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Chronic heart failure slows late sodium current in human and canine ventricular myocytes: Implications for repolarization variability

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Abstract

Background: Late Na^+ current (I_{NaL}) in human and dog hearts has been implicated in abnormal repolarization associated with heart failure (HF). HF slows inactivation gating of late Na^+ channels, which could contribute to these abnormalities.
Aims: To test how altered gating affects I_{NaL} time course, Na^+ influx, and action potential (AP) repolarization.
Methods: I_{NaL} and AP were measured by patch clamp in left ventricular cardiomyocytes from normal and failing hearts of humans and dogs. Canine HF was induced by coronary microembolization.
Results: I_{NaL} decay was slower and I_{NaL} density was greater in failing hearts than in normal hearts at 24 °C (human hearts: $\tau = 659 \pm 16$ *vs.* 529 ± 21 ms; $n = 16$ and 4 hearts, respectively; mean ± SEM; $p < 0.002$; dog hearts: 561 ± 13 *vs.* 420 ± 17 ms; and 0.307 ± 0.014 *vs.* 0.235 ± 0.019 pA/pF; $n = 25$ and 14 hearts, respectively; $p < 0.005$) and at 37 °C this difference tended to increase. These I_{NaL} changes resulted in much greater (53.6%) total Na^+ influx in failing cardiomyocytes. I_{NaL} was sensitive to cadmium but not to cyanide and exhibited low sensitivity to saxitoxin ($IC_{50} = 62$ nM) or tetrodotoxin ($IC_{50} = 1.2$ μM), tested in dogs. A 50% I_{NaL} inhibition by toxins or passing current opposite to I_{NaL}, decreased beat-to-beat AP variability and eliminated early afterdepolarizations in failing cardiomyocytes.
Conclusions: Chronic HF leads to larger and slower I_{NaL} generated mainly by the cardiac-type Na^+ channel isoform, contributing to larger Na^+ influx and AP duration variability. Interventions designed to reduce/normalize I_{NaL} represent a potential cardioprotective mechanism in HF *via* reduction of related Na^+ and Ca^{2+} overload and improvement of repolarization.

Keywords: Heart failure; Late Na^+ current inactivation; Action potential; Early afterdepolarizations

1. Introduction

Mortality in patients with heart failure (HF) remains high despite recent progress in treatment, with approximately 40% of patients dying suddenly [1]. Ventricular tachycardia and fibrillation have been documented in ~ 80% of these sudden deaths [2]. While the electrophysiological mechanisms leading to such arrhythmias remain unclear, erratic control of the action potential (AP) in the diseased myocardium is widely believed to be the proximate cause. Although the major cause of AP prolongation observed in HF [1] was previously thought to be downregulation of K^+ channels [3], there is a growing body of evidence that it could also be due to alteration of the late Na^+ current (I_{NaL}) produced by voltage-gated Na^+ channels (Na_v) that continue to be active during the AP plateau.

The significance of I_{NaL} in cardiac arrhythmias was demonstrated directly through the discovery of a genetic mutation in the cardiac Na^+ channel isoform (Na_v 1.5), resulting in aberrant inactivation in patients with this congenital defect (LQT3 syndrome) [4,5]. Further studies showed that wild type Na_v 1.5 could also produce an intrinsic I_{NaL}, which is present in ventricular cardiomyocytes (VCs) of normal and failing human

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hearts [6,7]. Because of its slow decay ($\tau \sim 0.5$ s), which is almost complete after 2 s, this I_{NaL} can be distinguished from I_{NaT}, as well as from the persistent Na^+ current reported in LQT3 patients.

I_{NaL} density is increased in VCs in experimental chronic HF in dogs [8], and I_{NaL} is implicated in the increased AP duration and early afterdepolarizations (EADs) observed in both canine and human failing VCs [6,8]. The increased density of I_{NaL} in HF was recently confirmed in VCs from a canine model of pacing-induced HF as well as in failing human hearts [9], and I_{NaL} has been suggested as one of the major mechanisms for arrhythmias in HF [1,10].

Despite the emerging importance of I_{NaL} in HF, some important data and conceptual links are still missing:

1) The molecular and gating mechanisms of the HF-related changes observed in whole-cell I_{NaL} are not well defined. A recent single-channel study showed that inactivation gating of individual late Na_v is altered in human failing VCs [7] and a new numerical model of Na_v operating in different gating modes predicted slower whole-cell I_{NaL} decay in HF [11], but this prediction has not been tested experimentally.
2) Changes in I_{NaL} density in HF were previously found only for the portion of I_{NaL} remaining after prolonged membrane depolarization (500 ms [8] or 750 ms [9]), much longer than the AP plateau in human and canine VCs. Since the entire time course of I_{NaL} has not been compared in normal and failing VCs, the role of I_{NaL} in AP abnormalities in HF remains unclear.

Accordingly, we conducted the present study in order to: 1) identify HF-related changes during almost entire time course of I_{NaL} in VCs of human and canine hearts, 2) characterize their importance for abnormal repolarization, and 3) delineate principles of cardioprotection in HF based on I_{NaL} correction.

2. Methods

Our investigation of human heart tissue conforms to the principles outlined in the Declaration of Helsinki, and was approved by the Henry Ford Health System Human Rights Committee (Institutional Review Board). The canine study conforms to the Guidelines for Care and Use of Laboratory Animals published by the US National Institutes of Health and was approved by the Institutional Animal Care and Use Committee (IACUC) of the Henry Ford Health System.

