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Review article

Regulation of intracellular Na^+ in health and disease: pathophysiological mechanisms and implications for treatment

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ABSTRACT

Transmembrane sodium (Na^+) fluxes and intracellular sodium homeostasis are central players in the physiology of the cardiac myocyte, since they are crucial for both cell excitability and for the regulation of the intracellular calcium concentration. Furthermore, Na^+ fluxes across the membrane of mitochondria affect the concentration of protons and calcium in the matrix, regulating mitochondrial function. In this review we first analyze the main molecular determinants of sodium fluxes across the sarcolemma and the mitochondrial membrane and describe their role in the physiology of the healthy myocyte. In particular we focus on the interplay between intracellular Ca^{2+} and Na^+. A large part of the review is dedicated to discuss the changes of Na^+ fluxes and intracellular Na^+ concentration($[Na^+]_i$) occurring in cardiac disease; we specifically focus on heart failure and hypertrophic cardiomyopathy, where increased intracellular $[Na^+]_i$ is an established determinant of myocardial dysfunction. We review experimental evidence attributing the increase of $[Na^+]_i$ to either decreased Na^+ efflux (e.g. via the Na^+/K^+ pump) or increased Na^+ influx into the myocyte (e.g. via Na^+ channels). In particular, we focus on the role of the "late sodium current" (I_{NaL}), a sustained component of the fast Na^+ current of cardiac myocytes, which is abnormally enhanced in cardiac diseases and contributes to both electrical and contractile dysfunction. We analyze the pathophysiological role of I_{NaL} enhancement in heart failure and hypertrophic cardiomyopathy and the consequences of its pharmacological modulation, highlighting the clinical implications.

The central role of Na^+ fluxes and intracellular Na^+ physiology and pathophysiology of cardiac myocytes has been highlighted by a large number of recent works. The possibility of modulating Na^+ inward fluxes and $[Na^+]_i$ with specific I_{NaL} inhibitors, such as ranolazine, has made Na^+a novel suitable target for cardiac therapy, potentially capable of addressing arrhythmogenesis and diastolic dysfunction in severe conditions such as heart failure and hypertrophic cardiomyopathy.

Keywords: sodium, sodium current, heart failure, calcium, hypertrophic cardiomyopathy, mitochondria

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INTRODUCTION

Sodium (Na^+) is the principal cation in the extracellular milieau and its highly regulated movement across the membrane of cardiac myocytes is the primary determinant of action potential (AP) upstroke and is essential for autoregenerative impulse propagation throughout the myocardium. In order to support cell excitability a large Na^+ gradient across the membrane needs to be maintained. Thus intracellular sodium concentration ($[Na^{2+}]_i$) within the cardiac myocyte must be accurately controlled and is finely regulated by a number of channels and transporters.[1] Despite that Na^+ has been classically seen solely as a regulator of cardiac excitability, $[Na^{2+}]_i$ is an important modulator of numerous aspects of cardiomyocyte function, from excitation-contraction coupling to energy production and consumption. In fact, $[Na^{2+}]_i$ is a major determinant of the intracellular concentrations of Ca^{2+}($[Ca^{2+}]_i$), as well as other ions and molecules. Moreover $[Na^{2+}]_i$ strictly regulates the concentration of Ca^{2+} within the mitochondria, a major determinant of the rate of ATP production and the generation of reactive oxygen species (ROS). Great interest has been recently devoted to the study of Na^+ homeostasis since $[Na^{2+}]_i$ can be dysregulated in cardiac disease and its dysregulation may strongly contribute to their pathophysiology. As an example, increased $[Na^{2+}]_i$ has been observed during ischemia[2–5] and abnormally high $[Na^{2+}]_i$ was identified as a contributor to ischemia-reperfusion injury. Increase of $[Na^{2+}]_i$ has also been observed in different models of heart failure (HF).[6–9] HF is characterized by a global dysregulation of Ca^{2+} homeostasis[10]: increased $[Na^{2+}]_i$ has been suggested to contribute to altered Ca^{2+} handling, which in turn is the main determinant of the altered contractility and arrhythmias in HF. In this pathological setting, intracellular Na^{2+} overload and Ca^{2+} overload go hand in hand and may constitute a vicious circle. $[Na^+]_i$ not only controls contractility and arrhythmogenicity but also regulates cardiomyocyte energetics via control of mitochondrial function: Na^{2+} overload may also contribute to energetic insufficiency and excessive production of ROS in cardiac diseases. For all these reasons, every therapeutic attempt aimed at normalizing Ca^{2+} homeostasis without addressing Na^{2+} overload is destined to fail. Actual therapeutic options for heart failure and other cardiac diseases mainly affect Ca^{2+} homeostasis and are incapable of reducing arrhythmogenesis and ameliorating contractile function of diseased hearts. Besides the direct electrophysiological effects, the possible global benefits of the inhibitors of Na^+ channels and carriers, are therefore of utmost interest and are being investigated in many disease settings.[11] This review will focus on the regulation of $[Na^{2+}]_i$ in healthy myocardium and on its alterations in cardiac diseases such as heart failure and hypertrophic cardiomyopathy. Moreover, we will review the evidence supporting novel therapeutic options aimed at normalizing Na^{2+} homeostasis in different cardiac diseases and their relevance for the clinical management of cardiac patients.

