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ORIGINAL RESEARCH ARTICLE

The functional consequences of sodium channel $Na_V1.8$ in human left ventricular hypertrophy

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Abstract

Aims In hypertrophy and heart failure, the proarrhythmic persistent Na^+ current (I_{NaL}) is enhanced. We aimed to investigate the electrophysiological role of neuronal sodium channel $Na_V1.8$ in human hypertrophied myocardium.

Methods and results Myocardial tissue of 24 patients suffering from symptomatic severe aortic stenosis and concomitant significant afterload-induced hypertrophy with preserved ejection fraction was used and compared with 12 healthy controls. We performed quantitative real-time PCR and western blot and detected a significant up-regulation of $Na_V1.8$ mRNA (2.34-fold) and protein expression (1.96-fold) in human hypertrophied myocardium compared with healthy hearts. Interestingly, $Na_V1.5$ protein expression was significantly reduced in parallel (0.60-fold). Using whole-cell patch-clamp technique, we found that the prominent I_{NaL} was significantly reduced after addition of novel $Na_V1.8$-specific blockers either A-803467 (30 nM) or PF-01247324 (1 μM) in human hypertrophic cardiomyocytes. This clearly demonstrates the relevant contribution of $Na_V1.8$ to this proarrhythmic current. We observed a significant action potential duration shortening and performed confocal microscopy, demonstrating a 50% decrease in proarrhythmic diastolic sarcoplasmic reticulum (SR)-Ca^{2+} leak and SR-Ca^{2+} spark frequency after exposure to both $Na_V1.8$ inhibitors.

Conclusions We show for the first time that the neuronal sodium channel $Na_V1.8$ is up-regulated on mRNA and protein level in the human hypertrophied myocardium. Furthermore, inhibition of $Na_V1.8$ reduced augmented I_{NaL}, abbreviated the action potential duration, and decreased the SR-Ca^{2+} leak. The findings of our study suggest that $Na_V1.8$ could be a promising anti-arrhythmic therapeutic target and merits further investigation.

Keywords Left ventricular hypertrophy; Sodium channels; Late sodium current; HFpEF; Arrhythmias; Calcium; SR-Ca^{2+} leak

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Introduction

Left ventricular remodelling caused by pressure overload can lead to myocardial hypertrophy. Left ventricular hypertrophy (LVH) is an adaptive response to increased chronic workload and a very common clinical finding.[1,2] It has been reported that LVH can potentially increase the incidents of congestive heart failure (HF) and sudden cardiac death.[3,4] Furthermore, it has been shown that structural modifications due to LVH are associated with HF with preserved ejection fraction (HFpEF).[5] LVH is also associated with an increased prevalence of cardiac arrhythmias and constitutes an important risk factor for cardiac morbidity and mortality.[6–9] The pathology of LVH shows not only mechanical but also

extensive cellular and molecular remodelling including cardiomyocyte growth changes, dysfunction of excitation–contraction coupling, certain metabolic dysfunctions, and fibrosis.[10,11]

Patients with severe aortic stenosis-dependent symptoms have a tremendous risk of sudden cardiac death if only medically treated.[12] In the past few decades, a great progress has been made in underpinning the cellular and molecular mechanisms of remodelling in myocardial hypertrophy. However, the involvement of voltage-gated sodium channel (Na_V) isoforms in HFpEF and/or myocardial hypertrophy with preserved ejection fraction leading to cardiac arrhythmias has not been elucidated comprehensively. Besides the peak sodium current causing the action potential (AP) upstroke, a small persistent sodium current is existing, also known as late sodium current (I_{NaL}).[13] In case of cardiac pathology, some I_{NaL} producing Na_V channels reopen or remain active throughout the whole AP. The amplitude of I_{NaL} is smaller when compared with peak sodium current but eventually with a larger Na integral due to longer persistence during the course of the AP plateau.[14] I_{NaL} has been reported to be enhanced in different clinically relevant cardiac pathologies like hypoxia, ischaemia, and HF.[15–17] In severe myocardial hypertrophy, I_{NaL} is also enhanced[18] and may cause intracellular Na^+ overload and prolongation of the action potential duration (APD).[19] Consequently, the resulting Na^+ accumulation may lead to intracellular Ca^{2+} overload because of reduced efflux and increased influx of Ca^{2+} through Na^+/Ca^{2+} exchanger.[20,21]

The voltage-gated sodium channel 1.5 ($Na_V1.5$) is considered the predominant cardiac Na_V isoform in the heart. In the past few years, different reports suggested the existence of other non-cardiac sodium channels including $Na_V1.8$ (*SCN10A*) in the heart, which like $Na_V1.5$ is tetrodotoxin resistant. In addition, genetic variants of *SCN10A* have been recently shown to influence cardiac conduction.[22,23] Further evidence about $Na_V1.8$ in cardiac conduction comes from genome-wide association studies reporting that $Na_V1.8$ could modulate cardiac conduction by effecting PR and QRS intervals.[23] However, the expression of $Na_V1.8$ in cardiac tissue and its direct involvement in human cardiac arrhythmias is still poorly understood. In the current study, we investigated the presence and the functional role of $Na_V1.8$ in cardiac hypertrophy with preserved contractility derived exclusively from patients suffering from severe aortic stenosis. To study the role of $Na_V1.8$ channel in the electrophysiological context of human LVH, we used two different $Na_V1.8$ blockers, which are described to be very specific for $Na_V1.8$ (A-803467 and PF-01247324). The blocker A-803467 is over 100-fold more selective for the $Na_V1.8$ channel vs. other human Na_V channels.[24] Similarly, the drugPF-01247324 is a highly specific inhibitor of $Na_V1.8$ over other Na_V channels, even when used at very high concentration.[25]

