Absence of Functional Na\textsubscript{v}1.8 Channels in Non-diseased Atrial and Ventricular Cardiomyocytes

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Abstract

\textbf{Purpose} Several studies have indicated a potential role for SCN10A/Na\textsubscript{v}1.8 in modulating cardiac electrophysiology and arrhythmia susceptibility. However, by which mechanism SCN10A/Na\textsubscript{v}1.8 impacts on cardiac electrical function is still a matter of debate. To address this, we here investigated the functional relevance of Na\textsubscript{v}1.8 in atrial and ventricular cardiomyocytes (CMs), focusing on the contribution of Na\textsubscript{v}1.8 to the peak and late sodium current (I\textsubscript{Na}) under normal conditions in different species.

\textbf{Methods} The effects of the Na\textsubscript{v}1.8 blocker A-803467 were investigated through patch-clamp analysis in freshly isolated rabbit left ventricular CMs, human left atrial CMs and human-induced pluripotent stem cell-derived CMs (hiPSC-CMs).

\textbf{Results} A-803467 treatment caused a slight shortening of the action potential duration (APD) in rabbit CMs and hiPSC-CMs, while it had no effect on APD in human atrial cells. Resting membrane potential, action potential (AP) amplitude, and AP upstroke velocity were unaffected by A-803467 application. Similarly, I\textsubscript{Na} density was unchanged after exposure to A-803467 and Na\textsubscript{v}1.8-based late I\textsubscript{Na} was undetectable in all cell types analysed. Finally, low to absent expression levels of SCN10A were observed in human atrial tissue, rabbit ventricular tissue and hiPSC-CMs.

\textbf{Conclusion} We here demonstrate the absence of functional Na\textsubscript{v}1.8 channels in non-diseased atrial and ventricular CMs. Hence, the association of SCN10A variants with cardiac electrophysiology observed in, e.g. genome wide association studies, is likely the result of indirect effects on SCN5A expression and/or Na\textsubscript{v}1.8 activity in cell types other than CMs.

\textbf{Keywords} SCN10A/Na\textsubscript{v}1.8 · Sodium channel · Patch-clamp · Cardiomyocytes · Late sodium current · hiPSC-CMs

Introduction

Sodium channels play a central role in the initiation and propagation of the action potential (AP) in excitable cells, including cardiomyocytes (CMs) and neurons. Cardiac sodium channel (Na\textsubscript{v}1.5) loss of function is a critical mediator of cardiac conduction slowing, predisposing to ventricular arrhythmias and sudden cardiac death (SCD), both in acquired pathologies (ischemia, hypertrophy, heart failure) and inherited cardiac disorders caused by mutations in SCN5A, the gene encoding Na\textsubscript{v}1.5 [1, 2]. On the other hand, impaired Na\textsubscript{v}1.5 inactivation may induce a small inward sodium current (I\textsubscript{Na}), the so-called late I\textsubscript{Na}, that persists during the plateau and repolarization phase of the AP. Enhanced late I\textsubscript{Na} can prolong AP duration (APD) and increase intracellular calcium (Ca\textsuperscript{2+}) via altered Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger activity, thus promoting arrhythmias [3]. While Na\textsubscript{v}1.5 is the main sodium channel isoform expressed in cardiac tissue, other sodium channel isoforms are also present in the heart, including Na\textsubscript{v}1.1/SCN1A, Na\textsubscript{v}1.2/SCN2A, Na\textsubscript{v}1.3/SCN3A and Na\textsubscript{v}1.6/SCN8A [4]. These isoforms are typically referred to as “neuronal” sodium channel isoforms due to their abundant expression and well-established function in neurons. While Na\textsubscript{v}1.5 is blocked only...
by micromolar concentrations of tetrodotoxin (TTX) [i.e. TTX-resistant], most neuronal isoforms are more TTX-sensitive and inhibited by nanomolar concentrations [4]. An exception is NaV1.8, encoded by the SCN10A gene. This isoform is mainly expressed in dorsal root ganglia, plays a role in pain perception [5] and is inhibited only by micromolar TTX concentrations, similar to NaV1.5 [4].