We used 4 normal donor human hearts, which for technical reasons were deemed unsuitable for transplantation, and 16 explanted failing hearts (Table 1). To complement our studies in humans, we used a canine model of chronic HF. Briefly; HF was produced in 25 canine hearts ~ 3 months after multiple sequential coronary artery microsphere embolizations [12]. By 3 months left ventricle (LV) ejection fraction had diminished to ~ 25–27%, and the dog hearts manifested ventricular arrhythmias and sudden death similar to human HF [13].

Table 1
Demography of human hearts used in the study

Patient/ heart	Aetiology	Age, y	Sex	τ, ms	Medications
1	ICM	48	M	663	ACE inhibitor, beta blocker, Digoxin, Furosemide
2	IDC	57	M	624	ACE inhibitor, Digoxin, Furosemide
3	ICM	57	M	665	Amiodarone, ACE inhibitor, Digoxin, Furosemide
4	ICM	39	M	723	ACE inhibitor, Digoxin, Isosorbide dinitrate, Furosemide
5	IDC	27	F	653	ACE inhibitor, Digoxin, Furosemide
6	IDC	44	M	633	ACE inhibitor, Digoxin, Furosemide, Milrinone
7	IDC	45	M	688	ACE inhibitor, Digoxin, Furosemide, Milrinone
8	ICM	55	M	623	Amiodarone, ACE inhibitor, Digoxin
9	ICM	62	M	728	Digoxin, Isosorbide dinitrate, Furosemide
10	IDC	54	M	692	Amiodarone, ACE inhibitor, Digoxin, Furosemide
11	ICM	52	M	675	Amiodarone, ACE inhibitor, Digoxin, Furosemide
12	ICM	53	M	536	ACE inhibitor, Spironolactone, Digoxin
13	IDC	56	F	611	Amiodarone, Diltiazem, Digoxin, Isosorbide dinitrate, Gemfibrozil, Atenolol
14	ICM	61	F	570	ACE inhibitor, Furosemide, Milrinone
15	IDC	63	M	641	Beta blocker, Digoxin, Furosemide, Losartan
16	IDC	39	F	820	ACE inhibitor, Spironolactone, Digoxin
17	*Normal	N/D	N/D	528	
18	*Normal	N/D	N/D	572	
19	Normal	N/D	N/D	546	
20	Normal	N/D	N/D	472	

τ indicates I_{NaL} decay time constant, IDC indicates idiopathic dilated cardiomyopathy; ICM, ischaemic cardiomyopathy. *Some data on the normal hearts have been reported previously [6]. N/D — not disclosed. Medications were given during preparation for transplantation.

Fourteen normal dog hearts served as controls. Cardiomyocytes were enzymatically isolated from the apical LV mid-myocardium as reported previously [6]. We used the mid-myocardial layer to avoid inconsistencies related to the transmural I_{NaL} density profile [14,15]. The yield of viable rod-shaped, Ca^{2+}-tolerant myocytes varied from 10% to 50%.

I_{NaL} was measured using a whole-cell patch-clamp technique [6]. It was assessed by 2-s membrane depolarizations to −30 mV from a holding potential of −140 mV applied with a stimulation frequency of 0.1 Hz. To avoid voltage-clamp problems, we inactivated I_{NaT} by a 5-ms pre-pulse to +50 mV (Fig. 1A, inset), which substantially diminishes I_{NaT} but does not change I_{NaL} [6]. The solution contained (in mM): 140 NaCl, 5 CsCl, 1.8 $CaCl_2$, 2 $MgCl_2$, 5 glucose, 0.002

nifedipine, and 5 HEPES-CsOH buffer (pH 7.3). The pipette solution contained (in mM): 5 NaCl, 133 CsCl, 2 MgATP, 20 tetraethylammonium chloride, 10 EGTA, and 5 HEPES-CsOH buffer (pH 7.3). Experiments were performed at room temperature (22–24 °C). I_{NaL} decay was evaluated by a single exponential fit starting 200 ms after the onset of depolarization [6]. The dose–response curve describing I_{NaL} block (B%) by cadmium (Cd^{2+}), tetrodotoxin (TTX), or saxitoxin (STX) was approximated by a one-binding-site model:

$$B\% = \frac{100\%}{1 + IC_{50}/[\text{Drug}]}$$

APs were recorded at a pacing frequency of 0.25 Hz and 37 °C using an amphotericin B-perforated patch in current-clamp mode [6].

2.1. Statistical methods

Multiple comparisons were made with one-way analysis of variance (ANOVA). Deviations from the mean in all measurements are reported in terms of standard error. In some cases (Fig. 4D) Student's paired *t* test was applied. A p value < 0.05 was considered significant.

The quality of the single-site binding model (Fig. 3A,B) was evaluated by *F*-test (StatMost 2.5 Software, DataMost, UT), testing the null hypothesis that variability of the experimental data and values predicted by the model are the same. The *F*-value was determined as the ratio of the larger over the smaller variance and compared with the tabulated *F*-values for the respective degrees of freedom and a confidence level of 0.95.

