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Late sodium current in failing heart: Friend or foe?

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Abstract

Most cardiac Na^+ channels open transiently upon membrane depolarization and then are quickly inactivated. However, some channels remain active, carrying the so-called persistent or late Na^+ current (I_{NaL}) throughout the action potential (AP) plateau. Experimental data and the results of numerical modeling accumulated over the past decade show the emerging importance of this late current component for the function of both normal and failing myocardium. I_{NaL} is produced by special gating modes of the cardiac-specific Na^+ channel isoform. Heart failure (HF) slows channel gating and increases I_{NaL}, but HF-specific Na^+ channel isoform underlying these changes has not been found. Na^+ channels represent a multi-protein complex and its activity is determined not only by the pore-forming α subunit but also by its auxiliary β subunits, cytoskeleton, calmodulin, regulatory kinases and phosphatases, and trafficking proteins. Disruption of the integrity of this protein complex may lead to alterations of I_{NaL} in pathological conditions. Increased I_{NaL} and the corresponding Na^+ flux in failing myocardium contribute to abnormal repolarization and an increased cell Ca^{2+} load. Interventions designed to correct I_{NaL} rescue normal repolarization and improve Ca^{2+} handling and contractility of the failing cardiomyocytes. This review considers 1) quantitative integration of I_{NaL} into the established electrophysiological and Ca^{2+} regulatory mechanisms in normal and failing cardiomyocytes and 2) a new therapeutic strategy utilizing a selective inhibition of I_{NaL} to target both arrhythmias and impaired contractility in HF.

Keywords

Late sodium current; heart failure; calcium; action potential; numerical model; sodium-calcium exchanger

1. Introduction

Congestive heart failure (HF) is associated with profound abnormalities in both cardiac rhythm and contractile function. Among numerous proteins involved in the cardiac cell alterations in HF, the voltage-gated Na^+ channels deserve special consideration, as they seem to be critically involved in abnormal conduction, repolarization, and Ca^{2+} handling (Bers et al., 2006; Tomaselli and Zipes, 2004). Most Na^+ channels open only transiently and are quickly inactivated resulting in the peak transient current, I_{NaT}, which determines excitation and conduction. However, some Na^+ channels remain active, carrying so-called persistent or late Na^+ current (I_{NaL}) throughout the action potential (AP) plateau (reviews (Carmeliet, 2006; Noble and Noble, 2006)).

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A growing body of evidence accumulated over the last decade shows that I_{NaL} provides a major contribution to the AP plateau in ventricular cardiomyocytes (VCs) in a variety of mammalian species including humans (Maltsev et al., 1998a). Single channel studies in human VCs and in heterologously expressed channels show that I_{NaL} is produced by the cardiac Na^+ channel isoform ($Na_v1.5$), operating in special gating modes (Undrovinas et al., 2002). I_{NaL} has a voltage-activated gating similar to I_{NaT} and a slow inactivation, which clearly separate I_{NaL} from background Na^+ currents.

Since late openings of Na^+ channel generate both electric current and Na^+ influx during the AP plateau, I_{NaL} is expected to contribute to at least two known HF cellular mechanisms (depicted in Fig.1): 1) electrophysiological alterations and 2) altered cell Na^+ cycling. The latter mechanism is tightly integrated with Ca^{2+} cycling, as Na^+ modulates the Na^+/Ca^{2+} exchanger (NCX) operation (Bers et al., 2006). These anticipated I_{NaL} contributions could be amplified at the state of chronic HF that reportedly slows the late Na^+ channel gating and increases the whole cell I_{NaL} (Maltsev et al., 2007;Maltsev and Undrovinas, 2006;Undrovinas et al., 1999). While mechanisms of the I_{NaL} change are mainly unknown, pharmacological properties of I_{NaL} remain unchanged in chronic HF and the late channel activity exhibits same gating modes (Maltsev et al., 1998a;Maltsev et al., 2007;Undrovinas et al., 2002;Undrovinas et al., 1999). This raises a possibility that HF alters I_{NaL} by affecting Na^+ channel environment factors known to modulate Na^+ channel gating, such as auxiliary subunits, Ca^{2+}/CaM/CaMKII, cytoskeleton, membrane phospholipid composition , and other scaffolding proteins such as caveolin.

The importance of I_{NaL} contribution into HF mechanisms has been demonstrated in experiments designed to “correct” I_{NaL}. It was shown that a partial I_{NaL} inhibition and/or acceleration of I_{NaL} decay rescue normal repolarization, decrease beat-to-beat AP duration variability, and improve Ca^{2+} handling and contractility of the failing cells (Maltsev et al., 1998b; Maltsev et al., 2007; Undrovinas et al., 2006; Undrovinas et al., 1999). Thus, I_{NaL} has emerged as a novel target for cardioprotection to treat the failing heart (Belardinelli et al., 2006; Maltsev et al., 2001; Noble and Noble, 2006).The focus of this review is to summarize the available data from experiment and numerical modeling of I_{NaL} in normal and failing myocardium in order to integrate I_{NaL} into the established electrophysiological and Ca^{2+} regulation mechanisms in normal and failing cardiomyocytes. We believe that such integration will improve our understanding of cardiac cell function under normal and pathological conditions and thus provide a basis for the development of new therapeutic strategies for cardioprotection.

2 The idea and first experimental evidence of a persistent Na^+ current from early AP studies

The concept of a persistent (“plateau”) Na^+ current in heart cells emerged when the first model of cardiac AP was developed by Denis Noble in 1960-1962 (Noble, 1960; Noble, 1962). At that time, Ca^{2+} current in cardiac cells (Reuter, 1967) had not yet been discovered, and persistent Na^+ current was suggested to explain a relatively long (vs. neuronal) AP plateau duration and cardiac AP shortening when external Na^+ is reduced (Weidmann, 1956) (see details in review (Noble, 2006)). Further experimental indication for the existence of a persistent Na^+ current and for its contribution to AP duration was found in microelectrode studies in cardiac Purkinje fibers in 1967 (Dudel et al., 1967). It was shown that tetrodotoxin (TTX), as well as many local anesthetic-type antiarrhythmic agents, shortens the cardiac AP (Carmeliet and Saikawa, 1982; Coraboeuf et al., 1979; Davis and Temte, 1969; Dudel et al., 1967; Gliklich and Hoffman, 1978). Coraboeuf and colleagues (Coraboeuf et al., 1979) found that TTX at concentrations lower than 1 μM shortenes AP and induces small hyperpolarization, but does not affect its $(dV/dt)_{max}$. They concluded that this effect was related to a slow

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component of Na^+ current flowing through a TTX-sensitive Na^+ channel. Accordingly, slow Na^+ current was believed to be a specific feature of cardiac Purkinje fibers, explaining the longer AP in Purkinje fibers compared to that in the working myocardium.

3. Classification of Na^+ currents: the late/persistent Na^+ current is different from the "window" current and "background" currents

Further voltage clamp studies have identified several types of single Na^+ channel activity and whole cell Na^+ currents that could contribute to AP duration. Late openings of Na^+ channels were found not only in Purkinje cells but also in myocardial cells. The variety of Na^+ channel activities identified so far can be classified (see review (Noble and Noble, 2006)) in terms of the "window" current, the late (persistent) Na^+ current i.e. I_{NaL} (I_{pNa}), and background Na^+ currents.

3.1. The "window" Na^+ current

This current was suggested as a theoretical mechanism to explain the persistent Na^+ current in cardiac cells (Attwell et al., 1979; Gadsby and Cranefield, 1977). According to the Hodgkin-Huxley formalism (Hodgkin and Huxley, 1952), the "window" current is a non-inactivating component of I_{NaT}, resulting from the crossover of its steady-state activation and inactivation curves.

3.2. The late Na^+ current, I_{NaL}

Voltage clamp studies, however, showed that the concept of the "window" current could not explain some fundamental properties of the late Na^+ current, such as its slow inactivation and voltage-dependence.

I_{NaL} exhibits slow inactivation—Gintant et al. (Gintant et al., 1984) and Carmeliet (Carmeliet, 1987), using two-microelectrode voltage clamp, demonstrated that I_{NaL} in canine and rabbit cardiac Purkinje fibers undergoes a slow inactivation, which deviates from the Hodgkin-Huxley formalism and thus does not support the "window" origin of the current. Furthermore, in single-channel patch-clamp studies, Patlak and Ortiz (Patlak and Ortiz, 1985) discovered bursts of openings of Na^+ channels that could underlie the late, slowly inactivated Na^+ current in rat VCs. Similar decaying burst activity of Na^+ channel was also observed in rabbit Purkinje cells (Zilberter et al., 1994). In addition to bursts, Kiyosue and Arita (Kiyosue and Arita, 1989) identified a sustained channel activity consisting of rare and brief openings in guinea pig VCs. This type of late openings was found to be slowly inactivating in human VCs (Undrovinas et al., 2002)(see section 5 for details). The current produced by the late openings of Na^+ channel seemed to play a role in AP plateau not only of Purkinje fibers but also of VCs, because TTX produced 10 to 20 % decrease in AP duration in these cells (Kiyosue and Arita, 1989; Maltsev et al., 1998a). Both TTX- and lidocaine-sensitive slowly inactivating whole-cell inward current in the AP plateau voltage range was reported by Wasserstrom and Salata in dog ventricular myocytes (Wasserstrom and Salata, 1988).

I_{NaL} is present within a wide range of voltages similar to that of I_{NaT}—The overlap of steady-state activation and inactivation curves occurs within a relatively narrow region of voltages close to I_{NaT} activation threshold. However, late openings of the Na^+ channel were found at voltages far out of the overlap (e.g. −10 mV, see Fig.5C) (Undrovinas et al., 2002) and significant whole cell I_{NaL} is present even at positive voltages (Fig.2B, filled circles) (Maltsev et al., 1998a;Sakmann et al., 2000), where the calculated "window" current is negligible.

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3.3 Background Na^+ currents

A Na^+-dependent current, called the Na^+ background current (I_{bNa}), was found in rabbit sinoatrial node (Hagiwara et al., 1992) and in guinea pig ventricular and atrial cells (Kiyosue et al., 1993). In contrast to I_{NaL}, it is a non-inactivating current with poor cation selectivity and no voltage dependence. The molecular and genetic origins of I_{bNa} remain unknown, but it could result from a leak form of Na^+–K^+ ATPase (Artigas and Gadsby, 2004) or of NCX (Hilgemann, 2004). This TTX-insensitive I_{bNa} is different from another "background" type Na^+ current, which is produced by openings of Na^+ channels. A non-inactivating, highly TTX sensitive "background" Na^+ channel activity was also recorded within the wide range of membrane potentials in the rabbit Purkinje cells (Zilberter et al., 1994) and in rat cardiomyocytes (Saint et al., 1992). In contrast to slowly inactivating I_{NaL}, this type of background Na^+ current has much higher sensitivity to TTX and exhibits neither voltage-gated activation, nor voltage-dependent steady-state inactivation; this current persists at potentials ranging from −120 to 0 mV with an almost linear I-V relationship. Experimental hypoxia and metabolic inhibitors, such as cyanide, reportedly augment the highly TTX sensitive background current (Ju et al., 1996). However, studies in human or canine VCs showed neither any TTX-blockable (25 μM) current at membrane potential < −80 mV nor any effect of cyanides, suggesting species-specific differences in the background current (Maltsev et al., 1998a; Maltsev et al., 2007).

A non-inactivating current with almost no voltage-dependency in the range of −60 to −20 mV and no voltage-dependent steady-state inactivation was reported in dog and human myocardium at a non-physiological $[Na^+]_o$=5 mM (Valdivia et al., 2005). All these properties are characteristic of a "background" type current rather than I_{NaL}. Being saxitoxin (STX) sensitive, it is supposed to be produced by Na^+ channel openings; however, the current consists of large, step-like fluctuations (±20 pA, i.e. ~30% of the current amplitude, see Fig. 3 in (Valdivia et al., 2005)), which are unlikely to originate from individual openings of the STX-sensitive Na^+ channels especially at the low $[Na^+]_o$. On the other hand, it has been recently demonstrated that low $[Na^+]_o$ destabilizes inactivation of Na^+ channel (Aman and Raman, 2006). It was suggested that occupancy of the channel pore by the permeant ion substantially affects slow inactivation process. Thus, molecular identity, ion selectivity, and gating properties of the ion channel underlying this "background" current remain unknown.

4. Whole-cell I_{NaL} in normal and failing human and canine ventricular myocardium

4.1. Biophysical characteristics of I_{NaL}

Our patch clamp studies characterized major biophysical and pharmacological characteristics of the whole-cell I_{NaL} in human VCs (Maltsev et al., 1998a; Maltsev et al., 2001; Maltsev et al., 2007) (summarized in Fig. 2). Many key properties of I_{NaL} turned out to be similar to those of I_{NaT}. The voltage dependencies of steady-state activation and availability of I_{NaL} are almost identical to those of I_{NaT}, and the I-V relationships of these currents almost coincide. Ion selectivity (Li^+-permeable and Cs^+-impermeable) and pharmacological properties for I_{NaL} (see section 6.3 for details) and I_{NaT} also proved to be the same. I_{NaL} time course measured 200 ms after membrane depolarization is well described by a single exponential decay with a time constant of about 0.5 sec at 24°C. Surprisingly and unlike I_{NaT}, the decay time course of I_{NaL} does not depend on the membrane potential. Similarly, I_{NaL} reactivation is very slow and voltage-independent. Furthermore, inactivation of the major portion of I_{NaT} by a short depolarizing prepulse does not affect I_{NaL}. This feature indicates that kinetic transitions between different states of the Na^+ channels responsible for I_{NaT} or I_{NaL} are independent. This property of I_{NaL} can be exploited for both experimental (separation of these two currents) and theoretical (modeling) purposes.

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A slowly inactivating I_{NaL} with aforementioned biophysical characteristics is produced by heterologously expressed cardiac Na^+ channel isoform main α-subunit $Na_v1.5$ (Fig.2F). It has also been identified in VCs of dogs (Maltsev et al., 2007;Undrovinas et al., 2006;Undrovinas et al., 1999;Zygmunt et al., 2001), guinea pigs (Belardinelli et al., 2006;La et al., 2006;Sakmann et al., 2000), rabbits (Wu et al., 2006) and rats (Chattou et al., 2000).

