



Inhibition of Na_v1.8 prevents atrial arrhythmogenesis in human and mice

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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 (Na_v1.8) in atrial electrophysiology. This study investigated the role and involvement of Na_v1.8 (SCN10A) in arrhythmia generation in the human atria and in mice lacking Na_v1.8. Na_v1.8 mRNA and protein were detected in human atrial myocardium at a significant higher level compared to ventricular myocardium. Expression of Na_v1.8 and Na_v1.5 did not differ between myocardium from patients with atrial fibrillation and sinus rhythm. To determine the electrophysiological role of Na_v1.8, we investigated isolated human atrial cardiomyocytes from patients with sinus rhythm stimulated with isoproterenol. Inhibition of Na_v1.8 by A-803467 or PF-01247324 showed no effects on the human atrial action potential. However, we found that Na_v1.8 significantly contributes to late Na⁺ current and consequently to an increased proarrhythmic diastolic sarcoplasmic reticulum Ca²⁺ leak in human atrial cardiomyocytes. Selective pharmacological inhibition of Na_v1.8 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 Na_v1.8). Pharmacological Na_v1.8 inhibition showed no effects in SCN10A^{-/-} mice. Importantly, in vivo experiments in SCN10A^{-/-} mice showed that genetic ablation of Na_v1.8 protects against atrial fibrillation induction. This study demonstrates that Na_v1.8 is expressed in the murine and human atria and contributes to late Na⁺ current generation and cellular arrhythmogenesis. Blocking Na_v1.8 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

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Introduction

Atrial arrhythmias, in particular atrial fibrillation (AF), contribute to the morbidity and mortality of western societies [4]. However, pharmacological therapeutic options are still

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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 $[\text{Na}^+]$ an enhanced I_{NaL} may lead to $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX)-mediated Ca^{2+} overload [26]. Consecutively, this induces arrhythmogenic Ca^{2+} release events (Ca^{2+} sparks) from the sarcoplasmic reticulum (SR) during diastole [12, 13]. Increasing diastolic Ca^{2+} levels may promote a depolarizing inward current (I_{ti}), 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 ($\text{Na}_v1.5$) are the predominant isoform in the heart [14], recent evidence suggested the involvement of SCN10A sodium channels ($\text{Na}_v1.8$) in atrial conduction [8]. Moreover, genome-wide association studies showed that variants of $\text{Na}_v1.8$ 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 $\text{Na}_v1.8$ in the human

and murine atria. Moreover, we studied the involvement of $\text{Na}_v1.8$ 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 $\text{Na}_v1.8$ 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

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 ($n = 10$) ^a
Male sex (%)	73.5	50.0	N/A
Age (Mean \pm SEM, y)	64.56 \pm 1.55	75.2 \pm 2.55	N/A
EF (Mean \pm SEM, %)	56.89 \pm 1.95	51.25 \pm 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 \pm SEM or n (%). Clinical data could not be completely obtained from every patient

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

^aBlinded 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 1, 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- $\text{Na}_v1.8$ antibodies (1:1,000, LSBio, LS-C109037), rabbit polyclonal anti- $\text{Na}_v1.5$ (1:2,000, Alomone 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 $\text{Na}_v1.8$, isolated cardiomyocytes were treated with either A-803467 (30 nmol/L, Sigma) or PF-01247324 (1 $\mu\text{mol/L}$, 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 $\mu\text{mol/l}$) 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 M Ω 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 Ca^{2+} spark measurements

Isolated atrial cardiomyocytes were loaded with the Ca^{2+} indicator Fluo 4-AM (10 $\mu\text{mol/L}$ 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 Ca^{2+} spark measurements were obtained with a laser scanning confocal microscope (Zeiss). Line scans were recorded during rest after loading the sarcoplasmic reticulum with Ca^{2+} by continuous field stimulation at 1 Hz. Ca^{2+} 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 \pm 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 Na_v1.8 in human atrial myocardium

To investigate whether 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 Na_v1.8 in the human atria. Moreover, 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 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 Na_v1.8 or the major cardiac sodium channel isoform 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 Na_v1.8 (SR: *n* = 14 patients, AF: *n* = 14 patients) nor 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 Na_v1.8 mRNA was lower compared to Na_v1.5, we found no changes between SR versus AF for Na_v1.8 (SR: *n* = 8 patients, AF: *n* = 8). 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, Na_v1.8 was confirmed to be present in the human atria without being regulated in patients with AF.

Effects of Na_v1.8 on the cardiac action potential

To investigate the effect of 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 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 Na_v1.8 has negligible effects on peak Na⁺ current (Fig. 2b, c). Also, RMP (-77.1 ± 2.3 mV) was not changed after Na_v1.8 inhibition (A-803467: -75.6 ± 2.3 mV; PF-01247324: -73.9 ± 2.5 mV, Fig. 2d). APD at 50% repolarization (APD₅₀) 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, APD₉₀ was slightly abbreviated (APD₉₀; 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 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 Na_v1.8 has no effects on APA (Fig. 3b), *dv/dt* (Fig. 3c), RMP (Fig. 3d) as well as APD₅₀ (Fig. 3e) or APD₉₀ (Fig. 3f). Accordingly, we observed no effects of pharmacological Na_v1.8 inhibition using PF-01247324 in SCN10A^{-/-} and WT. These experiments indicate that Na_v1.8 has negligible effects on the human and murine atrial action potential, which is of importance for further translational studies.

Role of Na_v1.8 for generation of I_{NaL}

The contribution of Na_v1.8 in I_{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 Na_v1.8 inhibition caused a significant reduction of I_{NaL} by $44.9 \pm 13.5\%$ after exposure to A-803467 (*n* = 12 cardiomyocytes/4 patients) and by $53.5 \pm 12.7\%$ 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_{NaL} compared to WT (*n* = 8 cells/ 4 mice, Fig. 4c–e). While pharmacological

