Inhibition of NaV1.8 prevents atrial arrhythmogenesis in human and mice

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Received: 26 December 2019 / Accepted: 10 February 2020 / Published online: 20 February 2020
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Abstract
Pharmacologic approaches for the treatment of atrial arrhythmias are limited due to side effects and low efficacy. Thus, the identification of new antiarrhythmic targets is of clinical interest. Recent genome studies suggested an involvement of SCN10A sodium channels (NaV1.8) in atrial electrophysiology. This study investigated the role and involvement of NaV1.8 (SCN10A) in arrhythmia generation in the human atria and in mice lacking NaV1.8. NaV1.8 mRNA and protein were detected in human atrial myocardium at a significant higher level compared to ventricular myocardium. Expression of NaV1.8 and NaV1.5 did not differ between myocardium from patients with atrial fibrillation and sinus rhythm. To determine the electrophysiological role of NaV1.8, we investigated isolated human atrial cardiomyocytes from patients with sinus rhythm stimulated with isoproterenol. Inhibition of NaV1.8 by A-803467 or PF-01247324 showed no effects on the human atrial action potential. However, we found that NaV1.8 significantly contributes to late Na+ current and consequently to an increased proarrhythmogenic diastolic sarcoplasmic reticulum Ca2+ leak in human atrial cardiomyocytes. Selective pharmacological inhibition of NaV1.8 potently reduced late Na+ current, proarrhythmic diastolic Ca2+ release, delayed afterdepolarizations as well as spontaneous action potentials. These findings could be confirmed in murine atrial cardiomyocytes from wild-type mice and also compared to SCN10A−/− mice (genetic ablation of NaV1.8). Pharmacological NaV1.8 inhibition showed no effects in SCN10A−/− mice. Importantly, in vivo experiments in SCN10A−/− mice showed that genetic ablation of NaV1.8 protects against atrial fibrillation induction. This study demonstrates that NaV1.8 is expressed in the murine and human atria and contributes to late Na+ current generation and cellular arrhythmogenesis. Blocking NaV1.8 selectively counteracts this pathomechanism and protects against atrial arrhythmias. Thus, our translational study reveals a new selective therapeutic target for treating atrial arrhythmias.

Keywords Antiarrhythmic drugs · Atrial arrhythmias · Na+ channel · Late sodium current

Introduction
Atrial arrhythmias, in particular atrial fibrillation (AF), contribute to the morbidity and mortality of western societies [4]. However, pharmacological therapeutic options are still...
limited due to moderate potency and severe side effects. Therefore, identification and evaluation of new targets involved in atrial arrhythmogenesis are of clinical interest. The mechanisms for atrial arrhythmogenesis include electrical remodelling and disturbances in ion homeostasis [16]. Both can cause focal triggered activity, which might evoke atrial arrhythmias or promote re-entry mechanisms. One potent substrate in promoting electrical disturbances and focal triggered activity in the atria is an increased late Na+ current ($I_{NaL}$), which is a persistent Na⁺ influx throughout the action potential [3, 16, 22, 33]. By prolonging the duration of the action potential, $I_{NaL}$ increases the probability of early afterdepolarizations (EADs), which constitute a trigger for arrhythmias. Moreover, by increasing cytosolic [Na⁺] an enhanced $I_{NaL}$ may lead to Na⁺/Ca²⁺ exchanger (NCX)-mediated Ca²⁺ overload [26]. Consecutively, this induces arrhythmogenic Ca²⁺ release events (Ca²⁺ sparks) from the sarcoplasmic reticulum (SR) during diastole [12, 13]. Increasing diastolic Ca²⁺ levels may promote a depolarizing inward current ($I_{\text{di}}$), resulting in delayed afterdepolarizations (DADs), which serve as a trigger for irregular action potentials and focal arrhythmias [32]. However, the mechanisms involved in $I_{NaL}$ generation in the atria are not fully understood.

While SCN5A sodium channels (Naᵥ1.5) are the predominant isoform in the heart [14], recent evidence suggested the involvement of SCN10A sodium channels (Naᵥ1.8) in atrial conduction [8]. Moreover, genome-wide association studies showed that variants of Naᵥ1.8 are associated with the development of atrial fibrillation [17, 21, 25]. Therefore, the aim of our study was to fundamentally investigate the molecular and functional role of Naᵥ1.8 in the human and murine atria. Moreover, we studied the involvement of Naᵥ1.8 in atrial arrhythmogenesis and evaluated the channel as a specific target for antiarrhythmic pharmacotherapy.