Several genome-wide association studies (GWAS) have suggested a role for SCN10A/NaV1.8 in modulating cardiac conduction parameters, such as PR and QRS interval [6–10]. SCN10A variants have also been associated with atrial fibrillation (AF) [11–13] and with Brugada syndrome [14–16], an inherited cardiac disease characterized by cardiac conduction slowing and increased risk for SCD. However, if and by which mechanism SCN10A/NaV1.8 impacts on cardiac electrical function is still a matter of debate. Inhibition of NaV1.8 by the blocker A-803467 has been reported to decrease late I_{Na} and shorten APD in mouse and rabbit cardiomyocytes [17], whereas we previously described the absence of functional NaV1.8 in murine cardiomyocytes [18]. Moreover, conflicting results have been reported in studies of mice deficient for Scn10a, with either similar or decreased APD observed in Scn10a−/− cardiomyocytes as compared to wild-type cardiomyocytes [17, 19]. We and others have shown that NaV1.8 is specifically expressed in murine, canine and human cardiac neurons [18, 20, 21], suggesting a function of the SCN10A gene product for cardiac conduction via modulation of AP firing in intracardiac neurons [18, 21, 22]. Additionally, the SCN10A variant rs6801957 has been shown to modulate SCN5A expression in cardiac tissue thereby potentially impacting on conduction [23]. Overall, the role of SCN10A/NaV1.8 in the heart and the mechanisms by which this gene and/or its gene product affects cardiac function remain only partially explained. In particular, electrophysiological studies in non-diseased human cardiomyocytes aimed at defining the physiological role of NaV1.8 in the human heart are limited. To address these issues, we here investigated the functional relevance of NaV1.8 in atrial and ventricular cardiomyocytes, focusing on the contribution of NaV1.8 to the peak and late I_{Na} under normal conditions in different species.

**Methods**

**Isolation of Rabbit Left Ventricular Cardiomyocytes**

Three–four-month-old male New Zealand White rabbits (Charles River Laboratories) were anaesthetized with 20 mg xylazine and 100 mg ketamine (intramuscularly) and heparinized with a bolus of 1000 IU heparin (intravenously). Subsequently, the animals were sacrificed, the thorax was opened and the heart was rapidly excised and connected to a Langendorff system. Left ventricular (LV) cardiomyocytes were isolated as previously described [24] (see Data Supplement).

**Isolation of Human Left Atrial Cardiomyocytes**

Human left atrial appendages (LAAs) were obtained from patients in sinus rhythm (SR) without a history of AF undergoing cardiac surgery (coronary bypass grafting or valve surgery) and included in the multicenter PREDICT AF study [25]. Patient characteristics are reported in Supplemental Table 1. Part of the LAA tissue was immediately frozen in liquid nitrogen to be subsequently used for molecular analysis, while the other part was transported to the laboratory on ice and single cells were obtained by an enzymatic isolation modified from Dobrev et al. [26]. An expanded Methods section is available in the Data Supplement.

**Differentiation of hiPSCs into Cardiomyocytes**

A human-induced pluripotent stem cell (hiPSC) control line (iC113) previously generated and characterized [27] was used to generate cardiomyocytes (hiPSC-CMs) by adaptation of a previously described protocol [28]. hiPSC-CMs were used for electrophysiological analysis and RT-PCR. An expanded Methods section is available in the Data Supplement.

**Electrophysiology**

**Data Acquisition and Analysis**

Membrane currents [(I_{Na}, late I_{Na} and L-type calcium currents (I_{CaL})) and APs were measured with the ruptured and perforated patch-clamp technique, respectively, using an Axopatch 200B amplifier (Molecular Devices, San Jose, CA, USA). Voltage control, data acquisition and analysis of currents and APs were performed with pClamp10.6/Clampfit (Molecular Devices, San Jose, CA, USA) or a custom-made software. Borosilicate glass patch pipettes (Harvard Apparatus, Holliston, MA, USA) with a tip resistance of 2–2.5 MΩ were used. Series resistance (Rs) and cell membrane capacitance (Cm) were compensated for 80%. Peak I_{Na}, I_{CaL} and APs were filtered at 5 kHz. I_{Na} and APs were digitized at 40 kHz, while I_{CaL} was digitized at 20 kHz. Finally, late I_{Na} was filtered and digitized at 2 kHz and 1 kHz, respectively.

