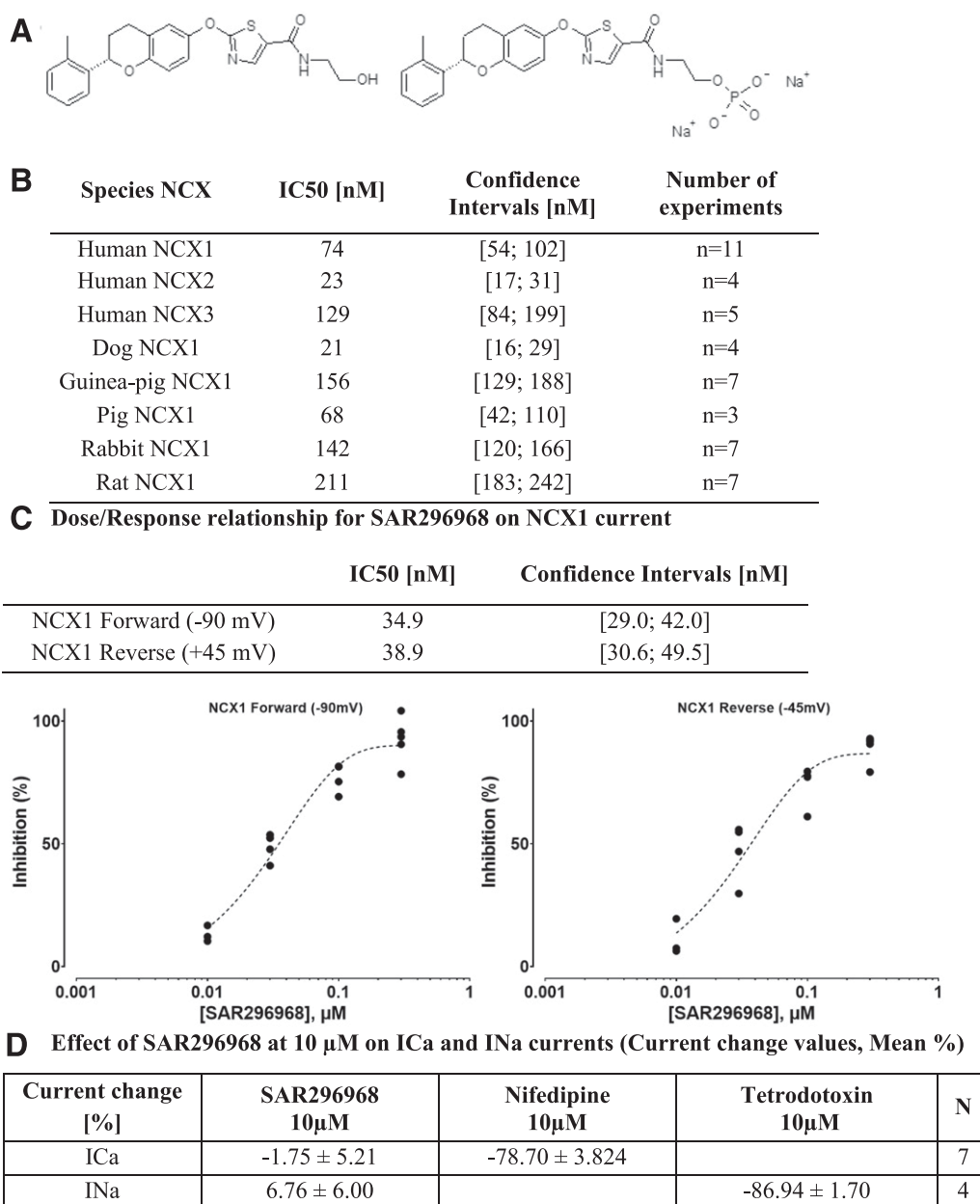
In voltage-clamp studies in guinea pig cardiomyocytes, SAR296968 inhibited both the forward and reverse mode of the NCX current in a concentration-dependent manner with similarly high potency. For the forward mode (recorded at the potential of  $-90$  mV), an  $\text{IC}_{50}$  value with 95% confidence interval of 34.9 nM (29.0; 42.0) was yielded. For the reverse mode (recorded at the potential of  $+45$  mV), the curve fit yielded an  $\text{IC}_{50}$  value with 95% confidence interval of 38.9 nM (30.6; 49.5) (Fig. 2).

**Selectivity of SAR296968.** At a concentration more than 100-fold higher than the  $\text{IC}_{50}$  on NCX, SAR296968 had no or only a minimal effect on the native voltage-dependent calcium and sodium currents in guinea pig cardiomyocytes (Fig. 2).

The target profiling showed that SAR296968 (10  $\mu\text{M}$ ) had only a weak antagonist effect on 5-Hydroxytryptamine receptor 2B ( $\text{IC}_{50} = 4$   $\mu\text{M}$ ) and benzodiazepine peripheral receptor ( $\text{IC}_{50} = 6$   $\mu\text{M}$ ) and weak inhibition of norepinephrine uptake ( $\text{IC}_{50} > 30$   $\mu\text{M}$ ) and dopamine uptake ( $\text{IC}_{50} = 17$   $\mu\text{M}$ ). No functional effects on PR ( $\text{IC}_{50}$  of 1.4  $\mu\text{M}$  in binding assay) were reported. There are no functional assays available for androgen receptor ( $\text{IC}_{50}$  of 0.18  $\mu\text{M}$  in binding assay), but potential effects should be limited because of the short duration of the anticipated treatment in patients (48 hours). The binding profile of SAR340835 is comparable to the one of SAR296968.

### Antiarrhythmic Properties of SAR296968

SAR296968 was effective to reduce DAD-related arrhythmias in both models at doses that increased cardiac contractility. Thus, in guinea pig papillary muscles, a positive inotropic effect was observed at all tested SAR296968 concentrations ( $dP/dt_{\text{max}}$  changes versus vehicle group: +29% and +47% at 1 and 3  $\mu\text{M}$  SAR296968, respectively; Supplemental Table 2). In parallel, the number of arrhythmic contractions triggered by high calcium/low potassium concentrations was strongly reduced from  $20.5 \pm 0.0$  (median  $\pm$  MAD,  $n = 12$ ) in the vehicle control group to  $0.0 \pm 0.0$  at 1  $\mu\text{M}$  (median  $\pm$  MAD,



**Fig. 2.** SAR296968 in vitro characterization. (A) Chemical structure of SAR296968 (active principle, left) and SAR340835 (prodrug, right). (B) Inhibition potency of SAR296968 on NCX isoforms in CHO cells. Effect of SAR296968 on the native NCX1 current (C) and on calcium current (I<sub>Ca</sub>) and sodium current (I<sub>Na</sub>) (D).