Table 1 Patient characteristics: values in the table represent the mean ± standard error of the mean

Male sex	50%
Age	66.38 ± 3.66 years
Ejection fraction	56.95 ± 1.81%
Dyspnoea	85.7%
Interventricular septum	15.0 ± 1.7 mm
Aortic valve area	0.8 ± 0.1 cm^2
Mean AV pressure gradient	44 ± 6.4 mm Hg
Diabetes	4.3%
ACE-inhibitors	17.4%
β-Blockers	65.2%
Diuretics	76.5%
Digoxin	0.0%
Amiodarone	0.0%
AT1 receptor antagonists	26.1%
Aldosterone antagonists	0.0%
Statins	30.4%
Ca channel blockers	26.1%

ACE, angiotensin-converting enzyme; AT1, angiotensin II receptor–type 1; AV, aortic valve.

Materials and methods

Human myocardial tissue

All procedures were conducted in compliance with the local ethics committee. The study conforms to the World Medical Association declaration of Helsinki. Written informed consent was received from all patients prior to inclusion. Myocardial tissue of the hypertrophied left ventricle was obtained from patients (n = 24; *Table 1*) with severe aortic valve stenosis and preserved ejection fraction undergoing an open-heart surgery for aortic valve replacement as a classic myectomy (Morrow resection). For molecular purposes, we utilized healthy left ventricular myocardium (n = 12) from healthy donor hearts that were not transplanted because of technical reasons.

Western blots

Myocardial tissue samples from patients with LVH (n = 12) compared with healthy control myocardium were homogenized in Tris buffer (pH 7.4) containing Tris–HCl (20 mM), NaCl (200 mM), NaF (20 mM), Na_3VO_4 (1 mM), dithiothreitol (1 mM), Triton X-100 (1%), and complete protease and phosphatase inhibitor cocktails (Roche Diagnostics, Germany). Protein concentration was determined by bicinchoninic acid assay (Pierce Biotechnology, United States). Denatured tissue homogenates (10 min, 70°C in 2% beta-mercaptoethanol) were separated on 7.5% sodium dodecyl sulfate–polyacrylamide gels, then transferred to a nitrocellulose membrane, and incubated with the following primary antibodies: mouse monoclonal anti-$Na_V1.8$ (1:1000, LSBio, United States, LS-C109037), rabbit polyclonal anti-$Na_V1.5$ (1:2000, Alomone Labs, Israel, ASC-005), and mouse monoclonal anti-GAPDH (1:20000, Biotrend, Germany, BTMC-A473-9) at 4°C overnight.

Secondary antibodies included horseradish peroxidase (HRP)-conjugated goat anti-rabbit and goat anti-mouse (1:10000, Jackson ImmunoResearch, United Kingdom, 111-035-144 and 115-035-062, respectively). The membrane was incubated with secondary antibodies for 1 h at room temperature. Immobilon™ Western Chemiluminescent HRP Substrate (Millipore, Germany) was used for the chemiluminescent detection.

Quantitative real-time PCR

Human cardiac tissues were snap-frozen in liquid nitrogen and stored at −80°C. RNA was isolated by use of the SV Total RNA Isolation System (Promega, Germany). DNA standards were generated by serial dilution in diethyl pyrocarbonate water containing yeast tRNA (30 μL/mL) as co-precipitant and used at varying concentrations between 0.125 and 100 000 fg/μL to ensure exponential growth of DNA amounts in the standard range. One hundred nanogram of RNA was reverse transcribed into cDNA using standard protocols. For quantitative PCR, 10 μL of SYBR Green PCR Master Mix (Thermo Fisher, Germany), 7 μL of nuclease-free water, 1 μL of forward and 1 μL of reverse primer, and 1 μL of cDNA were mixed. Quantitative PCR was carried out using the iQ5 Multicolor Real-time PCR Detection System (Bio-Rad, United States). Forty cycles of 15 s at 95°C followed by 1 min at 60°C were used, and fluorescence was measured after each cycle. After 40 cycles, melt curve analysis was performed to ensure specificity of the products. Thresholds cycles were evaluated and normalized to housekeeping genes and controls. The following primer sequences (5′-3′) were used for quantitative real-time PCR analyses: SCN10A, forward TGGCAGATGACCTGGAAGAACC and reverse CGATACGGTAGCAAGTCTTGCG (Origene, Cat. no. HP209444, NM_006514); and GAPDH, forward GTCTCCTCTGA CTTCAACAGCG and reverse ACCACCCTGTTGCTGTAGCCAA.

