Published in final edited form as:

Am J Physiol Heart Circ Physiol. 2008 April ; 294(4): H1597–H1608.

Modulation of the late sodium current by Ca^{2+} , calmodulin, and CaMKII in normal and failing dog cardiomyocytes: similarities and differences

Victor A. Maltsev, Vitaliy Reznikov, Nidas A. Undrovinas, Hani N. Sabbah, and Albertas Undrovinas

Department of Internal Medicine, Henry Ford Hospital, Detroit, Michigan

Abstract

Augmented and slowed late Na^+ current (I_{NaL}) was implicated in action potential duration variability, early afterdepolarizations, and abnormal Ca^{2+} handling in human and canine failing myocardium. **Objective** was to study I_{NaL} modulation by cytosolic Ca^{2+} ($[\text{Ca}^{2+}]_i$) in normal and failing ventricular myocytes.

Methods—Chronic heart failure was produced in 10 dogs by multiple sequential coronary artery microembolizations, 6 normal dogs served as a control. I_{NaL} fine structure was measured by whole-cell patch-clamp in ventricular myocytes and approximated by a sum of fast and slow exponentials produced by burst and late scattered modes of Na^+ channel gating, respectively.

Results— I_{NaL} greatly enhanced as $[\text{Ca}^{2+}]_i$ increased from “ Ca^{2+} free” to $1\mu\text{M}$: its maximum density increased, decay of both exponentials slowed, and steady-state-inactivation curve (SSI) shifted towards more positive potentials. Testing inhibition of CaMKII and CaM revealed similarities and differences of I_{NaL} modulation in failing vs. normal myocytes. Similarities: 1) CaMKII slows I_{NaL} decay and decreases the amplitude of fast exponential; 2) Ca^{2+} shifts SSI rightward. Differences: 1) slowing I_{NaL} by CaMKII is greater; 2) CaM shifts SSI leftward; 3) Ca^{2+} increases the amplitude of slow exponential.

Conclusions— Ca^{2+} /CaM/CaMKII signaling increases I_{NaL} and Na^+ influx in both normal and failing myocytes by slowing inactivation kinetics and shifting SSI. This Na^+ influx provides a novel Ca^{2+} positive feedback mechanism (via $\text{Na}^+/\text{Ca}^{2+}$ exchanger), enhancing contractions at higher beating rates, but worsening cardiomyocytes contractile and electrical performance in conditions of poor Ca^{2+} handling in heart failure.

Keywords

heart failure; late Na^+ current; calcium; arrhythmia

1. Introduction

Augmented and slowed late Na^+ current (I_{NaL}) in heart failure (HF) (21,46) contributes to action potential (AP) duration variability and development of early afterdepolarizations (EADs) in human and dog ventricular cardiomyocytes (VCs) isolated from failing hearts (16,

Address for correspondence: Albertas Undrovinas, Ph.D., Henry Ford Hospital, Cardiovascular Research, Education & Research Bldg. Room 4015, 2799 West Grand Boulevard, Detroit, MI 48202-2689, Phone: (313)-916-1321, Fax: (313)-916-3001, E-mail: aundrov1@hfhs.org.

Current address of Victor A. Maltsev is Gerontology Research Center, National Institute on Aging, NIH, 5600 Nathan Shock Drive, Baltimore, Maryland 21224, USA

Disclosures There are no disclosures

21,46). Interestingly, the amount of Na^+ carried by large but short (a few ms) transient Na^+ current (I_{NaT}) is almost equal to that carried by a much smaller but much longer I_{NaL} (hundreds of ms) (21,23). Since I_{NaT} is reportedly decreased (19,54) but I_{NaL} is increased in HF, the latter likely provides an important contribution to the increase in intracellular Na^+ ($[\text{Na}^+]_i$) and to a higher cell Ca^{2+} load in HF (26). Because of its pathophysiological significance, I_{NaL} recently emerged as a plausible target for cardioprotection therapies (reviews (8,26))

HF-related mechanisms leading to the I_{NaL} augmentation and slower decay are not clear. All previous comparisons of I_{NaL} in normal and failing hearts were performed at low intracellular Ca^{2+} in the presence of strong Ca^{2+} buffers, thus only Ca^{2+} -independent changes have been compared so far. Taking into account that Ca^{2+} handling in HF is altered (see review (6)), an intriguing question is then whether Ca^{2+} provides any modulatory feedback mechanism to I_{NaL} in HF.

The present study tested if intracellular Ca^{2+} modulates I_{NaL} in VCs and if this modulation is different in normal and failing hearts. For this purpose we employed an established model of canine chronic heart failure produced by the multiple sequential coronary artery microembolizations (34). This model produces array of morphological and functional changes similar to that for humans. In order to get a mechanistic insight to I_{NaL} modulation by Ca^{2+} , we tested how signaling via Ca^{2+} /calmodulin (CaM)/ Ca^{2+} -dependent CaM-kinase II (CaMKII) affects the whole-cell I_{NaL} in patch-clamped VCs isolated from normal and failing dog hearts. We found that while the intracellular Ca^{2+} signaling enhances I_{NaL} in both normal and failing VCs, its modulation range is larger in HF. This result indicates that previous evaluations of functional importance of I_{NaL} in normal and especially in failing hearts might be underestimated because they were based on Ca^{2+} independent I_{NaL} function. Preliminary data of this study have been reported as an abstract (18).

2. Materials and Methods

2.1. HF model and myocyte isolation

The 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 Animal Care and Use Committee (IACUC) of the Henry Ford Health System. Chronic heart failure that is similar by vast array of functional and pathophysiological parameters (33) to that in humans was produced in 10 dogs by multiple sequential coronary artery microsphere embolizations as previously described (34). Six normal dogs served as a control. At the time of harvesting the heart (~3 months after last embolization), left ventricular (LV) ejection fraction was approximately ~25%. Cardiomyocytes were enzymatically isolated from the apical LV mid-myocardial slices as previously reported (16). The yield of viable rod-shaped, Ca^{2+} -tolerant VCs varied from 40 to 70%.

2.2. Patch clamp technique and data analysis

I_{NaL} was measured using a whole-cell patch-clamp technique (16,46). I_{NaL} was assessed by 2s membrane depolarizations to various potentials from a holding potential of -130 mV applied with a stimulation frequency of 0.2 Hz, and presented in terms of the current density (pA/pF), i.e. $I_{\text{NaL}} = (\text{whole cell } I_{\text{NaL}}) / C_m$, where C_m is cell electric capacitance that was measured by a voltage ramp (19) in each cell. The composition of bath (B) and pipette (P) solution is shown in Table 1. Experiments were performed at room temperature (22-24°C). All measurements were made 8-25 min after the membrane rupture to complete cell dialysis with intracellular recording solutions (24,28,31). In previous studies peptides or small proteins such as protein kinase catalytic subunits (14) can quickly (within 5 minutes) reach their targets in adult ventricular myocytes during a whole cell dialysis via patch pipette.

The fine structure of I_{NaL} time course has been approximated by a double exponential fit to I_{NaL} decay starting at 40 ms after the onset of depolarization to -30 mV as previously suggested (23):

$$f(t) = I_{40} \cdot \{k_1 \cdot \exp(-t/\tau_1) + k_2 \cdot \exp(-t/\tau_2)\} \quad (1)$$

where τ_1 and τ_2 are the time constants, I_{40} is I_{NaL} instant value 40 ms after membrane depolarization, k_1 and k_2 are the contributions of each exponents ($k_1 + k_2 = 1$), respectively. 5-15 experimental traces were averaged to improve the quality of analysis.