$n = 8$ ,  $P = 0.0054$  vs. vehicle) and to  $0.0 \pm 0.0$  at  $3 \mu\text{M}$  (median  $\pm$  MAD,  $n = 8$ ,  $P = 0.0054$  vs. vehicle; Table 1). Similarly, guinea pig left atria treated with  $3 \mu\text{M}$  SAR296968 responded with a 1.28-fold increase in  $dP/dt_{\text{max}}$  from baseline versus a 0.84-fold change recorded under vehicle treatment, and a 90% reduction of the spontaneous arrhythmic contractions induced by isoprenaline [ $1.5 \pm 1.5$  spontaneous arrhythmic contractions after  $3 \mu\text{M}$  SAR296968 (median  $\pm$  MAD,  $n = 10$ ) vs.  $15.5 \pm 4.5$  in the separate vehicle control group (median  $\pm$  MAD,  $n = 10$ ) (Table 1)].

In isolated rabbit hearts, sotalol-induced TdP occurred in the six hearts tested when the pacing rate and the  $\text{K}^+$  concentration were decreased. Their occurrence was limited by SAR296968 pretreatment at  $0.3 \mu\text{M}$  (TdP in two of six hearts) or  $1 \mu\text{M}$  (TdP in one of six hearts) (Fig. 3). In parallel,

SAR296968 reduced in a concentration-dependent manner the sotalol prolonging effect on QT interval duration by  $-46$  and  $-95$  milliseconds for 80 bpm at  $0.3$  and  $1 \mu\text{M}$ , respectively. Moreover, SAR296968 blunted the sotalol-induced increase in dispersion of ventricular repolarization, although not significantly, except for the  $0.3 \mu\text{M}$  dose at 40 bpm (T wave peak to T wave end ) interval duration (Tp-Te) reduced by  $-27$  milliseconds at  $0.3 \mu\text{M}$  and  $-20$  milliseconds at  $1 \mu\text{M}$  SAR296968). The QRS interval duration was not affected by SAR296968 administration (Supplemental Table 3). The same level of antiarrhythmic efficacy was shown when TdP were induced by veratridine application (Fig. 3), which was associated with reduction of the veratridine-induced prolongation of the QT interval duration, without affecting the dispersion of ventricular repolarization or the QRS interval (Supplemental Table 3a).

TABLE 1

Antiarrhythmic effects of SAR296968 in animal models of arrhythmias related to early or late afterdepolarizations

In guinea pig papillary muscle and left atria, SAR296968 exhibited antiarrhythmic properties at 1 and 3  $\mu$ M. In a pig model of electrically induced episodes of atrial fibrillation, SAR296968 (1.5 mg/kg, i.v.) exhibited strong antiarrhythmic effects.

		<i>n</i>	Median	$\pm$	MAD
Number of arrhythmic contractions in guinea pig papillary muscle	Vehicle	12	20.5	$\pm$	0.0
	SAR296968 1 $\mu$ M	8	No arrhythmic contraction		
	SAR296968 3 $\mu$ M	8	No arrhythmic contraction		
Number of arrhythmic contractions in guinea pig left atria	Vehicle	10	15.5	$\pm$	4.5
	SAR296968 3 $\mu$ M	10	1.5	$\pm$	1.5
		<i>n</i>	Mean	$\pm$	S.E.M.
Number of inducible atrial fibrillation episodes in anesthetized pig	Baseline	8	25.5	$\pm$	1.75
	SAR296968 (1.5 mg/kg, i.v.)	8	6.56*	$\pm$	1.99

\* $P < 0.05$  vs. baseline.

In anesthetized pigs, SAR296968 1.5 mg/kg significantly increased left ventricular contractility by 49%, raising the  $dP/dt_{\max}$  from  $1423 \pm 144$  mm Hg/s at baseline to  $2115 \pm 189$  mm Hg/s after infusion completion. No changes in the left or right atrial effective refractory period were recorded after SAR296968 treatment regardless of the basic cycle length (Supplemental Table 3b). However, the incidence of atrial arrhythmias (LAV) was significantly reduced after SAR296968 administration by 74% ( $6.56 \pm 1.99$  events after SAR296968 administration vs.  $25.50 \pm 1.75$  events at baseline,  $n = 8$ ,  $P < 0.001$ ) (Table 1). No significant changes in either atrial effective refractory period (AERP) (Supplemental Table 3b) or LAV ( $23.29 \pm 6.02$  events after vehicle administration vs.  $27.00 \pm 3.66$  events at baseline,  $n = 7$ , non significant) were observed in the vehicle-treated group.

### Effects of SAR340835 in Normal Conscious Dogs

Infusion of SAR340835 had no effect on arterial pressure but dose-dependently decreased heart rate with a significant effect only at the highest dose ( $-23.4\%$ ,  $P = 0.0164$ ) (Table 2). Compared with vehicle, SAR340835 significantly increased  $dP/dt_{\max}$  at 250, 750, and 1500 by  $+24.4\%$  ( $P = 0.0479$ ),  $+38.7\%$  ( $P = 0.0054$ ), and  $+65.6\%$  ( $P = 0.0003$ ), respectively (Fig. 4; Table 2). In parallel, stroke volume (SV)