Myocyte isolation

Left ventricular myocardium was rinsed, cut into small pieces, and incubated at 37°C in a spinner flask filled with Joklik-MEM (JMEM) solution (PAN-Biotech, Germany) that contained 1.0 mg/mL collagenase (Worthington type 1, 185 U/mg, CellSystems, France) and 13% trypsin (Life Technologies, United States). After 45 min, the supernatant was discarded, and fresh JMEM solution containing collagenase was added. The solution was incubated for 10 min until myocytes were disaggregated. The supernatant that contained disaggregated cells was removed and centrifuged (700 rpm, 5 min).

Fresh JMEM with collagenase was added to the remaining tissue. This procedure was repeated four to five times. After every step, the centrifuged cells were resuspended in JMEM solution that contained bovine calf serum 10%, and pH was adjusted to 7.4 at room temperature. Only cell solutions that contained elongated, not granulated, cardiomyocytes with cross-striations were selected for experiments, plated on laminin-coated recording chambers, and left to settle for 30 min.

Whole-cell patch clamp

I_{NaL} measurements

Ruptured-patch whole-cell voltage-clamp was used to measure I_{NaL} in human ventricular cardiomyocytes isolated from hypertrophied hearts with microelectrodes (2–3 MΩ). Pipettes were filled with solution containing CsCl (95 mM), Cs-glutamate (40 mM), NaCl (10 mM), $MgCl_2$ (0.92 mM), Mg-ATP (5 mM), Li-GTP (0.3 mM), HEPES (5 mM), niflumic acid (0.03 mM; to block Ca^{2+}-activated chloride current), nifedipine (0.02 mM; to block Ca^{2+} current), strophanthidin (0.004 mM; to block Na^+/K^+-ATPase), EGTA (1 mM), and $CaCl_2$ (0.36 mM; free $[Ca^{2+}]_i$, 100 nM), and pH was adjusted to 7.2 with CsOH. The bath solution contained NaCl (135 mM), tetramethylammonium chloride (5 mM), CsCl (4 mM), $MgCl_2$ (2 mM), glucose (10 mM), and HEPES (10 mM), and pH was adjusted to 7.4 with CsOH. To minimize contaminating Ca^{2+} currents during I_{NaL} measurements, Ca^{2+} was omitted from the bath solution. Access resistance was <7 MΩ. Cardiomyocytes were held at −120 mV, and I_{NaL} was elicited using a train of pulses to −35 mV (1000 ms duration, 10 pulses, basic cycle length 2 s). Recordings were initiated 3 min after rupture. The measured I_{NaL} at −35 mV was leak subtracted before calculation of the I_{NaL} integral (between 100 and 500 ms) and was normalized to membrane capacitance of the measured cell. Cardiomyocytes were treated with either A-803467 (30 nM) or PF-01247324 (1 μM) for 10 min and compared with the control untreated cells. Measurements were conducted at a temperature of 37.7°C.

Action potential duration measurements

To record the AP from human ventricular cardiomyocytes, the whole-cell patch-clamp technique was used to measure membrane potential (current clamp configuration). Microelectrodes (7–8 MΩ) were filled with solution containing K-Aspartate (92 mM), KCl (48 mM), Mg-ATP (1 mM), HEPES (10 mM), EGTA (0.02 mM), GTP-Tris (0.1 mM), and Na_2-ATP (4 mM), and final pH was adjusted at 7.2 with KOH solution. Bath solution contained NaCl (140 mM), KCl (4 mM), $MgCl_2$ (1 mM), $CaCl_2$ (2 mM), glucose (10 mM), and HEPES (10 mM), and final pH was adjusted to 7.4 with NaOH. Action potentials were continuously elicited by square current pulses of 1–2 nA amplitude and 1–5 ms duration at increasing stimulation frequency (0.5–3 Hz). Access resistance was typically ~5–15 MΩ after patch rupture. Fast capacitance was compensated for in a cell-attached configuration. Recordings were commenced after cell stabilization, which was ~10 min after rupture. Human cell measurements were

conducted at a temperature of 37.7°C. Every cell was patched and measured before and after drug application.

In all patch-clamp experiments, cardiomyocytes were mounted on the stage of a microscope (Nikon T 300). Fast capacitance was compensated in cell-attached configuration. Membrane capacitance and series resistance were compensated after patch rupture. Signals were filtered with 2.9 and 10 kHz Bessel filters and recorded with an EPC10 amplifier (HEKA Elektronik, Germany). $Na_V1.8$ was inhibited using either A-803467 (30 nM for 10 min) or PF-01247324 (1 μM for 5 min). Both drugs were added to the bath solution.

Measurement of Ca^{2+} sparks

Isolated cardiomyocytes were incubated at room temperature for 30 min with a Fluo-4 AM loading buffer (10 μM; Molecular Probes, Life Technologies). Experimental solution contained NaCl (136 mM), KCl (4 mM), NaH_2PO_4 (0.33 mM), $NaHCO_3$ (4 mM), $CaCl_2$ (2 mM), $MgCl_2$ (1.6 mM), HEPES (10 mM), and glucose (10 mM), and final pH was adjusted to 7.4 by addition of NaOH solution at room temperature. $Na_V1.8$ inhibitors A-803467 (30 nM) and PF-01247324 (1 μM) were added to their respective groups in the experimental solution.