4.2. Whole cell I_{NaL} is larger and slower in chronic HF

The first evidence of importance of I_{NaL} in electrophysiological alterations induced by HF was found in 1995 in an experimental dog model of chronic HF (Maltsev et al., 1995). In VCs isolated from these failing hearts, the N-shaped I-V curve for the total current had a significantly increased contribution of a late/persistent, TTX and STX-blockable inward current within the AP plateau range. Experiments in human hearts demonstrated that TTX produces a similar effect on the I-V curve and AP shape of human failing VCs, indicating that I_{NaL} is present in humans and thus may have a clinical relevance (Maltsev et al., 1998a). Further patch clamp studies in human and canine hearts have conclusively shown that chronic HF increases I_{NaL} density and significantly slows inactivation kinetics of I_{NaL} in VCs (Maltsev et al., 2007; Undrovinas et al., 1999) (Fig.3A). Analysis of idealized I_{NaL} time course (Fig.3B) in canine normal and failing VCs of the same size (200 pF) shows that 1) absolute I_{NaL} difference between normal and failing canine VCs at 37°C is bell-shaped (top panel in Fig. 3B, gray area) with a maximum of 24.5 pA at 90 ms after membrane depolarization, i.e. within the time of AP plateau duration; 2) the relative difference between normal and failing cells increases progressively with membrane depolarization, doubling after 330 ms (Fig. 3B, inset); 3) both absolute and relative differences are much greater at 37°C than at 24°C (Maltsev et al., 2007).

5. Gating of late Na^+ channel openings

5.1. Separate Na^+ channel gating modes contribute to I_{NaL} and I_{NaT}

The I_{NaL} inactivation can be described in terms of channel gating modes each of which describes a separate set of gating parameters for the same channel type. To our knowledge, the idea and the experimental evidence for gating modes of cardiac Na^+ channel were first reported in 1985 by Patlak and Ortiz (Patlak and Ortiz, 1985). In patch-clamped rat VCs, they occasionally observed bursts of 10 or more sequential openings of a single channel that lasted for up to 150 ms. They concluded that “the single channel data cannot be explained by standard models, even those that have two inactivated states or two open states of the channel. Our results suggest that Na^+ channels can function in several different ‘modes,’ each with a different inactivation rate.” As I_{NaL} lasts hundreds of milliseconds, the numerous short-lived modes (a few milliseconds of membrane depolarization) identified by further patch clamp studies in myocardial cells of a variety of species (mice (Bohle and Benndorf, 1995), guinea pig (Nilius, 1988) and humans (Bohle et al., 2002)) contribute to I_{NaT} rather than I_{NaL}. More specifically, all 5 gating modes of Na^+ channel (F, M1, M2, S, and P-mode) identified by Böhle et al. (Bohle et al., 2002) in human VCs operate within 5 ms of membrane depolarization and thus have no contribution to I_{NaL}.

5.2. Gating modes underlying I_{NaL} in human VCs: bursts and late scattered openings

Most human cardiac Na^+ channels open transiently upon membrane depolarization and then are quickly inactivated (Fig.4A). However, some channels remain active, carrying I_{NaL}. In multi-channel cell-attached patches from heterologously expressed $Na_v1.5$ and human VCs, this late activity is arranged in two major gating modes: late scattered mode (LSM) and “burst” mode (Fig 4B,C). The channel openings of the two gating modes have almost identical single channel conductance and the reversal potential (Undrovinas et al., 2002). The latencies of LSM openings reveal voltage-independent ultraslow (hundreds of ms) inactivation, similar to that

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of whole-cell current decay (Undrovinas et al., 2002). LSM openings render one open voltage-independent state but burst mode openings exhibit one open (voltage-dependent) and two closed states (one voltage-dependent and another voltage-independent) (Undrovinas et al., 2002).

5.3 Alteration of late Na^+ channel gating in human failing VCs

Since whole cell studies identified slower and larger I_{NaL} in the failing VCs (Fig.3)(Maltsev et al., 2007;Undrovinas et al., 1999), one might expect that single channel activity underlying I_{NaL} is different in failing cells. However, no qualitative changes in Na^+ channel gating were found in failing human VCs (Undrovinas et al., 2002); they exhibit early openings (Fig.4A) and the two modes of late gating (Fig.4D) with the same single channel conductance (Undrovinas et al., 2002) as the late openings of both normal VCs and $Na_v1.5$ (Fig.4B,C). Quantitative characterization and comparison of late Na^+ channel gating is rather difficult because assessment of late openings from one and only one channel in the patch is technically impossible. Though it is possible to fabricate a small patch pipette covering a membrane patch with just one Na^+ channel (Benndorf, 1988), the probability that it will operate in a late gating mode (either burst mode or LSM) is extremely low. Accordingly, late Na^+ channel activity is recorded in relatively large multi-channel (8-15) patches (Maltsev and Undrovinas, 2006;Patlak and Ortiz, 1985;Undrovinas et al., 2002). However, even in such patches late openings of Na^+ channel are still insufficient to yield a smooth ensemble average current. Instead, the slow inactivation kinetics of the late channel has been assessed from a decay of the latencies of the channel openings (Undrovinas et al., 2002). This approach has shown a significantly slower inactivation of LSM in failing vs. normal VCs or heterologously expressed Nav1.5 (Fig.5A) (Maltsev and Undrovinas, 2006). As to the burst mode, the total burst length decreased with depolarization and was significantly larger in failing compared to normal myocytes and Nav1.5 (Fig.5B).

6. Molecular identity of I_{NaL} in normal and failing ventricular myocardium

6.1. Expression of multiple Na^+ channel isoforms in heart

Numerous Na^+ channel isoforms have been reported in heart, with $Na_v1.5$ dominating (Goldin et al., 2000). Molecular identity of I_{NaL} needs careful consideration especially for failing hearts where gene expression profile changes dramatically. Different transcripts of highly TTX-sensitive neuronal isoforms ($Na_v1.1$, 1.3, 1.6) have been discovered in mouse and dog heart ($Na_v1.1$, 1.2, 1.3) (Haufe et al., 2005; Maier et al., 2002). These studies suggested species-specific expression and protein localization within sarcolemmal compartments. These neuronal Na^+ channel isoforms are responsible for 10 to 20% of the Na^+ current peak in myocardial and Purkinje cells, respectively (Haufe et al., 2005). Furthermore, four splice variants of SCN5A gene that encodes $Na_v1.5$ have been reported in humans (Tan et al., 2005). These variants are present in human myocardium and differ in their biophysical properties (Tan et al., 2005). Contribution of these isoforms and/or variants in I_{NaL} and its modulation by different pathological conditions is not yet understood. The problem of molecular identity of I_{NaL} has been approached in many different ways including methods of biophysics, pharmacology, and molecular biology.

6.2. Whole cell and single channel data in human VCs and heterologously expressed $Na_v1.5$

Late Na^+ channel openings show similar gating modes and Na^+ conductance in normal human cardiomyocytes and in heterologously expressed $Na_v1.5$ (section 5.2). These data indicate the common molecular origin of the late channel openings i.e. they are produced by $Na_v1.5$ in human cardiomyocytes. The heterologously expressed $Na_v1.5$ produces the slowly inactivating whole cell I_{NaL} in tsA201 cells (Fig.2F) similar to that observed in human cardiomyocytes (Fig.2A). Studies of late Na^+ single-channel activity in failing human cardiomyocytes did not

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reveal any peculiar Na^+ channels, compared to normal hearts or heterologously expressed $Na_v1.5$ (sections 5.2, 5.3). Accordingly, the HF-related alterations in burst and late scattered openings (Fig. 5A,B) that underlie whole-cell I_{NaL} changes (Fig.3) (Maltsev et al., 2007) are likely due to a modulation of the same $Na_v1.5$ rather than to an isoform (or a splice variant) switch.

6.3. Pharmacological data in humans and dogs

Difference of 2 amino acids in the Na^+ channel pore region accounts for the high affinity of neuronal Na^+ channel and low affinity of cardiac Na^+ channel for TTX or STX, the feature commonly used to distinguish these Na^+ channel isoforms pharmacologically (Heinemann et al., 1992). Dose–response curves for blockade of I_{NaL} by TTX and STX reveal only a single-site binding with the half-concentration that is typical for the $Na_v1.5$ in both dog and human failing hearts (IC_{50} was 1.2 *vs*. 1.53 μM for TTX and 62 *vs*. 98 nM for STX comparing dog vs. human cardiomyocytes) (Maltsev et al., 1998a; Maltsev et al., 2007). Further evidence of $Na_v1.5$ origin of I_{NaL} is its sensitivity to Cd^{2+} (IC_{50}=104 μM) (Maltsev et al., 2007), as Cd^{2+} distinguishes between cardiac and neuronal Na^+ channel isoforms (Satin et al., 1992). Furthermore, if I_{NaL} did represent the activity of channels with different pharmacological and gating properties, the extent of TTX blockade of I_{NaL} would differ at different time points after membrane depolarization, interfering with the decay time course. On the contrary, in both humans and dogs the extent of toxin-induced blockade stayed approximately constant during I_{NaL} time course between 200 and 1000 ms. No significant “non-inactivated” or “steady-state” Na^+ current blocked by the toxins was observed as of 2 s of membrane depolarization (Maltsev et al., 1998a; Maltsev et al., 2007). These data suggest that within this time frame I_{NaL} is mainly determined by the activity of only one type of Na^+ channel. Thus, I_{NaL} in canine and human VCs has a common molecular origin (i.e. $Na_v1.5$) and, therefore, similar molecular mechanisms could be involved in alteration of I_{NaL} in HF in both species.

6.4. siRNA data

Silencing the SCN5A gene responsible for $Na_v1.5$ expression with siRNA decreases I_{NaL} by 75% in wide range of membrane potentials (including AP plateau), resulting in a significant reduction of AP duration and variability in dogs with chronic HF (Undrovinas et al., 2005) (Fig.6).

6.5. Summary for I_{NaL} molecular identity

All above findings support the hypothesis that $Na_v1.5$ is a major contributor in I_{NaL} in normal and failing myocardium. However, an unknown channel with pharmacological and gating properties similar to $Na_v1.5$ might still be contributing to I_{NaL}. The only reported mammalian Na^+ channel isoform, other than $Na_v1.5$, that can produce late openings and possesses a low affinity for TTX is $Na_v1.8$ (gene SCN10A) (Akopian et al., 1996). However, up to date mRNA encoding $Na_v1.8$ has not been detected in rat hearts (Akopian et al., 1996), failing human heart tissue, or isolated VCs (Maltsev et al., 2007).

7. Modulation of Na^+ channels as a possible mechanism for I_{NaL} alterations in pathological conditions

7.1. Na^+ channel structure has multiple links to modulatory proteins

The function of the Na^+ channel is not fully determined by its protein structure, but depends also on its environment. Na^+ channels represent a multi-protein complex comprising not only the main pore-forming α subunit and its auxiliary β subunits, but also components of cytoskeleton, Ca^{2+}-sensitive protein calmodulin, regulatory kinases and phosphatases, trafficking proteins, and extracellular matrix proteins embedded into lipid bilayer plasma

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membrane. A diagram of $Na_v1.5$ and interacting proteins is shown in Fig.7A. For elegant in-depth reviews on this topic please see (Abriel and Kass, 2005;Meadows and Isom, 2005;Nerbonne and Kass, 2005). Here we highlight only the components that seem to modulate I_{NaL} in different pathological conditions, including hypoxia and HF. The III-IV linker (see Fig. 7B) is responsible for Na^+ channel inactivation, and mutations in this region disrupt $Na_v1.5$ inactivation causing persistent Na^+ current linked to inherited LQT3 syndrome (Bennett et al., 1995). Recently COOH terminal that has binding sites for numerous regulatory proteins, has been implicated in $Na_v1.5$ inactivation (Cormier et al., 2002;Motoike et al., 2004).

7.2 Modulation of I_{NaL} by β subunits

Many of the mammalian voltage gated Na^+ channels are associated with auxiliary β subunits. The β-subunit gene family has four members β_1(SCN1B), β_2 (SCN2B), β_3 (SCN3B) β_4 (SCN4B) (see for review (Meadows and Isom, 2005; Nerbonne and Kass, 2005)). All these β-subunits are expressed in rodent hearts and are differently localized to specific subcellular domains and cell types. β_1 subunit is non-covalently attached to α subunit, and β_2 subunit is covalently linked to α subunit by a disulfide bond (Messner and Catterall, 1986). The protein of these β subunits contains an extracellular amino-terminus, a single transmembrane segment, and an intracellular COOH terminus (Fig. 7B). The extracellular N-terminus of all β subunits contain immunoglobulin domain found in cell adhesion molecules. Particularly, immunoglobulin domain for β_2 (and probably for β_4) is similar to contactin, whereas for β1 and its splice variant β_1A it is similar to myelin P_o This unique property allows interactions with variety of signaling molecules and components of the extracellular matrix. With the regard to the intracellular domain, direct interactions of β_1 subunit C terminus and ankyrin B in rat brain membranes have been demonstrated, indicating a role of this subunit in Na_v localization. Direct interaction between cytoplasmic C terminus domain of $Na_v1.1$ with β_1 and β_3 has been recently demonstrated (Spampanato et al., 2004). β subunits do not form an ion-conducting pore, but modulate Na^+ channel function, Na^+ channel protein expression at the plasma membrane (trafficking), and cell adhesion (Meadows and Isom, 2005; Nerbonne and Kass, 2005). More specifically, β_1-subunit: 1) is involved in abnormal Na^+ channel activity associated with the LQT3 mutation (An et al., 1998), 2) aggravates Na^+ channel dysfunction in Brugada syndrome (Makita et al., 2000), 3) modifies the blockade of Na^+ channel by fatty acids (Xiao et al., 2000) and lidocaine (Makielski et al., 1999), 4) modulates the trafficking of $Na_v1.5$ (Zhou et al., 2000), and 5) affects the burst mode of the heterologously expressed skeletal muscle Na^+ channel isoform in Xenopus oocytes (Chang et al., 1996).

Very few reports are currently available about modulation by β subunits of late openings of the cardiac Na^+ channel. Heterologous co-expression of β_1 subunit with $Na_v1.5$ in HEK293 cells diminishes a non-inactivating Na^+ current (Valdivia et al., 2002). Co-expression of β_1 subunit with $Na_v1.5$ in tsA201 cells increases I_{NaL} amplitude and significantly slows I_{NaL} decay, whereas co-expression of β_2 subunit does not affect I_{NaL} parameters (Undrovinas and Maltsev, 2001). The potency of β_1 subunit to modulate I_{NaL} has been confirmed in native cell environment. In normal dog VCs, knocking down of SCN1B by antisense nucleotides significantly accelerated I_{NaL} decay (Undrovinas and Maltsev, 2002)(Fig. 8A). In HF, $Na_v1.5$ protein level is downregulated, whereas β_1 has remains unchanged, indicating a relatively higher membrane content of β_1 (Zicha et al., 2004). This suggests, although indirectly, potential involvement of β_1 in the reported I_{NaL} alterations in HF. Possible involvement of other β subunits in I_{NaL} changes in HF awaits further studies.