**Sodium Current Measurements**

Peak I_{Na} and late I_{Na} were measured in single cells using a pipette solution containing (in mM) 3.0 NaCl, 133 CsCl, 2.0 MgCl2, 2.0 Na2ATP, 2.0 TEACl, 10 EGTA, 5.0 HEPES; pH 7.2 (CsOH). For late I_{Na} measurements, hiPSC-CMs, rabbit and human CMs were superfused with a bath solution...
containing (in mM) 130 NaCl, 10 CsCl, 1.8 CaCl₂, 1.2 MgCl₂, 11.0 glucose, 5.0 HEPES, 0.005 nifedipine; pH 7.4 (CsOH). For peak \( I_{\text{Na}} \) recordings, a similar bath solution was used with the exception of a lower NaCl concentration for proper voltage control. Hence, NaCl was replaced by CsCl (for rabbit and human CMs, we used 7 mM NaCl and 133 mM CsCl; for hiPSC-CMs: 20 mM NaCl and 120 mM CsCl). Peak \( I_{\text{Na}} \) was measured at room temperature in response to depolarizing voltage steps from a holding potential of \(-120\, \text{mV} \) (cycle length of 5 s). \( I_{\text{Na}} \) was defined as the difference between peak and steady-state current (at 500 ms). Voltage dependence of activation and inactivation curves was fitted with Boltzmann function \( (v = [1 + \exp.\{-(V-V_{1/2})/k]\}]^{-1}) \), where \( V_{1/2} \) is the half-maximal voltage of (in) activation and \( k \), the slope factor. \( Na_{\alpha} \)-based late \( I_{\text{Na}} \) and total late \( I_{\text{Na}} \) were measured at 36 °C, as A-803467 (100 nM) and TTX (30 \( \mu \)M)-sensitive currents, respectively, using descending ramp protocols (cycle length of 5 s) as depicted in Fig. 2 and Supplemental Fig. 4. Current densities were calculated by dividing current amplitude by \( C_m \). \( C_m \) was determined by dividing the decay time constant of the capacitive transient in response to 5 mV hyperpolarizing steps from \(-40\, \text{mV} \), by the Rs. Potentials for peak \( I_{\text{Na}} \) and late \( I_{\text{Na}} \) recordings were not corrected for the estimated change in liquid junction potential. The \( Na_{\alpha} \)-1.8 channel inhibitor A-803467 (Tocris Bioscience, Bristol, United Kingdom) was solubilized in DMSO at a stock solution of 10 mM and diluted to the final concentration of 100 nM before use. This dose was chosen based on previous IC₅₀ data and to ensure maximal inhibition of \( Na_{\alpha} \)-1.8-based current [29].

**L-Type Calcium Current Measurements**

\( I_{\text{Ca,l}} \) was measured in isolated rabbit left ventricular CMs at 36 °C. An expanded Methods section is available in the Data Supplement.

**Action Potential Measurements**

In single rabbit left ventricular CMs, human left atrial (LA) CMs and hiPSC-CMs, APs were measured at 36 °C using a modified Tyrode’s solution containing (in mM) 140 NaCl, 5.4 KCl, 1.8 CaCl₂, 1.0 MgCl₂, 5.5 glucose, 5 HEPES, pH 7.4 (NaOH). Pipettes were filled with (in mM) 125 K-gluconate, 20 KCl, 5 NaCl, 0.44 amphotericin-B, 10 HEPES, pH 7.2 (KOH). APs were elicited at 1 Hz by 3 ms, \( \approx 1.2\times \) threshold current pulses through the patch pipette. Typically, hiPSC-CMs have a small or even complete lack of the inward rectifying potassium current (\( I_{\text{K,1}} \)). Consequently, their resting membrane potential (RMP) is depolarized and they are frequently spontaneously active [30]. To overcome these conditions, which limit the functional availability of \( I_{\text{Na}} \), transient outward potassium current and L-type Ca²⁺ current [31], we injected an in silico \( I_{\text{K,1}} \) with kinetics of Kir2.1 channels through dynamic clamp [32]. Thus, cells became quiescent with a RMP of around \(-80\, \text{mV} \). We analysed RMP, AP amplitude (APA), maximal AP upstroke velocity (\( V_{\text{max}} \)) and AP duration (APD) at 50% and 90% repolarization (APD₉₀ and APD₅₀, respectively). Data from 10 consecutive APs were averaged and potentials were corrected for the calculated liquid junction potential of 15 mV [33].