3. Results

3.1. Chronic HF results in a slower and greater I_{NaL} in VCs

Fig. 1A shows superimposed representative raw I_{NaL} traces from VCs isolated from normal and failing hearts. Data on the I_{NaL} decay constant (τ) are summarized in Fig. 1B,C and shown for individual human hearts in Table 1. I_{NaL} was significantly and consistently slower in failing human hearts compared to normal hearts (by ~ 25%,

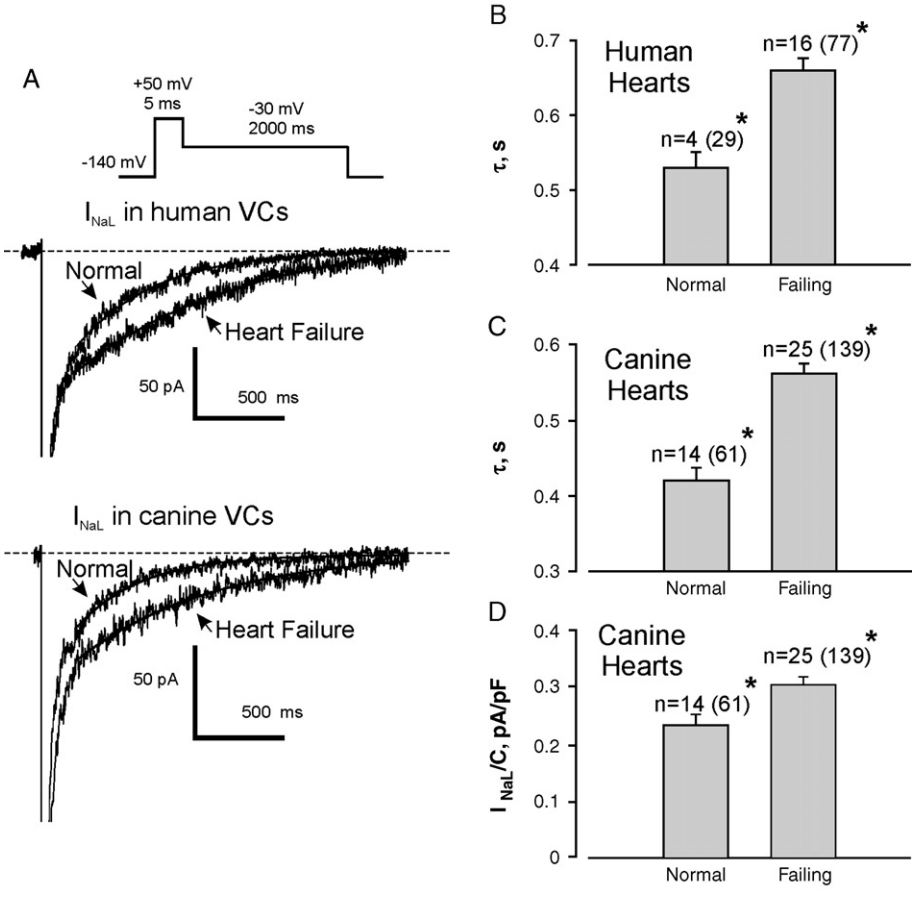

Fig. 1. Changes in whole-cell late Na^+ current (I_{NaL}) in left ventricular cardiomyocytes from normal and failing human and canine hearts. A, representative superimposed whole-cell traces recorded in normal and failing (patient #16, Table 1) human hearts (upper traces) and dog hearts (lower traces) using a voltage-clamp protocol (inset). Current was sent through a low-pass filter (200 Hz) and truncated at −150 pA. Dashed lines represent zero current. Solid lines show exponential fits to the experimental recordings, with decay time constants (τ) of 435 and 929 ms (human) or 351 and 739 ms (canine) for normal and failing myocardium, respectively. B and C, summarized data on τ for human and canine normal and failing hearts. D, average data on I_{NaL} density in normal and failing canine hearts. Plots show mean±SEM; n=number of hearts (with number of tested cells in parenthesis); *$p < 0.005$, normal *vs.* failing hearts (ANOVA).

i.e., $\tau = 659 \pm 16$ *vs*. 529 ± 21 ms; $n = 16$ and 4, respectively; $p < 0.002$). We also found a similar slowing of I_{NaL} decay (~ 34%) in canine failing cardiomyocytes (561 ± 13 *vs*. 420 ± 17 ms; $n = 25$ and 14 hearts, respectively; $p < 0.001$). The distribution of τ values in individual failing cells was broader, with a slight skew towards higher values compared to normal cells (Supplemental Fig. 1). Significantly, 14 out of 16 failing human hearts had $\tau > 600$ ms, whereas all normal hearts had $\tau < 600$ ms (Table 1). In some patients τ was relatively normal (536 and 570 ms for patients #12 and #14); others showed a dramatic increase, reaching as much as 1.5 times normal (723, 728, and 820 ms for patients #4, #9, and #16). Indeed, in some cells τ was close to 1 s (patient #16).

To describe I_{NaL} decay, in addition to time constants, we also measured an "initial" value of I_{NaL} density (I_{NaL}/C). However, identification and interpretation of HF-related I_{NaL}/C changes in humans proved complicated, as I_{NaL}/C varied greatly from patient to patient, probably due to differences in treatment, aetiology, sex, age, etc. (Table 1) [6]. Accordingly, we measured and analyzed HF-related changes in density using our reproducible canine HF model. I_{NaL}/C assessed 200 ms after membrane depolarization was significantly greater in the failing canine VCs (0.307 ± 0.014 pA/pF, $n = 25$ hearts *vs*. 0.235 ± 0.019 pA/pF, $n = 14$ normal hearts; $p < 0.005$) (Fig. 1D).