7.3. Modulation of I_{NaL} by cytoskeleton

The cytoskeleton is a collection of interacting proteins that forms a framework within the cytoplasm with connections to the membrane that include attachments to integral membrane proteins. This framework maintains cell shape, plasma membrane integrity, and localization

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of membrane proteins such as ion exchange carriers and ion channels (Fig 7, see for review (Abriel and Kass, 2005;Bennett and Baines, 2001;Meadows and Isom, 2005;Nerbonne and Kass, 2005)).

Ankyrin-B—An adapter protein ankyrin-B, which is expressed in heart, links $Na_v1.5$ to the cytoskeleton. A knockout of ankyrin B in mice affects late Na^+ channel openings in cardiomyocytes (Chauhan et al., 2000). Furthermore, mutations in ankyrin B cause the LQT4 syndrome in one French family (Mohler et al., 2003), indicating the importance of channel environment for the channel function. Accordingly, a disruption of any member of this multi-protein complex by pathological conditions may lead to alterations of I_{NaL}.

F-actin—Cytochalasin-D, an agent that interferes with F-actin polymerization, slows inactivation of cardiac Na^+ channel by inducing bursts of openings. It also affects coupling of steady state activation and availability of Na^+ current (Maltsev and Undrovinas, 1996; Undrovinas et al., 1995).

Fodrin—Fodrin (spectrin)-based cytoskeleton, another element of the Na^+ channel microenvironment in heart, is a dynamic structure that is altered under a variety of pathological conditions (e.g. ischemia or heart failure (Hein et al., 2000; Heling et al., 2000; Yoshida et al., 1995)). The role of the fodrin-based cytoskeleton in I_{NaL} modulation has been confirmed in experiments with anti-β-spectrin antibodies (Fig. 8B) and with the antisense oligonucleotides targeting mRNA's encoding α- and β-fodrin in dog VCs (Undrovinas and Maltsev, 2003).

The fodrin breakdown that occurs in some disease states featuring poor Ca^{2+} handling can be mediated by the Ca^{2+} - activated enzyme calpain (Matsumura et al., 2001; Yoshida et al., 1995). Therefore, disturbances of the ankyrin and/or fodrin-based cytoskeleton may affect the Na^+ channel inactivation process. Cytoskeletal elements such as ankyrin may bind directly to α and β subunits. The link to β subunits may thus modulate I_{NaL} indirectly (as discussed in section 7.2 for β_1 subunit).

7.4. Modulation of I_{NaL} by lysophosphatidylcholine (LPC)

LPC is the endogenous amphiphilic lipid metabolite that accumulates in ischemic myocardium and represents a major factor causing the electrophysiological alterations that contribute to arrhythmogenesis (Corr et al., 1987; DaTorre et al., 1991). A significant prolongation of QTc interval has been recently found during early transmural ischemia in patients undergoing balloon angioplasty (Kenigsberg et al., 2007). Experimentally, LPC causes depolarization, reduction of the maximal upstroke velocity of AP, sustained abnormal rhythmic activity in Purkinje fibers, and delayed afterpotentials (DADs) in isolated tissue (Arnsdorf and Sawicki, 1981; Corr et al., 1987). The mechanisms of the LPC effects include modifications of Na^+ current (Burnashev et al., 1991; Burnashev et al., 1989; Chattou et al., 2000; Shander et al., 1996; Undrovinas et al., 1992). Such modifications account for a reduction of I_{NaT} and an emergence of late openings that produce a sustained Na^+ current. Interestingly, the late openings caused by LPC form clusters of the synchronized multiple channel openings (Burnashev et al., 1989; Undrovinas et al., 1992); we believe that this functional cooperation of individual channels represents a new fundamental phenomenon and deserves further studies. One of the mechanisms underlying these LPC-induced modifications might be an integration of LPC into the lipid membrane, which would increase the membrane fluidity (Fink and Gross, 1984). This, in turn, enhances motility and interaction of proteins within the membrane. On the other hand, LPC can activate neuromodulation signaling via PKA and PKC (Obata, 2002; Scott et al., 2006), affecting Na^+ channel slow inactivation (Chen et al., 2006).

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7.5. Modulation of I_{NaL} by Ca^{2+}, CaM, and CaM-KII

Ca^{2+} dependent mechanisms of I_{NaL} modulation are discussed in detail later in the review in relation to altered Ca^{2+} handling in HF VCs (section 9.2).

7.6. Other recently emerged modulators

A novel protein partner of $Na_v1.5$ has been discovered using an yeast 2-hybrid screen (Allouis et al., 2006). This protein, called 14-3-3η, interacts with the cytoplasmic I interdomain of Na^+ channel (see Fig. 7B). Although its direct effect on I_{NaL} properties has not yet been studied, it was shown that this protein influences the inactivation process by delaying recovery from inactivation. Additionally, Src family tyrosine kinase Fyn has phosphorylation site on $Na_v1.5$A III-IV linker (Ahern et al., 2005), which is known to be responsible for the inactivation. The most recently emerged modulator is a membrane microdomain protein Caveolin-3. This protein was previously linked to Na^+ channel trafficking, (Yarbrough et al., 2002) but now is implicated in LQT syndrome (Vatta et al., 2006). Implementation of these new players in I_{NaL} modulation in different pathological conditions awaits further studies.

8. Experimental evidence of I_{NaL} importance for abnormal repolarization, Ca^{2+} handling, and contractility in failing myocardium

8.1 Inhibition of I_{NaL} normalizes AP duration and beat-to-beat variability and eliminates EADs in HF VCs

AP duration (APD) is extremely frequency dependent in failing VCs (Fig.9A; (Undrovinas et al., 1999)). At low pacing rates of 0.2-0.5 Hz the mean APD is significantly larger than in normal hearts (Fig.9A), and failing cells eventually exhibit EADs (Fig. 5C) (Maltsev et al., 1998a;Maltsev et al., 2007;Undrovinas et al., 1999). In addition to the prolongation, APD also exhibits significant beat-to-beat variability in failing VCs. Although AP prolongation is not evident at physiological frequencies (1Hz and higher), variability of APD in failing cells is substantially larger than in normal cells (compare APD distributions and their SD values in Fig. 9B) (Maltsev et al., 2007;Undrovinas et al., 1999). Increased and slowed I_{NaL} (Fig. 3) seems to be an important factor in APD prolongation, variability, and EADs. Partial reduction in the magnitude of I_{NaL} caused by specific Na^+ channel blockers (TTX,1.5 μM, STX, 100 nM), a new antianginal drug ranolazine (that turned out to be a specific I_{NaL} blocker), or injection of external current opposite to I_{NaL} during the AP plateau significantly shorten the prolonged APD, decrease beat-to-beat variability of APD, and abolish EADs (Maltsev et al., 1998a;Maltsev et al., 2007;Undrovinas et al., 2006;Undrovinas et al., 1999).

8.2. Blockade of I_{NaL} improves Ca^{2+} transient and contractility in HF

The contractile dysfunction in HF is related not only to ongoing loss of functional cardiac units, but also to abnormal function of cardiomyocytes. HF is characterized by systolic dysfunction due to depressed Ca^{2+} transients (Bers et al., 2006) and by abnormal relaxation of cardiac myocytes (Davies et al., 1995; Gwathmey et al., 1987). At low pacing rates, the prolonged relaxation is associated with the spike-dome configuration of contractile response (Fig.10A). The ratio between amplitudes of the spike and dome phases was suggested to be an index for the severity of HF (Gwathmey et al., 1987). Similar to the shape of abnormal contraction, abnormally prolonged Ca^{2+} transients have also been observed in both ventricular muscle strips (Gwathmey et al., 1987) and cardiomyocytes isolated from failing hearts (Beuckelmann and Erdmann, 1992; Maltsev et al., 1998b) (Fig.10A). At the higher frequencies, these abnormalities account for the reversal of the force-frequency relationship in failing myocardium, leading to an increase in diastolic $[Ca^{2+}]_i$ and diastolic tension (Fig.10B "Control") (Feldman et al., 1988; Undrovinas et al., 2006). A partial blockade of I_{NaL} by STX, TTX or ranolazine greatly improves performance of failing VCs; it abolishes the dome phase

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of both contraction-relaxation cycle and Ca^{2+} transient at low pacing rates and prevents the rising diastolic tension and $[Ca^{2+}]_i$ at the higher pacing rates in failing VCs (Maltsev et al., 1998b; Undrovinas et al., 2006) (Fig.10).

9. Integration of I_{NaL} into electrophysiological and Ca^{2+} mechanisms in HF (Fig.1).

Dramatic improvement of cardiomyocyte function due to inhibition of I_{NaL} described above is evidence for a substantial I_{NaL} contribution in the failing heart. However, the interpretation of these effects needs extreme care because electrophysiology, contraction, and Ca^{2+} dynamics in cardiomyocytes are interrelated via multiple feedback mechanisms. The extent of deterioration of cardiomyocyte function and these feedback mechanisms vary greatly with the progression of HF and etiology. Since late openings of Na^+ channels generate both an electric current and a Na^+ influx, I_{NaL} will contribute to at least two established HF mechanisms: 1) electrophysiological alterations (Tomaselli and Zipes, 2004); and 2) altered cell Na^+ and Ca^{2+} cycling (Bers et al., 2006).

9.1 The role of I_{NaL} in AP prolongation and EADs

Given the high membrane resistance during the AP plateau, I_{NaL} provides a critical contribution to the altered delicate balance of ion currents and thus to the AP duration. The relative contribution of I_{NaL} to the AP plateau in failing VCs is amplified by the reduced K^+ currents in HF (Tomaselli and Zipes, 2004). Since HF simultaneously increases and slows I_{NaL} (sections 4.2 and 5.3), I_{NaL} will contribute directly to AP prolongation in HF, thus explaining APD normalization with I_{NaL} inhibition described above. Prolonged APD allows more time for I_{CaL} reactivation and thus facilitates EADs (January and Riddle, 1989). Since I_{NaL} contributes to AP prolongation, it thus indirectly contributes to EADs (transitions 8-9-10 in Fig.1). Accordingly, inhibiting I_{NaL} eliminates EADs likely due to APD shortening.

9.2. I_{NaL} and elevated $[Na^+]_i$ increase Ca^{2+} entry via NCX and limit depression of systolic function

A major problem in HF is systolic dysfunction, which is associated with smaller Ca^{2+} transient and sarcoplasmic reticulum (SR) Ca^{2+} content. The extent of deterioration of systolic function is limited by multiple compensatory mechanisms (listed below), which are indirectly linked to I_{NaL} and elevated Na^+ (Fig.1).

1) NCX function depends on Na^+ and Ca^{2+} concentrations and membrane voltage. In HF VCs, increased Na^+ influx (including that via I_{NaL}) shifts the NCX operation from the predominant forward mode to the reverse mode i.e. from Ca^{2+} efflux to Ca^{2+} entry (Baartscheer et al., 2003). Elevated Na^+ in HF thus limits SR unloading and provides additional Ca^{2+} influx during the AP (Bers et al., 2006). Interestingly, in addition to preserving SR Ca^{2+} load, this operational shift in failing human myocardium results in the direct activation of contraction during the terminal phases of the AP via the reverse mode NCX Ca^{2+} influx (Weisser-Thomas et al., 2003).

2) The vast majority of studies demonstrated that NCX is upregulated in HF (Studer et al., 1994), therefore the above effect could be amplified by the enhanced NCX function.

3) I_{NaL} contributes to APD prolongation and thus indirectly prolongs Ca^{2+} influxes via I_{CaL} and the reverse mode NCX.

4) I_{NaL} in HF is positively modulated by intracellular Ca^{2+} (Maltsev et al., 2002a) which could yield a new possible amplification mechanism of the Ca^{2+} entry. This creates a positive

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feedback loop from I_{NaL} via NCX to larger cell Ca^{2+} load, then from the larger Ca^{2+} load back to I_{NaL}. What is known about the mechanisms of Ca^{2+} modulation of Na^+ channels and I_{NaL}? Structurally, the carboxyl terminus of Na^+ channels has binding sites for Ca^{2+} itself (Wingo et al., 2004) and for Ca^{2+}-binding protein calmodulin (CaM) that acts as a Ca^{2+} sensor translating changes in cytoplasmic Ca^{2+} into cellular responses (Mori et al., 2000). Discovery of these sites inspired multiple studies in heterelogously expressed Na^+ channels, including brain, skeletal and cardiac muscle isoforms (Deschenes et al., 2002; Herzog et al., 2003; Kim et al., 2004; Tan et al., 2002; Young and Caldwell, 2005). It was found that some inactivation states of I_{NaT} of heterologously expressed cardiac and skeletal Na^+ channel isoforms may be modulated directly by Ca^{2+}, CaM and/or *via* Ca^{2+} /CaM /CaM-kinase cascade. The most recent study showed that CaMKIIδ_c enhances I_{NaL} and increases $[Na]_i$ (Wagner et al., 2006). In normal and especially in failing VCs, elevated $[Ca^{2+}]_i$ slows the decay of I_{NaL} and increases I_{NaL} amplitude and integral (gray area in Fig.11A,B)(Maltsev et al., 2002a). CaM, and CaM-kinase seem to be involved in this modulation because specific antagonists of CaM and CaM-kinase (P209-309 and KN93, respectively) or CaM-KII blocker KN93 significantly accelerate I_{NaL} at high $[Ca^{2+}]_i$ (Fig.11C) (Maltsev et al., 2002a)

9.3. Adverse effects of increased $[Ca^{2+}]_i$ in HF. Role of I_{NaL} and NCX in diastolic dysfunction

While the increased cell Ca^{2+} load limits the depression of systolic function in HF, it also leads to diastolic dysfunction, especially at high rates as described in section 8.2. Relaxation of cardiac myocytes occurs when $[Ca^{2+}]_i$ declines, allowing Ca^{2+} dissociation from the myofilaments. Ca^{2+} is removed from cytosol, mainly via SERCA, which takes Ca^{2+} back into the SR, and by NCX operating in forward mode during diastole (Bers, 1991). It is believed that the diastolic dysfunction in HF is mainly due to a reduced SERCA function in HF. At the same time, increased expression and function of NCX in HF tends to offset the deficiency of Ca^{2+} removal by SERCA (review (Bers et al., 2006)). The contribution of the increased I_{NaL} to the Ca^{2+} removal could be twofold. First, as discussed in section 9.2, I_{NaL} and related increase of $[Na^+]_i$ facilitate Ca^{2+} influx. Secondly, higher $[Na^+]_i$ during diastole partially offsets the function of the forward mode NCX and thus worsens the problems of Ca^{2+} removal from the cytosol and diastolic dysfunction. The improvement of diastolic function by the inhibition of I_{NaL} (Fig.10B) can be attributed both to a decrease in Ca^{2+} load during the AP plateau and to improved removal of Ca^{2+} by forward mode NCX during diastole. The importance of Na^+ influx and of the forward mode NCX function for abnormal Ca^{2+} handling in HF VCs is illustrated in experiments with temporal substitution of Li^+ for Na^+ (Fig.12B). These conditions, designed to inhibit NCX function (at least in part, see Fig. 12 legend) and Na^+ influx, accelerate Ca^{2+} transient decay, decrease diastolic $[Ca^{2+}]_i$, and greatly improve overall contractile performance of failing VCs. Since the late current is almost equally carried by Na^+ and Li^+ (see Fig.2A, B), it is likely preserved under these conditions. This experiment thus illustrates the importance of I_{NaL}-related Na^+ influx (rather than the I_{NaL}-electric current per se) for abnormal Ca^{2+} handling and cell contraction in failing VCs. A partial blockade of the NCX also improves EC coupling in HF (Hobai et al., 2004) and reduces both EADs and DADs (Nagy et al., 2004; Pogwizd and Bers, 2002), indicating that NCX could be a promising therapeutic target in HF (Shah et al., 2005) (Sipido et al., 2006).