The steady-state inactivation (SSI) was evaluated by a double-pulse protocol with 2s-duration pre-pulses (V_p) ranging from -130 mV to -40 mV followed by a testing pulse to -30 mV. I_{Na} amplitudes were normalized to that measured at $V_p = -130$ mV and the data points were fitted to a Boltzmann function $A(V_p)$:

$$A(V_p) = 1 / (1 + \exp((V_{\frac{1}{2}A} - V_p) / k_A)) \quad (2)$$

The steady-state activation (SSA) parameters were determined from the current-voltage relationships by fitting data points of the normalized current with the function (19):

$$I_{\text{Na}}(V_t) = G_{\text{max}} \cdot (V_r - V_t) / \{1 + \exp[(V_{\frac{1}{2}G} - V_t) / k_G]\} \quad (3)$$

Where G_{max} is a normalized maximum Na^+ conductance, V_r is a reversal potential; $V_{\frac{1}{2}G}$, and k_G are the midpoint and the slope of the respective Boltzmann function underlying the steady-state Na^+ channel activation. The I_{NaL} data points in the current-voltage relationships were measured as the averaged current density within 200-220 ms after depolarization onset (vertical bar in Figs. 1A, B, 3A, B, 5A, B).

2.3. Statistical Analysis

Multiple comparisons between treatment groups were made using one-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test. Data are reported as mean $\pm$ SEM. The significance of SSA or SSI changes were evaluated using F-test (StatMost, DataMost Corp., Salt Lake City, UT) for tabulated values predicted by the model (Eqs. 2, 3) at a confidence level of 0.95. Differences for both experimental data and model predictions were considered statistically significant for $P < 0.05$.

2.4 Chemicals

Collagenase type II (291 U/mg) was from Worthington (Freehold, NJ). CaM, CaM binding domain, peptide 290-309, and CaMKII inhibitor KN93 and its inactive analog KN92 were purchased from Calbiochem (Darmstadt, Germany). All other chemicals and enzymes were purchased from Sigma (St. Louis, MO).

3. Results

3.1. Elevated intracellular Ca^{2+} slows decay of I_{NaL} , increases density, and shifts SSI in both normal and failing hearts

First we compared I_{NaL} decay time course, density, SSA and SSI at low (depicted as " 0Ca^{2+} ") and high (" $1\mu\text{M Ca}^{2+}$ ") intra-pipette $[\text{Ca}^{2+}]_i$ (solutions composition are depicted in Table 1). Two intracellular Ca^{2+} buffering agents were tested; EGTA and BAPTA (P_1 and P_2 in Table 1). Although BAPTA provides more stringent Ca^{2+} buffering and more rapid Ca^{2+} binding kinetics (50), no difference was found for I_{NaL} decay kinetics, density, or SSI

when using these buffers (not shown). Accordingly, “ 0Ca^{2+} ” refers further to EGTA buffer. Elevated $[\text{Ca}^{2+}]_i$ substantially increased amplitude and slowed inactivation of I_{NaL} in both normal and failing hearts (Fig.1). Both τ_1 and τ_2 significantly increased in the elevated Ca^{2+} (Fig.1C, D). For example, at -30 mV (maximum I_{NaL}) their average values in “ 0Ca^{2+} ” and “ $1\mu\text{M Ca}^{2+}$ ” in normal hearts were as follows: $\tau_1 = 31.3 \pm 0.7$; $\tau_2 = 417.7 \pm 9.1$ ms, $n = 27$ VCs (4 hearts) vs. $\tau_1 = 48.6 \pm 2.1$; $\tau_2 = 487 \pm 18$ ms, $n = 11$ (2) ($p < 0.005$, ANOVA), respectively. For HF: $\tau_1 = 35.2 \pm 1.9$; $\tau_2 = 505.7 \pm 19.2$ ms, $n = 12$ (4) vs. $\tau_1 = 57.4 \pm 3.7$; $\tau_2 = 625.6 \pm 42$ ms, $n = 28$ (8) ($P < 0.005$), respectively. Interestingly, both time constants were significantly different at the same $[\text{Ca}^{2+}]_i$ when compared normal vs. failing VCs. However, relative contribution of these exponents remained almost unchanged (normal heart, $k_2 = 0.43 \pm 0.02$ vs. 0.46 ± 0.05 , 0Ca^{2+} vs. $1\mu\text{M Ca}^{2+}$; HF, $k_2 = 0.41 \pm 0.05$ vs. 0.43 ± 0.04 , respectively, NS). Elevated $[\text{Ca}^{2+}]_i$ significantly increased I_{NaL} density (Table 2) in a wide range of voltages, including potentials of the AP plateau in both normal and failing hearts (Fig. 2A, B). Another noticeable effect of the Ca^{2+} elevation was a shift (~ 5.8 and ~ 4.4 mV for normal and failing hearts, respectively) of the SSI mid-potential towards positive potentials (Fig.2 C, D, Table 3) without changes in the SSA mid-potential (Fig. 2A, B).

3.2. Infusion of CaM does not affect I_{NaL}

To test if intrinsic intracellular amount of CaM can limit effects of elevated $[\text{Ca}^{2+}]_i$ on I_{NaL} , we infused additional CaM molecules in the presence of $1\mu\text{M} [\text{Ca}^{2+}]_i$. The I_{NaL} decay, density and SSI (not shown) parameters were not significantly affected by the intracellular infusion of CaM into the cells *via* the patch pipette. These data indicated that $[\text{CaM}]_i$ either is not involved in Ca^{2+} modulation of I_{NaL} or remains high enough during the whole cell recordings to mediate Na^+ channel modulation by Ca^{2+} . Thus, the absence of any effect of CaM infusion served as a control for our further experiments with peptide P290-309, which were designed to clarify the role of CaM in I_{NaL} modulation by testing effects of CaM inhibition (see below).

3.3. Effects of a CaMKII inhibitor KN93

In the presence of high $[\text{Ca}^{2+}]_i$, CaMKII inhibition by KN93 caused two major effects (see examples of recordings in Fig.3 A, B): 1) I_{NaL} decay acceleration (both fast and slow components of I_{NaL}) in both normal and failing hearts blunting the difference in terms of I_{NaL} inactivation kinetics between these two specimens (Figs.3C,D); 2) significant I_{NaL} density reduction observed at various levels of testing voltages within the range of action potential plateau (as the whole-cell conductance decreased) in failing but not in normal hearts (Fig.4 A, B, Table 2). The $V_{1/2}$ of the SSA in both normal and failing hearts changed insignificantly. There was no significant effect on SSI by KN93 in either normal or failing hearts compared with the augmented $[\text{Ca}^{2+}]_i$ (Fig. 4 C, D, Table 3).

To provide a control for the substantial KN93 effect on I_{NaL} kinetics, we also tested the effect of KN92 (an inactive analog of KN93): In the presence of $1\mu\text{M} [\text{Ca}^{2+}]_i$ and $10\mu\text{M}$ KN92 the I_{NaL} kinetics and SSA remained unchanged ($n = 16$ (4) HF, not shown)). However, we did not compare effects of KN93 and KN92 on SSI due to two reasons 1) CaMKII blockade by KN93 had no effect on SSI, 2) well-known nonspecific effects of KN92 on SSI (9).

3.4. Effects of interference to Ca^{2+} -dependent CaM binding

Infusion of P290-309, a potent CaM antagonist, in the presence of high $[\text{Ca}^{2+}]_i$ caused two effects described above for KN93, i.e. I_{NaL} decay acceleration and density decrease (Fig.5, Table 2); and this was expected because inhibition of CaM is a less specific intervention that includes CaMKII inhibition (CaMKII function is by definition is CaM dependent). However, a specific effect of CaM inhibition that was not observed under KN93 was a significant shift of SSI curve towards positive potentials in failing but not in normal hearts (Fig.6 C, D, Table 3).