### Materials and methods

#### Human myocardial tissue

All procedures were performed according to the Declaration of Helsinki and were approved by the local ethics committee. Informed consent was obtained from all patients. Human atrial myocardium from patients with sinus rhythm or atrial fibrillation was acquired from atrial resections during open heart surgery (for patient characteristics, see Table 1). For molecular purposes, we utilized left ventricular myocardium from healthy donor hearts that were not transplanted due to technical reasons.

#### SCN10A−/− and wild-type mice

SCN10A−/− and respective wild-type mice (WT) were studied to reveal the functional consequence of genetic Naᵥ1.8 ablation [2]. The animal investigations conform to the “Guide for the Care and Use of Laboratory Animals” published by the US National Institutes of Health (publication No. 85-23, revised 1996) and the guidelines from Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes. Murine atrial cardiomyocytes were isolated as previously reported [13]. Mice used for cardiomyocyte isolation were sacrificed under isoflurane 133 inhalation anesthesia (5%) by cervical dislocation. Mice

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**Table 1** Clinical characteristics of patients with sinus rhythm (n = 34) and patients with atrial fibrillation (n = 10)

| Patient data | Sinus rhythm (n = 34) | Atrial fibrillation (n = 10) | Non-failing ventricle (n = 10)* |
|--------------|-----------------------|-----------------------------|--------------------------------|
| Male sex (%) | 73.5                  | 50.0                        | N/A                            |
| Age (Mean ± SEM, y) | 64.56 ± 1.55         | 75.2 ± 2.55                | N/A                            |
| EF (Mean ± SEM, %) | 56.89 ± 1.95         | 51.25 ± 3.03               | N/A                            |
| Ischemic heart disease (%) | 100.0         | 100.0                      | N/A                            |
| Diabetes (%) | 41.4                  | 20.0                        | N/A                            |
| ACE inhibitors (%) | 83.3                  | 80.0                        | N/A                            |
| β-Blockers (%) | 81.8                  | 100.0                       | N/A                            |
| Digoxin (%) | 0.0                   | 0.0                         | N/A                            |
| Catecholamines (%) | 0.0                   | 0.0                         | N/A                            |
| Amiodaron (%) | 0.0                   | 30.0                        | N/A                            |
| PDE inhibitors (%) | 0.0                   | 0.0                         | N/A                            |
| Statin (%) | 83.9                  | 90.0                        | N/A                            |

Values are mean ± SEM or n (%). Clinical data could not be completely obtained from every patient

EF: ejection fraction, ACE: angiotensin-converting enzyme, PDE: phosphodiesterase

*Blinded due to ethical reasons
used for in vivo studies were anesthetized via intraperitoneal injection of medetomidine (0.5 mg/kg), midazolam (5 mg/kg) and fentanyl (0.05 mg/kg body weight) and killed by cervical dislocation after the procedure.

**Human atrial cardiomyocytes isolation**

Atrial myocardium from patients with sinus rhythm was used for cellular experiments. Before starting isolation, human atrial tissue was cleared from fat and blood, then cut into very small pieces and rinsed thoroughly. Cardiomyocytes were isolated using collagenase (Worthington type I, 370 U/mg) and proteinase (Sigma Type XXIV, 7.0–14.0 U/mg) as described previously [15]. Enzymatic digestion was stopped by adding BCS (2%). The supernatant containing dispersed cells was centrifuged (58 g, 5 min) and cells were resuspended in storage medium. Only cell solutions containing elongated cardiomyocytes with clear cross-striations were selected for experiments, plated on laminin-coated recording chambers, and left to settle for 30 min. A representative isolated human atrial cardiomyocyte is shown in Fig. 2a. Cellular experiments were performed at room temperature.

**Murine atrial cardiomyocytes isolation**

Atrial cardiomyocytes from SCN10A−/− and respective WT mouse hearts were isolated as previously described [13]. Cellular experiments were performed at room temperature.

**Quantitative real-time PCR (qPCR)**

Human atrial tissue or ventricular non-failing tissue were snap-frozen in liquid nitrogen and stored at − 80 °C. RNA was isolated by use of the SV total RNA isolation System (Promega). Primer sequences (forward and four reverse) of SCN10A (Origene, cat No HP209444), SCN5A and GAPDH were used for quantitative RT-PCR.

**Western blots**

Human atrial tissue samples from patients with sinus rhythm (SR) and atrial fibrillation (AF) as well as human ventricular samples from healthy donors (NF) were homogenized in Tris buffer and complete protease and phosphatase inhibitor cocktails (Roche Diagnostics). Protein concentration was determined by BCA assay (Pierce Biotechnology). Mouse monoclonal anti-NaV1.8 antibodies (1:1,000, LSBio, LS-C109037), rabbit polyclonal anti-NaV1.5 (1:2,000, Aloomne labs, ASC-005), and mouse monoclonal anti-GAPDH (1:20,000, BIOTREND, BTMC-A473-9) were used. ImmobilonTM Western Chemiluminescent HRP Substrate (Millipore) was used for the chemiluminescent detection.