9.4. Ca^{2+} overload and increased diastolic Na^+: potential importance of I_{NaL} for DADs

DADs occur due to spontaneous Ca^{2+} releases during diastole via the activation of the forward mode of NCX. I_{NaL} does not occur at low diastolic potentials in humans and dogs (see Fig. 2B, filled circles), so it cannot have a direct contribution to DADs. However, as discussed above, in HF VCs I_{NaL} increases Ca^{2+} influx leading to SR Ca^{2+} overload, which, in turn, is critical for initiation of spontaneous Ca^{2+} release (such as Ca^{2+} waves) during diastole. I_{NaL} involvement in DADs is thus limited to the contribution of the I_{NaL} to the Ca^{2+} overload. On the other hand, high diastolic $[Na^+]_i$ in HF (Despa et al., 2002) decreases the forward mode

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NCX current and hence can attenuate the amplitude of DADs. This positive factor of I_{NaL} can be counterbalanced by downregulation of I_{K1} in HF (Kaab et al., 1996), facilitating membrane excitations.

9.5. Role of I_{NaL} in dispersion of repolarization: a feedback from abnormal Ca^{2+} cycling?

The mechanisms of dispersion of repolarization in HF remain unclear. A possible mechanism involves beat-to-beat alternations and/or fluctuations in intracellular Ca^{2+} cycling transduced to abnormal repolarization by electrogenic feedback mechanisms (review (Wilson et al., 2006), Fig.1). Fluctuations of Ca^{2+} transient can be observed in failing VCs of a canine chronic HF model (Fig.12A). Listed are three possible indirect contributions of I_{NaL} to the beat-to-beat variability problem.

1) The I_{NaL} contribution to the abnormal Ca^{2+} cycling (section 9.2).

2) The I_{NaL} dependence on Ca^{2+}/CaM/CaMKII (section 9.2). Thus I_{NaL} can serve as one of the electrogenic Ca^{2+} feedback mechanisms along with other Ca^{2+} -dependent ion currents, such as I_{CaL} inactivation, I_{Ks}, I_{to}, Ca^{2+}-activated Cl^{-}-current, and NCX.

3) The I_{NaL} contributes to AP shape that, in turn, might cause fluctuations of Ca^{2+} transients. The synchronicity of Ca^{2+} release depends on the state of phosphorylation of Ca^{2+} cycling proteins (Song et al., 2001) and on the AP shape (Sah et al., 2002). More specifically, the faster the repolarization, the more synchronous is the Ca^{2+} release. Failing VCs isolated from the infarct border zone exhibit dyssynchronous SR Ca^{2+} release from junctional SR (Litwin et al., 2000). Also, SR Ca^{2+} release is spatially and temporally variable from beat to beat in feline failing VCs (Harris et al., 2005). Since voltage clamped normal and failing feline VCs showed almost the same Ca^{2+} dynamics, the abnormal AP shape (significant loss of phase 1 AP repolarization) is likely to be the reason for desynchronized Ca^{2+} release in this feline HF model (Harris et al., 2005). The contribution of I_{NaL} could be important for the abnormal early repolarization phase in HF via its increased burst mode (Maltsev and Undrovinas, 2006) (Fig. 5B), especially when I_{to} is decreased (Tomaselli and Zipes, 2004). Further, late scattered openings of Na^{+} channel also increase in human HF VCs (Maltsev and Undrovinas, 2006) (Fig. 5A); they persist on the AP plateau (Fig. 5C) and hence tend to prevent further repolarization. Thus late openings of both Na^{+} gating modes can indirectly (i.e. via the AP shape) contribute to the desynchronized, fluctuating Ca^{2+} release.

9.6. Summary of I_{NaL} role in failing myocardium: friend or foe?

I_{NaL} and its Na^{+} influx can directly and indirectly contribute to several important HF mechanisms related to electrophysiological alterations and ion homeostasis. These contributions could either improve or worsen performance of HF myocardium, i.e. being “friend” or “foe”, respectively (Fig.1). I_{NaL} is “friend” as it contributes to 1) APD prolongation as an adaptive and an anti-arrhythmic (anti-re-entry) response (Tomaselli and Zipes, 2004); and 2) Ca^{2+} entry to limit depression of systolic function. The latter mechanism is an intrinsic, adaptive, digitalis-like effect with all corresponding risks and benefits. Interestingly, a large slow component of the Na^{+} current decay (burst mode) has been identified in post-myocardial infarction-remodeled myocytes (Huang et al., 2001), i.e. in the transitional period from an infarction to HF. The increased I_{NaL} may indeed serve as an initial mechanism of adaptation to match an increased contractility demand for the survived, noninfarcted VCs.

Although APD prolongation can be beneficial in HF, the temporal and spatial dispersion of repolarization that accompanies AP prolongation is critical for arrhythmia and sudden cardiac death (Tomaselli and Zipes, 2004). Additionally, DADs and EADs have critical importance for nonreentrant arrhythmias or triggered activity (recent review (Bers et al., 2006)).

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Accordingly, I_{NaL} is "foe" as it contributes to EADs, DADs, dispersion of repolarization, and diastolic dysfunction.

10. I_{NaL} is a novel target for cardioprotection aiming at arrhythmias and Na^+-Ca^{2+} overload. Treatment strategies and requirements for new drugs, targeting Na^+ channels

After negative outcomes of Cardiac Arrhythmia Suppression Trial (CAST) (Epstein et al., 1991; Epstein et al., 1993) Class I antiarrhyrhmic drugs (Vaughan Williams, 1984) targeting Na^+ channels became close to a "taboo". The trial tested whether the suppression of ventricular arrhythmias by encainide, flecainide, moricizine after myocardial infarction improves survival. The conclusion was that the "treatment strategies designed solely to suppress these arrhythmias should no longer be followed" (Epstein et al., 1993). However, the discovery of inherited mutations in SCN5A gene that lead to an increased I_{NaL} (LQT3 syndrome, see for review (Shah et al., 2005)) and of increased and slowed I_{NaL} in acquired, chronic HF (see sections 4.2 and 5.3) gives rise to a revival of Na^+ channels as a therapeutic target. These studies suggest that not all Na^+ channels must be equally targeted. The emerging paradigm for Na^+ channels in HF is that I_{NaT} is decreased (Maltsev et al., 2002b; Valdivia et al., 2005; Zicha et al., 2004) but simultaneously I_{NaL} is increased (section 4.2). Blockers of I_{NaT} are proarrhythmic in HF because they slow conduction, thus worsening conduction problems (review (Shah et al., 2005)) and facilitating development of re-entry. Accordingly, new strategies for treatment must be considered: the new type of "smart" drugs should preferentially block I_{NaL} over I_{NaT}. This requirement calls for a new classification of Class 1 drugs in the future.

What are the potential benefits of a preferential I_{NaL} blockade in HF? Based on the summary of I_{NaL} integration into HF mechanisms shown in Fig.1, this could be both an antiarrhythmic effect and an improvement of diastolic function. A preferential blockade of I_{NaL} over I_{NaT} in failing human VCs was reported for amiodarone (Maltsev et al., 2001), suggesting an explanation of why amiodarone, classified as Class III anti-arrhythmic drug, shows an outstanding efficiency among K^+-channel blockers. Table 1 summarizes potencies of various drugs to block I_{NaT} and I_{NaL} based on their ability to differentially interact with Na^+ channel gating states. Based on a new index of the preferential blockade of I_{NaL} relative to I_{NaT}, the most promising drug is a new antianginal drug ranolazine (Fig.13) (Undrovinas et al., 2006).

The potential great benefits (preventing arrhythmias and Ca^{2+} overload) of the preferential I_{NaL} blockade can be expected not only in HF but in other cardiac diseases, such as ischemia, in which Na^+-Ca^{2+} overload is a major feature. Since LPC dramatically increases I_{NaL} (section 7.4), targeting I_{NaL} in ischemia is especially encouraged. Inhibition of I_{NaL} by ranolazine reduces Ca^{2+} overload and left ventricle mechanical dysfunction during ischemia/reperfusion (Fraser et al., 2006). On the other hand, the therapeutic strategy of a preferential I_{NaL} blockade should be exercised with care. The balanced and effective therapy targeting I_{NaL} should take into account that I_{NaL} might be involved in adaptive response (see section 9.6) at different states of cardiac disease, making the question whether I_{NaL} is "friend" or "foe" vitally important.

11. Quantitative integration of I_{NaL} into electrophysiological and Ca^{2+} regulatory mechanisms: present state and future perspectives

11.1. I_{NaL} simulation using a general function: predictions of I_{NaL} role in normal myocardium

The first quantitative integration of I_{NaL} into an AP model was done by Sakmann et al. (Sakmann et al., 2000). As described in sections 3.2 and 4.1, a distinctive property of I_{NaL} is its voltage-gated activation. In their model, the experimentally obtained *I*-V relations were

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fitted by the least-squares method to the general function: $I=G(V-E)/(1+\exp[V'-V]/k)$, where I is the current, V is the voltage, $G$is the maximum I_{NaL} conductance, E is the Na^+ equilibrium potential, V' is the voltage at half-maximal activation, and k is the slope factor. These I-V relationships were incorporated into the standard guinea pig ventricular cell model in OXSOFT Heart 4.8. This model predicted the role of I_{NaL} in normal myocardium. As discussed in sections 2 and 3, multiple electrophysiology studies demonstrated a significant contribution of I_{NaL} in APD. For example, in normal human VCs in the presence of 1.5 μM TTX, APD was reduced by 15 to 20% (Maltsev et al., 1998a). Computer modeling studies fit these data surprisingly well and predicted transmural differences in repolarization based on the I_{NaL} gradients across the ventricular wall (Sakmann et al., 2000; Zygmunt et al., 2001). TTX effect on APD duration (% change) in human VCs showed remarkable rate dependence (Maltsev et al., 1998a) indicating that I_{NaL} could be involved in the rate-dependent AP adaptation. This mechanism is thought to be related to a slow reactivation of I_{NaL} (0.5 s for human VCs (Maltsev et al., 1998a)) resulting in an incomplete recovery from inactivation, I_{NaL} reduction, and enhancement of repolarization as rate increases (see review (Carmeliet, 2006)). To test this mechanism in quantitative terms, an AP model will need to integrate the dynamics of I_{NaL} reactivation.

11.2. A Markovian chain-based model of late channel openings yielding whole cell I_{NaL}

The total Na^+ current including I_{NaT} and I_{NaL} in human VCs has been recently numerically modeled as a set of Markovian kinetic schemes, each of which represents a separate gating mode (Maltsev and Undrovinas, 2006). I_{NaT} was described by a single gating mode (Transient Mode, TM) and I_{NaL} was described by two gating modes: LSM and the burst mode (BM) (Fig. 14A-C). The transition rates for I_{NaL} gating modes were estimated from patch clamp single channel data in human VCs.

Based on the frequency of occurrence of different modes in patches with known numbers of channels, the populations of channels operating in each gating mode were estimated as follows: 79.8% for TM, 20% for LSM, and 0.2% for BM, yielding an apparent four exponential I_{Na} decay (τ=0.4, 4, 50, and 500 ms, at 24°C and −30 mV). In this model, the first two exponentials are produced by two inactivation states of TM, governed by rates c and e in Fig. 14A. The third exponential is related to apparent bursts inactivation, and the forth exponential is determined by inactivation of LSM reopenings governed by rate e in Fig14B. All four predicted components were clearly identified in whole-cell patch clamp recordings in human VCs (Maltsev and Undrovinas, 2006). The model also predicts the voltage and temperature dependence of the late gating modes and the dynamic contributions of each mode into the total Na^+ current. The early phase of Na^+ current decay at 24°C (<40 ms) involves all three Na^+ channel gating modes, the intermediate phase (from 40 to 300 ms) is produced by BM+LSM, although the contribution of BM decreases with depolarization, and ultra-late decay (>300 ms) is determined solely by LSM. This outcome is paradoxical as it means that most I_{NaL} is produced by relatively rare scattered openings rather than bursts characterized by abundant channel activity. However, the burst contribution is limited as bursts occur in a much smaller fraction of Na^+ channels than LSM (0.2% compared to 20%, respectively) and are inactivated much faster (with an apparent decay time constant of ~50 ms vs. 500 ms). Furthermore, the contribution from the bursting channels is expected to decrease with depolarization (Fig 5B).

11.3. I_{NaL} provides substantial Na^+ influx in HF VCs

Two numerical estimates suggest a substantial Na^+ influx via I_{NaL} (Maltsev and Undrovinas, 2006): 1) I_{NaL}-related Na^+ influx is similar in magnitude to that of I_{NaT} and 2) I_{NaL} integral significantly increases in HF. More specifically, a mathematical model based on single-channel data in human VCs described in section 11.2 shows that despite a much smaller channel population operating in LSM compared to TM (20% vs. 79.8%) and a much smaller scale of

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I_{NaL} compared to I_{NaT}, LSM and TM channels transfer almost the same amount of Na^+ through the plasma membrane during 2 s depolarization. Numerical integration of simulated currents for each mode in a cell with 10^5 Na^+ channels yields electrical charges of 42, 45 and 7 pC for TM, LSM, and BM, respectively. In other words, the I_{NaT} peak is about 3 orders of magnitude larger than I_{NaL} (50 nA compared to 50 pA), yet its span is about 3 orders of magnitude shorter (2 ms compared to 2 s), resulting in an almost equal total charge transfer.