3.2. Reconstitution of the I_{NaL} time course at a physiological temperature

Using a simple single exponential decay model for I_{NaL} time course, we then evaluated the effect of the identified changes in I_{NaL} density and inactivation gating on the I_{NaL} time course and total Na^+ influx into the cells. Based on the τ values and the densities from Fig. 1C,D, we calculated the time course of the average I_{NaL} (Fig. 2A) and its integral (Fig. 2C) for normal and failing canine cells of the same size (200 pF), thus excluding the effect of ventricular myocyte hypertrophy reported previously in this canine chronic HF model [8]. Using Q_{10} factors, we then calculated the time course of I_{NaL} and its integral at a physiological temperature, 37 °C (Fig. 2B,D).

Important findings are:

1) The absolute difference between failing and normal cells (dotted lines in Fig. 2) was bell-shaped, with the peak

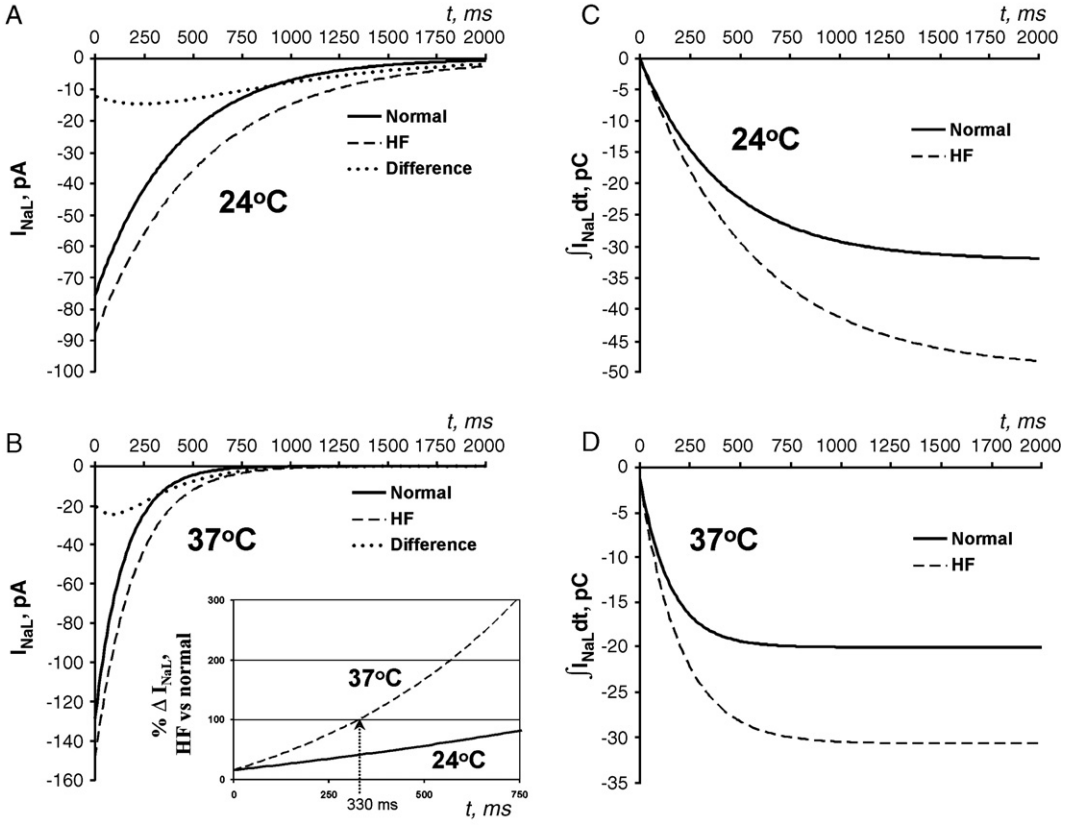

Fig. 2. Idealized total I_{NaL} time course calculated using an exponential decay function: $I(t) = C * d_{200\ ms} * \exp[-(t - 200\ ms)/\tau]$ with mean time constants (τ) and I_{NaL} densities, $d = I_{NaL}/C$ at 24 °C (A) and 37 °C (B) taken from Fig. 1C,D for normal (solid) and failing canine ventricular cardiomyocytes (dashed line) having the same electric capacitance C = 200 pF. The curves for 37 °C were calculated using Q_{10} factors $d_{0ms,37} = d_{0ms,24} * Q_{10d}^{\frac{37-24}{10}}$ and $\tau_{37} = \tau_{24} * Q_{10\tau}^{\frac{24-37}{10}}$ with $Q_{10d} = 1.5$ for Na_v conductance and $Q_{10\tau} = 2.2$ for late Na_v gating [11,38]. The absolute difference between normal and failing cells is shown by the dotted lines. The inset shows the relative difference (percent change) between normal and failing cells. C and D, total electrical charge transferred by I_{NaL}, assessed as an I_{NaL} integral at 24 °C and 37 °C (shown in A and B, respectively).

(−14.5 pA at 240 ms for 24 °C or −24.5 pA at 90 ms for 37 °C), occurring well within the AP plateau.
2) The relative difference between normal and failing cells increased progressively with membrane depolarization, doubling after 330 ms at 37 °C (Fig. 2B, inset).
3) Both absolute and relative differences were much greater at 37 °C, thus predicting a greater impact of I_{NaL} on membrane repolarization under physiological conditions.
4) The slower and greater I_{NaL} transferred far more Na^+ ions into failing cells (Fig. 2C,D). The integrals for I_{NaL} during 2 s were 48.3 *vs*. 31.9 pC (51.4% increase) at 24 °C and 30.66 *vs*. 19.96 pC (53.6% increase) at 37 °C.