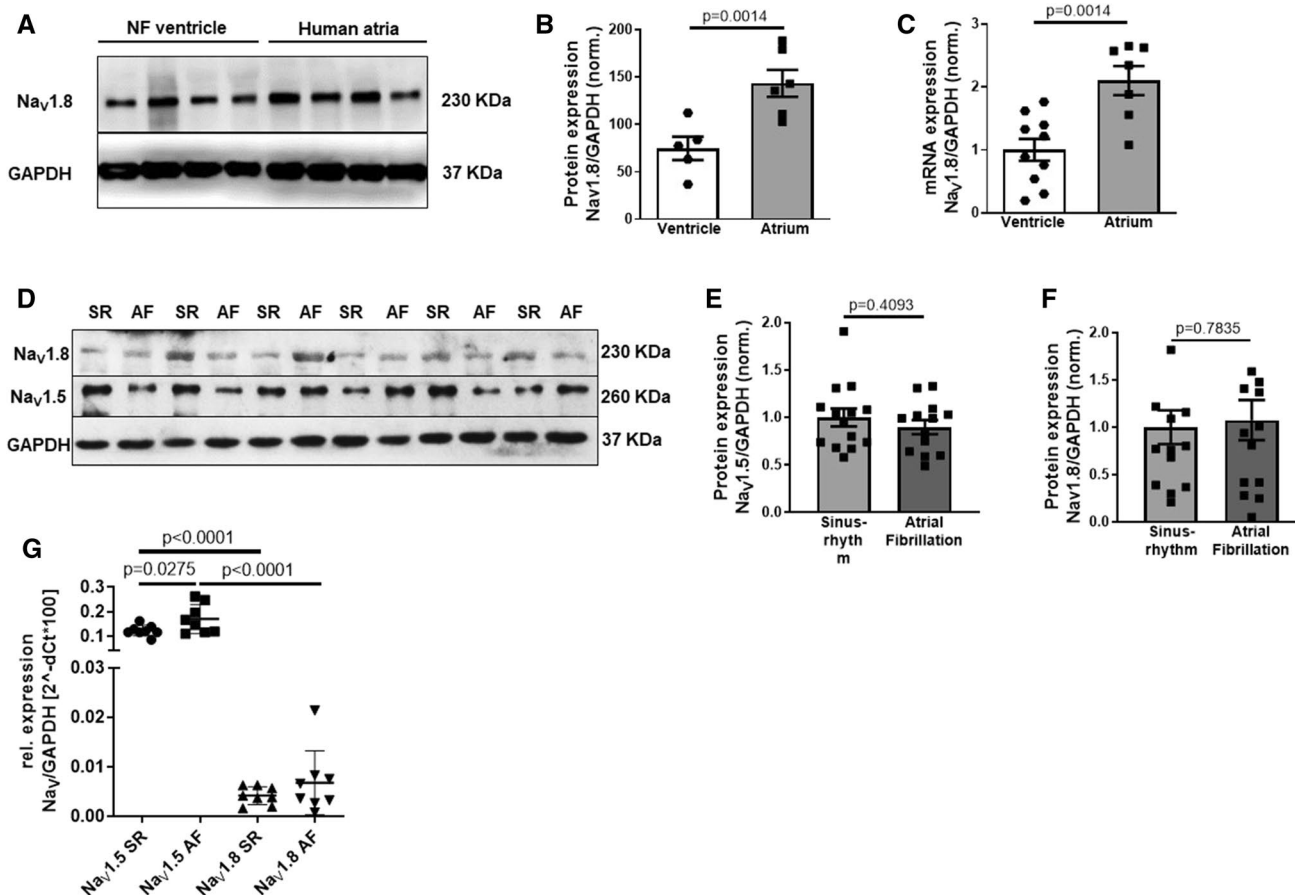


Fig. 1 Expression of $\text{Na}_v1.8$ in the human atrium. Data are presented as scatter plot with mean \pm SEM. *P* values were calculated using unpaired Student's *t* test. **a** Original Western blot for $\text{Na}_v1.8$ expression in atrial myocardium from patients with sinus rhythm compared to ventricular myocardium from non-failing donors (NF). **b** Normalized densitometry data comparing the protein expression of $\text{Na}_v1.8$ in human atria ($n=6$) and human ventricle ($n=5$). GAPDH was used as an internal loading control in all blots. **c** Normalized mRNA expression of $\text{Na}_v1.8/\text{GAPDH}$ in human atrial myocardium ($n=7$ patients) compared to ventricular myocardium from healthy subjects ($n=10$

patients). **d** Original Western Blot for $\text{Na}_v1.8$ and $\text{Na}_v1.5$ protein in human atria from patients with sinus rhythm (SR) or atrial fibrillation (AF). **e** Normalized densitometry data from Western Blots using atrial myocardium from patients with SR or AF showing the protein expression of $\text{Na}_v1.5$ (SR: $n=14$ patients, AF: $n=13$) and **f**) $\text{Na}_v1.8$ (SR: $n=14$ patients, AF: $n=14$). GAPDH was used as an internal loading control in all blots. **g** Normalized mRNA expression of $\text{Na}_v1.5/\text{GAPDH}$ (SR: $n=8$ patients, AF: $n=8$) and $\text{Na}_v1.8/\text{GAPDH}$ (SR: $n=8$ patients, AF: $n=8$) in human atrial myocardium from SR compared to AF

$\text{Na}_v1.8$ inhibition by PF-01247324 ($n=7$ cells/ 5 mice) exerted no effect on I_{NaL} in $\text{SCN10A}^{-/-}$, I_{NaL} could be significantly reduced by application of PF-01247324 in atrial WT cardiomyocytes ($n=7$ cells/ 4 mice, Fig. 4c–e). To determine the contribution of $\text{Na}_v1.8$ to I_{NaL} generation, we also performed measurements with TTX ($2 \mu\text{mol/L}$) to globally inhibit I_{NaL} . We observed a trend towards a lower I_{NaL} in TTX-treated $\text{SCN10A}^{-/-}$ cardiomyocytes and also compared to PF-01247324-treated WT cardiomyocytes (Fig. 4e) suggesting that other Na_v -dependent I_{NaL} is still relevant under these conditions.

Role of $\text{Na}_v1.8$ for sarcoplasmic Ca^{2+} -leak generation

It is well known that I_{NaL} can potentially induce arrhythmogenic diastolic SR Ca^{2+} release events [13]. To investigate whether the reduction of I_{NaL} caused by $\text{Na}_v1.8$ 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 $3.2 \pm 0.5 \times 100/\mu\text{m/s}$ ($n=84$ cardiomyocytes/13 patients) which could be significantly attenuated to 1.2 ± 0.2

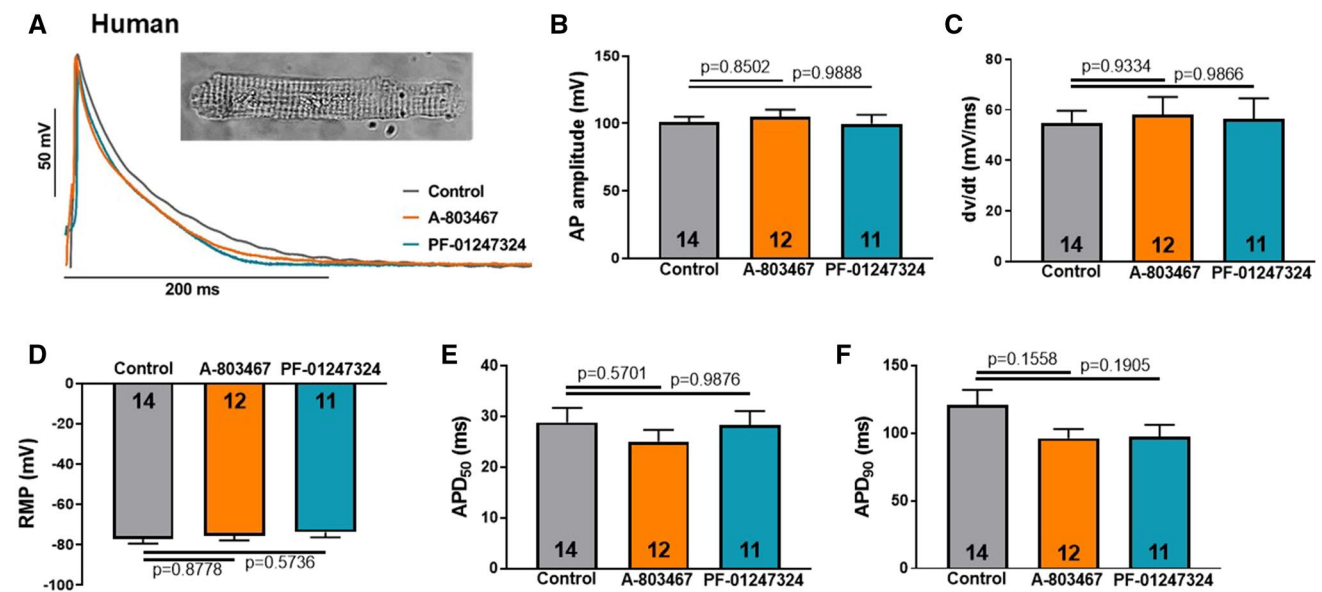


Fig. 2 Effects of $\text{Na}_V1.8$ on the human atrial action potential. Data are presented as mean \pm SEM. *P* values were computed using one-way ANOVA with Tukey's test for multiple comparisons. **a** Representative action potential recordings (1 Hz stimulation). Inset: isolated human atrial cardiomyocyte. **b** Effects of $\text{Na}_V1.8$ 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_{50}) and **(f)** 90% repolarization (APD_{90})