**Real-Time Polymerase Chain Reactions**

Total RNA was isolated from left atrial appendages of five patients undergoing cardiac surgeries using TRIzol Reagent (Invitrogen, Waltham, MA, USA) and from hiPSC-CMs obtained from four independent differentiations using NucleoSpin RNA (MACHEREY-NAGEL ref.: 740955.50, Duren, Germany) following manufacture protocol. cDNA was synthesized from total RNA by SuperScript™ II Reverse Transcriptase (Invitrogen, Waltham, MA, USA). Real-time PCR was performed on the platform of Light Cycler 480 (Roche, Basel, Switzerland) using SYBR green I master mix (Roche, Basel, Switzerland) and the sets of primers reported in Supplemental Table 2. Gene expression was determined according to linear regression analysis using LinRegPCR software and normalized by the expression of hypoxanthine phosphoribosyltransferase (HPRT).

**RNA Sequencing Data Analysis**

SCN5A and SCN10A expression in human right and left atria [34], ventricular and atrial hiPSC-CMs [35] and rabbit left ventricular tissue were extrapolated from the RNA sequencing (RNA-Seq) datasets GSE31999, GSE111007 and GSE115605, respectively, which are publicly available online https://www.ncbi.nlm.nih.gov/geo/. For the analyses, read counts for SCN5A and SCN10A transcripts were normalized to millions of total reads generated per sample and to SCN5A (ENST00000413689.1) and SCN10A (ENST00000449082.2) transcript size (i.e. Fragment Per Kilobase Million, FPKM).

**Statistical Analysis**

Values are shown as mean ± SEM. Paired Student’s \( t \) test, unpaired Student’s \( t \) test, one-way repeated measures ANOVA followed by Holm-Sidak test for post hoc analyses and two-way repeated measures ANOVA were used when appropriate. Mann-Whitney \( U \) test and one-way repeated measures ANOVA on Ranks (Friedman test) followed by Tukey test for post hoc analyses were used for data not
We next investigated the effects of A-803467 on late Na\textsubscript{V}1.8-based late CaL recordings under physiological conditions (baseline), in the presence of 100 nM A-803467 and after wash-out of the drug. On average, maximal upstroke velocity, AP amplitude and resting membrane potential were not affected by A-803467 exposure (Fig. 1b, d, f, Supplemental Table 3). In rabbit ventricular CMs, we observed a small, yet significant, AP shortening induced by A-803467 treatment (APD reduction of 4.8% for APD\textsubscript{50} and of 3.5% for APD\textsubscript{90}, Fig. 1b, Supplemental Table 3). However, as shown in detail in Supplemental Fig. 1a and b, the observed AP reduction was (partly) reversible upon wash-out only in a minority of cells. In the majority of cases, either A-803467 did not affect AP or the effect was non-reversible. Moreover, time-matched control experiments in rabbit ventricular CMs showed APD\textsubscript{50} and APD\textsubscript{90} shortening occurring over time similar to that observed with A-803467 (Supplemental Figs. 2 and 3). In human left atrial CMs, exposure to A-803467 did not change APD (Fig. 1e, f, Supplemental Fig. 1e, f and Supplemental Table 3), and in hiPSC-CMs, only APD\textsubscript{50} was significantly reduced but not APD\textsubscript{90} (Fig. 1c, d, Supplemental Table 3). The effect of A-803467 was reversible upon wash-out in the majority of hiPSC-CMs, but the blocker did not affect APD in all cells (Supplemental Fig. 1c and d).