3.3. Pharmacological characterization of I_{NaL}

TTX, STX and Cd^{2+} are valuable pharmacological tools to identify the specific Na^+ channel isoform underlying a Na^+ current. We found that I_{NaL} in human VCs was sensitive to Cd^{2+}; dose–response curves in one patient (patient #14, Table 1) revealed that Cd^{2+} blocked 50% of I_{NaL} at 104 μM (Fig. 3C). While it is known that I_{NaL} in human VCs exhibits low sensitivity to TTX and STX [6], the dose–response curves of these toxins for I_{NaL} in canine VCs has not been reported to our knowledge. We found that I_{NaL} of canine failing VCs exhibited a low affinity for TTX and STX (Fig. 3A,B). We also tested whether potassium cyanide (KCN) could modulate I_{NaL} in normal and failing human VCs. KCN did not influence I_{NaL} in failing human VCs either applied acutely during the patch-clamp experiment (40 min, 1 mM KCN; $n=5$ VCs) or after short-term pre-incubation of VCs before the patch-clamp experiment (2 h, 5 mM KCN) (Fig. 3D). KCN was also found to have no significant effect in normal VCs (not shown). In fact, the only remarkable effect of KCN was a significant increase in nonselective leak conductance (from 1.5- to 5-fold).

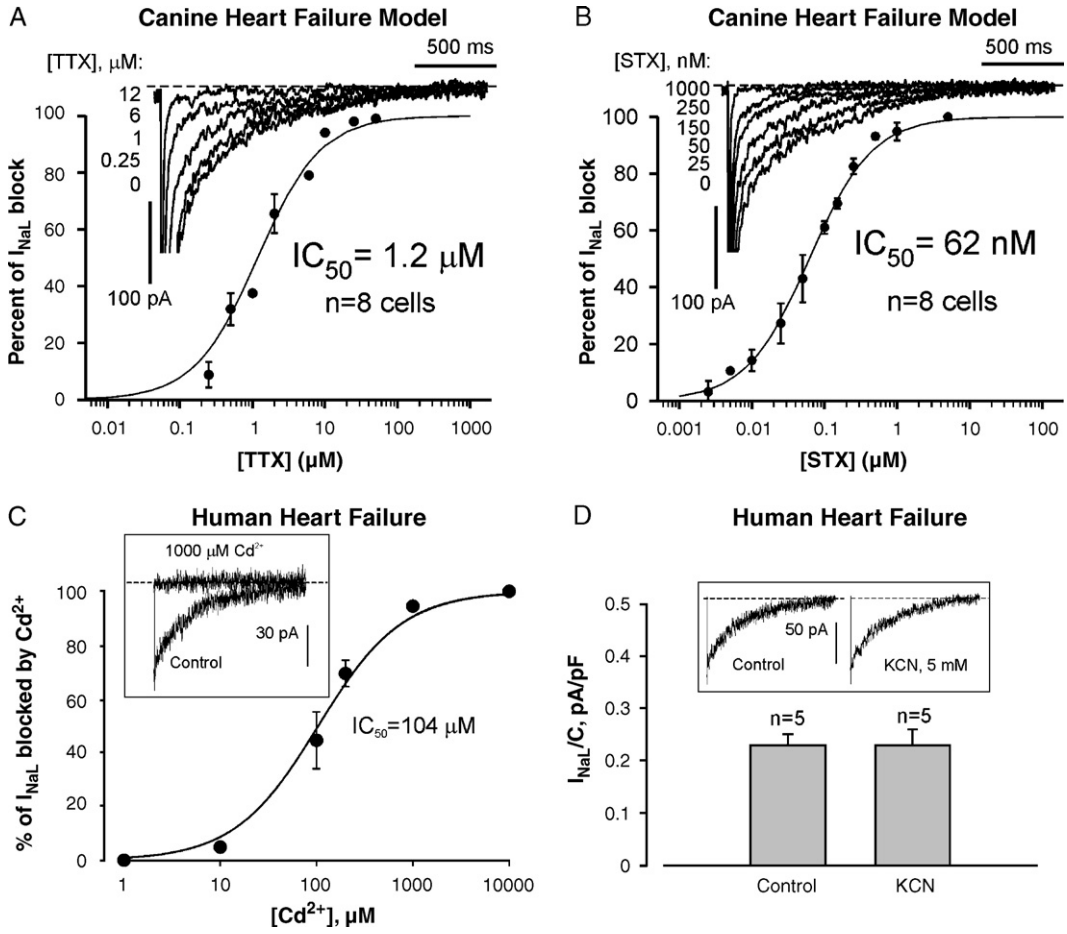

Fig. 3. Pharmacological characterization of I_{NaL}. A–C: Dose–response curve fitting percent of I_{NaL} blockade by TTX (A) and STX (B) in canine failing ventricular cardiomyocytes and by Cd^{2+} in human VCs (C, patient #3, Table 1) with a one-binding-site model (see Methods). Insets in A and B: superimposed whole-cell traces recorded in failing canine ventricular cardiomyocytes with different concentrations of tetrodotoxin (TTX) and saxitoxin (STX) in the bath (indicated at the traces), respectively. Traces were truncated at −200 pA, $V_h=-140$ mV, $V_t=-30$ mV, low-pass filter 50 Hz The inset in panel C shows superimposed I_{NaL} current trace fragments from 200 to 2000 ms before (control) and after application of 1 mM Cd^{2+}. D, summarized data on I_{NaL} current density recorded in control and cyanide-treated cardiomyocytes from human failing hearts. The inset shows representative raw I_{NaL} traces before (left) and after (right) exposure of cardiomyocytes isolated from a failing heart (patient #2, Table 1) to cyanide for 2 h. Room temperature was 22–23 °C in all experiments.