**Pharmacological interventions**

For selectively blocking NaV1.8, isolated cardiomyocytes were treated with either A-803467 (30 nmol/L, Sigma) or PF-01247324 (1 µmol/L, Sigma). Cells were incubated for 15 min before measurements were started. Isoproterenol (30 nmol/L, Sigma) was used for slight beta-adrenergic stimulation in all groups [10]. Moreover, we used tetrodotoxin (2 µmol/l) to inhibit I_{NaL}.

**Patch-clamp experiments**

I_{NaL} measurements

Ruptured-patch whole-cell voltage-clamp was used to measure I_{NaL} in human atrial cardiomyocytes (HEKA electronics). Cardiomyocytes were held at − 120 mV and I_{NaL} was elicited using a train of pulses to − 35 mV (1 s duration, ten pulses, BCL 2 s). Recordings were initiated 3 min after rupture. The measured current was integrated (between 100 and 500 ms) and normalized to the membrane capacitance (Suppl. Fig. 3).

**Action potential recordings**

For action potential recordings, the whole-cell patch-clamp technique was used (current clamp configuration, HEKA electronics). Access resistance was typically ~ 5–10 MΩ after patch rupture. Action potentials were continuously elicited by square current pulses of 0.5–1 nA amplitude and 1–5 ms duration at a frequency of 1 Hz. For assessing DADs and spontaneous action potentials, stimulation was paused for 15 s and for 30 s.

**Confocal Ca²⁺ spark measurements**

Isolated atrial cardiomyocytes were loaded with the Ca²⁺ indicator Fluo 4-AM (10 µmol/L for 15 min, Molecular Probes) at RT. The solution was substituted and cells were incubated for 15 min with Tyrode’s solution and the respective agents. Line scans for Ca²⁺ spark measurements were obtained with a laser scanning confocal microscope (Zeiss). Line scans were recorded during rest after loading the sarcoplasmic reticulum with Ca²⁺ by continuous field stimulation at 1 Hz. Ca²⁺ sparks were analysed with the program SparkMaster for ImageJ.

**In vivo arrhythmia studies**

For electrophysiological studies, SCN10A−/− and respective wild-type mice [2] were anesthetized and temperature controlled (37 °C). As previously described, a Millar 1.1F octapolar EP catheter (EPR-800; Millar Instruments) was
inserted via the right jugular vein [20]. Right atrial pacing was performed using 2 ms current pulses delivered by an external stimulator. Atrial capture was confirmed by atrial pacing prior to the arrhythmia protocol. Inducibility of atrial arrhythmias was tested by decremental burst pacing (5 episodes/mice). AF was defined as the occurrence of rapid and fragmented atrial electrograms with irregular AV nodal conduction and ventricular rhythm for at least 1 s.

Statistics

All data are presented as the mean values ± SEM. For statistical analysis of two groups containing parametric data Student’s t test was used, for non-parametric data Mann–Whitney test was used.

For analysis of parametric data comparing more than two groups, one-way ANOVA was used. P values were corrected for multiple comparisons by the Tukey method. For analysis of proportions, Fisher’s exact test was used. Analysis was performed using GraphPad Prism 8. P values are two-sided and considered statistically significant if \( P < 0.05 \).

Results

Expression of \( \text{Na}_{v1.8} \) in human atrial myocardium

To investigate whether \( \text{Na}_{v1.8} \) is expressed in the human atrium, we used myocardium from patients with SR and patients with AF for mRNA and protein analysis. At the protein level, we could confirm the existence of \( \text{Na}_{v1.8} \) in the human atra. Moreover, \( \text{Na}_{v1.8} \) protein expression is significantly higher in the human atria (\( n = 6 \)) compared to the human ventricle (\( n = 5 \) Fig. 1a, b). Using qPCR, we detected the expression of \( \text{Na}_{v1.8} \) mRNA in human atrial tissue, which was 3.0 ± 0.9-fold higher in the human atrium as compared to ventricular non-failing myocardium (ventricle: \( n = 10 \) patients, atria: \( n = 7 \) patients, Fig. 1c). To evaluate whether \( \text{Na}_{v1.8} \) or the major cardiac sodium channel isoform \( \text{Na}_{v1.5} \) might be differentially regulated in atrial fibrillation (AF) compared to sinus rhythm (SR), we investigated atrial myocardium from patients with SR or with AF. However, neither \( \text{Na}_{v1.8} \) (SR: \( n = 14 \) patients, AF: \( n = 14 \) patients) nor \( \text{Na}_{v1.5} \) (SR: \( n = 14 \) patients, AF: \( n = 13 \) patients) protein expression levels were different between myocardium from patients with AF and SR (Fig. 1d–f). Moreover, while \( \text{Na}_{v1.8} \) mRNA was lower compared to \( \text{Na}_{v1.5} \), we found no changes between SR versus AF for \( \text{Na}_{v1.8} \) (SR: \( n = 8 \) patients, AF: \( n = 8 \)). \( \text{Na}_{v1.5} \) mRNA levels differed between SR (\( n = 8 \) patients) and AF (\( n = 8 \), Fig. 1g), which however did not translate into protein expression differences. Therefore, \( \text{Na}_{v1.8} \) was confirmed to be present in the human atria without being regulated in patients with AF.