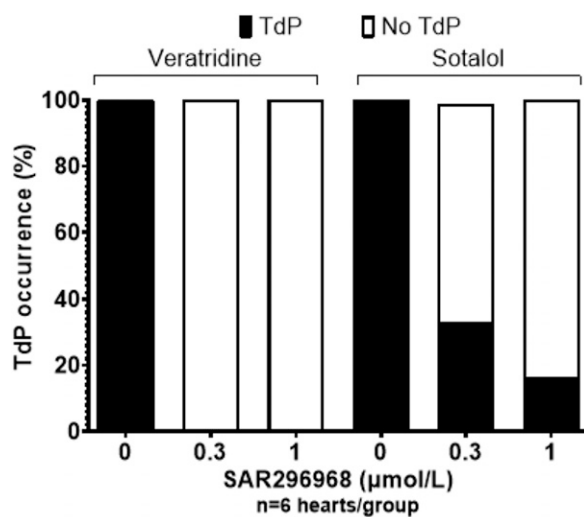
was significantly increased to an almost similar magnitude regardless of the dose [ $+16.5\%$  ( $P = 0.0144$ ),  $+21.7\%$  ( $P = 0.0026$ ), and  $+14.1\%$  ( $P = 0.0327$ ) at 250, 750, and 1500  $\mu$ g/kg per hour, respectively (Table 2)]. SAR340835 increased  $dP/dt_{\max}$  and left ventricular end-diastolic volume (LVEDV) in a dose-dependent manner, only reaching statistical significance at 750 and 1500  $\mu$ g/kg per hour [ $+27\%$  and  $+36.3\%$  increase compared with vehicle, respectively (Table 2)]. SAR340835 had no effect on calculated oxygen consumption (MVO<sub>2</sub>) (Table 2).

SAR340835 significantly increased  $dP/dt_{\min}$  at 750 and 1500  $\mu$ g/kg per hour by 7.2% ( $P = 0.0238$ ) and 9.6% ( $P = 0.0066$ ), respectively (Table 2). However, SAR340835 did not induce any changes in the other echocardiographic parameters of diastolic function (Table 2).

SAR340835 prolonged PR interval duration in a dose-dependent manner, reaching significance at 1500  $\mu$ g/kg per hour [ $+28.5\%$  ( $P = 0.0116$ )] (Fig. 4; Table 2). Second-degree AVBs (isolated P-waves) were attributed to SAR340835 in two of four normal mongrel dogs and occurred during infusions of both 750 and 1500  $\mu$ g/kg per hour. One dog did not exhibit any second-degree AVB whatever the dose, and one dog already had preexisting second-degree AVB, either in the vehicle-treated session or before treatment at each session. For the two other dogs, second-degree AVB occurred generally early after starting SAR340835 infusion (6–35 minutes for three experiments, and 3 hours in one animal) and with a frequency ranging from two to three isolated P-waves per 20 seconds to 6–12 isolated P-waves per 20 seconds.

### Effects of SAR340835 in Pacing-Induced HF Dogs

**HF Dog Model Characteristics.** After 4 weeks of rapid right ventricular pacing at 240 bpm, HF dogs' phenotype recapitulated the clinical signs of dilated cardiomyopathy and heart failure in humans (Table 3). The dogs exhibited a major increase in LVEDV from  $75 \pm 6$  ml at baseline (i.e., before starting the pacing) to  $119 \pm 6$  ml ( $P = 0.0010$ ), a depressed contractility illustrated by a marked decrease in  $dP/dt_{\max}$  and  $dP/dt_{\max}/LVEDV$  (Table 3), and a significant and profound decrease in LVEF from  $55\% \pm 3\%$  to  $28\% \pm 1\%$  ( $P = 0.0001$ ). This decrease in cardiac contractility is also reflected by a significantly reduced SV from  $64 \pm 2$  ml to  $31 \pm 3$  ml ( $P = 0.0012$ ). HF dogs also displayed a severe diastolic dysfunction illustrated by a restrictive pattern ( $E/A > 1$ ) and a reduced deceleration time (DT) from  $83.6 \pm 7.1$  to  $54.3 \pm 4.4$  milliseconds ( $P = 0.0076$ ). In addition, ANS modifications typical of human HF were also observed.



**Fig. 3.** Effect of SAR296968 on EAD-related arrhythmias in the isolated rabbit heart model SAR296968 in isolated rabbit heart: SAR296968 treatment at 0.3 or 1  $\mu$ M decreased the sotalol-induced TdP without any proarrhythmic activity by suppression of EAD induced by veratridine.

TABLE 2

Cardiac and hemodynamic effects of SAR340835 in normal dogs

Autonomic nervous system was evaluated by heart rate variability analysis. All data are expressed as means  $\pm$  SEM and median  $\pm$  MAD for Low Frequency/High Frequency ratio ( $P < 0.05$  vs. vehicle group;  $n = 3$ –5 dogs according to the group or parameter considered).

	<i>n</i>	Vehicle infusion	Normal Dogs		
			SAR340835 Infusion		
			250 µg/kg per hour	750 µg/kg per hour	1500 µg/kg per hour
Hemodynamics					
dP/dt <sub>max</sub> (mm Hg/s)	3 to 4	3091 ± 311	3856 ± 450*	4340 ± 264**	5143 ± 514***
dP/dt <sub>min</sub> (mm Hg/s)	3	−2648 ± 153	−2674 ± 106	−2840 ± 178*	−2902 ± 124**
LVEDP (mm Hg)	3	10 ± 0.7	9.5 ± 0.32	9.7 ± 0.5	9 ± 0.4
SBP (mm Hg)	4	119 ± 4.0	113 ± 6	131 ± 3	125 ± 4
DBP (mm Hg)	4	83 ± 2.8	75 ± 5	93 ± 1	84 ± 5
PR interval (ms)	4	100 ± 3	106 ± 5	124 ± 9	130 ± 13*
dP/dt <sub>max</sub> /LVEDV (mm Hg/s per milliliter)	3 to 4	40.8 ± 4.1	47.3 ± 5.6	56.7 ± 5.2**	57 ± 4.7**
MVO2 (ml O <sub>2</sub> /min per 100 gram)	4 to 5	13.85 ± 1.16	12.72 ± 0.84	13.97 ± 2.09	12.5 ± 0.86
Echocardiography					
SV (ml)	4 to 5	63 ± 2	73 ± 3*	77 ± 2**	72 ± 3*
HR (bpm)	4 to 5	93 ± 8	83 ± 5	76 ± 12	74 ± 6*
CO (l/min)	4 to 5	5.8 ± 0.6	6 ± 0.4	6 ± 1	5.3 ± 0.8
LVEF (%)	4 to 5	58 ± 3	62 ± 2	66 ± 3	64 ± 1
LVEDV (ml)	4 to 5	73 ± 2	77 ± 4	78 ± 4	82 ± 9
E (cm/s)	4 to 5	0.85 ± 0.02	0.825 ± 0.025	0.92 ± 0.04	0.86 ± 0.04
DT (ms)	4 to 5	80.1 ± 8.1	83.7 ± 10.5	93.8 ± 15.2	78.7 ± 9.0
A (cm/s)	4 to 5	0.58 ± 0.07	0.58 ± 0.06	0.59 ± 0.01	0.70 ± 0.075*
E/A	4 to 5	1.54 ± 0.16	1.58 ± 0.18	1.60 ± 0.17	1.28 ± 0.16
Heart rate variability					
Low Frequency (nu)	3 to 4	22 ± 3	14 ± 1	16 ± 4	14 ± 5
High Frequency (nu)	3 to 4	75 ± 3	83 ± 2	81 ± 5	83 ± 5
Low Frequency/High Frequency	3 to 4	0.32 ± 0.06	0.17 ± 0.03	0.15 ± 0.04	0.16 ± 0.10
BRS (mm Hg/s)	3 to 4	50 ± 2	53 ± 2	63 ± 10	55 ± 6