3.5. Interpretation of experimental data in terms of Ca^{2+} modulation of I_{NaL} gating modes and I_{NaL} -mediated Na^+ influx

While the experimental data (Figs. 3-6) of various interventions to Ca^{2+} regulatory pathways provide mechanistic insights into I_{NaL} regulation by those pathways (biochemical insights), additional analysis is required to evaluate numerically modulation by those pathways of various I_{NaL} gating modes (23,45) (biophysical insights) and of I_{NaL} mediated Na^+ influx into the cells (physiological insights). In our experiments the I_{NaL} density was measured 200 ms after membrane depolarization, and the I_{NaL} kinetics were evaluated starting from 40 ms. Thus, the observed changes in the density can be due to either I_{NaL} slowing or a decrease in the number of Na^+ channels operating in the late gating modes. To address this question, the fine structure of the entire I_{NaL} time course has been further analyzed based on I_{NaL} approximation by a double exponential function in Eq.2, which allows an easy interpretation of the whole cell data in terms of gating modes of late Na^+ channel openings. Contrary to a prediction of a non-inactivating I_{NaL} by a non-unique model of Na^+ channel gating (11), patch clamp studies in human ventricular myocytes showed that both the burst mode (BM) and the late scattered mode (LSM) openings do inactivate! (29,45). A recent unique numerical model (23) of Na^+ channel gating based on the single channel data of late openings describes the entire I_{NaL} time course as a sum of two exponentials: BM generates the fast, exponentially decaying I_{NaL} component (I_{BM}), whereas LSM openings generate a relatively slow I_{NaL} decaying component (I_{LSM}):

$$I_{\text{BM}}(t) = I_{40} \cdot k_1 \cdot e^{-(t-40\text{ms})/\tau_1} \quad (4)$$

$$I_{\text{LSM}}(t) = I_{40} \cdot k_2 \cdot e^{-(t-40\text{ms})/\tau_2} \quad (5)$$

Where I_{40} , k_1 , k_2 , τ_1 , and τ_2 are from Eq.1. Using the above approximations, we evaluated the following important parameters: amplitudes $I_{\text{BM}}(0)$ and $I_{\text{LSM}}(0)$ and integrals Q_{BM} and Q_{LSM} of the fast and slow I_{NaL} components, respectively:

$$Q_{\text{BM}} = \int_0^{2000\text{ ms}} I_{\text{BM}}(t) dt \quad (6)$$

$$Q_{\text{LSM}} = \int_0^{2000\text{ ms}} I_{\text{LSM}}(t) dt \quad (7)$$

The amplitudes thus reflect the total number of channels in respective gating modes and the integrals reflect the respective Na^+ influxes transferred by each mode. Since we found a complex modulation of SSI by Ca^{2+} and CaM, we also evaluated the total integral for I_{NaL} elicited from a physiological resting potential of $V_{\text{rest}} = -80$ mV:

$$Q_{\text{tot}} = A(-80) \cdot (Q_{\text{BM}} + Q_{\text{LSM}}) \quad (8)$$

where $A(-80)$ is Na^+ channel steady-state availability at -80 mV calculated using Eq.2. The results of the analysis are summarized in Table 4 and can be interpreted for each modulation pathway as follows:

CaMKII slows decay kinetics of both BM and LSM openings with the effect being greater in failing VCs, resulting in a substantially larger regulation range of Q_{LSM} by CaMKII in the failing cells, 56.8% vs. 35% (**CaMKII** modulates $I_{\text{LSM}}(0)$, i.e. the number of LSM channels, insignificantly). At the same time, CaMKII substantially decreases $I_{\text{BM}}(0)$, i.e. the number of BM channels, with Q_{BM} remaining almost unchanged in both normal and failing cells.

CaM produces no additional modulatory effects on the fine structure of I_{NaL} rather than those described above for CaMKII modulation. Hence, the only major and specific effect of CaM on I_{NaL} is the negative SSI shift in the failing cells.

Ca²⁺ itself, independently of CaM, increases $I_{LSM}(0)$ (reflecting the number of LSM channels) by ~30.7% in failing cells but produces no effect on this parameter in normal cells. Ca²⁺ also (independently of CaM) shifts SSI to negative potentials. The combined effect of this shift and $I_{LSM}(0)$ increase on Q_{tot} was substantially larger in the failing cells vs. normal cells (150% vs. 61.6%). Interestingly, that CaMKII contribution in Q_{tot} modulation is minor (only ~21%) in the failing cells.

Net Ca²⁺ effect on I_{NaL} and its integral is strong in both normal and failing VCs (Fig.7). The absolute modulation range of I_{NaL} is larger in failing cells, with the difference being greater at the beginning of depolarization (shaded area in Fig.7C). The relative instant increase of I_{NaL} produced by Ca²⁺ was larger in failing cells; in both cell types it exhibits an N-like shape with a local maximum at ~120 ms (Fig.7D). CaMKII-mediated slowing of LSM decay and rightward shift of SSI by Ca²⁺ (CaM-independent) turns out to be the major contributors to the I_{NaL} integral change (Fig.7 E-G, Table 4). In HF VCs, while Ca²⁺ increases the number of LSM channels and shifts SSI to more positive voltages, these effects are offset by CaM-induced negative SSI shift, so that the net Ca²⁺- induced increase of Q_{tot} is only moderately higher in HF (135% vs. 101%, respectively, Table 4, see also shaded area in Fig.7G “HF-NORM”)

4. Discussion

The present study has demonstrated that I_{NaL} in ventricular myocytes of normal and failing dog hearts is strongly enhanced by intracellular Ca²⁺ and this modulation is linked to complex Ca²⁺/CaM/CaMKII signaling (summarized in Table 4 and schematically illustrated in Fig.8). We identified Ca²⁺-dependent modulation mechanisms, which are common and different in normal and failing cells. The common mechanisms include 1) CaMKII slows the I_{NaL} decay kinetics and decreases the amplitude of the fast/bursts I_{NaL} component; 2) direct Ca²⁺ binding shifts SSI curve to more positive potentials. I_{NaL} modulation in failing VCs differs from that in normal cells: 1) slowing of both I_{NaL} components I_{NaL} by CaMKII is greater; 2) Ca²⁺ shifts SSI to more positive voltages; 3) CaM shifts SSI curve to negative potentials; 4) Ca²⁺ increases the amplitude of I_{NaL} slow/LSM component.

4.1 Molecular mechanisms of I_{NaL} modulation by Ca²⁺-CaM-CaMKII pathways

Previous studies of Na⁺ channels have established several pathways for Na⁺ channel modulation by intracellular Ca²⁺: 1) direct binding of Ca²⁺ ions to Ca²⁺ binding EF-hand motif found in Na_v1.5 C-terminus (50), 2) direct CaM binding to IQ motif of Na_v1.5 C-terminus (39), 3) activation CaMKII resulting in phosphorylation of the channel protein (9,49). Ca²⁺ modulation of Na⁺ channels is a rather complex phenomenon and continues to be a matter of debate (see review (1)) especially when heterologous expression systems and native myocytes are compared. Moreover, conclusions of aforementioned studies are mainly based on recordings of the transient Na⁺ current that may or may not be relevant to I_{NaL} modulation.

All three above molecular mechanisms can be potentially involved in the effects of complex Ca²⁺/CaM/CaMKII modulation of I_{NaL} characteristics found in the present study (Table 4, Fig. 8). More specifically, we found that late Na⁺ channel gating is regulated by CaMKII (Fig. 3) in both normal (in line with (49)) and failing hearts, and that Ca²⁺ shifts SSI towards positive potentials (Fig1 C, D) (in line with (50)). The latter effect is likely independent of CaM and CaMKII in normal myocytes because inhibition of either CaM or CaMKII has no effect on SSI (Fig. 6C).

However, specifically in failing cells CaM (independently of CaMKII) offsets, at least in part, the SSI shift associated with the major CaM-independent effect. The negative shift of SSI by CaM-dependent regulation is in line with Tan et al. (39). However, Wagner et al. (49) reported an opposite effect (compared with (50) and our study) of $[Ca^{2+}]_i$ elevation on SSI in rabbit and rat cardiomyocytes, including those in which CaMKII δ_C was over-expressed. The difference in results could be due to a) different cell types, e.g. tsA201 (50) cells vs. cardiomyocytes; b) perturbation of Ca^{2+} signaling by an over-expression of the signaling proteins; c) different currents used to assess SSI i.e. I_{NaT} or I_{NaL} (our study); d) different recording conditions, e.g. 10 mM (49) vs. 140 mM. In fact, occupancy of the Na^+ channel pore by the permeant ion substantially affects slow inactivation of the channel (3).