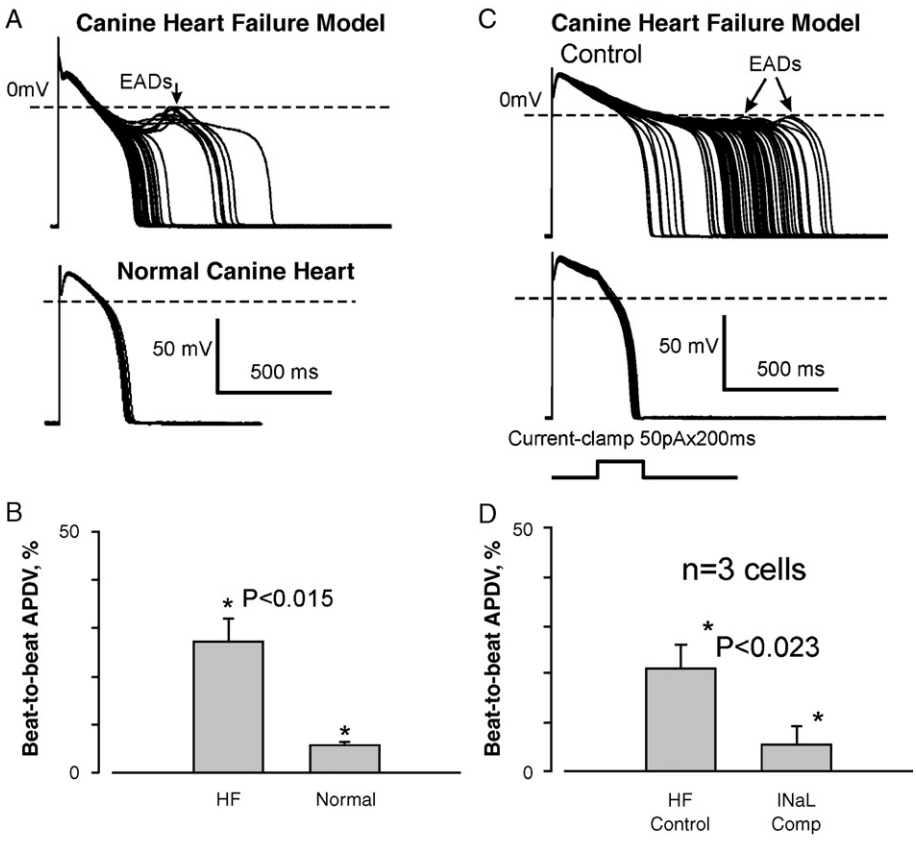

Fig. 4. Chronic HF increases beat-to-beat action potential duration variability (APDV) in canine ventricular myocytes that can be rescued by a reduction of I_{NaL}. A: Superimposed consecutive APs recorded by perforated patch clamp in representative normal and failing dog ventricular myocytes at 37 °C and 0.25 Hz pacing rate. B: Average data on APDV assessed as SD/mean. The figure shows the mean ± SEM for 18 failing hearts (61 cells, 908 APs) and 4 normal hearts (7 cells, 141 APs). *P* was evaluated by ANOVA. C and D: Beat-to-beat APDV in failing canine ventricular myocytes was decreased by electrical neutralization of the late Na^+ current with a delayed external current (200 ms after AP upstroke) at 37 °C and 0.25 Hz pacing rate. C: A representative example of a delayed external current effect on the consecutive APs (overlapped traces) in one cell. D: Average APDV data (mean ± SEM) assessed as SD/mean in 3 cells. Statistical comparison was made by Student's paired *t* test.

3.4. Partial I_{NaL} inhibition restores normal repolarization

We found that VCs from canine and human failing hearts exhibited variable beat-to-beat AP duration, culminating in early afterdepolarizations (EADs) [6,8] (Supplemental Fig. 2). Since HF results in a two-fold increase in I_{NaL} at the most critical time point of 330 ms (Fig. 2B, inset, 37 °C), corresponding to the repolarization phase in normal cells, we used 1.5 μM TTX which would block ~ 50% of I_{NaL} [6] and thus render it "normal". Partial I_{NaL} blockade shortened AP duration in failing human VCs [6,8] (Supplemental Fig. 2A). Pharmacological correction of I_{NaL} restored "healthy" APs in the failing cells: EADs ceased and both AP duration and variability returned to normal [6] (Compare Supplemental Fig. 2A, second trace and Fig. 2B). A similar effect was found in canine failing VCs [8,16,17] (Supplemental Fig. 2C, D) using STX (100 nM), another I_{NaL} blocker (Fig. 3B). We also tested the importance of I_{NaL} for AP duration and variability and EADs directly, electrically neutralizing I_{NaL} by current clamp. Injecting a 50-pA current at the AP plateau, 200 ms after the AP upstroke (see Fig. 2 B, dashed line for I_{NaL} prediction at 37 °C), normalized AP duration and variability and abolished EADs (Fig. 4).