High Frequency, parasympathetic modulation; Low Frequency, sympathetic modulation; Low Frequency/High Frequency, sympathovagal balance; nu, normalized unit. \* $P < 0.05$ , \*\* $P < 0.01$ , and \*\*\* $P < 0.001$  SAR340835 vs. vehicle.

Additional pathologic features of dogs included tachycardia, loss of the normally observed respiratory sinus arrhythmia, ANS imbalance as shown by an Low Frequency/High Frequency ratio that was increased from  $0.22 \pm 0.12$  in normal dogs to  $2.29 \pm 0.45$  in HF dogs ( $P < 0.0001$ ) and impaired baroreflex sensitivity ( $49 \pm 3$  in normal dogs vs.  $24 \pm 3$  in HF dogs,  $P < 0.0001$ ).

**Effects of SAR340835 and Dobutamine on Hemodynamics of Conscious HF Dogs.** The effects of SAR340835 and dobutamine on hemodynamic parameters in dogs with HF are shown in Table 4. Neither SAR340835 nor dobutamine altered systolic or diastolic arterial pressure compared with vehicle regardless of the dose tested (Table 4). SAR340835 infusion over 3 hours tended to decrease heart rate (HR) from 250  $\mu$ g/kg per hour (Fig. 4; Table 4), whereas dobutamine did not affect HR.

**Effects of SAR340835 and Dobutamine on Left Ventricular Systolic Function of Conscious HF Dogs.** Infusion of SAR340835 overall increased dP/dt<sub>max</sub> in a dose-dependent manner, but this effect reached statistical significance only at the highest dose tested. dP/dt<sub>max</sub> at the end of 1500  $\mu$ g/kg per hour infusion reached  $1699 \pm 208$  mm Hg/s in comparison with  $1276 \pm 79$  mm Hg/s at the completion of vehicle infusion ( $P = 0.0125$ ), (Fig. 5; Table 4). dP/dt<sub>max</sub>/LVEDV increased in a dose-dependent manner, but it reached statistical significance only at the highest dose tested ( $+26.4\%$ ,  $P = 0.0057$ ; Table 4).

Infusion of dobutamine significantly increased LV dP/dt<sub>max</sub> at 10  $\mu$ g/kg per minute ( $59.6\%$  vs. vehicle,  $P = 0.0177$ ) (Table 4). SAR340835 dose-dependently and significantly increased SV at 250, 750, and 1500  $\mu$ g/kg per hour by  $28.5\%$  ( $P = 0.0393$ ),  $48.8\%$  ( $P = 0.0022$ ), and  $62.2\%$  ( $P = 0.0002$ ), respectively (Fig. 5;

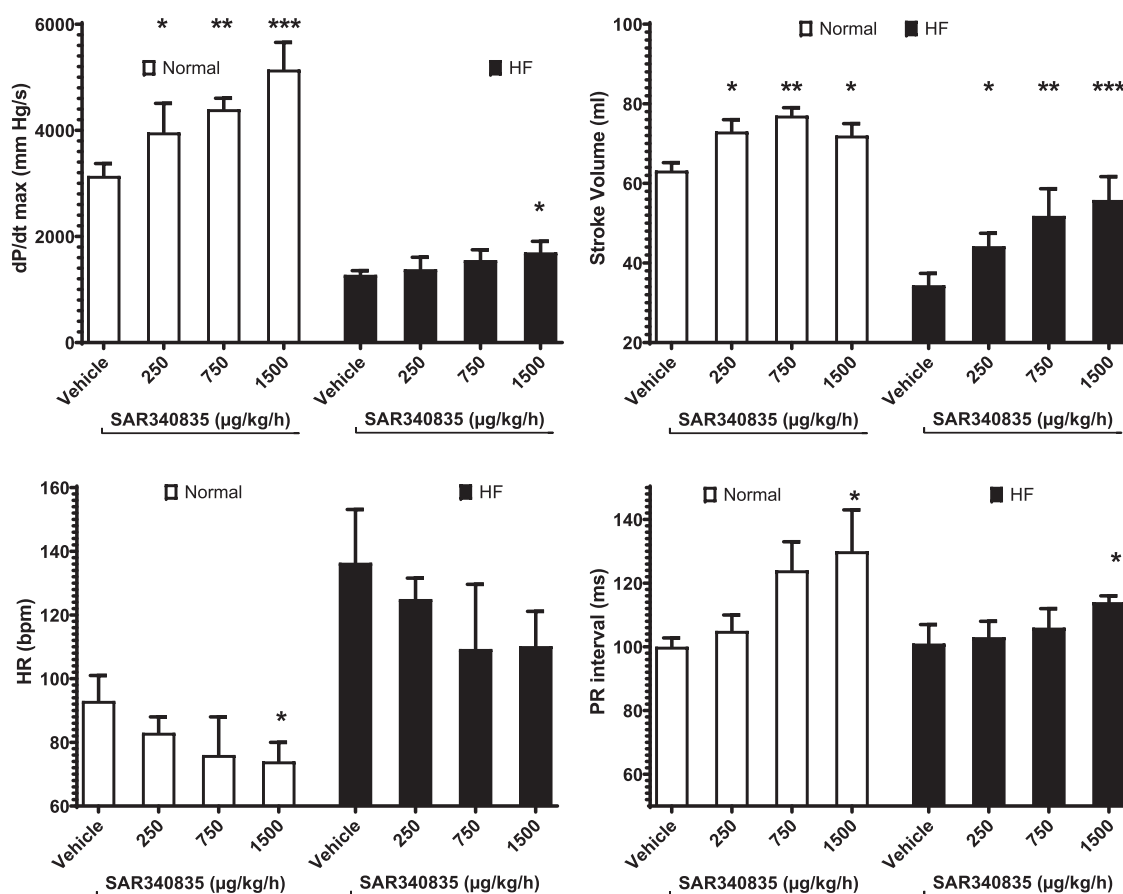
Table 4). As heart rate decreased with SAR340835 infusion, the increase in stroke volume translated into a moderate cardiac output (CO) increase ( $+21.7\%$ ,  $+5.5\%$ , and  $+26.1\%$  at 250, 750, and 1500  $\mu$ g/kg per hour, respectively) that did not reach statistical significance (Fig. 5; Table 4). SAR340835 dose-dependently increased LVEF ( $+33.9\%$ ,  $P = 0.0182$ , and  $+41\%$ ,  $P = 0.0034$ ) at 750 and 1500  $\mu$ g/kg per hour, respectively. SAR340835 did not change calculated oxygen consumption (MVO2) (Table 4).