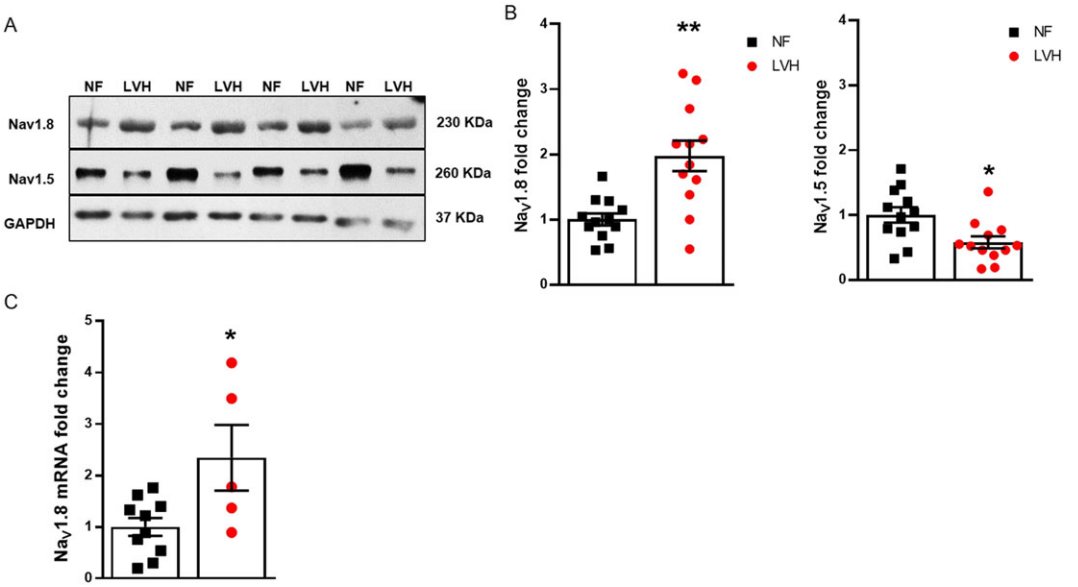
Cells were continuously superfused during experiments. To wash out the loading buffer and to remove any extracellular dye (as well as to provide enough time for complete de-esterification of Fluo-4 AM), cells were superfused with experimental solution for 5 min before the experiments were begun. Ca^{2+} spark measurements were performed with a laser scanning confocal microscope (LSM 5 Pascal, Zeiss, Germany) using a ×40 oil-immersion objective. Fluo-4 was excited by an argon ion laser (488 nm), and emitted fluorescence was collected through a 505 nm long-pass mission filter. Fluorescence images were recorded in the line scan mode with 512 pixels per line (width of each scan line: 38.4 μm) and a pixel time of 0.64 μs. One image consists of 10 000 unidirectional line scans, which equates to a measurement period of 7.68 s. Experiments were conducted at resting conditions after the sarcoplasmic reticulum (SR) was loaded with Ca^{2+} by repetitive field stimulation (10 pulses at 1 Hz, 20 V). Ca^{2+} sparks were analysed with the program SparkMaster for ImageJ. The mean spark frequency of the respective cell resulted from the number of sparks normalized to cell width and scan rate (100 $\mu m^{-1}\ s^{-1}$).

Results

Regulation of $Na_V1.5$ and $Na_V1.8$ in human left ventricular hypertrophy

To investigate the regulation of $Na_V1.8$ and $Na_V1.5$ protein expression in human LVH compared with healthy control ventricular myocardium tissue homogenates, western

Figure 1 Regulation of $Na_V1.8$ and $Na_V1.5$ expression in hypertrophy. (A) Western blots were performed utilizing left ventricular human tissue homogenates. (B) Densitometry data of $Na_V1.8$ and $Na_V1.5$ show a significant up-regulation of $Na_V1.8$ and down-regulation of $Na_V1.5$. GAPDH was used as an internal loading control in all blots [NF: $n = 12$; and left ventricular hypertrophy (LVH): $n = 12$]. (C) Real-time quantitative PCR showing the relative mRNA expression of $Na_V1.8$/GAPDH in left ventricle of human NF ($n = 10$) and LVH ($n = 5$). $^*P \leq 0.05$ and $^{**}P \leq 0.01$ vs. NF. Student's *t*-test. Data shown as mean ± standard error of the mean and individual values.



blots were performed. We found a significant up-regulation of $Na_V1.8$ protein expression (1.96- ± 0.31-fold) in LVH compared with healthy control ventricular myocardium (*Figure 1B*). In contrast, the expression of $Na_V1.5$ protein was significantly decreased (0.60- ± 0.10-fold) compared with Non-failing (NF) (*Figure 1B*). In accordance to our findings of $Na_V1.8$ expression at the protein level, we recorded a significant up-regulation of $Na_V1.8$ mRNA levels in LVH (2.34- ± 0.64-fold, n = 5) compared with NF (n = 10) by quantitative PCR. The mRNA expression of $Na_V1.8$ is shown as relative expression to the housekeeping gene GAPDH (*Figure 1C*).