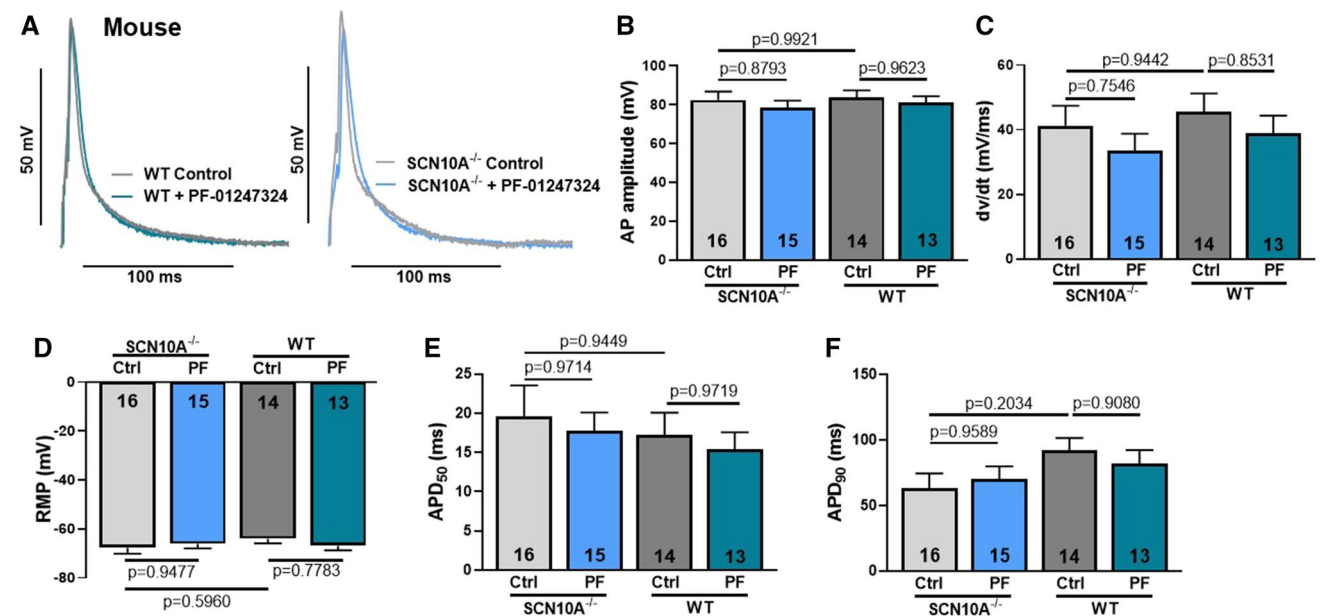


Fig. 3 Effects of $\text{Na}_V1.8$ on the murine atrial action potential using $\text{SCN10A}^{-/-}$ and WT mice. Data are presented as mean \pm SEM. *P* values were calculated using one-way ANOVA with Tukey's test for multiple comparisons. **a** Representative action potential recordings (1 Hz stimulation) of isolated murine atrial cardiomyocytes. **b** Effects of genetic ablation of $\text{Na}_V1.8$ ($\text{SCN10A}^{-/-}$; $n=16$ cells/10

mice) compared to WT ($n=14$ cells/8 mice) and effects of pharmacological inhibition of $\text{Na}_V1.8$ by PF-01247324 in each genotype ($\text{SCN10A}^{-/-}$: $n=15$ 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_{50}) and **(f)** 90% repolarization (APD_{90})

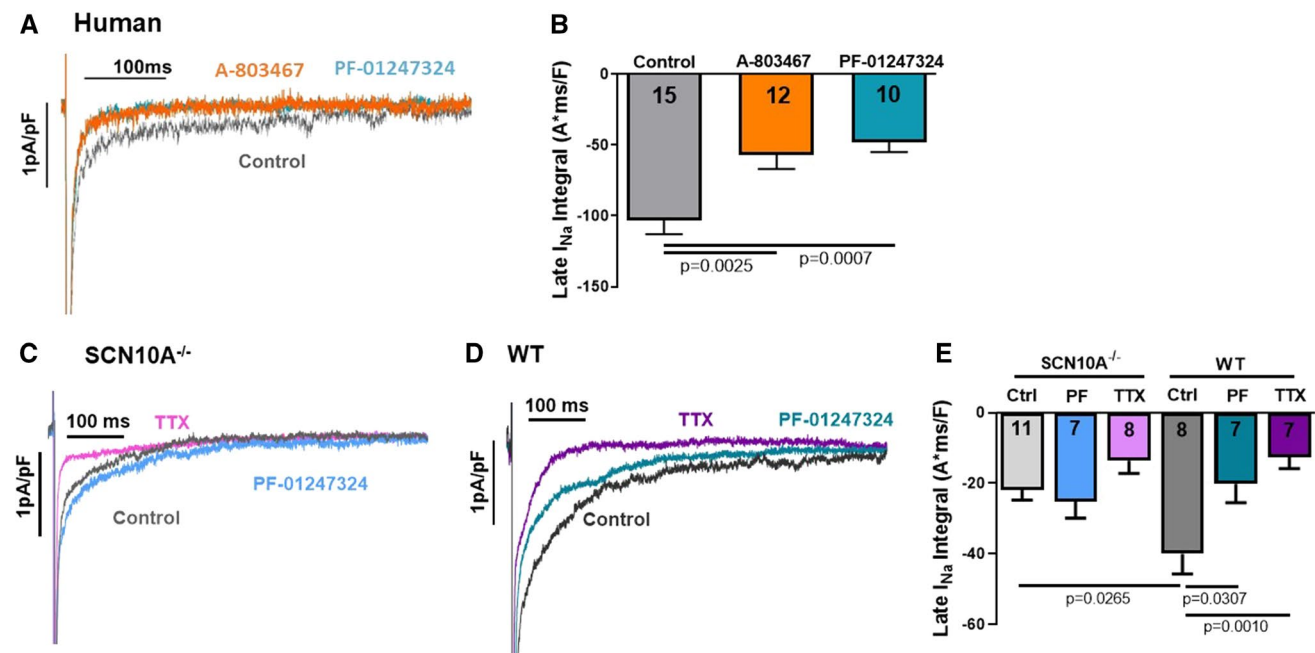


Fig. 4 Role of $\text{Na}_V1.8$ in I_{NaL} generation. Values are reported as mean \pm SEM. One-way ANOVA with Tukey's test for multiple comparisons was used to calculate P values. **a** Original traces of I_{NaL} in human atrial cardiomyocytes and **(b)** mean data of I_{NaL} (integral 100–500 ms) showing the effects of $\text{Na}_V1.8$ inhibition with either A-803467 ($n=12$ cardiomyocytes/4 patients) or PF-01247324 ($n=10/4$) on I_{NaL} compared to control ($n=15/6$). **c** Original I_{NaL} traces representing I_{NaL} in $\text{SCN10A}^{-/-}$ mice as well as **(d)** WT and

I_{NaL} after $\text{Na}_V1.8$ inhibition by PF-01247324 and tetrodotoxin treatment respectively. **e** Mean values of I_{NaL} (integral 100–500 ms) showing the effects of genetic ablation of $\text{Na}_V1.8$ ($\text{SCN10A}^{-/-}$; $n=11$ cells/5 mice) compared to WT ($n=8$ cells/4 mice) and effects of pharmacological inhibition of $\text{Na}_V1.8$ by PF-01247324 ($\text{SCN10A}^{-/-}$; $n=7$ cells/5 mice and WT: $n=7/4$) and TTX ($\text{SCN10A}^{-/-}$; $n=8$ cells/5 mice and WT: $n=7/4$) in each genotype

$\times 100/\mu\text{m/s}$ after addition of A-803467 ($n=73/9$) or PF-01247324 to $1.1 \pm 0.2 \times 100/\mu\text{m/s}$ ($n=88/11$, Fig. 5a, b). Moreover, the calculated diastolic SR Ca^{2+} leak was reduced by $64.3 \pm 22.2\%$ after addition of A-803467 and by $80.6 \pm 20.6\%$ 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 $\text{SCN10A}^{-/-}$ mice ($n=57$ cells/7 mice, Fig. 5d) showed a lower frequency of diastolic Ca^{2+} sparks as well as a lower diastolic Ca^{2+} leak compared to WT cells ($n=57$ cells/7 mice, Fig. 5e–g). While PF-01247324 ($n=62$ cells/7 mice) and TTX ($n=61$ cells/7 mice) treated cardiomyocytes had a significantly reduced Ca^{2+} spark frequency and Ca^{2+} leak in WT, both drugs had no further effects in $\text{SCN10A}^{-/-}$ cardiomyocytes ($n=57$ cells/7 mice and 64/7 respectively). Interestingly, we observed no further antiarrhythmic effect of TTX compared to $\text{Na}_V1.8$ inhibition alone. Thus, the reduction of both I_{NaL} and diastolic SR Ca^{2+} release reveal the significant role of $\text{Na}_V1.8$ for cellular arrhythmogenesis in the human atria. Of note, we observed no effects of pharmacological $\text{Na}_V1.8$ inhibition on systolic Ca^{2+} transient amplitude and SR Ca^{2+} load (Suppl. Figs. 1, 2).