After 3 hours of infusion, dobutamine at 10  $\mu$ g/kg per minute significantly increased SV by  $68.8\%$  ( $P = 0.0030$ ) and CO by  $75.1\%$  ( $P = 0.0031$ ) and increased LVEF by  $47.3\%$  without reaching significance ( $P = 0.0679$ ). Dobutamine significantly increased calculated MVO2 by  $28.14\%$  ( $P = 0.0013$ ) (Table 4).

**Effects of SAR340835 and Dobutamine on Left Ventricular Diastolic Function of Conscious HF Dogs.** Infusion of SAR340835 did not significantly change the indices of diastolic function. LVEDP was reduced to a similar extent ( $-7.5\%$ ,  $-21.4\%$ , and  $-16.3\%$  after 250, 750, and 1500  $\mu$ g/kg per hour, respectively), although these changes failed to reach the significance level as compared with vehicle-treated animals (Table 4).

Dobutamine infusion significantly improved dP/dt<sub>min</sub> by  $58.2\%$  ( $P = 0.0195$ ) and reduced LVEDP by  $17.7\%$  ( $P = 0.0249$ ) but did not modify other parameters of diastolic function (Table 4).

**Effects of SAR340835 and Dobutamine on Autonomic Nervous System in HF Dogs.** SAR340835 significantly improved sympathovagal imbalance as evidenced by the significant decrease in the Low Frequency/High Frequency ratio observed at all doses ( $P = 0.0112$ ,  $P = 0.0008$ , and  $P = 0.0007$  at 250, 750, and 1500  $\mu$ g/kg per hour, respectively)



**Fig. 4.** Cardiac function in conscious normal and heart failure mongrel dogs. SAR340835 was administered as an intravenous bolus, followed by an intravenous infusion (see Table 1). Dose-dependent effects of SAR340835 on normal (□) and heart failure dogs (■) on  $dP/dt_{max}$ , SV, HR, and PR interval ( $n = 3-5$  dogs). All data are expressed as means  $\pm$  SEM \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  vs. vehicle for each condition.

(Fig. 5; Table 4). SAR340835 significantly improved baroreflex sensitivity (BRS) in a dose-dependent manner (+55.6%,  $P = 0.0304$ ; +74.9%,  $P = 0.0048$ ; and +119.6%,  $P = 0.0003$ , compared with vehicle, respectively) (Fig. 5; Table 4). Dobutamine infusion did not change sympathovagal imbalance or baroreflex sensitivity as compared with vehicle (Table 4).

**Effects of SAR340835 and Dobutamine on ECG Parameters of HF Dogs.** SAR340835 infusion significantly increased PR interval duration after dose 3 in HF dogs (Table 2). Dobutamine infusion shortened PR interval significantly in HF dogs (Table 4). PR interval was  $90 \pm 2$  milliseconds versus  $101 \pm 5$  milliseconds in the dobutamine versus vehicle group, respectively. No AVBs were observed under SAR340835 infusion in HF dogs.

#### Effects of SAR296968 on HF Dog Cardiomyocytes

Detailed results of dog cardiomyocyte studies are provided in the Supplemental Material.

### Discussion

The current work aimed at establishing the potential therapeutic value of SAR340835, a novel NCX inhibitor, for the treatment of AHF. We first demonstrated that SAR340835 is a potent inhibitor of NCX across the different isoforms with no effects on  $Ca^{2+}$  or  $Na^{+}$  channels and with a similar potency

in reverse and forward mode. In vivo and in vitro studies showed that SAR340835 displayed antiarrhythmic effects, improved systolic function, reduced HR, and restored both sympathovagal balance and BRS in HF dogs, exerting a more pronounced beneficial cardiac effect in HF than in normal conditions without changing the calculated MVO<sub>2</sub>. Interestingly SAR340835 pharmacodynamic profile differed notably from dobutamine, the inotrope of reference for AHF.