4. Discussion

We believe the present study consistently shows for the first time that I_{NaL} decay is significantly slower in left ventricular cardiomyocytes taken from patients with chronic HF undergoing heart transplantation. Our complementary study of a reproducible experimental canine model of chronic HF revealed that in addition to this I_{NaL} slowing, the density of I_{NaL} significantly increased in the failing VCs. We then explored the biophysical and physiological consequences of altered I_{NaL} in HF myocytes, since a slower and larger I_{NaL} might influence intracellular Na^+/Ca^{2+} balance and the balance of ion currents at the AP plateau, thereby affecting cell contractile performance and AP repolarization, respectively. Finally, we tested pharmacologically which Na_v isoform could be responsible for the change in I_{NaL}.

4.1. Greater I_{NaL} in HF VCs: possible link to AP abnormalities

We previously showed a significantly longer AP in HF VCs using a canine chronic HF model [8], in line with data

from other experimental HF models as well as from human HF VCs [6,18]. In the present study, based on data from an extended number of canine hearts, we found that besides increased AP duration, beat-to-beat AP duration variability (assessed as SD/mean) was significantly increased in the failing VCs (Fig. 4A,B). This finding could explain greater variability of refractoriness in patients with HF, in turn believed to be a predictor of arrhythmic events and sudden death [1,19,20]. Such variability of AP duration could be at least partly owed to slower and greater I_{NaL} in failing VCs as reported in the present study. Computer simulations based on I_{NaL} data have confirmed that I_{NaL} contributes to the VC AP plateau [14], and late Na^+ channel openings were indeed observed on the AP plateau level in human VCs [7]. Accordingly, as I_{NaL} increases and becomes slower in HF cells (Figs. 1 and 2, and Supplemental Fig. 1), its contribution is expected to be both greater and more persistent, prolonging AP duration and ultimately causing abnormal repolarization, such as EADs and AP duration variability. We found that EADs ceased and both AP duration and variability returned to normal when I_{NaL} was partially inhibited with TTX or STX [6,8](Supplemental Fig. 2). We also observed similar effect by injecting current opposite to I_{NaL} during the AP plateau (Fig. 4C,D). In contrast to Na^+ current blockade by toxins or lidocaine (Supplemental Fig. 2, see also [6,8]), this protocol preserved I_{NaT} and hence, the I_{NaT}-related Na^+ contribution to function of the Na^+/Ca^{2+} exchanger (NCX) [21,22].

These findings indicate that the slower and greater I_{NaL} in HF indeed prolongs the AP plateau and contributes to the high incidence of EADs and greater AP duration variability in VCs of HF patients [6] (Supplemental Fig. 2A, B) and canine failing hearts [8,17] (Fig. 4A,B) that is in turn, considered a potential mechanism for ventricular arrhythmias in HF [1,19,20]. Thus interventions designed to block I_{NaL} and/or accelerate I_{NaL} decay [17,23] in patients may have a beneficial effect on spatial [15] and temporal repolarization abnormalities [19,20]. However, blockade of I_{NaL} should be performed with extreme caution: 1) it should be specific for I_{NaL}, because simultaneous blockade of I_{NaT} (already downregulated in HF [9,24,25]) could cause slowed conduction and therefore could be pro-arrhythmic; and 2) it may also shift the Na^+/Ca^{2+} balance so that more Ca^{2+} would be transferred from the cell by the NCX, thus decreasing SR load and worsening contractile performance.

4.2. Greater Na^+ influx via I_{NaL} in HF VCs: possible effects on Na^+/Ca^{2+} balance and contractility

A mathematical model based on single-channel data in human VCs predicted that although I_{NaL} has a much smaller amplitude than I_{NaT}, it lasts much longer; thus both currents transfer roughly the same amount of Na^+ during membrane depolarization [11], suggesting a substantial I_{NaL} contribution to intracellular Na^+ homeostasis. In the present study we found that I_{NaL}-associated Na^+ flux increased by 53.8% in failing cells *vs*. normal cells of same size (Fig. 2D). Moreover, taking into account that I_{NaT} is reduced by 30–40% in HF VCs [9,24,25], the role of I_{NaL} in Na^+ homeostasis should be even more substantial in the failing cells, in line with the recent finding that $[Na]_i$ was significantly increased in failing paced cardiomyocytes [26]. Accordingly, in addition to AP modulation, I_{NaL} in failing VCs would be expected to offset the enhanced function of the upregulated NCX in HF [27], thus preserving intracellular Ca^{2+} and SR load which in turn could either improve contractile function at low frequencies or lead to diastolic Ca^{2+} overload and poor myocyte contraction in failing hearts at higher frequencies [16,17,21,28]. Furthermore, the danger of Ca^{2+} overload is that it leads to spontaneous Ca^{2+} release and related EADs and delayed afterdepolarizations culminating in life-threatening arrhythmias [1,10,22]. In summary, a greater Na^+ influx *via* I_{NaL} in failing VCs can be interpreted as an intrinsic digitalis-like effect on Na^+/Ca^{2+} balance with all its known benefits and risks. Inhibition of I_{NaL} can reduce those risks and thus represent a potential cardioprotective mechanism.