Effects of \( \text{Na}_{v1.8} \) on the cardiac action potential

To investigate the effect of \( \text{Na}_{v1.8} \) on the action potential properties in human atrial cardiomyocytes, we performed ruptured-patch whole-cell current clamp experiments using freshly isolated cardiomyocytes from patients with sinus rhythm (Fig. 2a). Action potential amplitude (APA), maximum upstroke velocity (\( dv/dt \)), action potential duration (APD) as well as resting membrane potential (RMP) were investigated from five different patients with sinus rhythm (control: \( n = 14 \) cardiomyocytes; A-803467: \( n = 12 \); PF-01247324: \( n = 11 \)). APA (100.9 ± 4.1 mV) and \( dv/dt \) (54.88 ± 4.8 mV/ms) were not altered after inhibition of \( \text{Na}_{v1.8} \) by A-803467 (APA: 104.9 ± 5.5 mV; \( dv/dt \): 58.08 ± 7.0 mV/ms) or PF-01247324 (APA: 99.8 ± 6.7 mV; \( dv/dt \): 56.33 ± 8.2 mV/ms), which indicates that \( \text{Na}_{v1.8} \) has negligible effects on peak Na\(^+\) current (Fig. 2b, c). Also, RMP (-77.1 ± 2.3 mV) was not changed after \( \text{Na}_{v1.8} \) inhibition (A-803467: -75.6 ± 2.3 mV; PF-01247324: -73.9 ± 2.5 mV, Fig. 2d). APD at 50% repolarization (APD\(_{50}\)) was 28.8 ± 2.6 ms in control compared to A-803467 (25.0 ± 2.3 ms) and PF-01247324 (28.2 ± 2.8 ms, Fig. 2e). However, APD\(_{90}\) was slightly abbreviated (APD\(_{90}\): 121.0 ± 11.0 ms) after exposure to A-803467 (96.3 ± 6.9 ms) and PF-01247324 (97.2 ± 9.1 ms, Fig. 2f), which, however, did not reach statistical significance. We further evaluated the effects of \( \text{Na}_{v1.8} \) on the action potential in atrial cardiomyocytes from SCN10A\(^{-/-}\) (\( n = 10 \) mice, control: \( n = 16 \) cells, PF-01247324: \( n = 15 \) cells) and WT mice (\( n = 8 \) mice, control: \( n = 14 \) cells, PF-01247324: \( n = 13 \) cells, Fig. 3a). According to the human data, we could confirm that \( \text{Na}_{v1.8} \) has no effects on APA (Fig. 3b), \( dv/dt \) (Fig. 3c), RMP (Fig. 3d) as well as APD\(_{50}\) (Fig. 3e) or APD\(_{90}\) (Fig. 3f). Accordingly, we observed no effects of pharmacological \( \text{Na}_{v1.8} \) inhibition using PF-01247324 in SCN10A\(^{-/-}\) and WT. These experiments indicate that \( \text{Na}_{v1.8} \) has negligible effects on the human and murine atrial action potential, which is of importance for further translational studies.

Role of \( \text{Na}_{v1.8} \) for generation of \( I_{\text{NaL}} \)