$Na_V1.8$ contributes to I_{NaL} and action potential duration in human hypertrophy

Given the increased expression of $Na_V1.8$ in human LVH, we investigated the potential contribution to the augmentation of I_{NaL} and APD prolongation under hypertrophic condition. We adopted the approach using novel-specific blockers of $Na_V1.8$ channel A-803467 and PF-01247324 to inhibit the activity of this channel in human LVH cardiomyocytes for recording I_{NaL} and APDs by patch clamp. Cardiomyocytes were superfused with either A-803467 or PF-01247324. Our data show a significant reduction in I_{NaL} (*Figure 2*) and also shortening of APD_{90} (*Figure 3*) when cardiomyocytes were exposed to $Na_V1.8$ inhibitors compared with the control group. Furthermore, both $Na_V1.8$ inhibitors do not exert any effect on the upstroke velocity and amplitude of the AP (*Figure 4A* and *4B*). Therefore, it can be concluded that $Na_V1.8$ contributes to I_{NaL} in terms of a positive net inward current to APD prolongation in human hypertrophy.

Contribution of $Na_V1.8$ to proarrhythmic SR-Ca^{2+} leak

During pathological conditions such as HF, an enhanced I_{NaL} can lead to disturbed SR-Ca^{2+} metabolism and hence potentially proarrhythmic SR-Ca^{2+} leakiness. This can give rise to delayed after-depolarizations and thereby arrhythmias. However, the contribution of different Na_V isoforms to SR-Ca^{2+} leak in human LVH has not been evaluated. Therefore, we measured SR-Ca^{2+} leak in isolated cardiomyocytes from human hypertrophied hearts.

We detected a high incident of spontaneous diastolic Ca^{2+} release events in hypertrophied cardiomyocytes in the absence of inhibitors (*Figure 5A*). When cardiomyocytes were incubated with $Na_V1.8$ inhibitors either A-803467 or PF-01247324, a significant decrease in calcium spark frequency (CaSpF) and SR-Ca^{2+} leak was observed compared with control group (*Figure 5B* and *5E*). In control cells, CaSpF was 0.68 ± 0.12 $\mu m^{-1}\ s^{-1}$ (n = 145 cells/12 patients), while in A-806734-treated myocytes, CaSpF was reduced to 0.27 ± 0.06 $\mu m^{-1}\ s^{-1}$ (n = 124 cells/11 patients, $P \leq 0.01$) and in PF-01247324-treated myocytes to 0.26 ± 0.07 $\mu m^{-1}\ s^{-1}$ (n = 91 cells/9 patients, $P \leq 0.01$), respectively (*Figure 5B*). $Na_V1.8$ inhibition with PF-01247324 also resulted in a significant reduction of the Ca^{2+} spark duration (*Figure 5C*), while no differences were recorded for Ca^{2+} spark amplitude between control and drug treatment groups (*Figure 5D*). These data clearly demonstrate the important role of the $Na_V1.8$ channel in SR-Ca^{2+} leak regulation in hypertrophy. Taken together, proarrhythmic SR-Ca^{2+} release can significantly be decreased by targeting $Na_V1.8$ with specific inhibitors.

Discussion

LVH is a common clinical finding in daily practice caused, besides some other reason, by hypertension and aortic valve stenosis. At the early stages, myocardial hypertrophy serves as a compensatory mechanism, which can lead to systolic HF at later stages.[26] Importantly, patients with symptomatic aortic stenosis have a tremendous risk of sudden cardiac

Figure 2 (A) Original traces and (B) data showing individual and mean values ± standard error of the mean of I_{NaL} in human ventricular cardiomyocytes isolated from left ventricular hypertrophy patients (control: n = 8 cells; A-803467: n = 7 cells; PF-01247324: n = 7 cells). *$P \leq 0.05$. One-way analysis of variance and Bonferroni's post-test.