NCX expression and activity are upregulated in human and animal failing heart (Hasenfuss et al., 1999; Sipido et al., 2002), thereby contributing to cardiac dysfunction in HF (Hobai et al., 2004) and EAD- and DAD-related arrhythmias typically occurring in patients with HF (Sipido et al., 2002). Consistently NCX inhibition displayed antiarrhythmic properties (Tanaka et al., 2007; Milberg et al., 2008). This was confirmed with SAR340835, which strongly inhibits the occurrence of ventricular and atrial arrhythmias in various experimental conditions, stimulating NCX inward current in guinea pig, rabbit, or pig. Thus, SAR340835 suppressed DAD-related arrhythmias at concentrations that increased cardiac contractility in guinea pig isolated papillary muscles and atria. SAR296968 efficiently reduced long QT-related arrhythmias at concentrations matching those that increased SV in HF dog. Overall, these results suggested a marked antiarrhythmic profile that is probably devoid of proarrhythmic property since no direct activity was detected on  $K^{+}$ ,  $Na^{+}$ , or  $Ca^{2+}$  channels. Indeed, patch-clamp studies supported the

TABLE 3

Hemodynamics and LV function in conscious dogs before and after development of pacing-induced heart failure ( $n = 5$  to 6). Autonomic nervous system was evaluated by heart rate variability analysis. All data are expressed as means  $\pm$  SEM and median  $\pm$  MAD for Low Frequency/High Frequency ratio.

	Before Pacing Normal Dogs ( $n = 5$ to 6)	After Pacing HF Dogs ( $n = 5$ to 6)
<b>Hemodynamics</b>		
dP/dt <sub>max</sub> (mm Hg/s)	3082 $\pm$ 300	1249 $\pm$ 55**
dP/dt <sub>min</sub> (mm Hg/s)	-3104 $\pm$ 152	-1487 $\pm$ 112***
LVEDP (mm Hg)	11.0 $\pm$ 1.6	33.2 $\pm$ 3.7**
SBP (mm Hg)	122.3 $\pm$ 5.6	104.3 $\pm$ 4.7***
DBP (mm Hg)	92.0 $\pm$ 6.0	79.3 $\pm$ 5.5**
dP/dt <sub>max</sub> /LVEDV (mm Hg/s per milliliter)	43.5 $\pm$ 5.6	10.8 $\pm$ 1.1**
MVO <sub>2</sub> (ml O <sub>2</sub> /min per 100 gram)	13.0 $\pm$ 1.27	13.3 $\pm$ 1.02
<b>Echocardiography</b>		
SV (ml)	64 $\pm$ 2	31 $\pm$ 3**
HR (bpm)	87 $\pm$ 6	149 $\pm$ 9**
CO (l/min)	5.2 $\pm$ 0.5	4.3 $\pm$ 0.4
LVEF (%)	55 $\pm$ 3	28 $\pm$ 1***
LVEDV (ml)	75 $\pm$ 6	119 $\pm$ 6**
E (cm/s)	0.85 $\pm$ 0.051	0.872 $\pm$ 0.029
DT (ms)	83.6 $\pm$ 7.1	54.3 $\pm$ 4.4**
A (cm/s)	0.608 $\pm$ 0.042	0.423 $\pm$ 0.027**
E/A	1.41 $\pm$ 0.07	2.08 $\pm$ 0.07***
<b>Heart rate variability</b>		
Low Frequency (nu)	19 $\pm$ 4	61 $\pm$ 3***
High Frequency (nu)	78 $\pm$ 5	27 $\pm$ 2***
Low Frequency/High Frequency	0.22 $\pm$ 0.12	2.29 $\pm$ 0.45***
BRS (mm Hg/s)	49 $\pm$ 3	24 $\pm$ 3**

High Frequency, parasympathetic modulation; Low Frequency, sympathetic modulation; Low Frequency/High Frequency; sympathovagal balance; nu, normalized unit.

\* $P < 0.05$ , \*\* $P < 0.01$ , and \*\*\* $P < 0.001$  HF vs. Normal condition.

high selectivity of SAR296968 for NCX versus these ion channels.

Beyond its antiarrhythmic effects, prolonged infusion of SAR340835 markedly improved the systolic cardiac function. Such an effect was expected with NCX inhibition (O'Rourke et al., 1999; Otsomaa et al., 2020) but was not yet demonstrated in vivo in conscious large-species models of HF. SAR340835 increased systolic function at similar exposure in normal and HF dogs but with different associated cardiodynamic changes. In normal dogs, SAR340835 significantly and dose-dependently increased cardiac contractility as evaluated by dP/dt<sub>max</sub>, whereas the increase in SV plateaued from the first dose, probably because of the naturally high cardiac performance at baseline in normal conditions. Conversely, a marked and dose-dependent increase in SV revealed the improvement of systolic function in HF dogs more clearly than the increase in dP/dt<sub>max</sub>. The dP/dt<sub>max</sub> estimation of cardiac contractility depends on preload and afterload, and the large difference in cardiac load conditions at baseline between normal and HF dogs might have influenced the response on dP/dt<sub>max</sub>. Overall, SAR340835 was more efficient in improving cardiac pump function in HF than in normal condition.

In isolated HF dog cardiomyocytes, SAR296968 at the highest concentration tested significantly increased sarcomere shortening and contraction velocities to the same extent as dobutamine. Interestingly, SAR296968 had no contractile effect in normal dog cardiomyocytes, whereas dobutamine increased sarcomere shortening to the same magnitude in both preparations. This larger effect of NCX inhibition agrees with the literature. Often, the inotropic effects reported with NCX inhibitor are weak or nil in normal canine cardiomyocytes (Birinyi et al., 2008; Oravec et al., 2018). Interestingly, partial inhibition of NCX with the exchanger inhibitory

peptide XIP improved the Ca<sup>2+</sup> transient amplitude more in failing than in normal canine cardiomyocytes (Hobai et al., 2004). This was not unexpected considering the reported overexpression of NCX in human (Goldhaber and Philipson, 2013) and canine failing hearts (Winslow et al., 1999; O'Rourke et al., 1999), leading to premature Ca<sup>2+</sup> efflux, reduced sarcoplasmic reticulum Ca<sup>2+</sup> content, and impaired cell contractility. Then, partial inhibition of NCX could be enough to regulate disturbed calcium handling due to NCX overactivity and thus better improve the cardiac contractility than in normal condition.