The contribution of \( \text{Na}_{v1.8} \) in \( I_{\text{NaL}} \) generation was studied in human atrial cardiomyocytes from patients with sinus rhythm using ruptured-patch whole-cell voltage-clamp. Patch-clamp recordings of isolated human atrial cardiomyocytes showed that \( \text{Na}_{v1.8} \) inhibition caused a significant reduction of \( I_{\text{NaL}} \) by 44.9 ± 13.5% after exposure to A-803467 (\( n = 12 \) cardiomyocytes/4 patients) and by 53.5 ± 12.7% after PF-01247324 (\( n = 10/4 \)) compared to control (\( n = 15/6 \), Fig. 4a, b). Furthermore, isolated atrial cardiomyocytes from SCN10A\(^{-/-}\) mice (\( n = 11 \) cells/5 mice) showed a significantly lower \( I_{\text{NaL}} \) compared to WT (\( n = 8 \) cells/4 mice, Fig. 4c–e). While pharmacological
NaV1.8 inhibition by PF-01247324 \((n = 7 \text{ cells/5 mice})\) exerted no effect on \(I_{\text{NaL}}\) in atrial WT cardiomyocytes \((n = 7 \text{ cells/4 mice, Fig. 4c–e})\). To determine the contribution of NaV1.8 to \(I_{\text{NaL}}\) generation, we also performed measurements with TTX (2 µmol/L) to globally inhibit \(I_{\text{NaL}}\). We observed a trend towards a lower \(I_{\text{NaL}}\) in TTX-treated \(\text{SCN10A}^{-/-}\) cardiomyocytes and also compared to PF-01247324-treated WT cardiomyocytes (Fig. 4e) suggesting that other NaV-dependent \(I_{\text{NaL}}\) is still relevant under these conditions.

**Role of NaV1.8 for sarcoplasmic Ca\(^{2+}\)-leak generation**

It is well known that \(I_{\text{NaL}}\) can potently induce arrhythmogenic diastolic SR Ca\(^{2+}\)-release events \([13]\). To investigate whether the reduction of \(I_{\text{NaL}}\) caused by NaV1.8 inhibition may lead to a diminished incidence of diastolic SR Ca\(^{2+}\) release in human atrial cardiomyocytes we used confocal microscopy. The frequency of diastolic SR Ca\(^{2+}\) sparks (CaSpF) in line scans of human atrial cardiomyocytes was \(3.2 \pm 0.5 \times 100/\mu\text{m/s} (n = 84 \text{ cardiomyocytes/13 patients})\) which could be significantly attenuated to \(1.2 \pm 0.2\)
Fig. 2 Effects of Na\textsubscript{v}1.8 on the human atrial action potential. Data are presented as mean ± SEM. P values were computed using one-way ANOVA with Tukey’s test for multiple comparisons. a Representative action potential recordings (1 Hz stimulation). Inset: isolated human atrial cardiomyocyte. b Effects of Na\textsubscript{v}1.8 inhibition by A-803467 (n = 12 cardiomyocytes/5 patients) or PF-01247324 (n = 11/5) compared to control (n = 14/5) on action potential (AP) amplitude, (c) maximum upstroke velocity (dv/dt), (d) resting membrane potential (RMP) and action potential duration at (e) 50% (APD\textsubscript{50}) and (f) 90% repolarization (APD\textsubscript{90}).

Fig. 3 Effects of Na\textsubscript{v}1.8 on the murine atrial action potential using SCN10A\textsuperscript{-/-} and WT mice. Data are presented as mean ± SEM. P values were calculated using one-way ANOVA with Tukey’s test for multiple comparisons. a Representative action potential recordings (1 Hz stimulation) of isolated murine atrial cardiomyocytes. b Effects of genetic ablation of Na\textsubscript{v}1.8 (SCN10A\textsuperscript{-/-}: n = 16 cells/10 mice) compared to WT (n = 14 cells/8 mice) and effects of pharmacological inhibition of Na\textsubscript{v}1.8 by PF-01247324 in each genotype (SCN10A\textsuperscript{-/-}: n = 15 cells/10 mice and WT: n = 13/8) on action potential (AP) amplitude, (c) maximum upstroke velocity (dv/dt), (d) resting membrane potential (RMP) and action potential duration at (e) 50% (APD\textsubscript{50}) and (f) 90% repolarization (APD\textsubscript{90}).
× 100/µm/s after addition of A-803467 (n = 73/9) or PF-01247324 (n = 88/11, Fig. 5a, b). Moreover, the calculated diastolic SR Ca²⁺ leak was reduced by 64.3 ± 22.2% after addition of A-803467 and by 80.6 ± 20.6% after PF-01247324 (control: n = 84 cardiomyocytes/13 patients, A-803467: n = 73/9; PF-01247324: n = 88/11, Fig. 5c). In line with that, atrial cardiomyocytes from SCN10A−/− mice (n = 57 cells/7 mice) showed a lower frequency of diastolic Ca²⁺ sparks as well as a lower diastolic Ca²⁺ leak compared to WT (n = 80 cells/7 mice, Fig. 5d). While PF-01247324-(n = 62 cells/7 mice) and TTX- (n = 61 cells/7 mice) treated cardiomyocytes had a significantly reduced Ca²⁺ spark frequency and Ca²⁺ leak in WT, both drugs had no further effects in SCN10A−/−-cardiomyocytes (n = 57 cells/7 mice and 64/7 respectively). Interestingly, we observed no further antiarrhythmic effect of TTX compared to NaV1.8 inhibition alone. Thus, the reduction of both IₙaL and diastolic SR Ca²⁺ release reveal the significant role of NaV1.8 for cellular arrhythmogenesis in the human atria. Of note, we observed no effects of pharmacological NaV1.8 inhibition on systolic Ca²⁺ transient amplitude and SR Ca²⁺ load (Suppl. Figs. 1, 2).