MEASURING $[NA^+]_I$

The capability of measuring intracellular $[Na^+]_i$ and its variations in response to pathological changes of cell function is essential to understand the role of Na^+ in the heart. Early approaches to $[Na^+]_i$ measurement involved the use of Na^+ selective microelectrodes,[12] which are quantitatively precise but require direct probing of the desired cell with an impaling electrode, limiting its use in contracting tissue. Recently, the use of Na^+ selective fluorescent indicator dyes has become the approach of choice.

Up to 2011, the most widely used Na^+ indicator was SBFI,[13] which has been used both in single cells[4] and in intact myocardium.[14] The dye is intrinsically ratiometric, being similar to the Fura-2 Ca^{2+} dye and allows calculation of the actual $[Na^+]_i$ under different conditions. Its versatility is increased by the possibility to load intact cells and tissue with the membrane-permeant acetoxymethyl-ester form of the dye. However, SBFI used UV wavelength for excitation, making it unsuitable for use in some applications and in confocal microscopy. Na^+ dyes excited by blue light have been recently developed to be used in confocal systems and in all largely available fluorescence microscopes. CoroNa Green[15] suffered from a low light yield and a low signal stability. More recently, Asante-Natrium Green I and II dyes have been developed and employed in a number of studies on cardiac cells,[16,17] with outstanding results in terms of stability and signal intensity. The introduction of Asante-Natrium Green dyes is rendering $[Na^+]_i$ measurement available to all labs due to its remarkable ease of use. However, green dyes are not intrinsically ratiometric, rendering quantitation of $[Na^+]_i$ more difficult.

$[Na^+]_i$ REGULATION IN THE HEALTHY CARDIOMYOCYTE

$[Na^+]_i$ is maintained and regulated by a dynamic equilibrium of Na^+ influx and efflux, mediated by a number of channels and transporters. The influx of Na^+ into the myocyte occurs via multiple pathways, including Na^+ channels, Na^+/Ca^{2+} exchanger (NCX) and Na^+/H^+ exchanger (NHE). The efflux of Na^+ from the cell to the extracellular medium can occur in some conditions through the NCX (acting in "reverse mode") but is largely carried out by the Na^+/K^+ pump (NKA), which can act against Na^+ electrochemical gradient at rest. Additionally, $[Na^{2+}]_i$ is in equilibrium with Na^+ concentration within subcellular organelles, in particular the mitochondria, which can in turn contribute to $[Na^{2+}]_i$ modulation in disease conditions. We will now focus on the role of these influx and efflux pathways in the normal heart. A cartoon describing Na^+ homeostasis in the healthy cardiomyocyte is shown in Figure 1.