I_{NaL} transfers significantly more Na^+ into failing vs. normal VCs (Fig.14E, inset). The total charge transferred by I_{NaL} from 10 to 2000 ms is predicted to be 28.5 and 45 pC for normal and failing VCs, respectively, or a ~58% increase. This model estimate is in line with a 53.6% HF-induced increase of Na^+ influx via I_{NaL} evaluated from whole cell patch clamp measurements in canine normal and failing VCs (gray area in Fig.3B bottom panel; (Maltsev et al., 2007)). Taking into account that I_{NaT} is reduced by 30-40% in HF VCs (Maltsev et al., 2002b;Valdivia et al., 2005;Zicha et al., 2004), the role of I_{NaL} in Na^+ homeostasis should be even more substantial in the failing cells, in line with the recent finding that $[Na]_i$ is significantly increased in failing paced cardiomyocytes (Despa et al., 2002). Modulation of I_{NaL} by Ca^{2+} (section 9.2) is another possibility for a larger Na^+ transfer by I_{NaL} in HF, especially in cells with high $[Ca^{2+}]_i$.

11.4. Prediction of I_{NaL} importance for repolarization, EADs, and DADs

The functionality of integration of I_{NaL} into HF mechanisms (Fig.1, labeled by numbers) has been partly numerically tested by Denis Noble and co-authors in their several computer simulation studies, albeit in the models of normal (non-HF) cardiomyocytes. The critical importance of I_{NaL} in a hypothetical repolarization failure (transition 8 in Fig.1) has been demonstrated in numerical simulations (Noble and Noble, 2006) using the Sakmann's AP model of guinea pig VC (Sakmann et al., 2000) (described in section 11.1). More specifically, an inhibition of I_{NaL} (I_{pNa} in their paper) protects from a repolarization failure produced by a blockade of I_{Kr} at an external K^+ concentration of 3.5 mM. EADs in failing human VCs can be suppressed by a partial inhibition of I_{NaL} with TTX (Maltsev et al., 1998a). This result was reproduced in simulations when EADs were induced in the model by increasing I_{pNa} (Noble, 1999) (transitions 8-9-10 in Fig.1). Other numerical simulations (Noble and Varghese, 1998) performed in a model of atrial cells demonstrated the link of $[Na^+]_i$ to DADs (interactions 2-3-4-5-6-7 in Fig.1). It was shown that increasing $[Na^+]_i$ leads to increased Ca^{2+} loading via NCX. Beyond a threshold (when $[Na^+]_i$ exceeds 12–15 mM), increased Ca^{2+} loading triggers repetitive spontaneous release of Ca^{2+} from the SR. The oscillatory releases, in turn, activate forward mode NCX that produces an inward current and depolarizes cell membrane, resulting in DADs.

11.5. Summary and future perspective

A hypothetical integration of I_{NaL} into HF mechanisms, shown in Fig.1, was derived from established HF mechanisms and available data on I_{NaL}. It has not been systematically tested in quantitative terms. Quantitative integration of I_{NaL} into cell electrophysiological and Ca^{2+} regulatory mechanisms has been approached so far in non-HF and non-human cardiomyocytes. I_{NaL} was approximated with a general function that included only the voltage dependence of I_{NaL} activation, but missed the slow inactivation and the recovery from inactivation. These simulations mechanistically describe key phenomena (e.g. AP prolongation, EADs, and DADs) under specific conditions related to I_{NaL} and $[Na^+]_i$. Since biophysical properties of I_{NaL} and late Na^+ channel gating have been characterized in detail (sections 4 and 5) and numerically modeled (section 11.2), further studies on I_{NaL} integration can consider incorporation of these detailed I_{NaL} formulations. Our simulations of I_{NaL} demonstrate substantial Na^+ flux transferred by I_{NaL} that greatly increases in HF (section 11.3). They also describe complex effects of drugs on I_{NaL} based on the key parameters of their interactions

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with Na^+ channels (e.g. amiodarone (Maltsev et al., 2001)). Finally, the formulations are based on the data obtained in human VCs (both normal and failing) and thus can be incorporated into human AP models. We believe that the upgraded models will predict whether a particular disease manifestation (such as shown in Fig.1) will wax or wane at a given state of cardiac disease, therapeutic interventions, and genetic factors.

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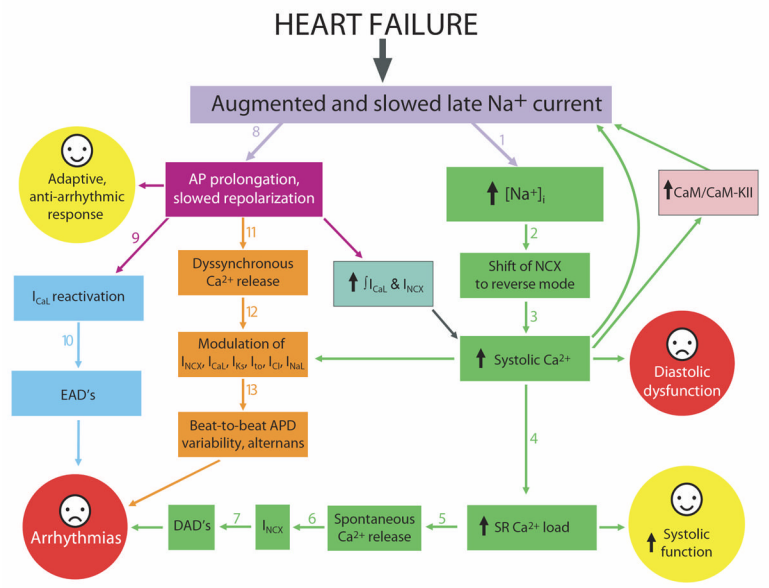

Figure 1.

Integration of the late Na^+ current into electrophysiological and Ca^{2+} regulatory mechanism in heart failure (see text for detail).

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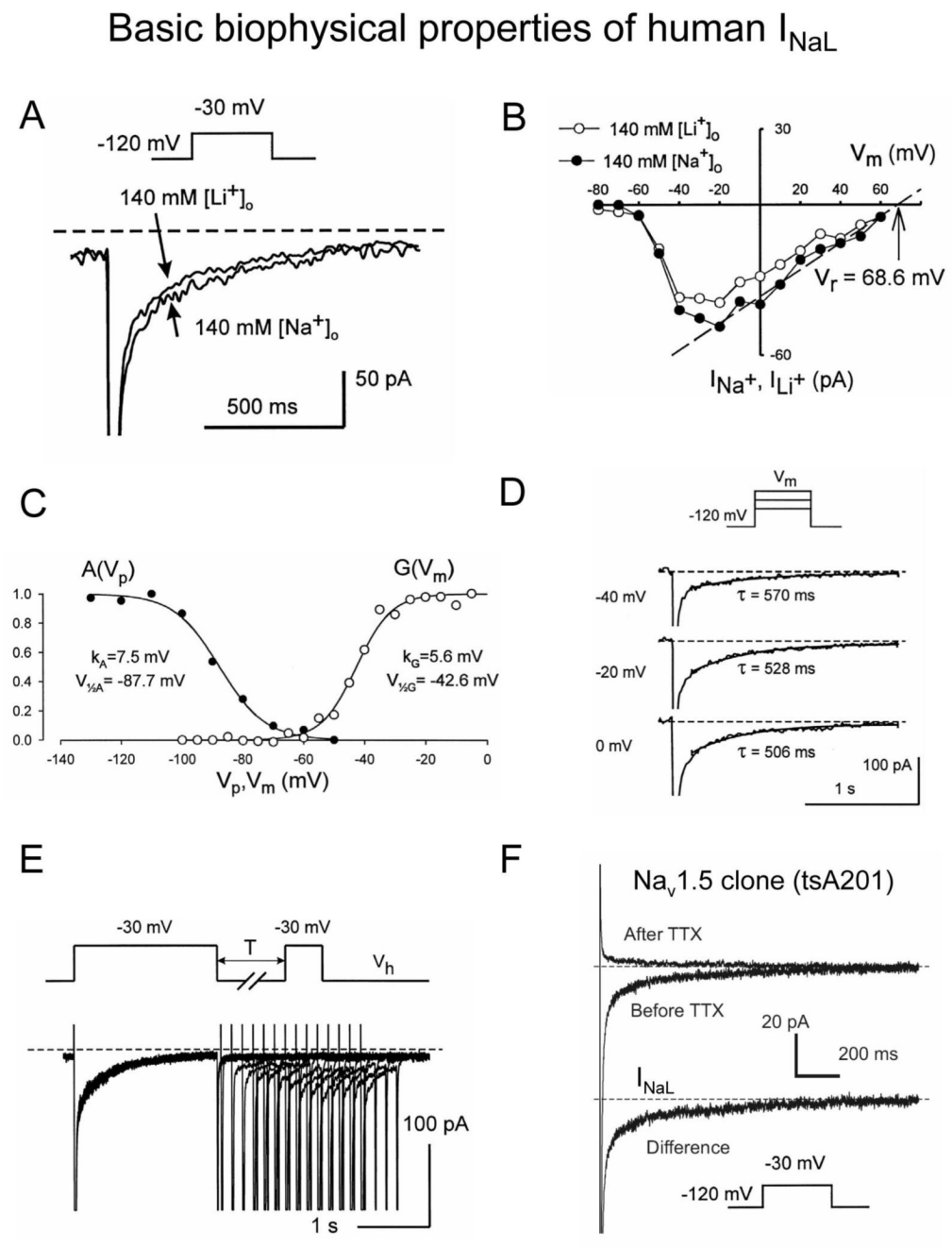

Figure 2.

Biophysical properties of the slowly inactivating, late Na^+ current (I_{NaL}) evaluated by whole cell patch clamp in human ventricular cardiomyocytes (A-E) and human cloned $Na_v1.5$ expressed in tsA201 cells (F). A-B: Late current can be carried either by Na^+ or Li^+. B: I-V relation for I_{NaL}. C: examples of steady-state activation and availability curves, $G(V_m)$ and $A(V_p)$, respectively. D: Examples of original traces illustrating voltage-independent I_{NaL} decay. E: slow reactivation of I_{NaL}. F: I_{NaL} produced by $Na_v1.5$ was assessed as difference current before application of a selective Na^+ channel blocker tetrodotoxin (TTX, 30 μM) (20 averaged traces) and after TTX (14 averaged traces). Voltage protocols are shown at the traces.

Recording was performed at 24°C. Reprinted from (Maltsev et al., 1998a) (A-E) and (Undrovinas et al., 2002)(F), used with permission.

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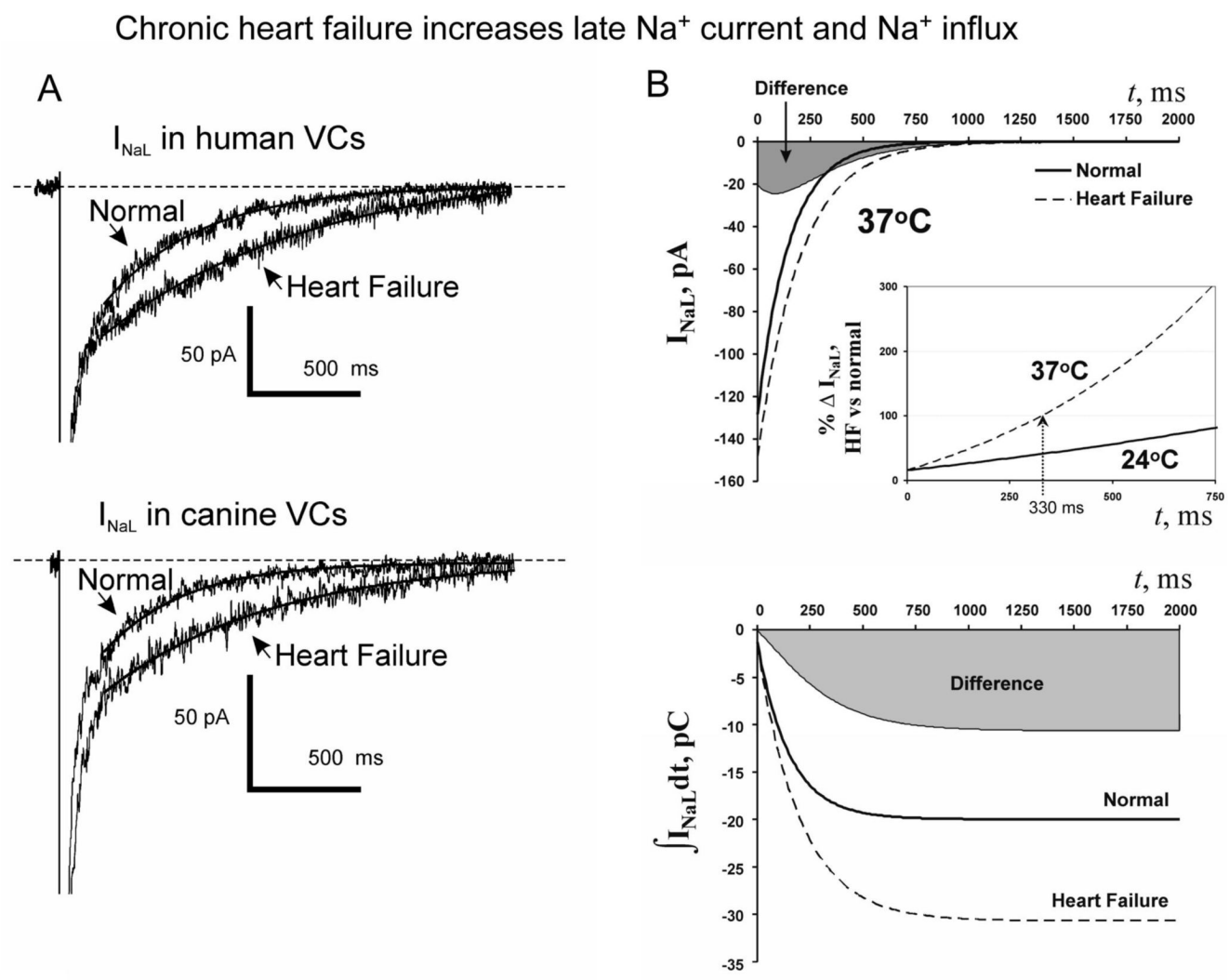

Figure 3.

A: Chronic heart failure slows and increases I_{NaL}. A: examples of whole cell I_{NaL} recordings in human and dog ventricular cardiomyocytes. B: Idealized I_{NaL} and their integrals in normal and failing dog cardiomyocytes of same size (200 pF) calculated using Q_{10} factors (37°C) and average parameters of I_{NaL} density and decay time constant. Larger and slower I_{NaL} in failing cardiomyocytes results in substantial increase in total charge (or Na^+) transfer by I_{NaL}. Gray areas illustrate difference between failing and normal cells. Adapted from (Maltsev et al., 2007), used with permission.