**Blocking NaV1.8 suppresses diastolic Ca²⁺ waves**

To elucidate potential antiarrhythmic effects of NaV1.8 inhibition, we have studied the effects of pharmacological inhibition and genetic ablation of NaV1.8 on the incidence of diastolic Ca²⁺ waves, which are major diastolic Ca²⁺ release events and constitute proarrhythmic triggers. Indeed, human atrial cardiomyocytes from patients with sinus rhythm treated with A-803467 or PF-01247324 showed a significantly reduced frequency of diastolic Ca²⁺ waves (Fig. 6a, b). Also, the percentage of cells developing diastolic Ca²⁺ waves, 26.1% under control conditions (n = 116 cardiomyocytes/13 patients), decreased after exposure to either A-803467 (to 8.4%, n = 90/9) or PF-01247324 (to 8.3%, n = 104/11, Fig. 6c). Also, SCN10A−/− mice (n = 67 cells/7 mice) had a reduced Ca²⁺ wave frequency as well as a reduced fraction of cardiomyocytes with arrhythmic events compared to WT (n = 80 cells/7 mice, Fig. 6d–g). Pharmacological NaV1.8 inhibition with PF-01247324 exerted no effects in SCN10A−/− mice (n = 67 cells/7 mice and 64/7), but significantly decreased Ca²⁺ wave frequency and the percentage of cells with arrhythmic events in WT (n = 70 cells/7 mice, Fig. 6f–g). IₙaL inhibition by TTX caused no further effects.
compared to Na\textsubscript{v}1.8 ablation (n = 68 cells/7 mice) or inhibition (n = 67 cells/7 mice Fig. 6f–g).

**Inhibition of Na\textsubscript{v}1.8 reduces proarrhythmic triggers in atrial cardiomyocytes**

Given that \(I_{\text{NaL}}\)-dependent diastolic SR Ca\textsuperscript{2+} release can induce an NCX mediated depolarizing current leading to cellular arrhythmias, we tested the effects of Na\textsubscript{v}1.8 on afterdepolarizations and spontaneous action potentials. The incidence of EADs in human atrial cardiomyocytes (3.1 ± 0.9/min, n = 14 cardiomyocytes/4 patients) could be significantly reduced by inhibiting Na\textsubscript{v}1.8 with either A-803467 (0.6 ± 0.4/min, n = 11/4) or PF-01247324 (0.4 ± 0.1/min, n = 11/4, Fig. 7a, b). Moreover, DADs and spontaneous action potentials during rest (13.6 ± 2.7/min in control, n = 11/4) were significantly less common when Na\textsubscript{v}1.8 was inhibited (A-803467: 4.5 ± 1.5/min, n = 9/4, PF-01247324: 5.5 ± 1.6/min, n = 10/4, Fig. 7c, d). Likewise, Na\textsubscript{v}1.8 inhibition by PF-01247324 (n = 13 cells/8 mice) strongly suppressed DAD occurrence and spontaneous action potentials during rest compared to control in WT (n = 14 cells/8 mice). Application of PF-01247324 (n = 13 cells/10 mice) had no effect in SCN10A\textsuperscript{−/−} mice, which
show an already reduced incidence of arrhythmic events ($n=13$ cells/10 mice, Fig. 7e, f). Thus, Na$_v$1.8 inhibition markedly prevented cellular arrhythmias in human and murine atrial cardiomyocytes.

**SCN10A$^{-/-}$ mice are protected against AF induction**

The role of Na$_v$1.8 for in vivo arrhythmias was investigated using SCN10A$^{-/-}$ mice and respective WT. After transjugular vein catheterization, five episodes of atrial burst stimulation were performed in anaesthetized mice (Fig. 8a). Electrocardiograms of SCN10A$^{-/-}$ mice showed no changes in cardiac conduction and repolarization compared to WT (Suppl. Table 1). In WT mice undergoing atrial burst stimulation AF was inducible in all animals ($n=5$ mice). However, only in two out of eight SCN10A$^{-/-}$ mice AF could be induced indicating a significantly lower susceptibility to AF ($n=8$ mice, Fig. 8b). Moreover, the AF duration after respective burst episodes was markedly shorter in SCN10A$^{-/-}$ mice ($12.0 \pm 3.8$ s) compared to WT ($33.2 \pm 5.5$ s, Fig. 8c). These data demonstrate that Na$_v$1.8 ablation is protective against AF.
induction and thereby confirm its arrhythmic potency in an in vivo system.