On the other hand, other factors besides the augmented cardiac contractility could promote an increase in SV. In HF dogs, we observed that SAR340835 corrected the autonomic tone and BRS deficiency from the first dose tested while reducing the HR, in contrast with dobutamine. These beneficial effects of SAR340835 were comparable to those reported after chronic electrical stimulation of the carotid sinus baroreflex in HF dogs (Zhang et al., 2009; Sabbah et al., 2011). Indeed, ANS disturbances have been studied extensively in the HF condition (Shen et al., 2002; Zucker et al., 2007; Zhang et al., 2009). Overall, the autonomic balance shifts from primarily dominant vagal tone in the normal condition to sympathetic predominance in HF. The pathophysiological role of sympathetic activation in HF is highlighted by the beneficial effects of  $\beta$ -blocker therapy. The functional weakness of parasympathetic activity was observed in clinical and experimental HF (Floras and Ponikowski, 2015), from the early stages of HF (Ishise et al., 1998), and rising with disease progression (van Bilsen et al., 2017). Furthermore, the diminished cardiac vagal activity, increased HR, and decreased BRS are predictors of high mortality rate in patients with myocardial infarction or HF (La Rovere et al.,

TABLE 4

Cardiac and hemodynamic effects of SAR340835 and dobutamine in HF dogs

Autonomic nervous system was evaluated by heart rate variability analysis. All data are expressed as means  $\pm$  SEM and median  $\pm$  MAD for Low Frequency/High Frequency ratio ( $P < 0.05$  vs. vehicle group;  $n = 3$ –5 dogs according to the group or parameter considered).

	<i>n</i>	HF Dogs				
		Vehicle Infusion	SAR340835 Infusion			Dobutamine
			250μg/kg per hour	750μg/kg per hour	1500 μg/kg per hour	
Hemodynamics						
dP/dt <sub>max</sub> (mm Hg/s)	3 to 4	1276 ± 79	1382 ± 225	1554 ± 195	1699 ± 208*	2037 ± 9 <sup>#</sup>
dP/dt <sub>min</sub> (mm Hg/s)	3 to 4	−1550 ± 42	−1643 ± 128	−1720 ± 117	−1726 ± 65	−2452 ± 194 <sup>#</sup>
LVEDP (mm Hg)	3 to 4	36.8 ± 2.8	34.0 ± 4.4	30.0 ± 3.5	30.8 ± 3.8	30.3 ± 2.3 <sup>#</sup>
SBP (mm Hg)	5	109.0 ± 4.4	105.8 ± 5.9	102.4 ± 5.2	111.0 ± 5.3	106.0 ± 2.2
DBP (mm Hg)	4 to 5	84.6 ± 4.2	80.0 ± 4.8	77.5 ± 4.7	83.4 ± 4.6	73.6 ± 3.5
dP/dt <sub>max</sub> /LVEDV (mm Hg/s per milliliter)	3 to 4	11.4 ± 0.7	12.2 ± 1.5	13.1 ± 1.2	14.4 ± 1.5**	18.8 ± 1 <sup>#</sup>
MVO <sub>2</sub> (mlO <sub>2</sub> /min per 100 gram)	4 to 5	13.2 ± 1.06	13.51 ± 0.52	12.51 ± 0.99	14.01 ± 1.03	16.93 ± 1.19 <sup>##</sup>
PR interval (ms)	4 to 5	101 ± 5	103 ± 5	106 ± 6	114 ± 2*	90 ± 2 <sup>#</sup>
Echocardiography						
SV (ml)	4 to 5	34 ± 3	44 ± 3*	52 ± 7**	56 ± 6***	60 ± 5 <sup>#</sup>
HR (bpm)	4 to 5	136 ± 17	125 ± 7	109 ± 20	110 ± 11	133 ± 5
CO (l/min)	4 to 5	4.6 ± 0.2	5.6 ± 0.2	5.3 ± 0.5	5.8 ± 0.6	8.0 ± 0.7 <sup>##</sup>
LVEF (%)	4 to 5	29 ± 3	34 ± 3	40 ± 3 *	41 ± 2**	43 ± 3
LVEDV (ml)	4 to 5	111 ± 2	110 ± 4	116 ± 4	118 ± 2	109 ± 2
E (cm/s)	4 to 5	0.912 ± 0.027	0.958 ± 0.071	0.965 ± 0.108	1.012 ± 0.078	1.088 ± 0.083
DT (ms)	4 to 5	56.2 ± 7.1	56.2 ± 3.0	61.8 ± 5.1	65.6 ± 3.0	57.3 ± 6.6
A (cm/s)	4 to 5	0.446 ± 0.027	0.472 ± 0.035	0.465 ± 0.044	0.472 ± 0.061	0.485 ± 0.031
E/A	4 to 5	2.07 ± 0.11	2.05 ± 0.13	2.16 ± 0.38	2.22 ± 0.22	2.25 ± 0.15
Heart rate variability						
Low Frequency (nu)	4 to 5	55 ± 4	31 ± 4**	21 ± 2 ***	24 ± 5***	48 ± 3
High Frequency (nu)	4 to 5	35 ± 4	66 ± 4**	74 ± 4 ***	73 ± 5***	43 ± 6
Low Frequency/High Frequency	4 to 5	1.51 ± 0.40	0.44 ± 0.06*	0.28 ± 0.06 ***	0.31 ± 0.09***	0.95 ± 0.14
BRS (mm Hg/s)	4 to 5	32 ± 3	50 ± 4*	55 ± 4**	70 ± 4***	29 ± 2

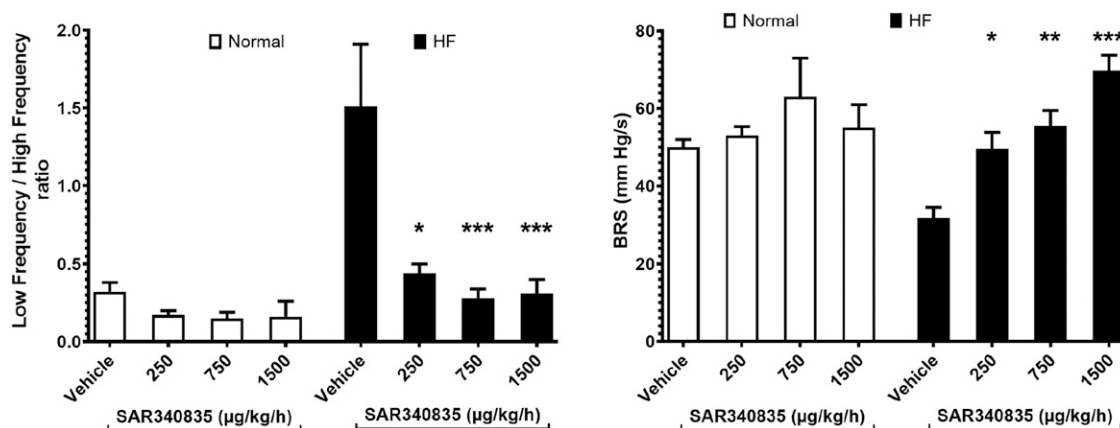
High Frequency, parasympathetic modulation; Low Frequency, sympathetic modulation; Low Frequency/High Frequency, sympathovagal balance; nu, normalized unit.