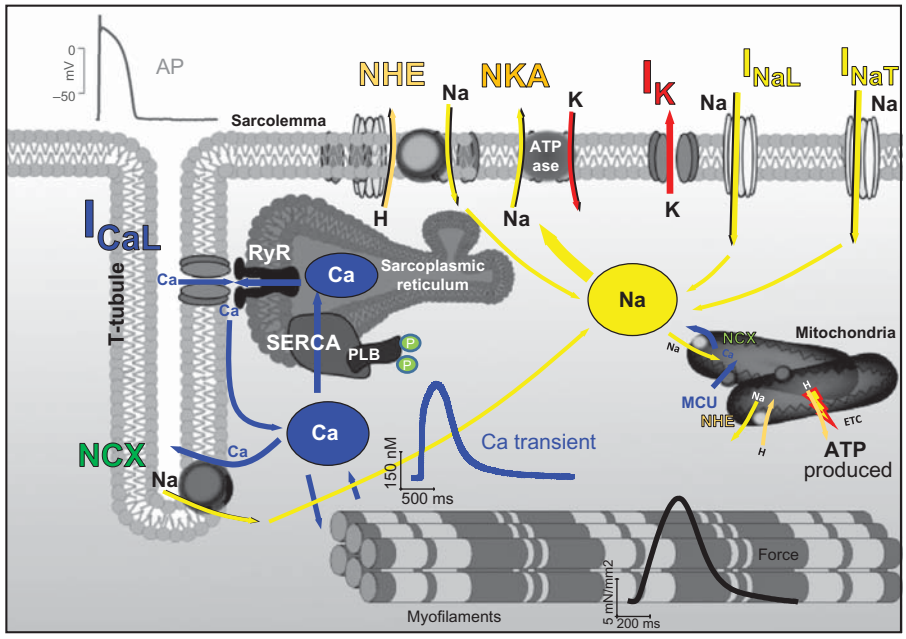

Figure 1. Na^+ homeostasis in the healthy cardiomyocyte. Cartoon showing the cellular and molecular determinants of Na^+ homeostasis in healthy cardiac cells and their role in the regulation of Ca^{2+} handling. Note that yellow arrows mark Na^+ fluxes while blue arrows indicate Ca^{2+} fluxes. Representative APs from reference [18], representative Ca^{2+} transient and force twitch from Coppini et al.[17]

Na^+ channels: peak and late Na^+ current

When electrotonic propagation from the neighboring cells depolarizes the cell membrane to reach the threshold for Na^+ channel voltage-dependent activation, a large number of channels open giving rise to a huge depolarizing current (~20–30 nA/pF at peak), which determines the upstroke phase of the AP. Therefore, the principal role of Na^+ channels is to allow the AP to initiate and permit propagation of the AP throughout the myocardium. This current is generally short lived because the intrinsic time-dependent inactivation of Na^+ channels determines their fast closure at the beginning of AP plateau. However, since the current is very large, a non-negligible amount of Na^+ is transferred inside the cytosol at each activation. Their contribution to $[Na^+]_i$ is obviously directly dependent on the rate of activation: the higher the heart rate, the larger the total amount of Na^+ entering through the Na^+ channel. The most represented isoform of Na^+ channel in the heart is the so called "cardiac" Na^+ channel NaV1.5 (SCN5a gene), which has a relatively low affinity for the selective blocker Tetrodotoxin (TTX). However, recent work identified other isoforms of Na^+ channels in the heart, in particular neuronal isoforms such as NaV1.1, NaV1.3 and NaV1.7,[19] which are all largely more sensitive to TTX. Meier et al. found that neuronal channels, despite contributing for only 10% to total peak Na^+ current, have a high density in the membrane of t-tubules and may thus be important for t-tubular propagation of the AP.[20] However, selective inhibition of neuronal isoform in the heart with TTX at low concentration ($<0.5\ \mu M$) showed that neuronal channels are not essential for a proper AP upstroke and efficient

excitation-contraction coupling in the cardiomyocyte.[21] These investigations are made difficult by the fact that, in stimulated preparations (such as isolated myocytes), the sodium channel might not be necessary for cell contraction since the electrical stimulation might also activate the calcium channel directly.[22] Therefore, the exact role of neuronal Na^+ channels in the normal heart remains to be clarified.