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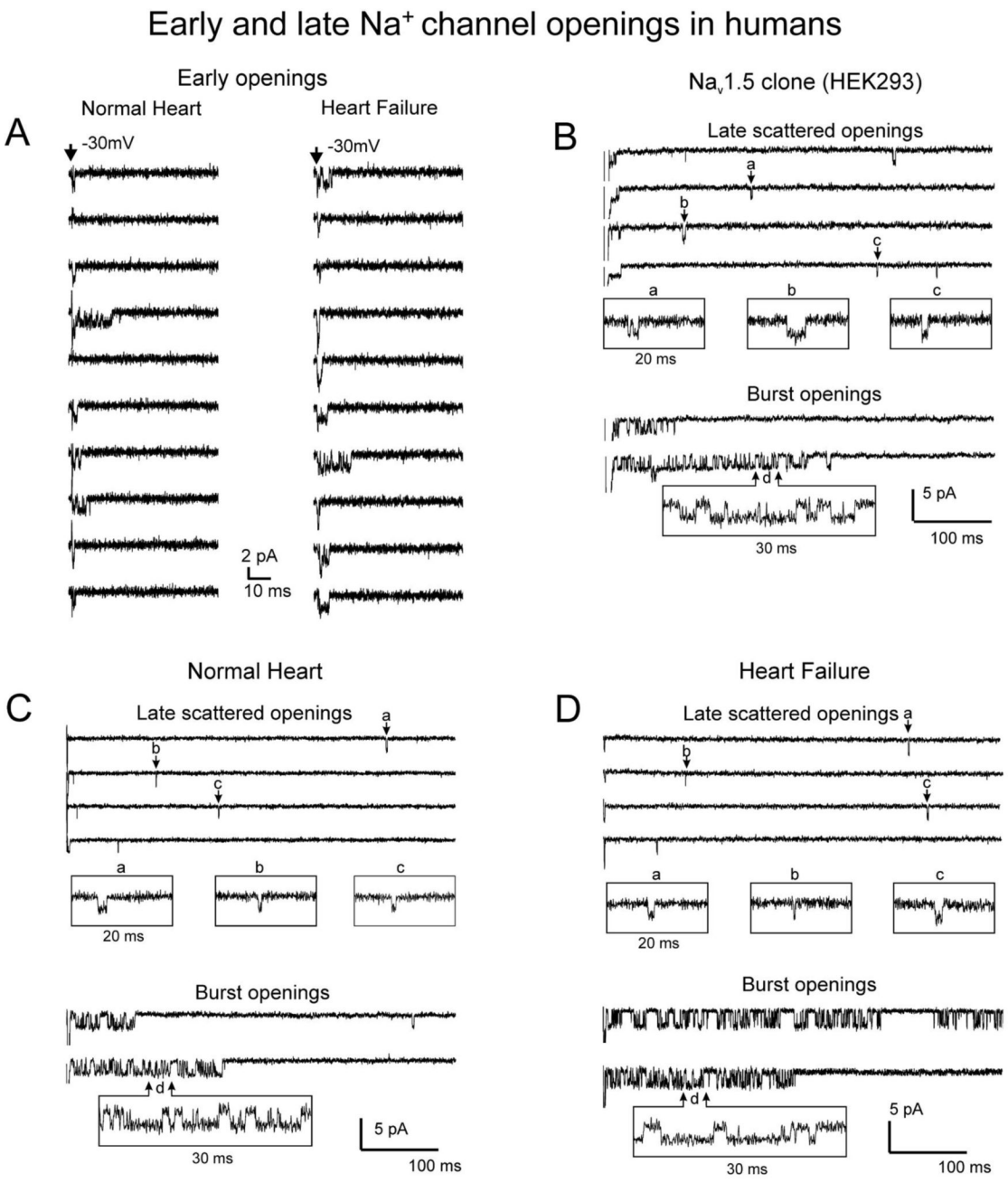

Figure 4.

Examples of Na^+ channel activities that underlie peak ("early openings", panel A) and late (panels B-D) Na^+ currents in normal and failing human cardiomyocytes as well as in cardiac channel clone ($Na_v1.5$) expressed in HEK293 cells. Note similarity between modes of late activity in normal hearts, failing hearts, and the clone. Cell-attached patches were held at −130 mV and step clamped to −30 mV at 23°C. Reprinted from (Undrovinas et al., 2002), used with permission from European Society of Cardiology.

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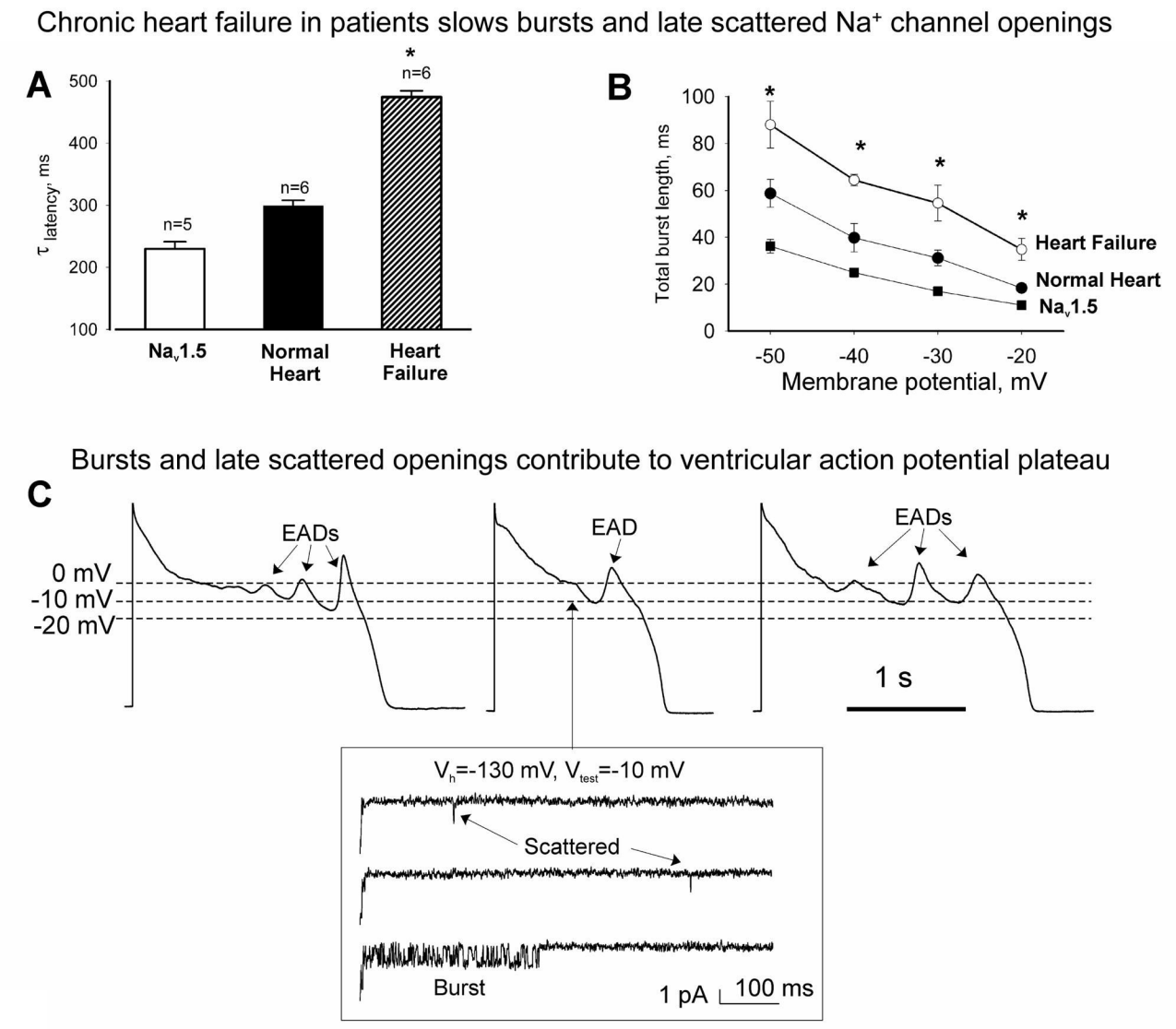

Figure 5.

Inactivation of both late scattered mode (A) or burst mode (B) of the late openings of Na^+ channel was slowest in failing human cardiomyocytes compared with those from normal human hearts or heterologously expressed $Na_v1.5$. *P<0.05, heart failure *vs*. normal heart or clone (Mean±SEM). Cell-attached patches: V_h = −140 mV, 24°C. (Reprinted from (Maltsev and Undrovinas, 2006) with permission from European Society of Cardiology). C: recordings of action potentials in failing human cardiomyocytes are shown along with late scattered mode and burst mode openings occurring at −10 mV, i.e. within the voltages of the action potential plateau. Reprinted from (Undrovinas et al., 2002), used with permission from European Society of Cardiology.

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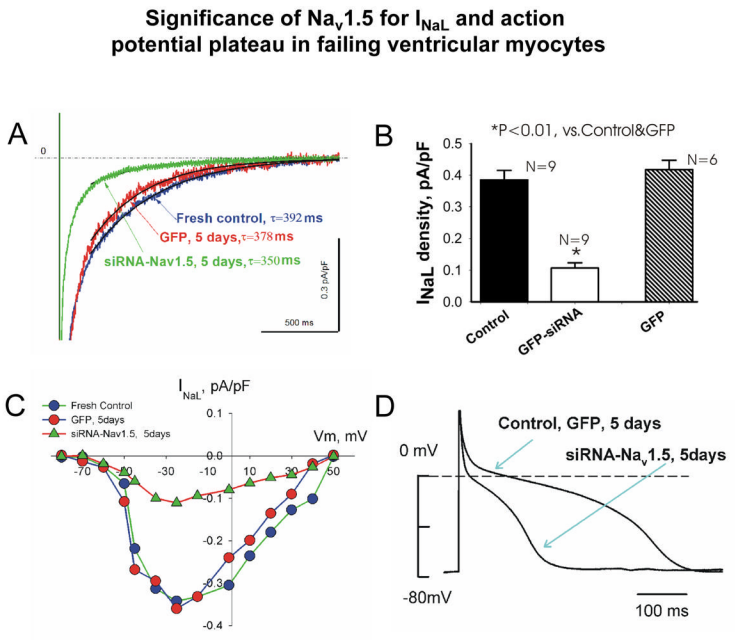

Figure 6.

SCN5A gene regulates late sodium current (I_{NaL}) and action potential duration in failing heart. Silencing of SCN5A gene expression in ventricular cardiomyocytes of dogs with HF by adenovirally-transferred siRNA causes significant reduction in I_{NaL} density (A and B) in wide range of membrane potentials (C) and reduction of action potential duration (D). Shown are typical examples of I_{NaL} recordings, current-voltage relationships (whole cell patch clamp, 24° C) and action potential recordings (perforated patch, 35°C, 0.5 Hz pacing rate). Labels: “fresh control”- freshly isolated cells; “GFP” –cultured cells expressing reporter gene GFP, and “siRNA”- cultured cells with silenced SCN5A. Average data for action potential duration: fresh control, 515±51 ms, n=18; GFP control, 479±73 ms, n=8; siRNA, 181±17 ms, n=11; mean±SEM; n is number of cells; * P<0.01, vs. fresh control and GFP, ANOVA. From (Undrovinas et al., 2005).

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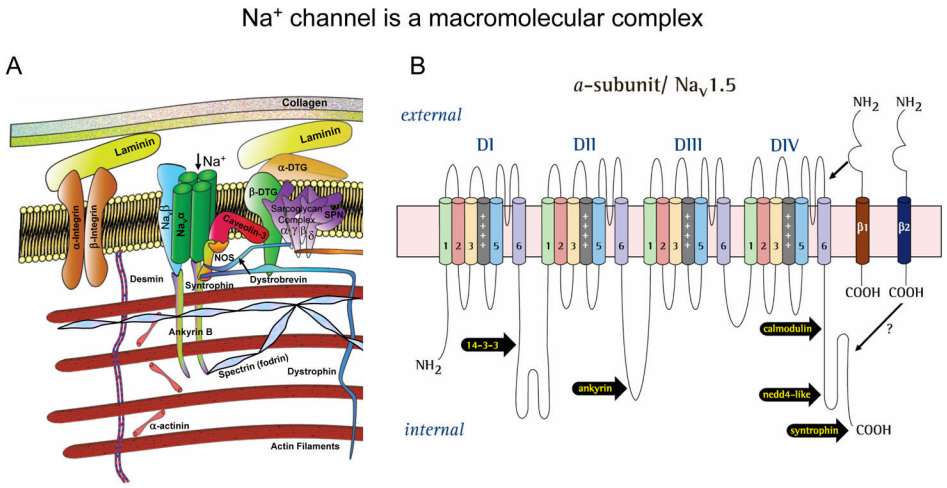

Figure 7.

Schematic illustration of Na^+ channel as a macromolecular complex. A: The pore forming α subunit of the channel interacts with β-subunits, cytoskeleton and the extracellular matrix (Modified after (Nerbonne and Kass, 2005), used with permission). B: schematic presentation of the α subunit of the cardiac Na^+ channel isoform ($Na_v1.5$) with reported sites of interaction with β subunits (restricted only to β1 and β2) and other regulatory proteins.