\* $P < 0.05$ , \*\* $P < 0.01$ , and \*\*\* $P < 0.001$  SAR340835 vs. vehicle.\* $P < 0.05$ , \*\* $P < 0.01$ , and \*\*\* $P < 0.001$  dobutamine vs. vehicle.

1998; Lechat et al., 2001). Accordingly, several clinical studies have used stimulation devices, like vagal nerve stimulation or baroreceptor activation therapy, to reduce sympathetic nervous system reflex activity or promote vagal activity to restore a proper ANS balance (Singh et al., 2014). However, while the vagal nerve stimulation or baroreceptor activation therapy worked in animal models, clinical studies have been disappointing (van Bilsen et al., 2017).

The mechanisms provided by SAR340835 that lead to ANS balance restoration and the slight but consistent negative

chronotropic effect are probably the end results of complex changes into electromechanical machinery underlying cardiac activity. Since HR was unaltered in NCX Knock Out mice or in mice overexpressing NCX, a direct role of NCX on pacemaker cells is unlikely (Gao et al., 2013; Kaese et al., 2017). However, NCX is an essential effector in  $\beta$ -adrenergic-mediated chronotropy (Gao et al., 2013; Kaese et al., 2017). Therefore, in HF conditions combining NCX and sympathetic overactivity, one might expect that NCX inhibition is likely to reduce HR, as observed in our HF dogs. However, we could not exclude that



**Fig. 5.** Autonomic function in conscious normal and in heart failure mongrel dogs. SAR340835 was administered as an intravenous bolus, followed by an intravenous infusion (see Table 1). Dose-dependent effects of SAR340835 on normal (□) and heart failure dogs (■) on the Low Frequency/High Frequency ratio and BRS ( $n = 4$  to 5 dogs). All data are expressed as means  $\pm$  SEM for BRS and median  $\pm$  MAD for the Low Frequency/High Frequency ratio. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  vs. vehicle for each condition.

SAR340835-mediated bradycardia could be the consequence of restored sympathovagal balance and BRS, as previously reported for other positive inotropes that increased intracellular  $\text{Ca}^{2+}$  concentrations.  $\text{Na}^+$  channel enhancer (Shen et al., 2002) and  $\text{Ca}^{2+}$  channel activator (Uechi et al., 1998) both reduced HR and enhanced BRS in pacing-induced HF dogs but not in normal dogs. Using ganglionic or  $\beta$ -adrenergic blockade, these authors demonstrated that the negative chronotropic effect was mainly due to an increased parasympathetic tone. The  $\text{Ca}^{2+}$  channel activator was hypothesized either to enhance BRS due to local alterations in the  $\text{Na}^+$  content of the vessel wall (Kunze and Brown, 1978) or to exert a central neuronal mechanism increasing the vagal tone through intracellular  $\text{Ca}^{2+}$  elevation (Uechi et al., 1998). A close mode of action can reasonably be hypothesized for SAR340835, which similarly increased intracellular  $\text{Ca}^{2+}$  and showed the same pharmacological in vivo profile, but further studies are warranted to confirm this hypothesis. Additional beneficial effects of NCX inhibitors would be related to their antiarrhythmic properties, as they did not affect the refractory periods in ECG time intervals but slightly accelerated repolarization. This contrasts with  $\text{Na}^+$  channel enhancers, which prolong action potential duration and can promote EADs and torsade de pointes, and with  $\text{Ca}^{2+}$  channel activators also known to induce EADs and DADs (Chang et al., 2012; Shryock et al., 2013).

The attractiveness of NCX inhibition for the treatment of AHF could be compromised by the occurrence of second-degree AVB reported in normal dogs. However, SAR340835 at the highest dose tested showed only a limited prolongation effect on the PR interval in HF dogs and did not induce second-degree AVB. The high vagal tone characterizing normal dogs and the profound electrophysiological remodeling of failing hearts (including NCX overactivity) could partly explain these contrasting results between HF and normal conditions.

Overall, SAR340835 displays an attractive therapeutic profile for patients with HF, contrasting with current inotropes, reducing afterdepolarization-related arrhythmias, and improving systolic cardiac function without changing blood pressure or impairing the diastolic function. SAR340835 restored the impaired baroreflex sensitivity in HF dogs, thereby improving the balance between sympathetic and parasympathetic tone while maintaining potent  $\beta$ -adrenergic-independent inotropic effects. In addition, the slight bradycardia reported with SAR340835 could contribute to a reduction in myocardial oxygen consumption and improve cardiac efficiency, a valuable property in the setting of AHF. Accordingly, in HF dogs, the calculated MVO<sub>2</sub> remained unchanged regardless of the dose of SAR340835, whereas it was raised with dobutamine infused in a therapeutic dose range. These promising results warrant further studies comparing SAR340835 and dobutamine at doses inducing a similar increase in cardiac contractility. Altogether, these results show that potent selective NCX inhibitors offer new therapeutic opportunities for the treatment of patients with AHF. Whether this efficacy would be observed in HF with preserved ejection fraction (HFpEF) as well as in HF with reduced ejection fraction conditions remains to be determined. Experimental studies (Kamimura et al., 2012; Primessnig et al., 2019) suggested that chronic partial NCX inhibition could be beneficial in HFpEF through normalization of overactivity of NCX. Moreover, several publications support the rationale for NCX overactivity in

patients with HFpEF (Pieske et al., 2002; Ashrafi et al., 2017). Next steps with SAR340835 would be to explore the potential benefit against diastolic dysfunction in HFpEF models and to characterize the safety risk and determine the safety margin before considering any clinical development in AHF.

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