4.2. Why is Ca^{2+} modulation of I_{NaL} different in heart failure?

Na^+ channel environment and composition—The function of Na^+ channels (especially their late openings) is complex and not fully determined by their protein structure, but also greatly depends on interactions with multiple molecules of the channel environment, the function of which could be, in turn, also Ca^{2+} -dependent and greatly altered in HF. In addition to the pore-forming α subunit and its auxiliary β subunits, the multi-protein Na^+ channel complex includes components of cytoskeleton, regulatory kinases and phosphatases, trafficking proteins, and extracellular matrix proteins embedded into lipid bilayer plasma membrane (see reviews (1,25)). Subsarcolemmal actin-fodrin cytoskeleton is involved in modulation of the $Na_v1.5$ inactivation gating (44,47). Breakdown of actin-fodrin cytoskeleton integrity leads to the activation-inactivation uncoupling (22) similar to that reported here (Fig. 2 A, B vs. C, D). The fodrin breakdown that occurs in some hearts disease states featuring poor Ca^{2+} handling, can be mediated by the Ca^{2+} /CaM via enzymes calpain and caspase (32,51). Accordingly, these **indirect** mechanisms can contribute to the CaM effect on SSI in failing myocytes reported here.

Possible specific mechanisms for the differences—While the greater modulation range for CaMKII in failing cells found in the present study is likely due to a higher expression of CaMKII in HF (see review (53)), the mechanism of CaM effect on SSI in failing hearts is not clear. As to $Na_v1.5$, it is still ambiguous if its SSI is (39) or is not (9,50,52) regulated by CaM. Our data in normal cardiomyocytes rather supports the latter hypothesis. Thus, possibilities for the specific I_{NaL} regulation by CaM in HF include indirect effect of CaM (via molecules of the Na^+ channel complex, see above) or expression of other Na^+ channel isoforms modulated by CaM. Different transcripts of highly TTX-sensitive neuronal isoforms ($Na_v1.1$, 1.3, 1.6) have been indeed identified in mouse and dog heart ($Na_v1.1$, 1.2, 1.3) (12,15). These neuronal Na^+ channel isoforms are responsible for 10 to 20% of the Na^+ current peak in myocardial and Purkinje cells, respectively (12). CaM also shifts SSI curve for the skeletal muscle isoform $Na_v1.4$ (9,52). Furthermore, four splice variants of SCN5A gene that encodes $Na_v1.5$ have been reported in humans and they differ in their biophysical properties (38). Contributions of these variants and/or isoforms into cardiac I_{NaL} and their modulation by different pathological conditions are not yet understood and need further studies.

4.3. Significance of I_{NaL} modulation by Ca^{2+} for normal heart function

Ca^{2+} modulation of I_{NaL} likely contributes to cell Na^+ / Ca^{2+} balance, which is critical for normal and enhanced cardiomyocyte performance. More specifically, Ca^{2+} dependent increase of I_{NaL} may provide a novel mechanism contributing to a well known Bowditch-Treppe effect postulating that an increase in the heart rate leads to an increase in the force of contraction (inotropic effect of rate). This fundamental law defines the net heart performance when demand for blood pumping increases. Higher cell Ca^{2+} levels, which are required for stronger contractions, can be preserved with increased Na^+ influx via I_{NaL} during AP plateau by the Ca^{2+} -dependent positive feedback mechanism discovered in the present study. The

contribution of this mechanism is thus linked to a higher cellular Na^+ , which, in turn, decreases Ca^{2+} extrusion by NCX.

4.4. Significance of I_{NaL} and its modulation by Ca^{2+} in heart failure

Chronic HF is characterized by both cardiac arrhythmias and reduced contractility, and I_{NaL} likely contributes to these problems, as partial blockade of I_{NaL} normalizes AP repolarization, inhibits EADs, and greatly improves contractility of failing VCs (16,21,46). I_{NaL} decay is significantly slowed and I_{NaL} density is increased in VCs of chronic human and canine HF (21,46). The importance of I_{NaL} relative to other currents can be greater in HF, because K^+ current and I_{NaT} are downregulated in failing myocardium (7,19,48,54). For example, after 500 ms of membrane depolarization I_{NaL} becomes comparable with I_{CaL} ($I_{\text{NaL}} = 0.68 \cdot I_{\text{CaL}}$) in canine failing VCs (46). Thus, I_{NaL} greatly contributes to the ion current balance on the AP plateau (36,45) as well as to cell Na^+ influx (21), and thereby indirectly to cell Ca^{2+} regulation via NCX ((17,42), review (5)).

The increased net I_{NaL} provides additional Na^+ influx in failing cardiomyocytes by ~54 to 58% (21,23) and the total I_{NaL} -mediated Na^+ influx is almost equal to that mediated by I_{NaT} (23). The rise of I_{NaL} -related Na^+ influx can explain, at least in part, the increased cell Na^+ load documented in HF (4,10,30). The I_{NaL} contribution might be even larger, because I_{NaL} was always measured at low $[\text{Ca}^{2+}]_i$ in the previous studies, but the present study identified strong positive I_{NaL} modulation by $[\text{Ca}^{2+}]_i$ (Fig. 1, Table . The effect of Ca^{2+} modulation was observed within the voltage range of AP plateau (Fig.2 B). Accordingly, the Na^+ overload, in turn, is expected to shift the operation of NCX (upregulated in HF (37)) during the AP plateau from the forward to the reverse mode of operation, leading to a higher cell Ca^{2+} load.

This Na^+ -driven higher Ca^{2+} load limits depression of systolic function of failing VCs (5) and thus can be considered as an intrinsic, adaptive, digitalis-like effect with all corresponding risks and benefits. Interestingly, a large burst mode component of the Na^+ current has been identified in post-myocardial infarction-remodeled myocytes (13), i.e. in the transitional period from an infarction to HF. The increased I_{NaL} may indeed serve as an initial adaptation mechanism to match an increased contractility demand for the survived VCs.

On the other hand, Ca^{2+} overload has substantial adverse effects in failing hearts including: 1) arrhythmias via increased probability of spontaneous Ca^{2+} release and DADs, and 2) failing contractility due to diastolic dysfunction, especially at high heart rates. In addition to the problems of Ca^{2+} overload, the increased I_{NaL} prolongs AP plateau and leads to beat-to-beat variability of AP duration, and EADs (16,21,42,46), which may also cause arrhythmias (40). Interestingly, decay kinetics of I_{CaL} and I_{NaL} are regulated in the opposite ways by Ca^{2+} , i.e. accelerating (2) and slowing (this study), respectively. As failing VCs are loaded with Ca^{2+} , the mechanism of the Ca^{2+} -induced- I_{NaL} increase may thus provide a positive feedback mechanism to increase further Na^+ influx and facilitate cell Ca^{2+} overload, thus exaggerating further the aforementioned HF-related problems.

4.5. Ca^{2+} modulates gating modes of late Na^+ channel openings

We interpret our results in terms of channel gating based on previous single channel studies and numerical modeling of I_{NaL} (see section 3.5). CaMKII is expected to slow inactivation kinetics of both burst mode and LSM (to a greater extent in HF) and to decrease the number of burst mode channels; Ca^{2+} (independently on CaM) increases the number of LSM channels specifically in failing myocytes. The effect of Ca^{2+} on bursts is in line with that the cytoskeleton integrity and lysophosphatidylcholine affect the burst mode of Na^+ channel openings (43,47). These modulators are Ca^{2+} -dependent: the cytoskeleton is affected by calpain and caspase (see section 4.1), and lysophosphatidylcholine is a substrate for protein kinase C (27). We found

that instant Ca^{2+} modulation in failing cells is much greater at the beginning of depolarization (shaded area in Fig. 7C), indicating that Ca^{2+} regulates not only Na^+ total influx via I_{NaL} but also complex I_{NaL} dynamics, contributed by different gating modes of late channel openings. More specifically, while the integral of the burst mode (i.e. Q_{BM}) remains almost the same at various $[\text{Ca}^{2+}]_i$ (Table 4), the dynamic contribution of bursts into I_{NaL} is greatly modulated by $[\text{Ca}^{2+}]_i$ in HF. Since bursts are active early after depolarization onset (45), this modulation, in turn, could be important for early repolarization phase (the “notch” phase), which is altered in HF (in our canine model and humans (16,46)) and determines the synchronicity of the Ca^{2+} release (see review (35)), and thereby the quality of EC coupling.