**Discussion**

This study comprehensively investigated Na\(_v\)1.8 in human atrial myocardium and its role in cellular electrophysiology and arrhythmogenesis. We could detect relevant Na\(_v\)1.8 mRNA and protein levels in the human atrium. While pharmacological Na\(_v\)1.8 modulation showed no significant effects on action potentials, it depicted a contribution to \(I_{\text{Na}}\) generation and thereby to diastolic SR Ca\(^{2+}\) leak in human atrial cardiomyocytes. Importantly, selective inhibition of Na\(_v\)1.8 with two agents potently reduced cellular arrhythmogenic triggers. These findings could be confirmed in mice lacking Na\(_v\)1.8 (SCN10A\(^{-/-}\)). Finally, in vivo studies revealed that SCN10A\(^{-/-}\) mice are protected against AF induction.

We not only found that Na\(_v\)1.8 is expressed in the human atrium but could show that mRNA and protein expression is higher in atrial compared to ventricular myocardium. The presence of Na\(_v\)1.8 in the human atria was indirectly suggested by genome-wide association studies (GWAS) reporting that the SCN10A gene (encoding Na\(_v\)1.8) impacts atrial conduction, in particular PR interval and P wave duration [8, 18]. Data from mice further support our findings by showing a higher Na\(_v\)1.8 expression in the atria compared to the ventricle [34]. Of note, one previous study reported a generally lower Na\(_v\)1.8 mRNA expression in the atria compared to other Na\(_v\) isoforms [19] and other studies described difficulties in the detection of Na\(_v\)1.8, which may be due to a high
Recent genetic studies demonstrated an involvement of SCN10A in atrial cellular electrophysiology and could associate SCN10A variants with AF [17, 18, 25]. We therefore investigated whether NaV1.8 compared to NaV1.5 expression might be differentially regulated in patients with SR or with AF. However, we observed no differences in NaV1.8 protein or mRNA expression levels between SR and AF myocardium.

We therefore investigated human atrial cardiomyocytes from patients with sinus rhythm to elucidate the cellular role of NaV1.8 in the human atria. In patch clamp experiments, pharmacological inhibition of NaV1.8 did not change APA, RMP or \( dv/dt \) in human atrial cardiomyocytes, which could be confirmed in SCN10A−/− mice. Since \( dv/dt \) is a surrogate for the fast Na⁺ influx and hence peak Na⁺ current [5], these observations suggest that the involvement of NaV1.8 in the peak Na⁺ current is negligible and therefore atrial conduction may not be affected. We observed a trend towards a reduced APD after NaV1.8 inhibition, which however did not reach statistical significance. Thus, while we previously showed a distinct APD abbreviation upon NaV1.8 inhibition in ventricular cardiomyocytes [10], the impact on atrial APD appears minor. However, APD is abbreviated in AF due to NaV1.8 inhibition [16]. SCN10A variants associated with AF were also found to modulate \( I_{NaL} \) after transfection in ND7/23 cells, which further strengthens findings about the role of NaV1.8 for \( I_{NaL} \) [23]. Of note, we observed a clear trend towards a further \( I_{NaL} \) reduction in SCN10A−/− cardiomyocytes after exposure to TTX suggesting that other NaV isoforms still contribute to \( I_{NaL} \) generation. Since \( I_{NaL} \) directly impacts atrial arrhythmogenesis [3, 13, 28], we consecutively evaluated whether specific NaV1.8 inhibition could prevent cellular arrhythmias. We have previously shown that in the human atrium \( I_{NaL} \)-mediated Na⁺ influx can induce Ca²⁺ influx via reverse-mode NCX leading to an increased cytosolic [Ca²⁺] and an enhanced incidence of Ca²⁺ sparks [13]. In the present study, selective inhibition or ablation of NaV1.8 markedly suppressed SR Ca²⁺ spark frequency and the total calculated diastolic Ca²⁺ leak in atrial cardiomyocytes. Most importantly, the incidence of major diastolic Ca²⁺ release events like Ca²⁺ waves, which are considered as a proarrhythmic trigger, was significantly blunted after NaV1.8 inhibition/ablation. Interestingly, \( I_{NaL} \) inhibition by TTX showed similar antiarrhythmic effects compared to NaV1.8 inhibition/ablation. Thus, NaV1.8-dependent \( I_{NaL} \) inhibition alone might be sufficient enough for disrupting...
the vicious circle of $I_{\text{nat}}$-dependent SR Ca$^{2+}$ leak. The electrogenic exchange of Ca$^{2+}$ against Na$^+$ via NCX can induce a transient inward current ($I_{\text{t}}$) leading to depolarization of the cell, which serves as a trigger for spontaneous action potentials [32]. In human atrial cardiomyocytes, both Na$\nu_1$1.8 blockers significantly diminished the incidence of EADs and prevented the generation of DADs and spontaneous action potentials during rest. Accordingly, SCN10A$^{-/-}$ mice and PF-01247324-treated WT cells also showed a lower incidence of triggered activity. Cellular afterdepolarizations as well as irregular action potentials are considered as a potent underlying mechanism for triggered ectopic activity/ectopic firing, which may promote and/or maintain atrial arrhythmias [16].