Several experiments pointed out that inhibition of the Na^+ current with TTX not only reduces the maximal upstroke velocity of the AP but also shortens the plateau: these observations mean that a sustained component of Na^+ current is somewhat involved in determining the balance of inward and outward currents that influence AP plateau.[23] Two main mechanisms underlie the sustained component of Na^+ current:

1) "Window currents", i.e. steady state Na^+ currents that are present in a voltage interval (−30 to −50 mV) where channel activation is still possible and channel inactivation is incomplete, so that a number of inactivated channels can recover from inactivation and immediately reopen.[24,25] Window currents are not largely represented in normal cardiac myocytes[26,27] but may be favored by a prolonged repolarization. Moreover, mutations of the SCN5a gene associated with Long QT Syndrome Type 3 (LQT3) are associated with increased window Na^+ currents.[28]
2) "Late Na^+ current (I_{NaL})", i.e. TTX-sensitive Na^+ current that can be recorded outside the window voltage range. Such currents can be recorded from several types of normal cardiac cells from different species, such as rabbit and human atrial myocytes,[29,30] ventricular myocytes from dog, guinea pig and human,[17,31,32] purkinje fibers[33,34] and sinus node cells.[35] In normal myocytes, I_{NaL} is generally very small (30 pA at 0 mV) but it may be enhanced 3 to 5 fold in pathological conditions (see below).[36] I_{NaL} has been demonstrated to be brought about by the same Na^+ channels that are responsible for the peak current, i.e. NaV1.5. Indeed, when only NaV1.5 channels are re-expressed in cell vectors, I_{NaL} can be recorded.[37] However, a recent work pointed out that 44% of the late sodium current in cardiac myocytes from dog ventricle is due to non-cardiac-type Na^+ channels, such as neuronal isoforms NaV1.1 or NaV1.3 or skeletal muscle isoform NaV1.4.[38] Single channel studies demonstrated that two mechanisms are involved in determining I_{NaL}: (i) burst openings, which undergo voltage-dependent inactivation and (ii) scattered openings, which inactivate extremely slowly and therefore are able to determine a truly "background" Na^+ current. The first mechanism is the most represented in the human.[37] I_{NaL} is also strongly rate dependent, in that it is reduced at faster rates of activation, suggesting inactivation and incomplete recovery of the current.[34] Albeit very small when compared to peak current, I_{NaL} can significantly contribute to determine $[Na^+]_i$ since its duration is several orders of magnitude longer. During a single AP, a normal I_{NaL} may increase the amount of Na^+ flowing into the cell by 50% on top of the inflow occurring during the AP upstroke. As discussed later, this is of utmost importance in conditions where I_{NaL} is increased and its contribution to the total Na^+ inflow increases significantly.

In summary, the total amount of Na^+ entering the cell through the Na^+ channels is essentially proportional to the rate of activation and to the density of I_{NaL}.