Blocking $\text{Na}_V1.8$ suppresses diastolic Ca^{2+} waves

To elucidate potential antiarrhythmic effects of $\text{Na}_V1.8$ inhibition, we have studied the effects of pharmacological inhibition and genetic ablation of $\text{Na}_V1.8$ on the incidence of diastolic Ca^{2+} waves, which are major diastolic Ca^{2+} 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 Ca^{2+} waves (Fig. 6a, b). Also, the percentage of cells developing diastolic Ca^{2+} 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, $\text{SCN10A}^{-/-}$ mice ($n=67$ cells/7 mice) had a reduced Ca^{2+} 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 $\text{Na}_V1.8$ inhibition with PF-01247324 exerted no effects in $\text{SCN10A}^{-/-}$ mice ($n=67$ cells/7 mice), but significantly decreased Ca^{2+} wave frequency and the percentage of cells with arrhythmic events in WT ($n=70$ cells/7 mice, Fig. 6f–g). I_{NaL} inhibition by TTX caused no further effects

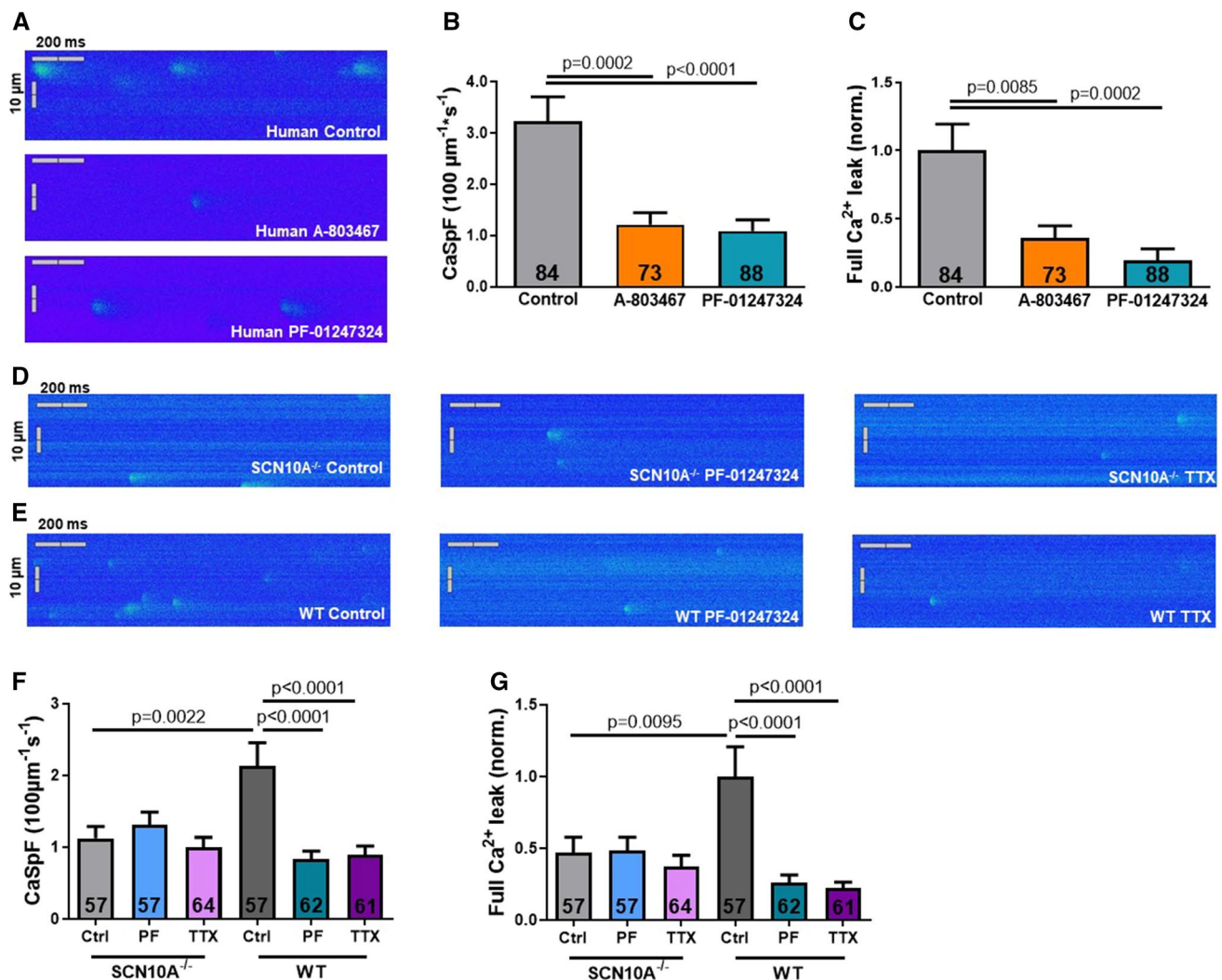


Fig. 5 Relevance of $\text{Na}_v1.8$ for diastolic sarcoplasmic Ca^{2+} leak. Values are given as mean \pm SEM. One-way ANOVA with Tukey's test for multiple comparisons was used to calculate *P* values. **a** Representative confocal line scan images of human atrial cardiomyocytes loaded with the Ca^{2+} indicator Fluo-4 showing Ca^{2+} sparks during diastole. **b** Mean values of the frequency of Ca^{2+} sparks (CaSpF) and (**c**) the total calculated diastolic Ca^{2+} leak in atrial cardiomyocytes ($n=84$ cardiomyocytes/13 patients) and after blocking $\text{Na}_v1.8$ with

A-803467 ($n=73/9$) or PF-01247324 ($n=88/11$). **d** Original line scan images of murine atrial cardiomyocytes from SCN10A^{-/-} and (**e**) WT mice. **f** Mean values of CaSpF and (**g**) the total calculated diastolic Ca^{2+} leak in SCN10A^{-/-} ($n=57$ cells/7 mice) and WT ($n=57$ cells/7 mice) mice and, respectively, effects of $\text{Na}_v1.8$ inhibition by PF-01247324 (SCN10A^{-/-}: $n=57$ cells/7 mice and WT: $n=62/7$) and TTX (SCN10A^{-/-}: $n=64$ cells/7 mice and WT: $n=61/7$)

compared to $\text{Na}_v1.8$ ablation ($n=68$ cells/7 mice) or inhibition ($n=67$ cells/7 mice Fig. 6f–g).

Inhibition of $\text{Na}_v1.8$ reduces proarrhythmic triggers in atrial cardiomyocytes

Given that I_{NaL} -dependent diastolic SR Ca^{2+} release can induce an NCX mediated depolarizing current leading to cellular arrhythmias, we tested the effects of $\text{Na}_v1.8$ on afterdepolarizations and spontaneous action potentials. The incidence of EADs in human atrial cardiomyocytes ($3.1 \pm 0.9/\text{min}$, $n=14$ cardiomyocytes/4 patients)

could be significantly reduced by inhibiting $\text{Na}_v1.8$ with either A-803467 ($0.6 \pm 0.4/\text{min}$, $n=11/4$) or PF-01247324 ($0.4 \pm 0.1/\text{min}$, $n=11/4$, Fig. 7a, b). Moreover, DADs and spontaneous action potentials during rest ($13.6 \pm 2.7/\text{min}$ in control, $n=11/4$) were significantly less common when $\text{Na}_v1.8$ was inhibited (A-803467: $4.5 \pm 1.5/\text{min}$, $n=9/4$, PF-01247324: $5.5 \pm 1.6/\text{min}$, $n=10/4$, Fig. 7c, d). Likewise, $\text{Na}_v1.8$ 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^{-/-} mice, which