To translate our cellular experimental findings in an in vivo model, we here demonstrate that SCN10A$^{-/-}$ mice were protected against AF induction by rapid pacing and the duration of induced AF was significantly shorter in these mice. Ca$^{2+}$ sparks and DAD-related ectopic activity have been shown to trigger ectopic beats, re-entry mechanisms [7] and may also lead to dispersion of repolarization, which further increases the susceptibility to arrhythmias/AF [31]. Accordingly, Ca$^{2+}$ sparks and DAD-related ectopic activity could previously be linked to pacing induced AF in mice [20, 27]. Thus, our in vivo data in SCN10A$^{-/-}$ mice may serve as a translation of our mechanistic findings into an in vivo system.

Using genetic ablation, the proarrhythmic role of Na$\nu_1$1.8 in the absence of pharmacological approaches and also our findings based on the Na$\nu_1$1.8 inhibitor PF-01247324 could be confirmed. Interestingly, few association studies in patients with early onset AF also report that SCN10A variants are associated with AF susceptibility [17, 23]. Of note, as Na$\nu_1$1.8 was discussed to modulate cardiac conduction [6, 29] the influence of SCN10A expressed in cardiac neurons/ganglia [30] may theoretically contribute to our in vivo findings. However, we demonstrate a distinct functional proarrhythmogenic role of Na$\nu_1$1.8 on human and murine cardiomyocyte level. Notably, Na$\nu_1$1.8 did not change $dv/dt$ and amplitude of action potentials in atrial cardiomyocytes in our study as well as in ventricular cardiomyocytes [1, 10]. In addition, the QRS complex in the ECG was also unchanged in SCN10A$^{-/-}$ mice. In sharp contrast, Na$\nu_1$1.5 inhibition (e.g., by flecainide) and reduction of peak Na$^+$ influx causing changes in cardiac conduction can adversely affect mortality by promoting arrhythmogenic mechanisms [11].

We propose Na$\nu_1$1.8-dependent selective $I_{\text{nat}}$ reduction and prevention of atrial arrhythmogenesis to constitute a novel antiarrhythmic approach in the human, in particular for atrial arrhythmias involving focal and/or ectopic activity. Importantly, the current study investigated atrial cardiomyocytes from patients with sinus rhythm (or murine atrial cardiomyocytes) stressed with isoproterenol. From a clinical point of view, patients with permanent or long-standing AF, which are characterized by advanced structural atrial remodeling, are likely not the optimal patients for a pharmacological rhythm strategy. Therefore, we believe that atrial samples from patients at high risk for triggered/ectopic activity or paroxysmal AF may be more appropriate to investigate from a translational point of view. Nevertheless, Na$\nu_1$1.8 dysregulation might also have functional implications in long-standing AF.

Taken together, the herein presented functional evidence of Na$\nu_1$1.8 in human atrial cardiomyocytes and, most importantly, the potent antiarrhythmic effects of Nav1.8 inhibition and deletion in vitro and in vivo, could lay the foundation development towards a novel therapeutic option for atrial rhythm disorders.

Acknowledgements Open Access funding provided by Projekt DEAL. We gratefully acknowledge the technical assistance of D. Riedl, Y. Metz, T. Schulte and J. Heine. We thank Prof. John Wood (Institute for Biomedical Research, University College London, UK) for providing us the SCN10A$^{-/-}$ mice.

Funding This work was supported by the University Hospital Regensburg (ReFoRm C and A programs) (to L.S.M., S.S. and J.M. respectively); by the German Heart Foundation/German Foundation of Heart Research (S.S. and PB); by the Marga und Walter Boll-Stiftung (to S.A. and S.S.); by the German Society of Internal Medicine (S.P.); by the Else Kröner-Fresenius Stiftung (S.P.); by the German Cardiac Society (M.K.); and the Deutsche Forschungsgemeinschaft (DFG, SFB 1002) (to N.D. and G.H.).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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