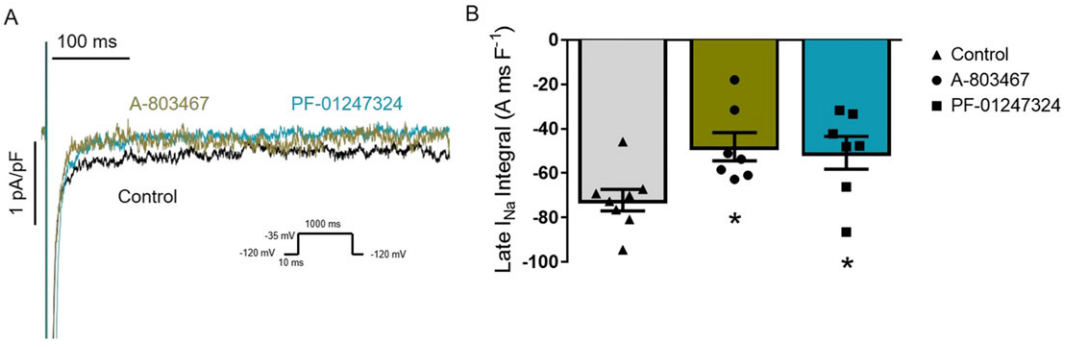
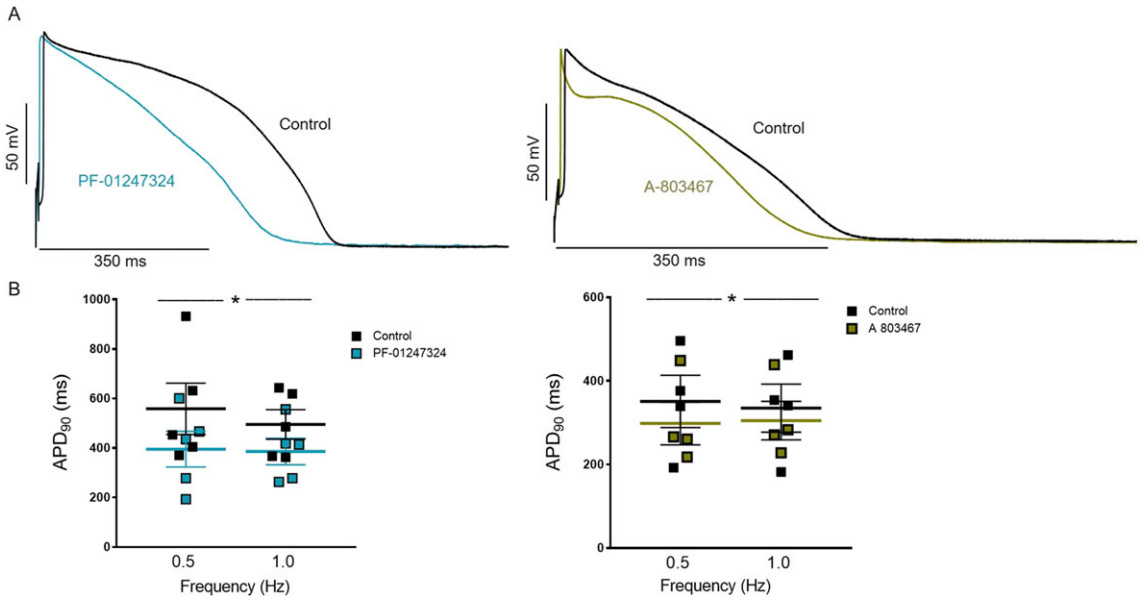


Figure 3 (A) Original action potential recording (0.5 Hz) and (B) data showing individual and mean values ± standard error of the mean of APD_{90} in left ventricular cardiomyocytes from patients with left ventricular hypertrophy. A-803467 or PF-01247324 paced at 0.5 and 1 Hz (n = 5 cells and n = 4 cells, respectively; $^*P \leq 0.05$ vs. control). Two-way repeated measures analysis of variance and Bonferroni's post-test. All the action potential duration (APD) measurements were performed pairwise by wash-in.