**Absence of Na\textsubscript{V}1.8-Based Late I\textsubscript{Na} in Ventricular and Atrial Cardiomyocytes**

We next investigated the effects of A-803467 on late I\textsubscript{Na} in rabbit left ventricular CMs, hiPSC-CMs and human left atrial CMs using descending ramps after a 200 ms pre-pulse to 40 mV (see inset of Fig. 2a, c, e). The advantage of using a ramp protocol instead of a single step protocol is that the ramp protocol allows measurements of late I\textsubscript{Na} across a dynamic voltage range simulating a plateau and repolarization phase of an AP [36]. Figure 2 a, c, and e show typical examples of Na\textsubscript{V}1.8-based late I\textsubscript{Na} recordings under basal conditions (baseline, black line) and after 5 min wash-in of 100 nM A-803467 (red line). Na\textsubscript{V}1.8-dependent late I\textsubscript{Na}, measured as A-803467-sensitive current, was obtained by subtraction of the current recorded in the presence of A-803467 from the current recorded in the absence of the compound. Na\textsubscript{V}1.8-dependent late I\textsubscript{Na} was not detected in any of the three cell types (Fig. 2b, d, f). In a subset of rabbit left ventricular CMs, A-803467 perfusion was followed by 30 \mu M TTX application, and total late I\textsubscript{Na} was measured as TTX-sensitive current obtained by subtraction of the current recorded in the presence of TTX from the current recorded earlier in the absence of TTX (Supplemental Fig. 4). Average total late I\textsubscript{Na} was around −0.2 pA/pF, while the A-803467 sensitive current was undetectable. Hence, these experiments demonstrate that functional Na\textsubscript{V}1.8-based late I\textsubscript{Na} is absent under basal conditions in atrial and ventricular cardiomyocytes.

### Absence of Na\textsubscript{V}1.8-Based Peak I\textsubscript{Na} in Ventricular and Atrial Cardiomyocytes

In addition to late I\textsubscript{Na}, we also investigated the effects of A-803467 on peak I\textsubscript{Na} density and voltage dependency of activation and inactivation. Figure 3a, c, e shows typical peak I\textsubscript{Na} recordings obtained from rabbit left ventricular CMs, hiPSC-CMs and human left atrial CMs under basal conditions (baseline) and after 5 min wash-in of 100 nM A-803467. Average peak I\textsubscript{Na} densities were unchanged after exposure to A-803467 in all cell types analysed (Fig. 3b, d, f, Supplemental Table 4).

I\textsubscript{Na} voltage dependence of activation and inactivation, assessed as the half voltage of (in)activation (V\textsubscript{1/2}) and the slope factor k, was not affected by A-803467 in rabbit CMs and hiPSC-CMs (Fig. 4a–d, Supplemental Table 4). A-803467 caused a small negative shift in V\textsubscript{1/2} of activation and inactivation (−2.8 mV for both activation and inactivation curve) in human LA cardiomyocytes (V\textsubscript{1/2} activation −40.5 ± 1.1 mV vs −43.3 ± 1.2 mV, p < 0.05, paired Student’s t test; V\textsubscript{1/2} inactivation −90.6 ± 0.9 mV vs −93.4 ± 1.0 mV, p < 0.05, paired Student’s t test; Supplemental Table 4). Although significant, the biological meaning of such a small change is questionable. Moreover, in a subset of cells where wash-out experiments were also performed, we were unable to reverse these effects of A-803467, and a further negative shift of V\textsubscript{1/2} of (in)activation was observed upon wash-out of the compound (Supplemental Fig. 5). These results suggest a time-dependent shift of (in)activation, rather than a A-803467-dependent effect on I\textsubscript{Na} kinetics [37]. Taken together, these findings demonstrate the absence of functional Na\textsubscript{V}1.8-based peak I\textsubscript{Na} in atrial and ventricular cardiomyocytes.