4.6. Targeting I_{NaL} is a novel approach in prevention of ventricular arrhythmia and diastolic dysfunction in HF

Based on the discovery that amiodarone effectively and selectively reduces I_{NaL} compared to I_{NaT} in human failing myocardium, we have previously suggested a new therapeutic strategy for cardioprotection by a selective inhibition of I_{NaL} (20). Our most recent finding in failing VCs was that ranolazine blocks I_{NaL} (41) even more selectively than amiodarone, and this new strategy of I_{NaL} blockade is now seriously considered by clinical cardiologists in future therapies to prevent cardiac arrhythmias and Ca^{2+} overload both in HF and cardiac ischemia (8). The results of the present study indicate that I_{NaL} can be also targeted indirectly via Ca^{2+} signaling. More specifically, as CaMKII was found to be upregulated in human HF (review (53)), I_{NaL} decay can be potentially accelerated by a CaMKII inhibition as well as by intracellular Ca^{2+} buffering, or by prevention of CaM binding.

Study limitations

Although the $1\ \mu\text{M}$ $[\text{Ca}^{2+}]_i$ used in the study is high (but still within a physiological range for VCs), its effect on I_{NaL} can be exaggerated because of a prolong influence of Ca^{2+} at the steady-state that is different from dynamic Ca^{2+} variations in intact beating cells. Studies of the dynamic modulation of I_{NaL} by Ca^{2+} (including numerical modeling of AP in HF) as well as of detailed molecular mechanisms of the modulation merit further consideration. Although we suggest I_{NaL} and its Ca^{2+} regulation as potential targets for cardioprotection, this should be considered with caution as I_{NaL} inhibition can decrease systolic function and in some cases can be pro-arrhythmic, when the I_{NaL} -mediated AP prolongation is associated with an anti-reentry effect.

Acknowledgements

Grants This study was supported by grants from the National Heart, Lung and Blood Institute HL-53819, HL074238 (A Undrovinas), and by a grant-in-aid from the American Heart Association 0350472Z (A Undrovinas).

References

1. Abriel H, Kass RS. Regulation of the voltage-gated cardiac sodium channel Nav1.5 by interacting proteins. *Trends Cardiovasc Med* 2005;15:35–40. [PubMed: 15795161]
2. Alseikhan BA, DeMaria CD, Colecraft HM, Yue DT. Engineered calmodulins reveal the unexpected eminence of Ca^{2+} channel inactivation in controlling heart excitation. *Proc Natl Acad Sci U S A* 2002;99:17185–90. [PubMed: 12486220]
3. Aman TK, Raman IM. Subunit dependence of Na channel open-channel block and slow inactivation in cerebellar neurons. *Biophys J* 2006;22:22.
4. Baartscheer A, Schumacher CA, Belterman CN, Coronel R, Fiolet JW. $[\text{Na}^+]_i$ and the driving force of the $\text{Na}^+/\text{Ca}^{2+}$ -exchanger in heart failure. *Cardiovasc Res* 2003;57:986–95. [PubMed: 12650876]
5. Bers DM. Altered cardiac myocyte Ca regulation in heart failure. *Physiology (Bethesda)* 2006;21:380–7. [PubMed: 17119150]

6. Bers DM, Despa S. Cardiac myocytes Ca^{2+} and Na^{+} regulation in normal and failing hearts. *J Pharmacol Sci* 2006;100:315–22. [PubMed: 16552170]
7. Beuckelmann DJ, Nabauer M, Erdmann E. Alterations of K^{+} currents in isolated human ventricular myocytes from patients with terminal heart failure. *Circ Res* 1993;73:379–85. [PubMed: 8330380]
8. Conti CR. Inhibition of sodium-dependent calcium overload to treat myocardial ischemia. *Clin Cardiol* 2006;29:141–3. [PubMed: 16649721]
9. Deschenes I, Neyroud N, DiSilvestre D, Marban E, Yue DT, Tomaselli GF. Isoform-specific modulation of voltage-gated Na^{+} channels by calmodulin. *Circ Res* 2002;90:E49–57. [PubMed: 11884381]
10. Despa S, Islam MA, Weber CR, Pogwizd SM, Bers DM. Intracellular Na^{+} concentration is elevated in heart failure but Na/K pump function is unchanged. *Circulation* 2002;105:2543–8. [PubMed: 12034663]
11. Dumaine R, Wang Q, Keating MT, Hartmann HA, Schwartz PJ, Brown AM, Kirsch GE. Multiple mechanisms of Na^{+} channel-linked long-QT syndrome. *Circ Res* 1996;78:916–24. [PubMed: 8620612]
12. Haufe V, Cordeiro JM, Zimmer T, Wu YS, Schiccitano S, Benndorf K, Dumaine R. Contribution of neuronal sodium channels to the cardiac fast sodium current I_{Na} is greater in dog heart Purkinje fibers than in ventricles. *Cardiovasc Res* 2005;65:117–27. [PubMed: 15621039]
13. Huang B, El-Sherif T, Gidh-Jain M, Qin D, El-Sherif N. Alterations of sodium channel kinetics and gene expression in the postinfarction remodeled myocardium. *J Cardiovasc Electrophysiol* 2001;12:218–25. [PubMed: 11232622]
14. Kameyama M, Hofmann F, Trautwein W. On the mechanism of beta-adrenergic regulation of the Ca channel in the guinea-pig heart. *Pflugers Arch* 1985;405:285–93. [PubMed: 2415919]
15. Maier S, Westenbroek R, Yu FH, Vien T, Scheuer T, Catterall WA. Functional expression and localization of brain $\text{Na}_v1.1$ sodium channel α -subunits in single cardiac myocytes. *Circulation* 2001;104:II–309.
16. Maltsev VA, Sabbah HN, Higgins RSD, Silverman N, Lesch M, Undrovinas AI. Novel, ultraslow inactivating sodium current in human ventricular cardiomyocytes. *Circulation* 1998;98:2545–2552. [PubMed: 9843461]
17. Maltsev VA, Sabbah HN, Tanimura M, Lesch M, Goldstein S, Undrovinas AI. Relationship between action potential, contraction-relaxation pattern, and intracellular Ca^{2+} transient in cardiomyocytes of dogs with chronic heart failure. *Cell Molec Life Sci* 1998;54:597–605. [PubMed: 9676578]
18. Maltsev VA, Sabbah HN, Undrovinas AI. Calmodulin modulates late sodium current in normal and failing myocardium. *Circulation* 2002;106:II–227.
19. Maltsev VA, Sabbah HN, Undrovinas AI. Down-regulation of sodium current in chronic heart failure: effects of long-term therapy with carvedilol. *Cell Mol Life Sci* 2002;59:1561–8. [PubMed: 12440776]
20. Maltsev VA, Sabbah HN, Undrovinas AI. Late sodium current is a novel target for amiodarone: Studies in failing human myocardium. *J Mol Cell Cardiol* 2001;33:923–932. [PubMed: 11343415]
21. Maltsev VA, Silverman N, Sabbah HN, Undrovinas AI. Chronic heart failure slows late sodium current in human and canine ventricular myocytes: Implications for repolarization variability. *Eur J Heart Fail* 2007;9:219–227. [PubMed: 17067855]
22. Maltsev VA, Undrovinas AI. Cytoskeleton modulates coupling between availability and activation of cardiac sodium channel. *Am J Physiol Heart Circ Physiol* 1997;273:42:H1832–H1840. [PubMed: 9362250]
23. Maltsev VA, Undrovinas AI. A multi-modal composition of the late Na^{+} current in human ventricular cardiomyocytes. *Cardiovasc Res* 2006;69:116–127. [PubMed: 16223473]
24. Mathias RT, Cohen IS, Oliva C. Limitations of the whole cell patch clamp technique in the control of intracellular concentrations. *Biophys J* 1990;58:759–70. [PubMed: 2169920]
25. Meadows LS, Isom LL. Sodium channels as macromolecular complexes: implications for inherited arrhythmia syndromes. *Cardiovasc Res* 2005;67:448–58. [PubMed: 15919069]
26. Noble D, Noble PJ. Late sodium current in the pathophysiology of cardiovascular disease: consequences of sodium-calcium overload. *Heart* 2006;92:iv1–iv5. [PubMed: 16775091]