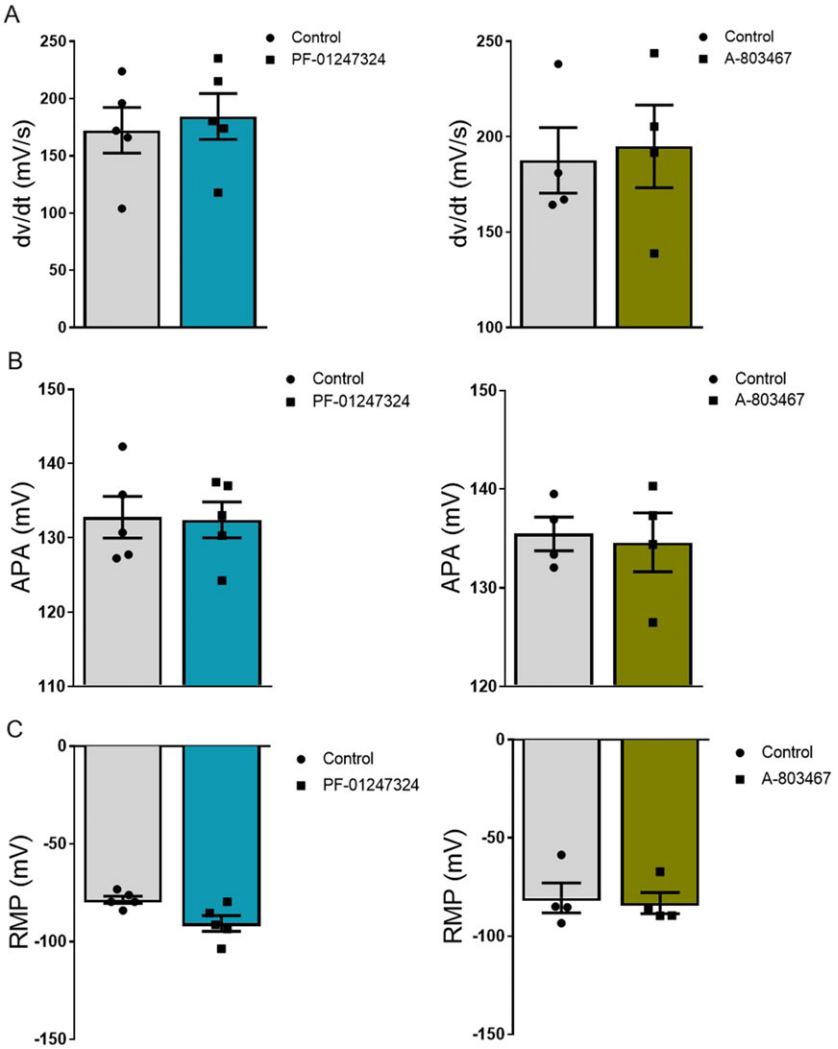


death if only medically treated.[12] In the past few decades, a great progress has been made in underpinning the cellular and molecular mechanisms of remodelling during hypertrophy. However, the mechanistic links between dysregulated proteins and in particular arrhythmogenesis, especially in human hypertrophy, still remain elusive. We herein investigated the regulation of neuronal sodium channel $Na_V1.8$ and its role in pathophysiology of human significant hypertrophy. We demonstrate that $Na_V1.8$ expression is up-regulated during LVH with preserved ejection fraction compared with healthy left ventricles, while $Na_V1.5$ expression is down-regulated in parallel. To the best of our knowledge, this is the first report of such an ion channel switch in human hypertrophy. Furthermore, inhibition of $Na_V1.8$ by specific blockers causes a significant reduction of the enhanced I_{NaL} and consequently leads to abbreviated APDs in LVH. Moreover, we observed a significant decrease of the proarrhythmic SR-Ca^{2+} leak by inhibition of $Na_V1.8$ in human hypertrophied cardiomyocytes. Therefore, inhibition of $Na_V1.8$ modulates well-accepted proarrhythmogenic triggers such as I_{NaL}, APD prolongation, and diastolic SR-Ca^{2+} leak.

In the current study, we found increased expression of $Na_V1.8$ at both mRNA and protein level in human LVH with preserved contractility when compared with NF. Transcripts of many non-cardiac Na_V channels ($Na_V1.1$, $Na_V1.3$, $Na_V1.2$, and $Na_V1.6$) including $Na_V1.8$ were detected in mouse and dog heart, while there was no previous evidence of $Na_V1.8$ expression in the human ventricle during LVH.[27,28] Moreover, there have been conflicting reports about the involvement of these non-cardiac Na_V isoforms in I_{NaL} enhancement in animal models due to species-specific expression. We already reported that the protein expression of other non-cardiac Na_V channels ($Na_V1.1$ and $Na_V1.6$) was down-regulated while $Na_V1.5$ was increased showing no significant I_{NaL} enhancement in a transverse aortic constriction mouse model of compensated hypertrophy.[17] Similar findings were reported in a dog and rat HF model where $Na_V1.5$ protein expression was found to be down-regulated, whereas a significant increase in I_{NaL} was observed.[29,30] These findings from animal models suggest the involvement of other non-cardiac sodium channels in I_{NaL} augmentation and arrhythmogenesis under hypertrophy and HF conditions. However, animal models represent a very artificial variability in hypertrophy, which cannot be compared directly with human hypertrophy with severe aortic stenosis. Moreover, these animal-derived findings cannot be directly translated into the human because of severe differences in cellular electrophysiology. Therefore, the translational data of our current study add novel knowledge on I_{NaL} regulation in hypertrophy and show for the first time that I_{NaL} augmentation is also caused by over-expression of $Na_V1.8$ in human hypertrophy.

Electrophysiological recordings also described that non-cardiac Na_V channels contribute to I_{NaL} up to 44% in canine ventricular cardiomyocytes under normal physiological conditions[31] and $Na_V1.8$ was suggested to be a major contributor of I_{NaL} in healthy mouse ventricular cardiomyocytes.[32] However, $Na_V1.8$ was not investigated before in animal models

Figure 4 (A) Individual and mean values ± standard error of the mean showing upstroke velocity of action potential in control and drug groups from left ventricular hypertrophy cardiomyocytes. (B) Data showing individual and mean values ± standard error of the mean of action potential amplitude (APA) measurements from isolated human left ventricular hypertrophy cardiomyocytes. (C) Graph shows the resting membrane potential (RMP) of cardiomyocytes measured under control condition and drug treatments (PF-01247324: *n* = 5 cells; and A-804367: *n* = 4 cells). All measurements were performed pairwise by wash-in. Paired Student's *t*-test was performed.



with present heart disease, for example, hypertrophy. It was shown previously by us and other groups that increased I_{NaL} is implicated as potential contributor to proarrhythmogenic triggers and thereby to the occurrence of arrhythmias.[33–35] During different pathological conditions associated with heart diseases, over-expression of these non-cardiac Na_V channels was also shown to be responsible for the prolonged cardiac APD.[31] The functional properties of $Na_V1.8$ are characterized by a long AP duration with preserved excitability during sustained stimulation in the dorsal root ganglion.[36] In accordance with an enhanced I_{NaL}, we observed prolongation of APD in ventricular cardiomyocytes of hypertrophy patients. Inhibition of $Na_V1.8$ with specific blockers showed a significant abbreviation of APD. Up-regulated $Na_V1.8$ expression in human hypertrophy may cause increased I_{NaL} potentially leading to a pathological prolongation of APD in this disease. This prolonged APD can give rise to early after-depolarizations, thereby posing increased fatal risk for ventricular arrhythmias.[13] The partial inhibition of I_{NaL} up to 50% not only restored healthy APD in failing cardiomyocytes but also ceased after-depolarizations.[13]