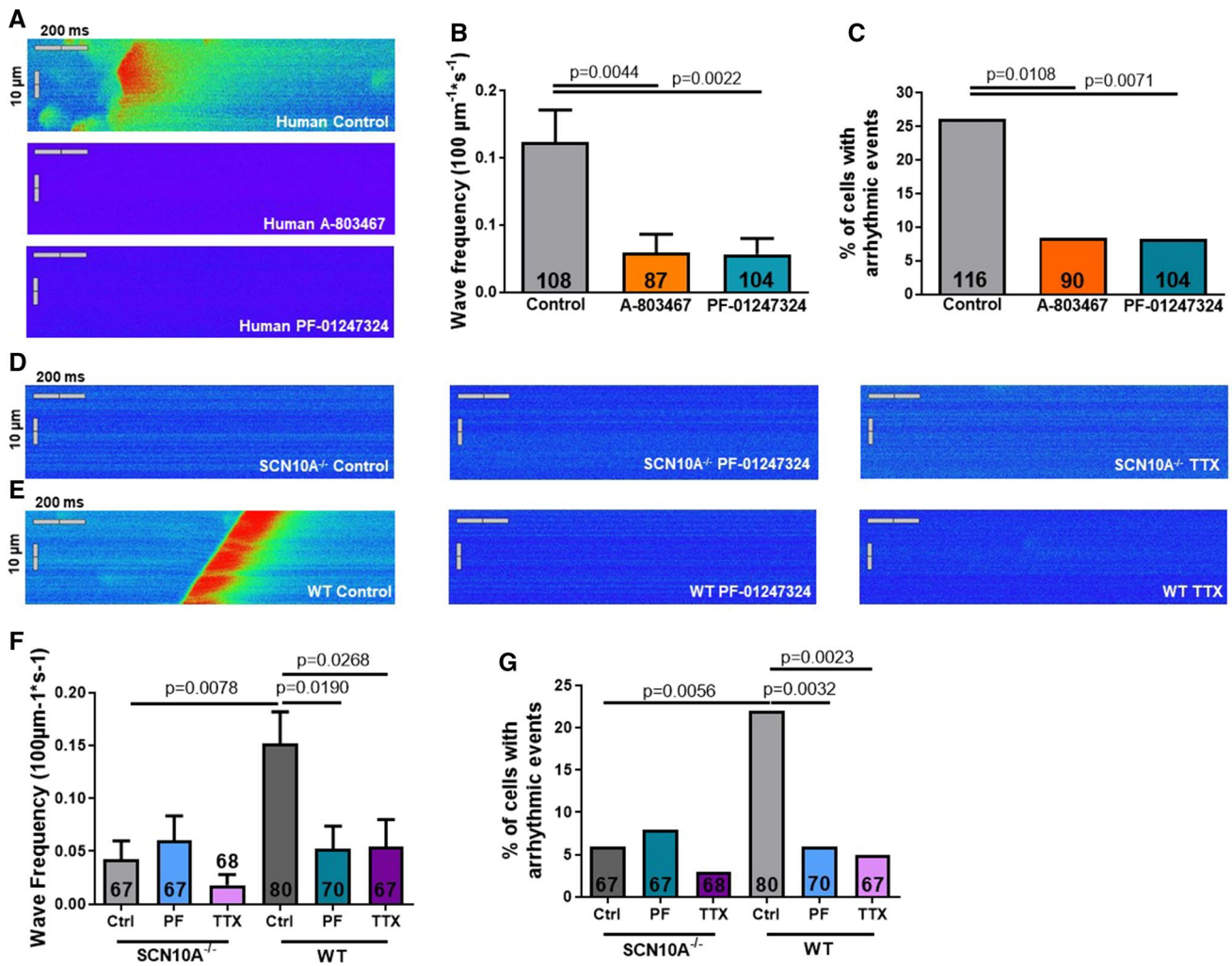


Fig. 6 Effects of Na_v1.8 on diastolic Ca²⁺ waves. Data are presented as mean ± SEM. P values were calculated using one-way ANOVA with Tukey’s test for multiple comparisons or Fisher’s exact test (for c, g). **a** Confocal line scan images of human atrial cardiomyocytes loaded with the Ca²⁺ indicator Fluo-4 representing the occurrence of major diastolic arrhythmogenic Ca²⁺ release events. **b** Mean frequency of Ca²⁺ waves in control (n = 108 cardiomyocytes/13 patients) and after treatment with A-803467 (n = 87/9) or PF-01247324 (n = 104/11). **c** Percentage of cells developing arrhythmic events (Ca²⁺ waves or spontaneous transients; n = 24 of 116 cardiomyo-

cytes/13 patients) and effects of Na_v1.8 blockade with A-803467 (n = 7 of 90/9 patients) or PF-01247324 (n = 8 of 104/11 patients). **d** Representative original line scans of murine atrial cardiomyocytes from SCN10A^{-/-} and (e) WT mice. **f** Mean values of Ca²⁺ wave frequency and (g) proportion of cells showing arrhythmic events in SCN10A^{-/-} (n = 67 cells/7 mice) and WT (n = 80 cells/7 mice) mice and effects of Na_v1.8 inhibition by PF-01247324 (SCN10A^{-/-}: n = 67 cells/7 mice and WT: n = 70/7) and TTX (SCN10A^{-/-}: n = 68 cells/7 mice and WT: n = 67/7) in both genotypes

show an already reduced incidence of arrhythmic events (n = 13 cells/10 mice, Fig. 7e, f). Thus, Na_v1.8 inhibition markedly prevented cellular arrhythmias in human and murine atrial cardiomyocytes.

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

The role of Na_v1.8 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 ± 3.8 s) compared to WT (33.2 ± 5.5 s, Fig. 8c). These data demonstrate that Na_v1.8 ablation is protective against AF

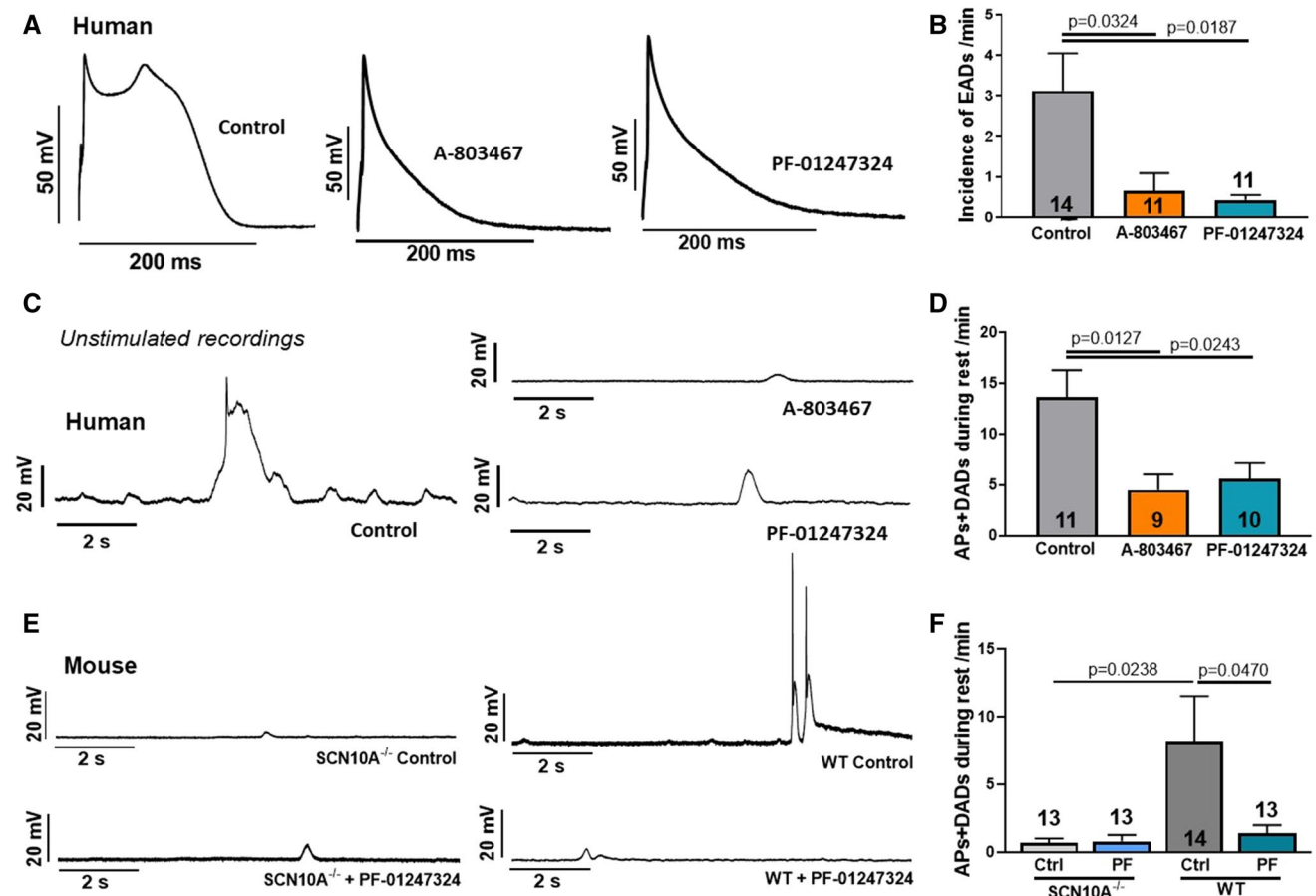


Fig. 7 Effects of $\text{Na}_V1.8$ inhibition on cellular arrhythmogenic trigger. Data are reported as mean \pm SEM. *P* values were calculated using one-way ANOVA with Tukey's test for multiple comparisons. **a** Action potential recordings (1 Hz) representing the occurrence of an early afterdepolarization (EAD). **b** Incidence of EADs/min ($n=14$ cardiomyocytes/4 patients) and effects of inhibiting $\text{Na}_V1.8$ with A-803467 ($n=11/4$) or PF-01247324 ($n=11/4$). **c** Original unstimulated recordings of human atrial cardiomyocytes during 10 s rest after a series of 30 stimulated action potentials (1 Hz). **d** Mean

incidence of spontaneous action potentials (APs) and delayed afterdepolarizations (DADs) during rest ($n=11/4$) and after treatment with A-803467 ($n=9/4$) or PF-01247324 ($n=10/4$). **e** Original unstimulated recordings of murine atrial cardiomyocytes during 10 s rest after a series of 30 stimulated action potentials (1 Hz) from $\text{SCN10A}^{-/-}$ and WT mice. **f** Mean values of spontaneous APs and DADs during rest in $\text{SCN10A}^{-/-}$ ($n=13$ cells/ 10 mice) and WT ($n=14$ cells/ 8 mice) and effects of PF-01247324 in $\text{SCN10A}^{-/-}$ ($n=13$ cells/ 10 mice) and WT ($n=13$ cells/ 8 mice)

induction and thereby confirm its arrhythmic potency in an in vivo system.