Na^+/Ca^{2+} exchanger (NCX)

NCX employs the energy provided by Na^+ entering the myocyte following its electrochemical gradient to transport Ca^{2+} outside the cell. It is essentially aimed at maintaining a regular Ca^{2+} homeostasis by allowing the excess of Ca^{2+} to flow out of the cell against its electrochemical gradient.[39] In fact, NCX is the major outflow pathway for Ca^{2+} and is crucial to maintain an equilibrium between Ca^{2+} entering the cell through the Ca^{2+} channel and Ca^{2+} leaving the cell via the exchanger, which must be equal in steady-state conditions. Rate and function of the NCX depend on the intracellular and extracellular concentrations of both Na^+ and Ca^{2+} and are also influenced by the membrane potential. At negative resting potentials, when most of the Ca^{2+} outflow occurs, the electrochemical gradient of Na^+ and Ca^{2+} favors the "forward" mode of the NCX: three sodium ions are brought inside the cell to extrude a single Ca^{2+} with a net inflow of one positive change. If Ca^{2+} inside the cell increases for any reason, such as an increase of Ca^{2+} current, so does the activity of the NCX, thereby increasing the amount of Na^+ flowing into the cell through the exchanger.[40] Moreover, the activity of NCX can be reduced by decreased pH and potentiated by ATP: in ischemic conditions, when pH and ATP are relatively lower,

NCX activity can be seriously impaired.[41,42] Though NCX mainly operates in the forward mode, at potentials positive to the NCX reverse potential (~ − 10 mV), the exchanger can also operate in the reverse mode, producing entry of calcium ions coupled to outflow of Na^+. This phenomenon however is likely to play a minor role in the healthy myocyte: in fact, though membrane depolarization during upstroke and plateau could potentially support reverse mode, the concurrent increase of intracellular $[Ca^{2+}]$ due to Ca^{2+}-induced Ca^{2+}-release from the SR antagonizes Ca^{2+} inflow through the NCX. In conditions where $[Na^+]_i$ is higher and Ca^{2+} release from the SR is reduced NCX reverse mode may play an increased role, as discussed later.

Na^+/H^+ exchanger (NHE)

NHE is also capable of exploiting the energy of Na^+ gradient to pump H^+ out of the cell by letting Na^+ flow into the cell.[43–45] When pH is reduced, the activity of NHE increases in order to bring back pH to normal levels, making the NHE one of the main molecular modulators of cellular pH. Protons directly stimulate the exchanger, but NHE has been shown to be modulated by the intracellular signaling cascade mediated by a number of hormones, neurohormonal agonists and growth factors.[46] In conditions of intracellular acidosis, NHE can significantly contribute to increase Na^+ entry and $[Na^+]_i$ within the myocyte.

Na^+/K^+ ATPase (NKA)

Na^+ removal through the NCX is non-relevant in normal conditions, therefore the main mechanism able to pump the excess of intracellular Na^+ out of the cell is the NKA.[47] NKA is essential to maintain the gradients of Na^+ and K^+ across the membrane and to allow myocardial excitability. Using the energy liberated by the hydrolysis of ATP, the NKA is able to extrude 3 intracellular Na^+ ions while bringing 2 extracellular K ions inside the cytosol, thus moving one net charge per cycle. The NKA is characterized by two major subunits, α and β: the α subunit features the binding site for ATP, K^+, Na^+ and cardiac glycosides, while the β is essential for a proper membrane localization and insertion. Different α subunit isoforms with different affinity for ATP and ions exist in the heart and their relative expression can be modulated by cardiac diseases.[47] Interestingly, the rate of NKA can be actively modulated by a small membrane bound protein similar to phospholamban (the main modulator of sarcoplasmic reticulum Ca^{2+} ATPase −SERCA-), called phospholemman (PLM). PLM, like phospholamban, is abundant in the heart and can be phosphorylated following activation of β-adrenergic cascade.[48] When PLM interacts with the NKA, it reduces the activity of the α subunit, ultimately reducing Na^+ extrusion rate. This has been demonstrated via phospholemman knock-out in cardiac cells, which led to an increase of NKA activity of the same extent as maximal β-adrenergic stimulation.[49] These observations suggest that PLM, like phospholamban, detaches from the NKA when phosphorylated by protein kinase A, relieving the specific inhibition.