Isolated cardiomyocytes from patients with hypertrophic cardiomyopathy showed an augmented I_{NaL} with a subsequent increase in intracellular Ca^{2+} load.[18] Enhanced I_{NaL} might lead to diastolic Ca^{2+} overload through the Na^+/Ca^{2+} exchanger operation in reverse mode.[37] An increase in diastolic Ca^{2+} not only results in impaired relaxation but also plays a key role in activating pro-hypertrophic signalling pathways,

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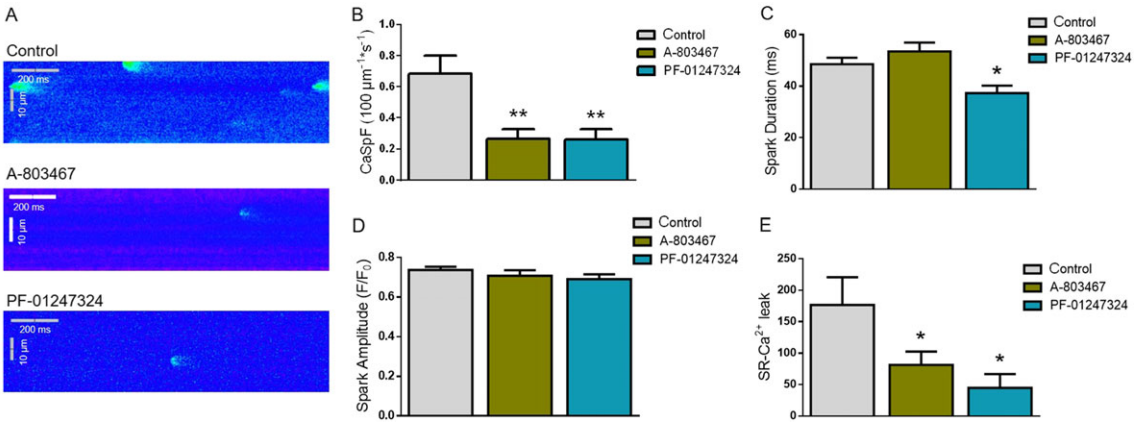
Figure 5 (A) Representative line scan images and (B) calcium spark frequency (CaSpF) in left ventricular cardiomyocytes from patients with left ventricular hypertrophy under control condition and $Na_V1.8$ inhibition. Pre-incubation with either A-803467 or PF-01247324 resulted in a significant decrease of CaSpF in hypertrophy compared with control. (C) Mean values of spark duration and (D) spark amplitude of cardiomyocytes. (E) Calculated full SR-Ca^{2+} leak in left ventricular cardiomyocytes from left ventricular hypertrophy patients (control: n = 145; A-803467: n = 124 cells; PF-01247324: n = 91 cells). Data shown as mean ± standard error of the mean. One-way analysis of variance and Bonferroni's post-test. *$P \leq 0.05$, **$P \leq 0.01$.



leading to increased stiffness of the left ventricle.[38,39] These data suggest that I_{NaL} is not only the consequence of hypertrophy but is also involved in inducing diastolic intracellular Ca^{2+} overload. The resulting Ca^{2+} load is thought to trigger intracellular spontaneous Ca^{2+}-release events, leading to cytosolic Ca^{2+} oscillations, automaticity, and triggered activity. In addition to animal studies, we have previously demonstrated that inhibition of an enhanced I_{NaL} with tetrodotoxin or ranolazine reduces the SR-Ca^{2+} leak in human diseased cardiomyocytes.[37,40] In the present study, we extend this evidence to human cardiomyocytes with very significant hypertrophy and HFpEF. The highly specific inhibition of $Na_V1.8$ potently suppressed the diastolic SR-Ca^{2+} leak in ventricular cardiomyocytes of patients with severe LV hypertrophy. Taken together, the data of our present study define a significant contribution of $Na_V1.8$ in the initiation of proarrhythmic triggers via I_{NaL}-induced SR-Ca^{2+} leak and also APD prolongation. The differences between the activation of $Na_V1.5$ and $Na_V1.8$ suggest that the selective inhibition of $Na_V1.8$-mediated I_{NaL} can be antiarrhythmic.

Patients with severe aortic stenosis and concomitant LV hypertrophy including HFpEF are at high risk of sudden cardiac death, and one of the probable causes is lethal arrhythmias. Our current study provides better understanding of electrophysiological disturbances that occur in human severe hypertrophy. We identified a potential new ion channel target ($Na_V1.8$) and provide a respective possible pharmacological antiarrhythmic approach. Therefore, these findings provide basic evidence for *in vivo* studies.

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Conflict of interest

None declared.

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