Discussion

This study comprehensively investigated $\text{Na}_V1.8$ in human atrial myocardium and its role in cellular electrophysiology and arrhythmogenesis. We could detect relevant $\text{Na}_V1.8$ mRNA and protein levels in the human atrium. While pharmacological $\text{Na}_V1.8$ modulation showed no significant effects on action potentials, it depicted a contribution to I_{NaL} generation and thereby to diastolic SR Ca^{2+} leak in human atrial cardiomyocytes. Importantly, selective inhibition of $\text{Na}_V1.8$ with two agents potently reduced cellular arrhythmogenic triggers. These findings could be confirmed

in mice lacking $\text{Na}_V1.8$ ($\text{SCN10A}^{-/-}$). Finally, in vivo studies revealed that $\text{SCN10A}^{-/-}$ mice are protected against AF induction.

We not only found that $\text{Na}_V1.8$ 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 $\text{Na}_V1.8$ in the human atria was indirectly suggested by genome-wide association studies (GWAS) reporting that the SCN10A gene (encoding $\text{Na}_V1.8$) impacts atrial conduction, in particular PR interval and *P* wave duration [8, 18]. Data from mice further support our findings by showing a higher $\text{Na}_V1.8$ expression in the atria compared to the ventricle [34]. Of note, one previous study reported a generally lower $\text{Na}_V1.8$ mRNA expression in the atria compared to other Na_V isoforms [19] and other studies described difficulties in the detection of $\text{Na}_V1.8$, which may be due to a high

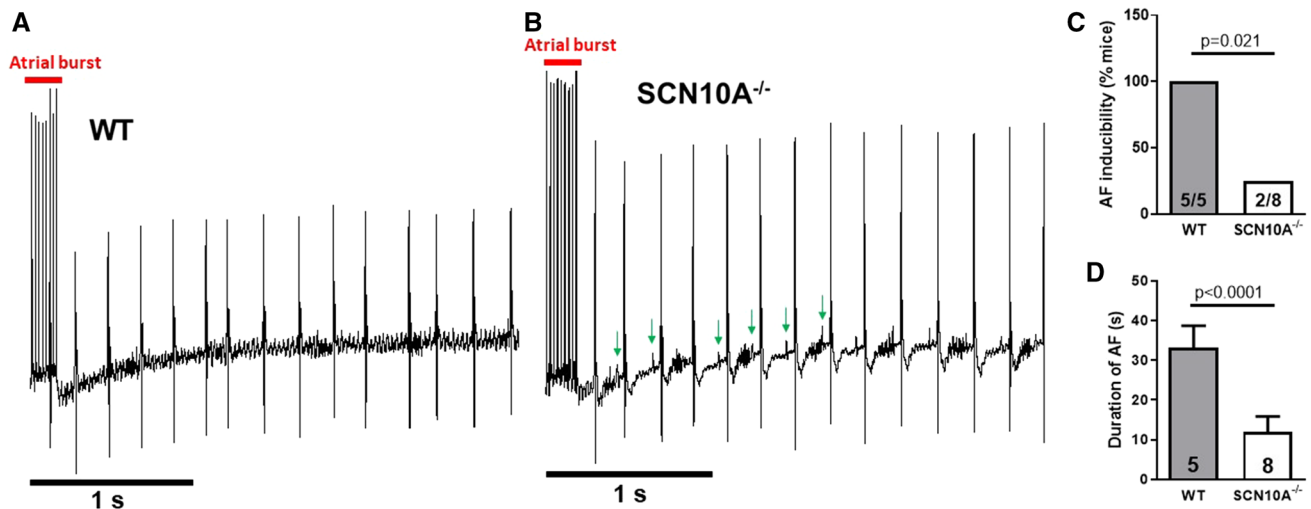


Fig. 8 $SCN10A^{-/-}$ mice are protected against AF induction. Data are presented as mean \pm SEM. **a**, **b** Representative electrocardiogram recordings of wild type (WT) and $SCN10A^{-/-}$ mice undergoing burst stimulation protocol. Arrows indicate regular P waves. **c** Percentage

of inducible WT ($n=5$) or $SCN10A^{-/-}$ mice ($n=8$). *P* value was calculated using Fisher's exact test. **d** AF duration after burst stimulation (5 episodes/mice) in WT ($n=5$) and $SCN10A^{-/-}$ mice ($n=8$). *P* value was calculated using Mann–Whitney test

rate of alternate splicing [6, 9]. 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 $Na_V1.8$ compared to $Na_V1.5$ expression might be differentially regulated in patients with SR or with AF. However, we observed no differences in $Na_V1.8$ 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 $Na_V1.8$ in the human atria. In patch clamp experiments, pharmacological inhibition of $Na_V1.8$ 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 $Na_V1.8$ in the peak Na^+ current is negligible and therefore atrial conduction may not be affected. We observed a trend towards a reduced APD after $Na_V1.8$ inhibition, which however did not reach statistical significance. Thus, while we could previously show a distinct APD abbreviation upon $Na_V1.8$ inhibition in ventricular cardiomyocytes [10], the impact on atrial APD appears minor. However, APD is abbreviated in AF and APD shortening may not be a suitable strategy for the treatment of AF [16]. A critical issue is that many previous experimental reports on AF treatment strategies investigated permanent AF atria with a very short action potential. However, patients with permanent or long-standing AF are probably not suitable patients for a pharmacological rhythm strategy due to advanced remodeling. Since atrial APD is differentially regulated in different cardiac diseases, i.e.,

atrial APD is prolonged in patients with left-ventricular dysfunction [24], further patient-specific studies are needed.

We here demonstrate that pharmacological and genetic $Na_V1.8$ inhibition markedly reduced I_{NaL} in human and murine atrial cardiomyocytes. Previous studies in animal ventricular cardiomyocytes by Yang et al. and in human ventricular cardiomyocytes by our group described a reduction of I_{NaL} as well as an abbreviation of APD due to $Na_V1.8$ inhibition [10, 34]. *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 $Na_V1.8$ 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 Na_V isoforms still contribute to I_{NaL} generation. Since I_{NaL} directly impacts atrial arrhythmogenesis [3, 13, 28], we consecutively evaluated whether specific $Na_V1.8$ inhibition could prevent cellular arrhythmias. We have previously shown that in the human atrium I_{NaL} -mediated Na^+ influx can induce Ca^{2+} influx via reverse-mode NCX leading to an increased cytosolic $[Ca^{2+}]$ and an enhanced incidence of Ca^{2+} sparks [13]. In the present study, selective inhibition or ablation of $Na_V1.8$ markedly suppressed SR Ca^{2+} spark frequency and the total calculated diastolic Ca^{2+} leak in atrial cardiomyocytes. Most importantly, the incidence of major diastolic Ca^{2+} release events like Ca^{2+} waves, which are considered as a proarrhythmic trigger, was significantly blunted after $Na_V1.8$ inhibition/ablation. Interestingly, I_{NaL} inhibition by TTX showed similar antiarrhythmic effects compared to $Na_V1.8$ inhibition/ablation. Thus, $Na_V1.8$ -dependent I_{NaL} inhibition alone might be sufficient enough for disrupting

the vicious circle of I_{NaL} -dependent SR Ca^{2+} leak. The electrogenic exchange of Ca^{2+} against Na^+ via NCX can induce a transient inward current (I_{ti}) leading to depolarization of the cell, which serves as a trigger for spontaneous action potentials [32]. In human atrial cardiomyocytes, both $\text{Na}_v1.8$ blockers significantly diminished the incidence of EADs and prevented the generation of DADs and spontaneous action potentials during rest. Accordingly, $\text{SCN10A}^{-/-}$ 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 $\text{SCN10A}^{-/-}$ 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 $\text{SCN10A}^{-/-}$ mice may serve as a translation of our mechanistic findings into an in vivo system.

Using genetic ablation, the proarrhythmic role of $\text{Na}_v1.8$ in the absence of pharmacological approaches and also our findings based on the $\text{Na}_v1.8$ 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 $\text{Na}_v1.8$ 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 proarrhythmic role of $\text{Na}_v1.8$ on human and murine cardiomyocyte level. Notably, $\text{Na}_v1.8$ 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 $\text{SCN10A}^{-/-}$ mice. In sharp contrast, $\text{Na}_v1.5$ 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 $\text{Na}_v1.8$ -dependent selective I_{NaL} 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, $\text{Na}_v1.8$ dysregulation might also have functional implications in long-standing AF.