To explore a potential off-target effect of A-803467, we also measured its effects on the L-type calcium current (I\textsubscript{Ca,L}) in rabbit left ventricular CMs. Typical example of I\textsubscript{Ca,L} traces recorded under basal conditions (baseline) and in the presence
of A-803467 are shown in Supplemental Fig. 6a. Exposure to A-803467 affected neither $I_{CaL}$ density (Supplemental Fig. 6b, Supplemental Table 5) nor $I_{CaL}$ voltage dependence of activation and inactivation (Supplemental Fig. 6c, Supplemental Table 5).
Low SCN10A mRNA Transcript Levels in hiPSC-CMs and in Human Left Atrial Appendages

We finally assessed the mRNA expression levels of SCN10A in hiPSC-CMs and human LAAs using quantitative real-time PCR (RT-PCR). In LAAs tissue, RT-PCR was performed on the same samples used for AP and late I\textsubscript{Na} measurements. As expected, both hiPSC-CMs and LAAs tissue showed robust expression of the cardiac sodium channel isoform SCN5A (relative to the reference gene HPRT). In contrast, SCN10A transcript levels were very low in both hiPSC-CMs and human LAAs (Fig. 5). Similarly, low to almost absent expression of SCN10A as compared to SCN5A was observed in online RNA-Seq datasets of rabbit left ventricular tissue (GSE115605) (Fig. 6a), atrial and ventricular hiPSC-CMs (GSE111007) (Fig. 6b, c) [35] and human left and right atria (GSE31999) (Fig. 6d, e) [34]. These observations are in line with our patch-clamp data showing the absence of functional Na\textsubscript{v}1.8-based sodium channels under basal conditions in atrial and ventricular CMs.

![Fig. 2 Absence of Na\textsubscript{v}1.8-based late sodium current (I\textsubscript{Na}) in ventricular and atrial cardiomyocytes. a, c, e Representative Na\textsubscript{v}1.8-based late I\textsubscript{Na} traces recorded during a ramp protocol (see insets) in rabbit left ventricular (LV) cardiomyocytes (CMs) (a), human-induced pluripotent stem cell-derived CMs (hiPSC-CMs) (c) and human left atrial (LA) CMs (e) at baseline and after 5-min application of 100 nM A-803467. A-803467 sensitive current was obtained by subtraction of the current recorded in the presence of A-803467 from the current recorded earlier in the absence of the compound. b, d, f Average current-voltage (I-V) relationships for Na\textsubscript{v}1.8-based late I\textsubscript{Na} measured as A-803467 sensitive current in rabbit LV CMs (b), hiPSC-CMs (d) and human LA CMs (f).](image-url)
**Discussion**

While several studies have previously implicated Na\textsubscript{V}1.8 in modulating cardiac electrophysiology and arrhythmia susceptibility, the underlying mechanism(s) are still a matter of debate. To address this, we here investigated the functional relevance of Na\textsubscript{V}1.8 in atrial and ventricular cardiomyocytes, focusing on the contribution of Na\textsubscript{V}1.8 to the peak and late \(I_{Na}\) under physiological conditions. Using detailed patch-clamp analyses of atrial and ventricular myocytes from different species, we observed a lack of effect of the Na\textsubscript{V}1.8 blocker A-803467 on peak and late \(I_{Na}\) in cardiomyocytes. In line with these observations, molecular investigation showed a virtual absence of SCN10A mRNA in human atrial tissue and hiPSC-
CMs. Similarly, analysis of online RNA-Seq datasets of rabbit ventricular tissue, ventricular and atrial hiPSC-CMs and human right and left atria revealed low to almost absent expression of SCN10A as compared to SCN5A. Hence, our results demonstrate the absence of functional NaV1.8 channels in non-diseased atrial and ventricular cardiomyocytes, which is of particular relevance when extrapolating findings on SCN10A mutations and (common) variants.