4.3. Molecular basis of the altered I_{NaL} in HF

Our previous studies of late Na^+ single-channel activity [7] did not reveal any peculiar Na_v activity that might be responsible for I_{NaL} in failing compared to normal hearts. Furthermore, heterologous expression of Na_v 1.5 produced late openings and gating modes similar to human VCs [7,11], indicating that Na_v 1.5 seems to be the main source of I_{NaL} in human VCs. However, this does not exclude the possibility that another voltage-sensitive Na_v α subunit isoform (Na_v x) could contribute to I_{NaL}.

A difference of 2 amino acids in the Na_v pore region accounts for the high affinity of neuronal Na_v and low affinity of cardiac Na_v for TTX or STX, a feature used to distinguish these Na_v isoforms [29]. A highly TTX-sensitive Na_v isoform has been suggested as a source of late Na_v openings in rat cardiomyocytes [30]. Different transcripts of highly TTX-sensitive brain isoforms (Na_v 1.1, 1.3, 1.6) have been discovered in the mouse heart and recently in dogs (Na_v 1.1, 1.2, 1.3) [31,32], suggesting species-specific expression. These neuronal Na_v isoforms were responsible for 10% to 20% of peak I_{Na} in myocardial and Purkinje cells, respectively [32]; however, our previous study showed that the total I_{NaL} in human VCs had a low affinity for TTX or STX [6]. The only reported mammalian Na_v isoform with a low affinity for TTX other than Na_v 1.5 is Na_v 1.8 (gene SCN10A) [33]; however, as far as we know, the mRNA encoding Na_v 1.8 has not been detected in rat hearts [33], failing human heart tissue or isolated LV cardiomyocytes (Undrovinas and Kyle, unpublished data). In the present study, we found that I_{NaL} of failing canine hearts had a low affinity for TTX and STX (Fig. 3A,B) similar to human VCs [6]; IC_{50} was 1.2 *vs*. 1.53 μM for TTX and 62 *vs*. 98 nM for STX comparing dog vs. human cells.

The experiments blocking I_{NaL} with different concentrations of TTX and STX were crucial to understand the origin I_{NaL}. If I_{NaL} represented the activity of channels with different pharmacological and gating properties, the extent of blockade would differ at different time points after membrane depolarization. However from our original I_{NaL} traces in dogs (see example in Fig. 3A,B) and humans (Fig. 3A,B from our previous paper [6]), it is clearly seen that the extent of the toxin-induced blockade was about the same during I_{NaL} time course, ranging from 200 to 1000 ms. There was no significant "non-inactivated" or "steady-state" Na^+ current blocked by the toxins as of 2 s of membrane depolarization. Thus our data suggest that within this time frame I_{NaL} is mainly defined by the activity of only one type of Na_v so that I_{NaL} in canine and human VCs has a common molecular origin (i.e. Na_v 1.5) and similar molecular mechanisms could be involved in slowing of I_{NaL} in HF.

A possible role for other Na_v isoforms in I_{NaL} might be substantiated by the use of divalent cations. TTX-sensitive Na_v isoforms are blocked poorly by divalent cations, whereas TTX-resistant forms of Na_v are blocked by micromolar Cd^{2+} [34]. The present study indicates that in human VCs I_{NaL} is sensitive to Cd^{2+} (Fig. 3C), supporting our supposition that the Na_v isoform with low TTX affinity is primarily responsible for I_{NaL} in humans [6]. We recently demonstrated that silencing the SCN5A gene responsible for Na_v 1.5 expression with a siRNA decreased I_{NaL} by 75% and reduced AP duration in dogs with chronic HF [35]. Therefore Na_v 1.5 generates most of the total I_{NaL}.

Experimental hypoxia and metabolic inhibitors such as cyanide can reportedly augment the persistent Na^+ current in rats [36]. These authors suggested that a different Na_v isoform sensitive to both hypoxia and cyanide is responsible for this persistent Na^+ current [30,36]. However, our data show that I_{NaL} is not sensitive to cyanide (Fig. 3D). These negative results do not support the hypothesis that a novel cyanide-sensitive Na_v isoform is involved in I_{NaL} generation in both normal and failing human hearts.

A persistent background I_{Na} has been reported in rabbit [37] and rat [30] cardiomyocytes at potentials ranging from −120 to 0 mV. However, we did not find any TTX-blockable (25 μM) I_{Na} at potentials less than −80 mV in human or canine VCs (not shown), suggesting species-specific differences in background I_{Na}, so that the possible role of Na_v in the regulation of resting potential suggested for some animals has not been confirmed for humans and dogs. Our previous finding also supports this result that TTX or STX decreased AP duration but did not change resting potential in human [6] or canine VCs [8].

4.4. Study limitations

There are two major limitations for studies that use live human tissue: 1) the great difficulty of obtaining normal human hearts, and 2) patient-to-patient variability due to various treatments, aetiologies, sex, age, etc. It is well known that gene expression (in our case SCN5A) can be treatment-dependent; for example, the beta adrenoreceptor blockers commonly used in patients with HF can alter I_{Na} density [24].

4.5. Conclusions

Chronic HF alters late Na^+ current in VCs; the current increases and becomes slower, thereby carrying a significantly greater Na^+ influx and contributing to abnormal repolarization and beat-to-beat AP duration variability. Interventions normalizing I_{NaL} can rescue functional AP profile in HF VCs. The mechanism that causes slowing of I_{NaL} decay in HF is related to modulation of gating (e.g., late channel gating modes [11]) of the predominant cardiac channel isoform Na_v 1.5 rather than expression of a different Na_v isoform.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejheart.2006.08.007.

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