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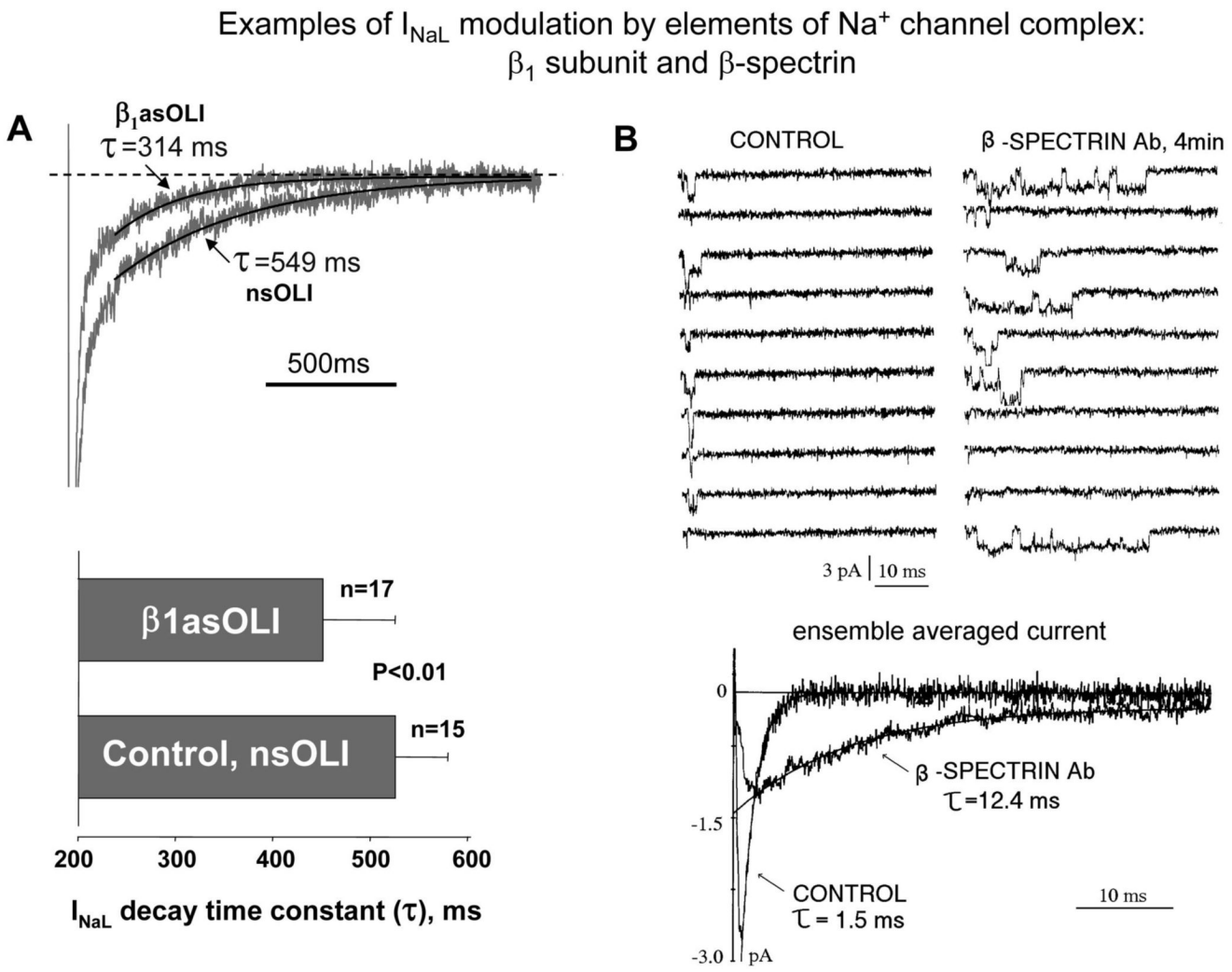

Figure 8.

Modulation of I_{NaL} by the channel environment elements: auxilliary β_1-subunit and β-spectrin-based cytoskeleton. A: In normal dog ventricular myocytes, knocking down of SCN1B by antisense oligonucleotide ($\beta_1$asOLI) significantly accelerates I_{NaL} decay compared to control nonsence oligonucleotide (nsOLI) (Undrovinas and Maltsev, 2002). B: Exposure of the cytoplasmic side of Na^+ channel to the specific anti-β-spectrin antibody dramatically enhances activity of late Na^+ channel openings culminating in the increased ensemble averaged late current. (rat ventricular myocytes, excised inside-out patch, from Undrovinas, Dubreuil and Makielski, unpublished)

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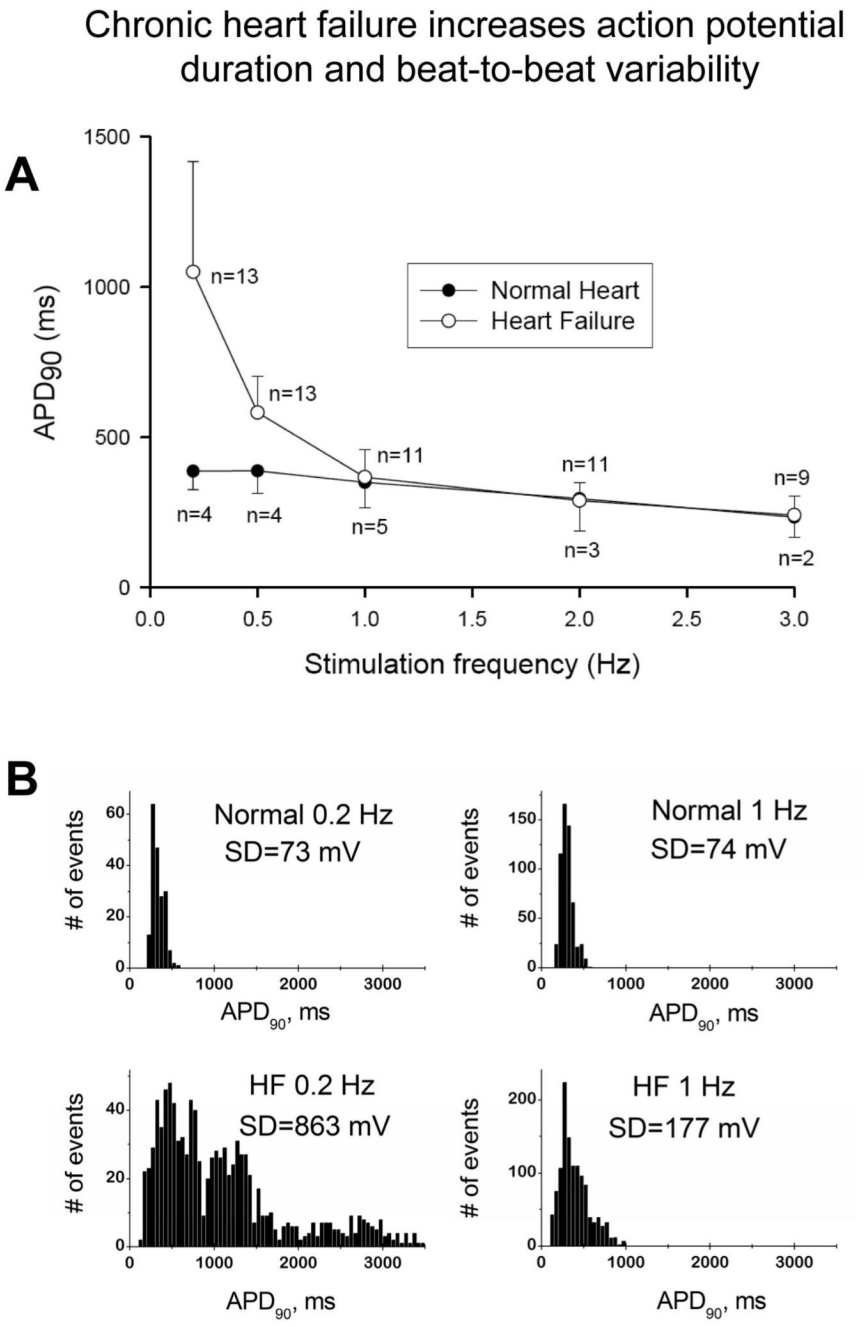

Figure 9.

A, Frequency-dependence of action potential duration in ventricular cardiomyocytes of normal dogs and dogs with chronic heart failure. Note that largest difference occurs at low pacing rates. B: at the low (0.2 Hz) and the physiologic (1 Hz) pacing rates, AP duration in failing myocytes exhibits significant beat-to beat variability (see respective SD values in the APD_{90} distribution histograms) Adapted from (Undrovinas et al., 1999) used with permission.

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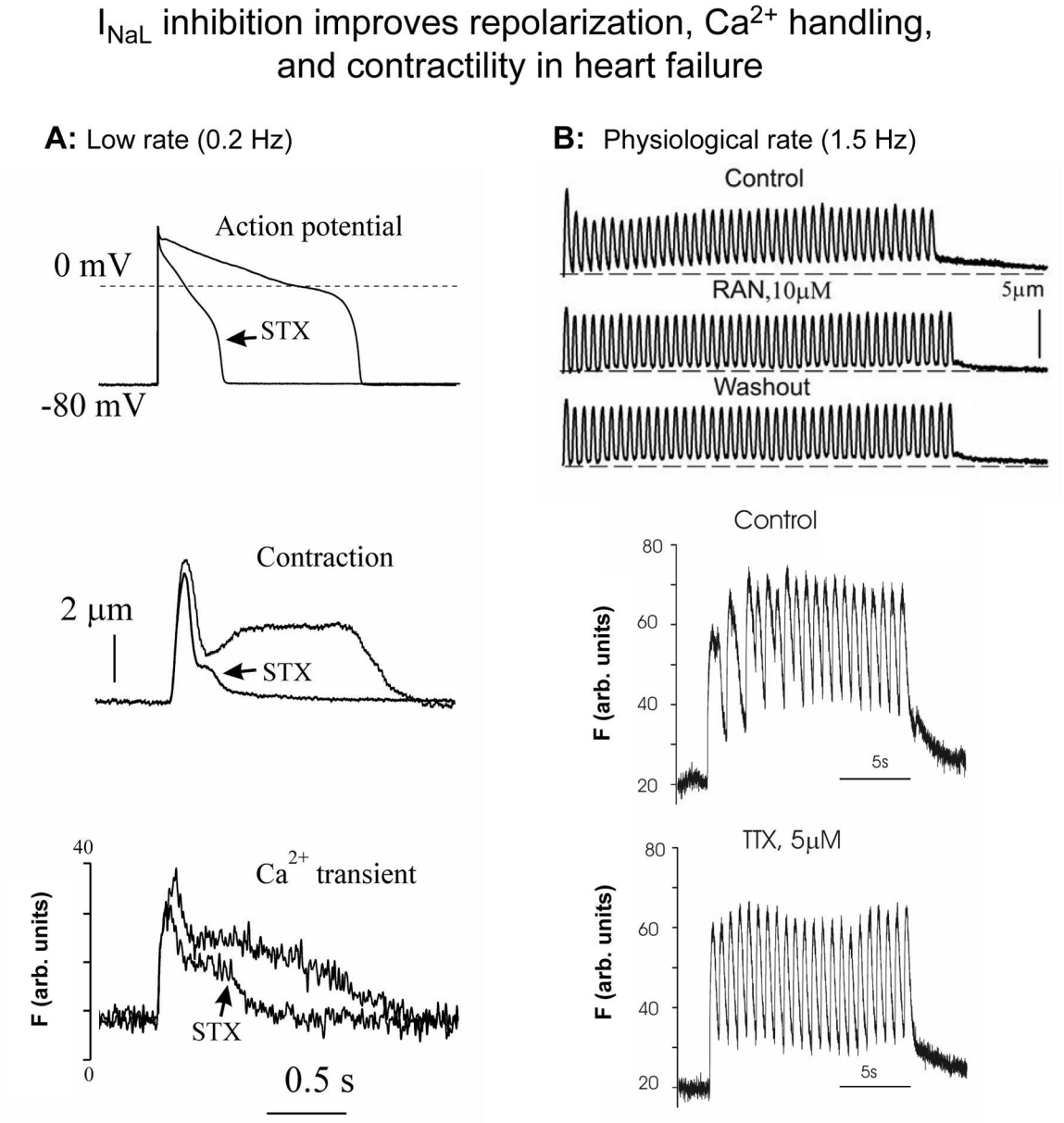

Figure 10.

A: Examples of effects of a specific Na^+ channel blocker saxitoxin (STX) on AP duration, contraction and Ca^{2+} transient in ventricular cardiomyocytes of dogs with chronic heart failure at a low pacing rate of 0.2 Hz STX reduces AP duration, abolishes "dome" phase of contraction and of Ca^{2+} transient in failing cells. B: At higher pacing rates a specific I_{NaL} blockers ranolazine reduces diastolic tension, and a specific Na^+ channel blocker tetrodotoxin (TTX) reduces Ca^{2+} accumulation (Fluo-4 signals). Adapted from (Maltsev et al., 1998b; Undrovinas et al., 2006), used with permission.

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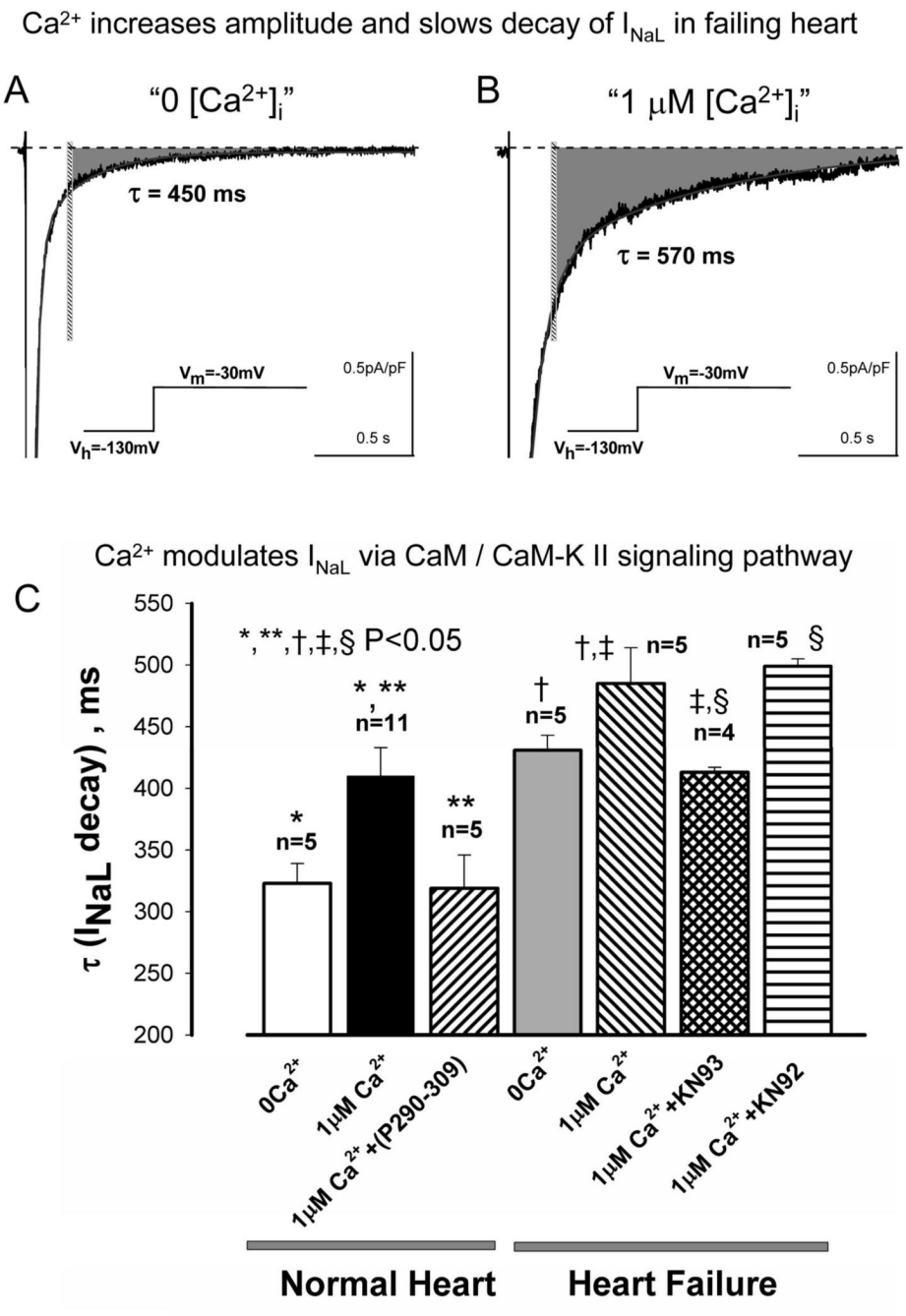

Figure 11.