Our findings are in contrast to the study of Yang et al. [17], which suggested that NaV1.8 is a component of late \( I_{Na} \) in non-diseased atrial and ventricular cardiomyocytes, and as such may modulate arrhythmia susceptibility [17]. Yang et al. showed that in mouse and rabbit ventricular cardiomyocytes, application of A-803467 reduced late \( I_{Na} \) and shortened APD, without affecting peak \( I_{Na} \) density [17]. In contrast, we did not detect any NaV1.8-based late \( I_{Na} \) in our cardiomyocytes. This discrepancy could be due to species differences (mouse vs human) and/or to different experimental conditions such as different temperature (room temperature [17] versus physiological temperature used by us). Indeed, in a recent study, Poulet et al. [38] reported a significant increase in late \( I_{Na} \) in human right atrial cardiomyocytes from AF patients as compared to patients in SR when experiments were conducted at room temperature. However, at physiological temperature, the difference in late \( I_{Na} \) amplitudes between SR and AF cells was less pronounced and did not reach the level of statistical significance [38]. In our study, A-803467 reduced APD_{90} by only 3.5% in rabbit ventricular CMs, while in the study of Yang et al. [17], the reduction was ~ 30%. Again, different experimental conditions may underlie these discrepancies, for instance the use...
of the perforated patch vs rupture patch, differences in recording solutions and temperature. Moreover, in the study of Yang et al., wash-out of A-803467 was not investigated, leaving the possibility that part of the observed effect was a time-dependent effect. Indeed, in time-matched AP recordings in rabbit cardiomyocytes, we observed an APD reduction over time similar to that observed with A-803467 suggesting that the apparent APD shortening induced by A-803467 in this species is a non-specific effect independent of the blocker. A similar small APD reduction was also observed in mouse ventricular CMs (but not in mouse atrial CMs) in our previous study [18]. Finally, despite the absence of NaV1.8-based peak and late I\textsubscript{Na}, we still observed a small yet significant and mostly reversible decrease in APD in hiPSC-CMs induced by A-803467. This AP shortening could be due to a potential off-target effect of A-803467. Although we observed no effects of A-803467 on I\textsubscript{CaL} in rabbit, we cannot completely rule out effects of A-803467 on other ion channels, cautioning its use in electrophysiological studies aimed at establishing the functional relevance of Na\textsubscript{V}1.8 in, e.g. arrhythmogenesis.

A number of previous studies have suggested a role for SCN10A/Na\textsubscript{V}1.8 in modulating cardiac conduction and arrhythmogenesis. Perhaps the most compelling evidence on a potential role for Na\textsubscript{V}1.8 came from studies in mice deficient for Scn10a (Scn10a\textsuperscript{−/−}). In ventricular CMs isolated from Scn10a\textsuperscript{−/−} mice, APs were shorter than those in wild-type mice and A-803467 had no effect on peak or late I\textsubscript{Na}, nor on APD, thus supporting the idea of a contribution of Na\textsubscript{V}1.8 to late I\textsubscript{Na} [17]. However, in a follow-up study, the same authors reported similar APD in wild-type and Scn10a\textsuperscript{−/−} ventricular cardiomyocytes at baseline conditions, and only under extreme experimental conditions, e.g. after pre-treatment with the late I\textsubscript{Na} enhancer ATX II, a reduced late I\textsubscript{Na} was observed in knockout ventricular CMs [19]. Nevertheless, ATX-II administration in anesthetized mice and Langendorff-perfused hearts prolonged QTc and induced arrhythmias to the same extent in wild-type and Scn10a\textsuperscript{−/−} mice. Finally, no Scn10a transcript was detected in either wild-type or Scn10a\textsuperscript{−/−} ventricular CMs and ECG parameters were similar in both wild-type Scn10a\textsuperscript{−/−} mice [19], further underlining the limited relevance of Na\textsubscript{V}1.8 in cardiomyocytes under physiological conditions.
Mutations or variants in SCN10A (associated with both gain and loss of Na\textsubscript{v}1.8-based sodium channel function) have been associated with inherited arrhythmia syndromes such as Brugada syndrome [14–16], as well as increased AF susceptibility [11–13]. However, functional relevance of these identified mutations and variants has only been assessed in heterologous expression systems such as HEK-293 cells which differ significantly from the cardiomycyte environment [10, 12, 13, 15, 16]. Nonetheless, even if a mutation in SCN10A is found to alter function of Na\textsubscript{v}1.8-based channels in expression systems, this does not automatically imply a functional impact on the cardiomycyte level. In fact, our current and previous findings demonstrate very low expression levels of SCN10A and the consequent absence of functional Na\textsubscript{v}1.8-based current in atrial and ventricular CMs. We therefore propose that the potential electrophysiological and pro-arrhythmic effects of SCN10A variants/mutations do not occur on the cardiomycyte level, but instead are consequent to the actions of SCN10A/Na\textsubscript{v}1.8 in other cell types such as intracardiac neurons. Indeed, we and others have previously demonstrated that Na\textsubscript{v}1.8 is specifically expressed and functionally relevant in murine, canine and human cardiac neurons [18, 20, 21], suggesting a function of the SCN10A gene product for cardiac conduction via regulation of action potential firing in intracardiac neurons [18, 21, 22].