Taken together, the herein presented functional evidence of $\text{Na}_v1.8$ in human atrial cardiomyocytes and, most importantly, the potent antiarrhythmic effects of $\text{Nav}1.8$ inhibition and deletion in vitro and in vivo, could lay the foundation development towards a novel therapeutic option for atrial rhythm disorders.

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Compliance with ethical standards

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

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References

- Ahmad S, Tirilomis P, Pabel S, Dybkova N, Hartmann N, Molina CE, Tirilomis T, Kutschka I, Frey N, Maier LS, Hasenfuss G, Streckfuss-Bomeke K, Sossalla S (2019) The functional consequences of sodium channel $\text{Na}_v1.8$ in human left ventricular hypertrophy. *ESC Heart Fail* 6:154–163. <https://doi.org/10.1002/ehf2.12378>

2. Akopian AN, Souslova V, England S, Okuse K, Ogata N, Ure J, Smith A, Kerr BJ, McMahon SB, Boyce S, Hill R, Stanfa LC, Dickenson AH, Wood JN (1999) The tetrodotoxin-resistant sodium channel SNS has a specialized function in pain pathways. *Nat Neurosci* 2:541–548. <https://doi.org/10.1038/9195>
3. Antzelevitch C, Belardinelli L (2006) The role of sodium channel current in modulating transmural dispersion of repolarization and arrhythmogenesis. *J Cardiovasc Electrophysiol* 17(Suppl 1):S79–s85. <https://doi.org/10.1111/j.1540-8167.2006.00388.x>
4. Benjamin EJ, Wolf PA, D'Agostino RB, Silbershatz H, Kannel WB, Levy D (1998) Impact of atrial fibrillation on the risk of death: the Framingham Heart Study. *Circulation* 98:946–952. <https://doi.org/10.1161/01.cir.98.10.946>
5. Berecki G, Wilders R, de Jonge B, van Ginneken AC, Verkerk AO (2010) Re-evaluation of the action potential upstroke velocity as a measure of the Na⁺ current in cardiac myocytes at physiological conditions. *PLoS ONE* 5:e15772. <https://doi.org/10.1371/journal.pone.0015772>
6. Chambers JC, Zhao J, Terracciano CM, Bezzina CR, Zhang W, Kaba R, Navaratnarajah M, Lotlikar A, Sehmi JS, Kooner MK, Deng G, Siedlecka U, Parasramka S, El-Hamamsy I, Wass MN, Dekker LR, de Jong JS, Sternberg MJ, McKenna W, Severs NJ, de Silva R, Wilde AA, Anand P, Yacoub M, Scott J, Elliott P, Wood JN, Kooner JS (2010) Genetic variation in SCN10A influences cardiac conduction. *Nat Genet* 42:149–152. <https://doi.org/10.1038/ng.516>
7. Chelu MG, Sarma S, Sood S, Wang S, van Oort RJ, Skapura DG, Li N, Santonastasi M, Muller FU, Schmitz W, Schotten U, Anderson ME, Valderrabano M, Dobrev D, Wehrens XH (2009) Calmodulin kinase II-mediated sarcoplasmic reticulum Ca²⁺ leak promotes atrial fibrillation in mice. *J Clin Invest* 119:1940–1951. <https://doi.org/10.1172/jci37059>
8. Christophersen IE, Magnani JW, Yin X, Barnard J, Weng LC, Arking DE, Niemeijer MN, Lubitz SA, Avery CL, Duan Q, Felix SB, Bis JC, Kerr KF, Isaacs A, Muller-Nurasyid M, Muller C, North KE, Reiner AP, Tinker LF, Kors JA, Teumer A, Petersmann A, Sinner MF, Buzkova P, Smith JD, Van Wagener DR, Volker U, Waldenberger M, Peters A, Meitinger T, Limacher MC, Wilhelmssen KC, Psaty BM, Hofman A, Uitterlinden A, Krijthe BP, Zhang ZM, Schnabel RB, Kaab S, van Duijn C, Rotter JI, Sotoodehnia N, Dorr M, Li Y, Chung MK, Soliman EZ, Alonso A, Whitsel EA, Stricker BH, Benjamin EJ, Heckbert SR, Ellinor PT (2017) Fifteen genetic loci associated with the electrocardiographic P wave. *Circ Cardiovasc Genet*. <https://doi.org/10.1161/circgenetics.116.001667>
9. Duff MO, Olson S, Wei X, Garrett SC, Osman A, Bolisetty M, Plocik A, Celniker SE, Graveley BR (2015) Genome-wide identification of zero nucleotide recursive splicing in *Drosophila*. *Nature* 521:376–379. <https://doi.org/10.1038/nature14475>
10. Dybkova N, Ahmad S, Pabel S, Tirilomis P, Hartmann N, Fischer TH, Bengel P, Tirilomis T, Ljubojevic S, Renner A, Gummert J, Ellenberger D, Wagner S, Frey N, Maier LS, Streckfuss-Bomeke K, Hasenfuss G, Sossalla S (2018) Differential regulation of sodium channels as a novel proarrhythmic mechanism in the human failing heart. *Cardiovasc Res* 114:1728–1737. <https://doi.org/10.1093/cvr/cvy152>
11. Echt DS, Liebson PR, Mitchell LB, Peters RW, Obias-Manno D, Barker AH, Arensberg D, Baker A, Friedman L, Greene HL et al (1991) Mortality and morbidity in patients receiving encainide, flecainide, or placebo. The Cardiac Arrhythmia Suppression Trial. *N Engl J Med* 324:781–788. <https://doi.org/10.1056/nejm199103213241201>
12. Eiringhaus J, Herting J, Schatter F, Nikolaev VO, Sprenger J, Wang Y, Köhn M, Zabel M, El-Armouche A, Hasenfuss G, Sossalla S, Fischer TH (2019) Protein kinase/phosphatase balance mediates the effects of increased late sodium current on ventricular calcium cycling. *Basic Res Cardiol* 114:13. <https://doi.org/10.1007/s00395-019-0720-7>
13. Fischer TH, Herting J, Mason FE, Hartmann N, Watanabe S, Nikolaev VO, Sprenger JU, Fan P, Yao L, Popov AF, Danner BC, Schondube F, Belardinelli L, Hasenfuss G, Maier LS, Sossalla S (2015) Late INa increases diastolic SR-Ca²⁺-leak in atrial myocardium by activating PKA and CaMKII. *Cardiovasc Res* 107:184–196. <https://doi.org/10.1093/cvr/cvv153>
14. Goldin AL (2001) Resurgence of sodium channel research. *Annu Rev Physiol* 63:871–894. <https://doi.org/10.1146/annurev.physiol.63.1.871>
15. Hartmann N, Pabel S, Herting J, Schatter F, Renner A, Gummert J, Schotola H, Danner BC, Maier LS, Frey N, Hasenfuss G, Fischer TH, Sossalla S (2017) Antiarrhythmic effects of dantrolene in human diseased cardiomyocytes. *Heart Rhythm* 14:412–419. <https://doi.org/10.1016/j.hrthm.2016.09.014>
16. Iwasaki YK, Nishida K, Kato T, Nattel S (2011) Atrial fibrillation pathophysiology: implications for management. *Circulation* 124:2264–2274. <https://doi.org/10.1161/circulationaha.111.019893>
17. Jabbari J, Olesen MS, Yuan L, Nielsen JB, Liang B, Macri V, Christophersen IE, Nielsen N, Sajadieh A, Ellinor PT, Grunnet M, Haunso S, Holst AG, Svendsen JH, Jespersen T (2015) Common and rare variants in SCN10A modulate the risk of atrial fibrillation. *Circ Cardiovasc Genet* 8:64–73. <https://doi.org/10.1161/hcg.0000000000000022>
18. Pfeufer A, van Noord C, Marcicante KD, Arking DE, Larson MG, Smith AV, Tarasov KV, Müller M, Sotoodehnia N, Sinner MF, Verwoert GC, Li M, Kao WHL, Köttgen A, Coresh J, Bis JC, Psaty BM, Rice K, Rotter JI, Rivadeneira F, Hofman A, Kors JA, Stricker BHC, Uitterlinden AG, van Duijn CM, Beckmann BM, Sauter W, Gieger C, Lubitz SA, Newton-Cheh C, Wang TJ, Magnani JW, Schnabel RB, Chung MK, Barnard J, Smith JD, Van Wagener DR, Vasani RS, Aspelund T, Eiriksdottir G, Harris TB, Launer LJ, Najjar SS, Lakatta E, Schlessinger D, Uda M, Abecasis GR, Müller-Miyhok B, Ehret GB, Boerwinkle E, Chakravarti A, Soliman EZ, Lunetta KL, Perz S, Wichmann HE, Meitinger T, Levy D, Gudnason V, Ellinor PT, Sanna S, Kääh S, Witteman JCM, Alonso A, Benjamin EJ, Heckbert SR (2010) Genome-wide association study of PR interval. *Nat Genet* 42:153–159. <https://doi.org/10.1038/ng.517>
19. Poulet C, Wettwer E, Grunnet M, Jespersen T, Fabritz L, Matschke K, Knaut M, Ravens U (2015) Late sodium current in human atrial cardiomyocytes from patients in sinus rhythm and atrial fibrillation. *PLoS ONE*. <https://doi.org/10.1371/journal.pone.0131432>
20. Purohit A, Rokita AG, Guan X, Chen B, Koval OM, Voigt N, Neef S, Sowa T, Gao Z, Luczak ED, Stefansdottir H, Behunin AC, Li N, El-Accaoui RN, Yang B, Swaminathan PD, Weiss RM, Wehrens XH, Song LS, Dobrev D, Maier LS, Anderson ME (2013) Oxidized Ca²⁺/calmodulin-dependent protein kinase II triggers atrial fibrillation. *Circulation* 128:1748–1757. <https://doi.org/10.1161/circulationaha.113.003313>
21. Ritchie MD, Denny JC, Zuvich RL, Crawford DC, Schildcrout JS, Bastarache L, Ramirez AH, Mosley JD, Pulley JM, Basford MA, Bradford Y, Rasmussen LV, Pathak J, Chute CG, Kullo IJ, McCarty CA, Chisholm RL, Kho AN, Carlson CS, Larson EB, Jarvik GP, Sotoodehnia N, Manolio TA, Li R, Masys DR, Haines JL, Roden DM (2013) Genome- and phenome-wide analyses of cardiac conduction identifies markers of arrhythmia risk. *Circulation* 127:1377–1385. <https://doi.org/10.1161/circulationaha.112.000604>
22. Ronchi C, Torre E, Rizzetto R, Bernardi J, Rocchetti M, Zaza A (2017) Late sodium current and intracellular ionic homeostasis in acute ischemia. *Basic Res Cardiol* 112:12. <https://doi.org/10.1007/s00395-017-0602-9>