27. Obata T. Adenosine production and its interaction with protection of ischemic and reperfusion injury of the myocardium. *Life Sci* 2002;71:2083–103. [PubMed: 12204768]
28. Oliva C, Cohen IS, Mathias RT. Calculation of time constants for intracellular diffusion in whole cell patch clamp configuration. *Biophys J* 1988;54:791–9. [PubMed: 3242629]
29. Patlak JB, Ortiz M. Slow currents through single sodium channels of the adult rat heart. *J Gen Physiol* 1985;86:89–104. [PubMed: 2411848]
30. Pieske B, Maier LS, Piacentino V 3rd, Weissner J, Hasenfuss G, Houser S. Rate dependence of $[Na^+]_i$ and contractility in nonfailing and failing human myocardium. *Circulation* 2002;106:447–53. [PubMed: 12135944]
31. Pusch M, Neher E. Rates of diffusional exchange between small cells and a measuring patch pipette. *Pflügers Arch* 1988;411:204–11. [PubMed: 2451806]
32. Rotter B, Kroviarski Y, Nicolas G, Dhermy D, Lecomte MC. AlphaII-spectrin is an in vitro target for caspase-2, and its cleavage is regulated by calmodulin binding. *Biochem J* 2004;378:161–8. [PubMed: 14599290]
33. Sabbah HN, Goldberg AD, Schoels W, Kono T, Webb C, Brachmann J, Goldstein S. Spontaneous and inducible ventricular arrhythmias in a canine model of chronic heart failure: relation to haemodynamics and sympathoadrenergic activation. *Eur Heart J* 1992;13:1562–72. [PubMed: 1281453]
34. Sabbah HN, Stein PD, Kono T, Gheorghiade M, Levine TB, Jafri S, Hawkins ET, Goldstein S. A canine model of chronic heart failure produced by multiple sequential coronary microembolizations. *Am J Physiol* 1991;260:H1379–84. [PubMed: 1826414]
35. Sah R, Ramirez RJ, Oudit GY, Gidrewicz D, Trivieri MG, Zobel C, Backx PH. Regulation of cardiac excitation-contraction coupling by action potential repolarization: role of the transient outward potassium current (I_{to}). *J Physiol* 2003;546:5–18. [PubMed: 12509475]
36. Sakmann BF, Spindler AJ, Bryant SM, Linz KW, Noble D. Distribution of a persistent sodium current across the ventricular wall in guinea pigs. *Circ Res* 2000;87:910–4. [PubMed: 11073887]
37. Studer R, Reinecke H, Bilger J, Eschenhagen T, Böhm M, Hasenfuss G, Just H, Holtz J, Drexler H. Gene expression of the cardiac $Na^+ - Ca^{2+}$ exchanger in end-stage human heart failure. *Circ Res* 1994;75:443–53. [PubMed: 8062418]
38. Tan BH, Valdivia CR, Rok BA, Ruwaldt KM, Tester DJ, Ackerman MJ, Makielski JC. Common human SCN5A polymorphisms have altered electrophysiology when expressed in Q1077 splice variants. *Heart Rhythm* 2005;2:741–747. [PubMed: 15992732]
39. Tan HL, Kupersmidt S, Zhang R, Stepanovic S, Roden DM, Wilde AA, Anderson ME, Balser JR. A calcium sensor in the sodium channel modulates cardiac excitability. *Nature* 2002;415:442–447. [PubMed: 11807557]
40. Tomaselli GF, Zipes DP. What causes sudden death in heart failure? *Circ Res* 2004;95:754–763. [PubMed: 15486322]
41. Undrovinas AI, Belardinelli L, Undrovinas NA, Sabbah HN. Ranolazine improves abnormal repolarization and contraction in left ventricular myocytes of dog with heart failure by inhibiting late sodium current. *J Cardiovasc Electrophys* 2006;17:S1–S9.
42. Undrovinas AI, Belardinelli L, Undrovinas NA, Sabbah HN. Ranolazine improves abnormal repolarization and contraction in left ventricular myocytes of dogs with heart failure by inhibiting late sodium current. *J Cardiovasc Electrophysiol* 2006;17:S169–S177. [PubMed: 16686675]
43. Undrovinas AI, Fleidervish IA, Makielski JC. Inward sodium current at resting potentials in single cardiac myocytes induced by the ischemic metabolite lysophosphatidylcholine. *Circ Res* 1992;71:1231–41. [PubMed: 1327577]
44. Undrovinas AI, Maltsev VA. Spectrin-based cytoskeleton modulates late sodium currents in heart. *Biophys J* 2003;84:25A.
45. Undrovinas AI, Maltsev VA, Kyle JW, Silverman NA, Sabbah HN. Gating of the late Na^+ channel in normal and failing human myocardium. *J Mol Cell Cardiol* 2002;34:1477–1489. [PubMed: 12431447]
46. Undrovinas AI, Maltsev VA, Sabbah HN. Repolarization abnormalities in cardiomyocytes of dogs with chronic heart failure: Role of sustained inward current. *Cell Mol Life Sci* 1999;55:494–505. [PubMed: 10228563]

47. Undrovinas AI, Shander GS, Makielski JC. Cytoskeleton modulates gating of voltage-dependent sodium channel in heart. *American Journal of Physiology* 1995;269:H203–14. [PubMed: 7631850]
48. Valdivia CR, Chu WW, Pu J, Foell JD, Haworth RA, Wolff MR, Kamp TJ, Makielski JC. Increased late sodium current in myocytes from a canine heart failure model and from failing human heart. *J Mol Cell Cardiol* 2005;38:475–83. [PubMed: 15733907]
49. Wagner S, Dybkova N, Rasenack EC, Jacobshagen C, Fabritz L, Kirchhof P, Maier SK, Zhang T, Hasenfuss G, Brown JH, Bers DM, Maier LS. Ca/calmodulin-dependent protein kinase II regulates cardiac Na channels. *J Clin Invest* 2006;22:22.
50. Wingo TL, Shah VN, Anderson ME, Lybrand TP, Chazin WJ, Balser JR. An EF-hand in the sodium channel couples intracellular calcium to cardiac excitability. *Nat Struct Mol Biol* 2004;11:219–25. [PubMed: 14981509]
51. Yoshida K, Inui M, Harada K, Saido TC, Sorimachi Y, Ishihara T, Kawashima S, Sobue K. Reperfusion of rat heart after brief ischemia induces proteolysis of caldesmon (nonerythroid spectrin or fodrin) by calpain. *Circ Res* 1995;77:603–10. [PubMed: 7641330]
52. Young KA, Caldwell JH. Modulation of skeletal and cardiac voltage-gated sodium channels by calmodulin. *J Physiol* 2005;565:349–70. [PubMed: 15746172]
53. Zhang T, Brown JH. Role of Ca²⁺/calmodulin-dependent protein kinase II in cardiac hypertrophy and heart failure. *Cardiovasc Res* 2004;63:476–86. [PubMed: 15276473]
54. Zicha S, Maltsev VA, Nattel S, Sabbah HN, Undrovinas AI. Post-transcriptional alterations in the expression of cardiac Na⁺ channel subunits in chronic heart failure. *J Mol Cell Cardiol* 2004;37:91–100. [PubMed: 15242739]

Fig. 1.