Modulation of I_{NaL} by intracellular Ca^{2+} and CaM/CaM-Kinase signaling pathway in cardiomyocytes from normal and failing dog hearts. Elevated intracellular Ca^{2+} concentration up to 1 μM dramatically increases and slowes I_{NaL}. A, B: representative traces recorded in cardiomyocytes of failing hearts at "low" and "high" intracellular Ca^{2+}. Gray areas indicate total integrals of I_{NaL} that proportionate to Na^+ influx by I_{NaL} (beginning from 200 ms after depolarization onset depicted by the vertical bar). C: statistical data on I_{NaL} decay changes produced by perturbations of Ca^{2+}/CaM/CaM-KII cascade in cardiomyocytes from normal and failing dog hearts. N corresponds to the number of cells tested. Adapted from (Maltsev et al., 2002a), used with permission.

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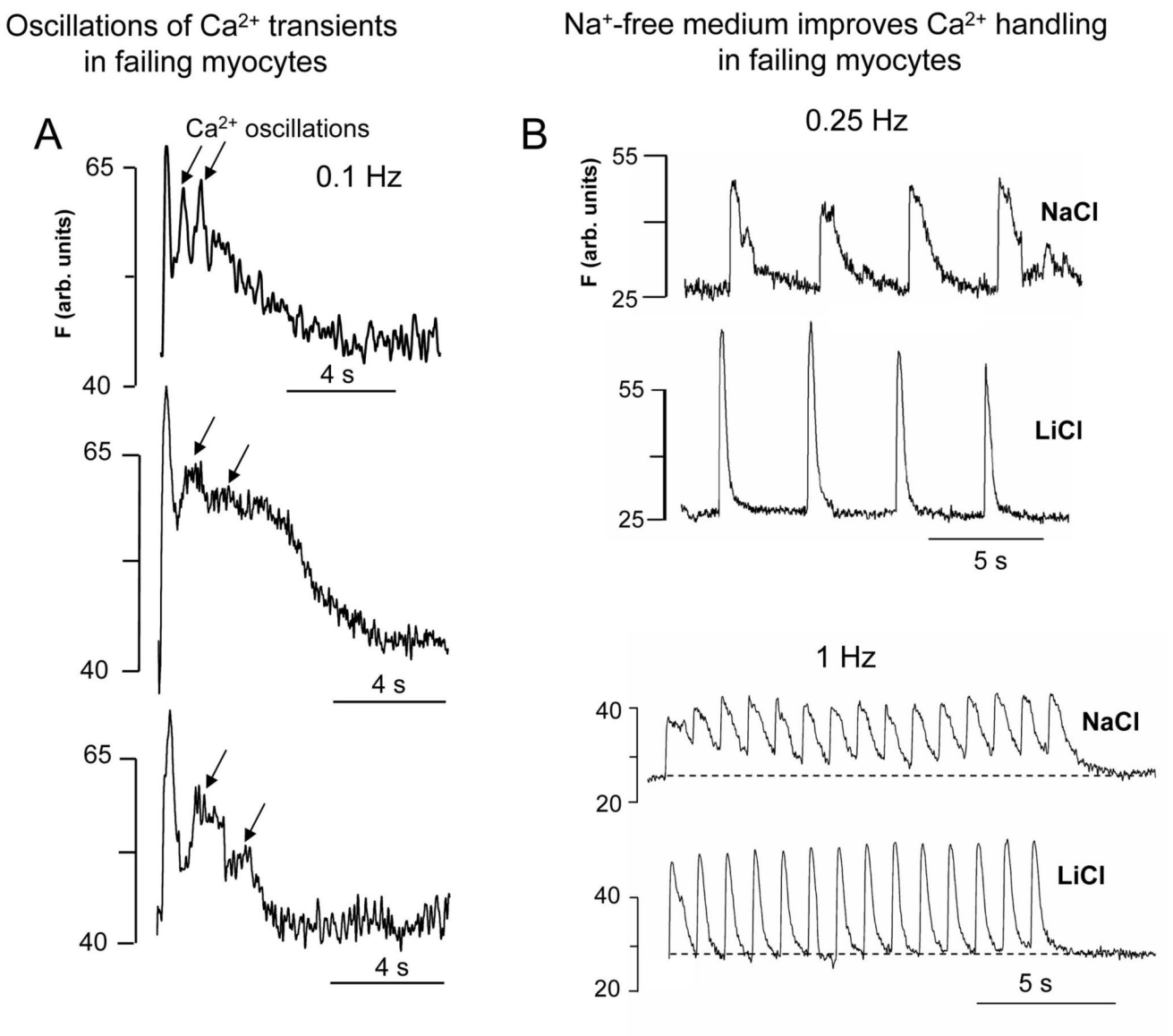

Figure 12.

A: Examples of fluctuations of Ca^{2+} transient (Fluo 4 signals) observed in ventricular myocytes of a canine chronic HF model at low pacing rates. B: Replacement of external Na^+ by Li^+ accelerates Ca^{2+} transients, abolishes Ca^{2+} oscillations, and decreases diastolic Ca^{2+} level in canine failing cardiomyocytes at low and high pacing rates, respectively (Undrovinas N., Sabbah H., Undrovinas A., unpublished). To avoid Li^+ accumulation in the cells, the Na^+-free solution was applied only for a few contraction cycles. Also, a new member NCLX of NCX superfamily has been recently discovered in a variety of tissues including heart (Palty et al., 2004). A distinct feature of NCLX is its ability to slowly transport Li^+ in exchange to Ca^{2+}, indicating that Li^+ can be no longer considered as an "ideal" NCX blocker. Accordingly, if NCLX is functional in the failing canine myocytes, it could also prevent Li^+ accumulation in the experimentation illustrated in panel B.

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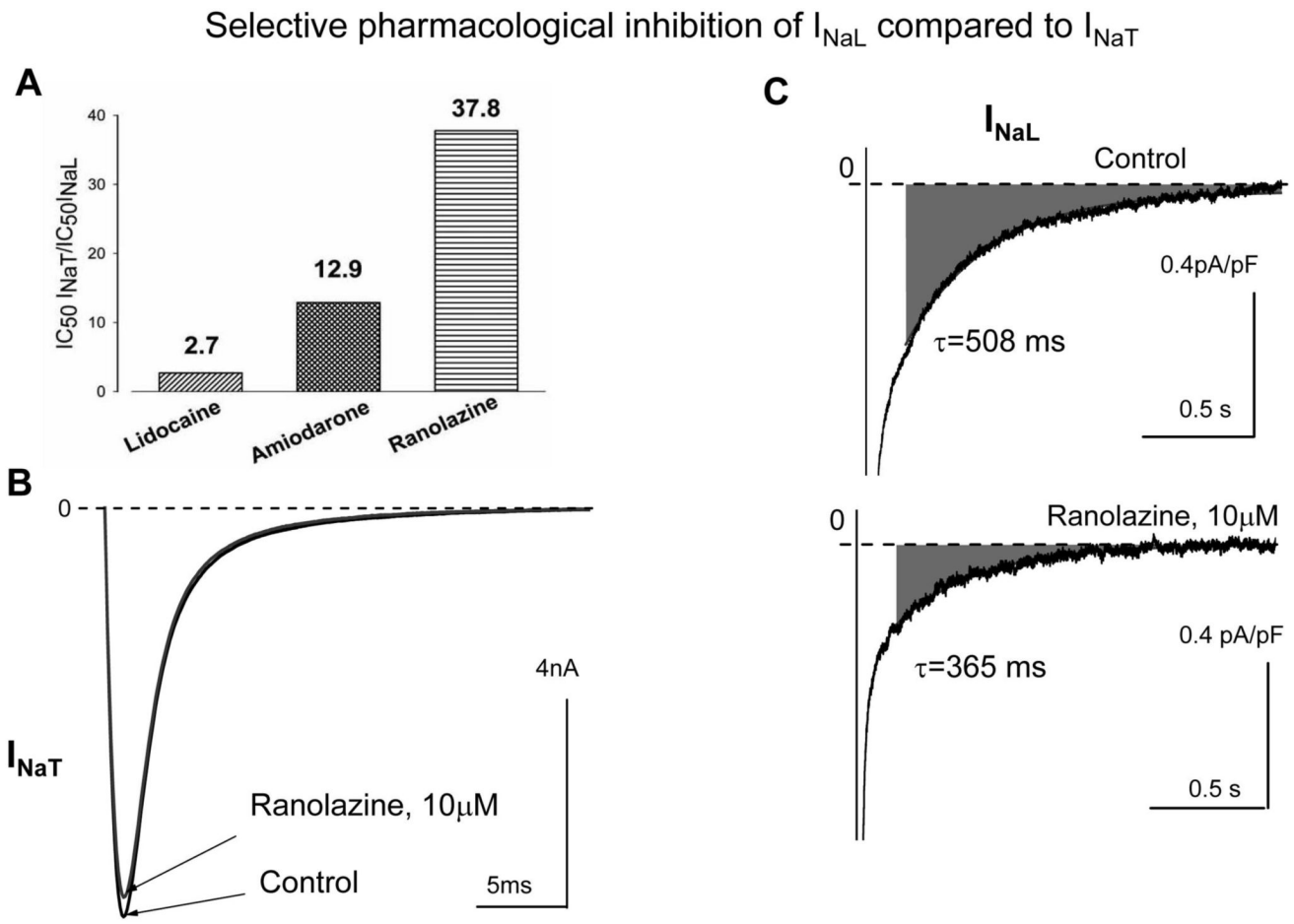

Figure 13.

A: Comparison of the potencies of lidocaine, amiodarone and ranolazine to selectively inhibit I_{NaL} over the peak transient Na^+ current (I_{NaT}). Shown are the ratios of IC_{50}, i.e. the drug concentrations causing 50% resting block of the currents. The higher numbers correspond to the higher selectivity to block I_{NaL}. B, C: examples of a highly selective blockade of I_{NaL} over I_{NaT} produced by ranolazine in canine ventricular cardiomyocytes. Reprinted from (Undrovinas et al., 2006), used with permission.

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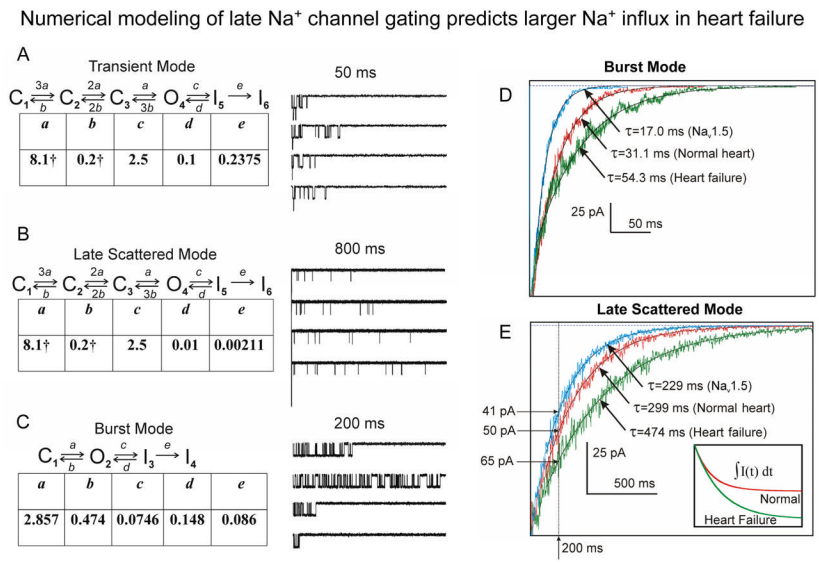

Figure 14.

A-C: Markov chain kinetic models describing the three major modes of Na^+ channel gating in human cardiomyocytes: transient mode (A), late scattered openings (B) and bursts (C), together with simulated traces and transition rates; traces were simulated from 3, 5, and 1 channel(s), respectively. Total simulation times are indicated above the traces. D,E: The model predicts increased and slowed I_{NaL} in a failing human ventricular myocyte vs. a normal human myocyte and heterologously expressed $Na_v1.5$ for BM (F) and LSM (G). Inset in panel E shows a substantially larger integral of the LSM current in a failing myocyte vs. a normal myocyte; the box size is 50 pC ×1990 ms. Shown are simulated cumulative activities of 20000 LSM channels or 193 BM channels. Channel numbers were chosen to correspond to a typical human ventricular myocyte. Reprinted from (Maltsev and Undrovinas, 2006) with permission from European Society of Cardiology.

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Table1

Interaction of different drugs with resting, inactivated and activated sodium channel responsible for peak transient (I_{NaT}) or late (I_{NaL}) currents in ventricular cardiomyocytes isolated from failing human and dog hearts: experimental results and theoretical predictions.

Parameter	Protocol/ drug		Value
$K_{d,peak}$, (μM)	I_{NaT} dose-response, IC_{50}	Lidocaine	291
		Amiodarone	87±28
		Ranolazine	294
$K_{d,late}$, (μM)	I_{NaL} dose-response	Lidocaine	110.6
		Amiodarone	6.7±1.1
		Ranolazine	6.5
K_{dr}, (μM)	Calculated from SSI shift for I_{NaL}	Lidocaine	178
		Amiodarone	14.1±4.2
		Ranolazine	7.47
K_{di}, (μM)	Calculated from SSI shift for I_{NaL}	Lidocaine	32
		Amiodarone	0.15±0.02
		Ranolazine	1.71
K_{block}, ($\mu M^{-1}s^{-1}$)	Calculated from I_{NaL} decay acceleration	Amiodarone	0.239

$K_{d,peak}$ $K_{d,late}$ - half-blocking dose (IC_{50}) was obtained from the single-site binding model fit to dose-response experimental data points for I_{NaT} and I_{NaL}, respectively, K_{dr} and K_{di} are dissociation constant for the resting and inactivated state obtained from steady-state-inactivation (SSI) shifts in the presence of different drug concentrations ($\Delta V_{½} = 6.27*\ln((1+[DRUG]/K_{dr})/(1+[DRUG]/K_{di}))$. K_{block}, association rate constant for the activated state. Methods used to obtain these values are referred as following protocols: SSI shift corresponds to a mid-point potential shift of Boltzmann function fit to the SSI, K_{block} was calculated using a bimolecular model fit to the dose-dependent I_{NaL} acceleration assessed by the single-exponential model. Data sources: for amiodarone (Maltsev et al., 2001), lidocaine (Jia et al., 1993), Maltsev&Undrovinas, unpublished, and ranolazine (Undrovinas et al., 2006), accordingly.