In recent years, various GWAS studies have suggested a potential modulatory effect of SCN10A common genetic variants on ECG parameters such as PR and QRS interval [6–10] in addition to susceptibility to AF [11–13] and Brugada syndrome [14–16]. However, it was subsequently demonstrated that the SCN10A variant rs6801957 (associated with QRS duration [9]) is located within a cardiac enhancer region which interacts with the promotor of SCN5A. As such, rs6801957 was shown to decrease SCN5A expression in the heart, explaining the observed associations of this SCN10A variant with cardiac conduction [23]. Based on these observations and our current findings, we therefore propose that the SCN10A locus identified in various GWAS studies exerts its modulatory effects indirectly through their impact on SCN5A expression and/or neuronal activity, rather than through a direct effect on cardiomycyte electrophysiology.

While our findings indicate a lack of functional relevance for Na\textsubscript{v}1.8 in CMs under physiological conditions, they do not rule out a potential function during pathophysiological situations. Recently, increased SCN10A/Na\textsubscript{v}1.8 expression in human ventricular tissue isolated from heart failure and hypertrophic patients as compared to non-failing and healthy myocardium, has been demonstrated [39, 40]. Na\textsubscript{v}1.8 inhibition with the specific blockers A-803467 and PF-01247324 decreased late I\textsubscript{Na} magnitude, abbreviated APD and reduced cellular-spontaneous Ca\textsuperscript{2+} release and proarrhythmic events in human failing and hypertrophic CMs [39, 40]. Of note, in both these studies, no electrophysiological experiments were performed in non-failing and non-hypertrophic CMs, thus precluding comparison of the effects of Na\textsubscript{v}1.8 inhibition in human non diseased CMs under comparable experimental settings [39, 40]. A modulatory role for SCN10A/Na\textsubscript{v}1.8 has furthermore been suggested in AF, with A-803467 administration preventing AF recurrence in a fast-pacing canine model [21]. Increased late I\textsubscript{Na} has been observed in right atrial appendage cardiomycocytes from AF patients as compared to individuals in SR [38, 41]. Whether alterations in SCN10A/Na\textsubscript{v}1.8-based contribute to this increased late I\textsubscript{Na} in the setting of AF will require further investigation. Interestingly, injection of A-803467 into canine cardiac ganglioneoned plexi (GP) [22] and canine left stellate ganglion (LSG) [42] suppressed vagal-mediated AF and ischemia-induced ventricular arrhythmia, respectively, most likely by inhibiting the neuronal activity of GP and LSG. This further underlines the potential functional involvement of SCN10A/Na\textsubscript{v}1.8 in intracardiac neurons.

In conclusion, our study demonstrates the (functional) absence of SCN10A/Na\textsubscript{v}1.8-based channels in human and rabbit atrial and ventricular CMs under basal, non-remodeled conditions. We therefore propose that the association of SCN10A variants with cardiac electrophysiology is likely the result of indirect effects on SCN5A expression and/or Na\textsubscript{v}1.8 activity in cell types other than CMs, including (intracardiac) neurons.

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Compliance with Ethical Standards

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

Ethical Approval All applicable international, national and/or institutional guidelines for the care and use of animals were followed. All procedures performed in this study involving human participants were in accordance with the ethical standards of the Amsterdam UMC, Amsterdam, The Netherlands and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Left atrial appendages were obtained from subjects undergoing cardiac surgery for coronary or valvular disease, included in the multicenter PREDICT AF study [25] (clinicaltrials.gov: NCT03130985).

Informed Consent Informed consent was obtained from all individual participants included in the study.
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