Ca^{2+} modulates I_{NaL} kinetics and amplitude in ventricular cardiomyocytes of normal and failing dog hearts. Representative raw traces were recorded at different $[\text{Ca}^{2+}]_i$ in normal (A) and failing (B) cells. Exponential fits (Eq.1, see Methods) are shown by solid lines together with their parameters. Inset shows voltage-clamp protocol. Vertical bars indicate (here and in other figures) the time window (200-220 ms of depolarization) used to evaluate I_{NaL} density. Statistical data for the decay time constants τ_1 (C) and τ_2 (D), as well as I_{NaL} density (E) at different $[\text{Ca}^{2+}]_i$ in normal and failing hearts. Bars represent average data mean $\pm$ SEM, N=number of cells (hearts). Statistical significant difference ($P < 0.05$, ANOVA followed by Bonferroni's post hoc test): *,** Normal and failing heart 0 Ca^{2+} vs. 1 μM Ca^{2+} , respectively, &, ' Normal vs. failing heart at 0 Ca^{2+} and. 1 μM Ca^{2+} , respectively.

Fig.2.

Elevation of the intracellular Ca^{2+} from 0 to $1\mu M$ uncouples the steady-state activation (SSA) and inactivation (SSI) of I_{NaL} in both normal and failing hearts. (A,B), I_{NaL} -voltage relationship at different $[Ca^{2+}]_i$ in normal and failing hearts, respectively. Data represent mean $\pm$ SEM pooled from 4-11 cells. The solid lines show theoretical curves of SSA (Eq.3, Methods) fitted to data points. Maximum I_{NaL} conductance G_{max} significantly increased from 2.34 to 3.68 pS/pF (normal hearts) and from 3.65 to 5.38 pS/pF (HF) ($P < 0.001$, F-test). Other fit parameters ($V_{1/2}$ and k are shown at the traces) remained almost unchanged in augmented $[Ca^{2+}]_i$. Action potential (AP) plateau range from -15 to 20 mV (46) is depicted by a box (B). SSI was significantly shifted rightward in response to the elevated $[Ca^{2+}]_i$ in both normal (C) and failing (D) hearts ($P < 0.001$, F-test). Solid lines represent theoretical curve fit (Eq. 2, Methods). Statistical comparison of all SSI parameters for experiments is given in the text. Data points were pooled from the 5-20 cells (3 normal and 6 HF dogs). Inset in D shows voltage-clamp protocol.

Fig.3.

Inhibition of CaMKII accelerates I_{NaL} decay in normal and failing hearts. Representative raw traces were recorded at high $[\text{Ca}^{2+}]_i$ alone or in the presence of CaMKII inhibitor KN93 ($10 \mu\text{M}$) in normal (A) and failing (B) cells. Exponential fits (Eq.1, see Methods) are shown by solid lines together with their parameters. Inset shows voltage-clamp protocol. C, D - Statistical data for the decay time constants τ_1 and τ_2 in normal and failing hearts, respectively. Bars represent average data mean $\pm$ SEM, N=number of cells (hearts). For decay time constants (C, D) statistical significant difference ($P < 0.05$, ANOVA followed by Bonferroni's post hoc test): *,** Normal and failing heart $1 \mu\text{M} \text{Ca}^{2+}$ vs. $1 \mu\text{M} \text{Ca}^{2+} + \text{KN93}$, respectively. & Normal vs. Failing heart at $1 \mu\text{M} \text{Ca}^{2+}$.

Fig. 4. Effects of CaMKII inhibition on SSA and SSI of I_{NaL} in both normal and failing hearts. (A,B) I_{NaL} -voltage relationship at high $[Ca^{2+}]_i$ and in the presence of CaMKII inhibitor KN93 (10 μM) in normal (A) and failing (B) cells, respectively. Data represent mean $\pm$ SEM pooled from 4-5 cells (2 normal and 2 failing hearts). The solid lines show theoretical curves of SSA (Eq. 3, Methods) fitted to data points. In response to CaMKII inhibition maximum I_{NaL} conductance G_{max} does not significantly change 3.7 vs. 4.0 pS/pF in normal hearts (A), but significantly reduced from 5.4 to 4.62 pS/pF in HF (B) ($P < 0.05$, F-test). Other fit parameters ($V_{1/2}$ and k) are shown at the traces. SSI was not affected by the CaMKII inhibition in either normal (C) or failing hearts (D). Detailed statistical comparison of all SSI parameters for large number of experiments is given in the text (Table 3). Asterisks in D show statistically significant ($P < 0.05$) differences in data points. Data points were pooled from the 5-10 cells (2 normal and 3 HF dogs). Inset in D shows voltage-clamp protocol.

Fig.5.

Peptide P290-309 antagonist of Ca^{2+} -dependent CaM binding accelerates I_{NaL} decay, and reduces its amplitude in normal and failing hearts. Representative raw traces were recorded at high $[\text{Ca}^{2+}]_i$ alone or in the presence of P290-309 in normal (A) and failing (B) cells. Exponential fits (Eq.1, see Methods) are shown by solid lines together with their parameters. Inset shows voltage-clamp protocol. Statistical data for the decay time constants τ_1 (C) and τ_2 (D) at different $[\text{Ca}^{2+}]_i$ in normal and failing hearts. Bars represent average data mean $\pm$ SEM, N=number of cells (hearts). Statistical significant difference ($P < 0.05$, ANOVA followed by Bonferroni's post hoc test): *,** Normal and failing heart $1\mu\text{M}\text{Ca}^{2+}$ vs. $1\mu\text{M}\text{Ca}^{2+} + \text{P290-309}$, respectively. & Normal vs. Failing heart at $1\mu\text{M}\text{Ca}^{2+}$ (C, D). No significant difference: Normal vs. failing heart at $1\mu\text{M}\text{Ca}^{2+} + \text{P290-309}$ (C, D)

Fig.6.

Effects of blockade of Ca^{2+} -dependent CaM binding by the peptide P290-309 on the steady-state activation (SSA) and inactivation (SSI) of I_{NaL} in both normal and failing hearts. (A, B) I_{NaL} -voltage relationship at high $[Ca^{2+}]_i$ alone or in the presence of P290-309 in normal and failing hearts, respectively. Data represent mean $\pm$ SEM pooled from 4-10 cells (2 normal and 2 failing hearts). The solid lines show theoretical curves of SSA (Eq.3, Methods) fitted to data points. Maximum I_{NaL} conductance G_{max} significantly decreased 3.68 to 2.17 pS/pF (normal hearts) and from 5.38 to 4.12 pS/pF (HF) ($P < 0.001$, F-test) in the presence of P290-309. Other fit parameters ($V_{1/2}$ and k are shown at the traces) remained almost unchanged in augmented $[Ca^{2+}]_i$. Action potential (AP) plateau range from -15 to 20 mV (46) is depicted by a box (B). The theoretical curve of SSI was significantly shifted rightward in the presence of P290-309 in HF (D, $P < 0.001$ F-test), but not in normal (C) hearts. Statistical comparison of all SSI parameters for experiments is given in the text. Data points were pooled from the 5-20 cells (3 normal and 6 HF dogs). Inset in D shows voltage-clamp protocol.

Fig. 7.

Theoretical evaluation of difference in the cumulative effects of the elevated Ca^{2+} on I_{NaL} and its integral ($\sim Na^+$ influx) in cardiomyocytes of normal dogs (NORM) and dogs with chronic heart failure (HF). Parameters for idealized, two-exponential I_{NaL} decay time course were assigned from the average experimental data measured in two extreme conditions: $[Ca^{2+}]_i=0$, and $[Ca^{2+}]_i=1 \mu M$ (see section 3.5 for details). The average maximum I_{NaL} density at -30 mV was corrected for SSI voltage-dependence assuming a resting/holding potential of -80 mV. A and B, idealized I_{NaL} in normal and failing ventricular myocytes, respectively. C and D, the absolute and relative ranges of Ca^{2+} regulation of I_{NaL} are larger in failing cells (the absolute difference is shown by shaded area in C). E and F: substantial modulation of I_{NaL} -related Na^+ influx by Ca^{2+} assessed as an absolute value of I_{NaL} integral in normal and failing myocytes. G: the modulation of I_{NaL} -related Na^+ influx by Ca^{2+} is greater in failing myocytes (shaded area "HF-NORM").

Figure 8.