23. Savio-Galimberti E, Weeke P, Muhammad R, Blair M, Ansari S, Short L, Atack TC, Kor K, Vanoye CG, Olesen MS, LuCamp YT, George JAL, Roden DM, Darbar D (2014) SCN10A/Nav1.8 modulation of peak and late sodium currents in patients with early onset atrial fibrillation. *Cardiovasc Res* 104:355–363. <https://doi.org/10.1093/cvr/cvu170>
24. Schmidt C, Wiedmann F, Zhou XB, Heijman J, Voigt N, Ratte A, Lang S, Kallenberger SM, Campana C, Weymann A, De Simone R, Szabo G, Ruhparwar A, Kallenbach K, Karck M, Ehrlich JR, Baczko I, Borggrefe M, Ravens U, Dobrev D, Katus HA, Thomas D (2017) Inverse remodelling of K2P3.1 K⁺ channel expression and action potential duration in left ventricular dysfunction and atrial fibrillation: implications for patient-specific antiarrhythmic drug therapy. *Eur Heart J* 38:1764–1774. <https://doi.org/10.1093/eurheartj/ehw559>
25. Sigurdsson MI, Saddic L, Heydarpour M, Chang TW, Shekar P, Aranki S, Couper GS, Shernan SK, Muehlschlegel JD, Body SC (2017) Post-operative atrial fibrillation examined using whole-genome RNA sequencing in human left atrial tissue. *BMC Med Genom.* <https://doi.org/10.1186/s12920-017-0270-5>
26. Song Y, Shryock JC, Belardinelli L (2008) An increase of late sodium current induces delayed afterdepolarizations and sustained triggered activity in atrial myocytes. *Am J Physiol Heart Circ Physiol* 294:H2031–2039. <https://doi.org/10.1152/ajpheart.01357.2007>
27. Sood S, Chelu MG, van Oort RJ, Skapura D, Santonastasi M, Dobrev D, Wehrens XH (2008) Intracellular calcium leak due to FKBP12.6 deficiency in mice facilitates the inducibility of atrial fibrillation. *Heart Rhythm* 5:1047–1054. <https://doi.org/10.1016/j.hrthm.2008.03.030>
28. Sossalla S, Kallmeyer B, Wagner S, Mazur M, Maurer U, Toischer K, Schmitto JD, Seipelt R, Schondube FA, Hasenfuss G, Belardinelli L, Maier LS (2010) Altered Na⁺ currents in atrial fibrillation effects of ranolazine on arrhythmias and contractility in human atrial myocardium. *J Am Coll Cardiol* 55:2330–2342. <https://doi.org/10.1016/j.jacc.2009.12.055>
29. Sotoodehnia N, Isaacs A, de Bakker PI, Dorr M, Newton-Cheh C, Nolte IM, van der Harst P, Muller M, Eijgelsheim M, Alonso A, Hicks AA, Padmanabhan S, Hayward C, Smith AV, Polasek O, Giovannone S, Fu J, Magnani JW, Marcianti KD, Pfeufer A, Gharib SA, Teumer A, Li M, Bis JC, Rivadeneira F, Aspelund T, Kottgen A, Johnson T, Rice K, Sie MP, Wang YA, Klopp N, Fuchsberger C, Wild SH, Mateo Leach I, Estrada K, Volker U, Wright AF, Asselbergs FW, Qu J, Chakravarti A, Sinner MF, Kors JA, Petersmann A, Harris TB, Soliman EZ, Munroe PB, Psaty BM, Oostra BA, Cupples LA, Perz S, de Boer RA, Uitterlinden AG, Volzke H, Spector TD, Liu FY, Boerwinkle E, Dominiczak AF, Rotter JJ, van Herpen G, Levy D, Wichmann HE, van Gilst WH, Witteman JC, Kroemer HK, Kao WH, Heckbert SR, Meitinger T, Hofman A, Campbell H, Folsom AR, van Veldhuisen DJ, Schwenbacher C, O'Donnell CJ, Volpato CB, Caulfield MJ, Connell JM, Launer L, Lu X, Franke L, Fehrmann RS, te Meerman G, Groen HJ, Weersma RK, van den Berg LH, Wijmenga C, Ophoff RA, Navis G, Rudan I, Snieder H, Wilson JF, Pramstaller PP, Siscovick DS, Wang TJ, Gudnason V, van Duijn CM, Felix SB, Fishman GI, Jamshidi Y, Stricker BH et al (2010) Common variants in 22 loci are associated with QRS duration and cardiac ventricular conduction. *Nat Genet* 42:1068–1076. <https://doi.org/10.1038/ng.716>
30. Verkerk AO, Remme CA, Schumacher CA, Scicluna BP, Wolswinkel R, de Jonge B, Bezzina CR, Veldkamp MW (2012) Functional Nav1.8 channels in intracardiac neurons: the link between SCN10A and cardiac electrophysiology. *Circ Res* 111:333–343. <https://doi.org/10.1161/circresaha.112.274035>
31. Voigt N, Dobrev D (2012) Cellular and molecular correlates of ectopic activity in patients with atrial fibrillation. *EP Europace* 14:97–105. <https://doi.org/10.1093/europace/eus282>
32. Voigt N, Li N, Wang Q, Wang W, Trafford AW, Abu-Taha I, Sun Q, Wieland T, Ravens U, Nattel S, Wehrens XH, Dobrev D (2012) Enhanced sarcoplasmic reticulum Ca²⁺ leak and increased Na⁺–Ca²⁺ exchanger function underlie delayed afterdepolarizations in patients with chronic atrial fibrillation. *Circulation* 125:2059–2070. <https://doi.org/10.1161/CIRCULATIONAHA.111.067306>
33. Wagner S, Maier LS, Bers DM (2015) Role of sodium and calcium dysregulation in tachyarrhythmias in sudden cardiac death. *Circ Res* 116:1956–1970. <https://doi.org/10.1161/circresaha.116.304678>
34. Yang T, Atack TC, Stroud DM, Zhang W, Hall L, Roden DM (2012) Blocking Scn10a channels in heart reduces late sodium current and is antiarrhythmic. *Circ Res* 111:322–332. <https://doi.org/10.1161/circresaha.112.265173>

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