Simplified hypothetical diagram of the intracellular Ca^{2+} signaling pathways modulating late sodium current in normal and failing canine ventricular myocytes based on the results of the present study. Pathways 1, 2 and 3 (marked by black digits) represent Ca^{2+} binding to: the E-F domain, CaM-binding site (IQ motif) on C-terminus of Na channel (NaCh) as well as CaM/CaMKII complex, respectively. Arrows with plus/minus signs indicate modulation in terms of increase/decrease of I_{NaL} integral. Biophysical mechanisms of the the modulations are shown as follows: SSI, the shift of the voltage dependency of steady-state inactivation. #LSM and #BM, the number of channels in late scattered or burst mode, respectively. Gating kinetics, slowing inactivation gating for LSM and BM. Please note that pathways shown by dashed lines are effective only in failing myocytes. Also shown are the inhibitory sites of the CaM antagonist peptide P290-309 and KN93 - inhibitor of CaMKII employed in the present study to test the signaling.

Table 1

Composition of bath (B) and pipette (P) solutions used in the study. Abbreviations: TEA- tetraethylammonium, EGTA- ethylene glycolbis(beta-amonoethyl ether)N,N,N',N'-tetraacetic acid, HEPES-N-[2-hydroxyethyl] piperazine-N'- [2ethanesulfonic acid], BAPTA-Ethylenedioxybis(o-phenylenenitrilo)tetraacetic acid.

	Pipette solution, mM			Bath solution, mM
	P1 (0 Ca ²⁺ , EGTA)	P2 (0 Ca ²⁺ , BAPTA)	P3 (1μM Ca ²⁺)	B
NaCl	5	5	5	140
CsCl	133	133	133	5.4
CaCl ₂			0.9	1.8
MgCl ₂				2
MgATP	2	2	2	
TEA	20	20	20	
Nifedipine				0.002
EGTA	10		1	
BAPTA		10		
HEPES	5	5	5	5
pH	7.3	7.3	7.3	7.3
	CsOH	CsOH	CsOH	NaOH

Table 2

Effects of intracellular Ca^{2+} signaling on the density of I_{NaL} in failing dog cardiomyocytes. The amplitude of I_{NaL} was determined as an averaged current density within 200-220 ms after the onset of a 2 second long depolarization step at $-30mV$.

Intracellular conditions	I_{NaL} density, pA/pF		N, cells (dogs)	
	HF	NH	HF	NH
0 Ca^{2+}	0.31 ± 0.02	0.24 ± 0.01	26(5)	37(5)
1 μM Ca^{2+}	$0.49 \pm 0.03^{**}$	$0.34 \pm 0.03^{*}$	12(3)	13(2)
1 μM Ca^{2+} +KN93 (10 μM)	$0.37 \pm 0.03^{**}$	$0.26 \pm 0.025^{*}$	9(4)	11(2)
1 μM Ca^{2+} +P290-309 (10 μM)	$0.36 \pm 0.01^{\dagger}$	$0.25 \pm .02^{\dagger}$	9(4)	6(1)

Data are mean $\pm$ SEM, (N)- first number is number of cells and the second number is the number of dogs. Statistical significant difference ($P<0.05$, ANOVA followed by Bonferroni's post hoc test); * Normal and failing heart vs. 0 Ca^{2+} ; **, $\dagger$ Normal and failing heart vs. 1 μM Ca^{2+} ; Normal vs. HF significant difference compared at the same conditions (not indicated).

Effects of intracellular Ca^{2+} signaling pathways on the steady-state inactivation parameters in normal and failing dog cardiomyocytes. Steady state inactivation of I_{NaL} was assessed by a double pulse protocol, and was fitted to Boltzmann model (Equation 2, Methods) with mid-potential $V_{1/2}$ and slope K_A .

Intracellular conditions	$V_{1/2}$, mV		K_A , mV		N, cells (dogs)	
	HF	NH	HF	NH	HF	NH
0 Ca^{2+}	-81.3 ± 3.9	-80.1 ± 0.7	-5.6 ± 0.4	-6.3 ± 0.3	14 (3)	38 (5)
1 μM Ca^{2+}	-75.7 ± 3.7*	-73.9 ± 1.9*	-7.2 ± 0.4*	-6.2 ± 0.8	15 (4)	6 (2)
1 μM Ca^{2+} + KN93 (10 μM)	-74.6 ± 1.5	-77.9 ± 1.6	-5.2 ± 0.4	-5.8 ± 0.9	7 (3)	7 (2)
1 μM Ca^{2+} + P290-309 (10 μM)	-70.2 ± 1.5**	-73.3 ± 1.2	-5.4 ± 0.5	-5.4 ± 0.8	7 (2)	6 (1)

Data are mean ± SEM, N- first and second numbers are number of cells and dogs, respectively. Statistical comparison for $V_{1/2}$: *significantly different from 0 Ca^{2+} ; ** significantly different from 1 μM Ca^{2+} and 1 μM Ca^{2+} + KN93. For K_A : *significantly different from 0 Ca^{2+} (ANOVA followed by Bonferroni's post-hoc test). Differences were considered statistically significant for $P < 0.05$. Abbreviations: NH-normal heart, HF-heart failure

Results of numerical evaluation and comparison of different Ca^{2+} -dependent mechanisms of I_{NaL} modulation in normal and failing cardiomyocytes. The components describing I_{NaL} fine structure and I_{NaL} integrals presented in the table are described in section 3.5. Shaded cells indicate regulation components, which are substantially different in normal and failing cells. Each numerical estimate is accompanied by plus or minus signs reflecting semi-quantitatively the strength of the change (just for guidance). NS denotes not significant change. Abbreviations for pipette solution: 0Ca , $[\text{Ca}^{2+}]=0$; 1Ca , $[\text{Ca}^{2+}]=1\mu\text{M}$; P, Peptide 290-309; KN, KN93. Q_{tot} was calculated (Eq. 8, section 3.5) at a physiological resting potential $V_h=-80\text{mV}$, and thus includes effects of corresponding SSI shifts. All other parameters, including Q_{BM} and Q_{LSM} (Eqs. 6, 7, section 3.5), were calculated at $V_h=-130\text{mV}$. Values of the integrals are given in pC/pF.

Modulatory mechanism	Component	Normal		Heart failure	
		Parameter change	Effect on the respective Integral	Parameter change	Effect on the respective Integral
CaMKII	All components	See Fig. 7A	(+++) Q_{tot} : 0.103 (0Ca) to 0.211 (1Ca) by 101%	See Fig. 7B	(++++) Q_{tot} : 0.126 (0Ca) to 0.297 (1Ca) by 135%
	$I_{\text{LSM}}(0)$, pA/pF	(NS) 0.407 to 0.483	(++) Q_{LSM} : 0.172 to 0.232 by 35% (NS) Q_{BM} : 0.0675 to 0.0584	NS 0.677 to 0.603	(++) Q_{LSM} : 0.231 to 0.361 by 56.8% (+) Q_{tot} : 0.246 (1Ca&KN) to 0.297 (1Ca) by 20.7%
	τ_2 , ms	(+) 425 to 487 by 14.6%		(+++) τ_2 341 to 625 by 83.3%	
	$I_{\text{BM}}(0)$, pA/pF	(-) 1.94 to 1.19		(-) τ_2 3.53 to 1.70	
	τ_1 , ms	(+) 34.3 to 48.6 by 41.7%		(+++) τ_1 28.5 to 57.5 by 202%	(NS) Q_{BM} : 0.102 to 0.099
CaM	SSI shift, mV	No shift	(NS)	(-) -5.3 mV -70.2 to -75.5	(-) Q_{tot} : 0.246 (1Ca&KN) to 0.317 (1Ca&P) by 28.8%
Ca^{2+} (CaM independent)	$I_{\text{LSM}}(0)$, pA/pF	(NS) 0.383 to 0.394	(++) Q_{tot} : increase 0.105 (0Ca) to 0.169 (1Ca&P) by 61.6%	(++) 0.452 to 0.591 by 30.7%	(++++) Q_{tot} : 0.126 to 0.317 (1Ca&P) by 150%
	SSI shift, mV	(+) +6.8 mV: -80.1 to -73.3		(++) +11.1 mV: -81.3 to -70.2	