Mitochondrial Na^+ outflow and inflow mechanisms

The main Na^{2+} efflux mechanism from the cytosol to the mitochondria in the cardiac myocyte is the mitochondrial Na^+/H^+ exchanger (mNHE).[50] The electron transport chain leads to extrusion of protons from the mitochondrial matrix to the cytosol. The mNHE senses the excess of protons on the cytosolic side of mitochondrial membrane and brings back H^+ ions into the matrix while extruding Na^+ ions from the mitochondria. From this observation stems the assumption that mitochondrial $[Na^+]$ is around 2 times lower than cytosolic $[Na^+]$ in the intact cell, an assumption that has been experimentally confirmed in isolated mitochondria and permeabilized myocytes.[50] In line with this observation, metabolic inhibition, via reduction of the activity of the electron transport chain, abolishes the net flow of Na^+ across the mitochondrial membrane and leads to an equal $[Na^+]$ in mitochondria and cytosol. Under normal conditions, it is unlikely that mitochondria contribute substantially to the regulation of $[Na^+]_i$. However, in conditions of increased metabolic activity, due to the higher pH gradient across the mitochondrial membrane, the increased activity of the mNHE could actually lead to transient changes of $[Na^+]_i$.

The major mechanism involved in Na^+ influx to the mitochondria is the mitochondrial Na^+/Ca^{2+} exchanger (mNCX). Like the sarcolemmal NCX, mNCX is electrogenic and transports 3 sodium ions from the cytosol to the mitochondrial matrix while extruding a single Ca^{2+} from the mitochondrion to the cytosol.[51–55] What provides the driving force for such an exchange given the fact the $[Na^{2+}]$ gradient

across the mitochondrial membrane is relatively small? Active mitochondria have a very large membrane potential with inward direction, ranging from −150 mV to −180 mV. This large ΔV provides the driving force for mNCX function in the forward direction. Interestingly, the mNCX never reaches the equilibrium in the intact cell: considering $[Na^+]_i = 8$ mM, mitochondrial $[Na^+] = 5$ mM and cytosolic $[Ca^{2+}] = 300$nM (average values for an active myocyte), mitochondrial $[Ca^{2+}]$ would be as low as 1 nM at equilibrium.[56] However, the measured mitochondrial $[Ca^{2+}]$ in the intact active myocardium is usually above 150 nM.[57] There are multiple reasons explaining why the mNCX does not operate at equilibrium: (i) the Ca^{2+} uniporter continuously brings calcium ions inside the mitochondrion following the favorable electrochemical gradient for Ca^{2+} across the mitochondrial membrane and (ii) the activity of mNCX is much lower than that of the Ca^{2+} uniporter. When the uniporter is blocked by Ruthenium or by an excess of magnesium, mNCX approaches the equilibrium and mitochondrial $[Ca^{2+}]$ is strongly reduced.[54,55] Mitochondrial $[Ca^{2+}]$ is an important regulator of energetics: increased $[Ca^{2+}]$ inside the mitochondria activates the enzymes of the oxidative metabolism thus leading to an increased production of NADH, which in turn increases the function of the electron transport chain, ultimately determining higher rate of ATP production.[56] Ca^{2+} can also directly activate the macromolecular components of the electron transport chain.[58] mNCX can play a significant role in regulating mitochondrial $[Ca^{2+}]$ and thus cardiomyocyte energetics. Increased $[Na^+]_i$ leads to increased influx of Na^+ to the mitochondria via the mNCX, accompanied by the concurrent extrusion of Ca^{2+}, ultimately resulting in lower mitochondrial $[Ca^{2+}]$ and thus decreased energetics. This phenomenon may be relevant in disease conditions where $[Na^+]_i$ is elevated (see below).

NA^+ REGULATION IN HEART FAILURE (HF)

HF involves a large number of pathophysiological pathways occurring at the level of the single myocyte. Depending on the model employed, some mechanisms may be different but there is a general consensus on the presence of two main features: (i) electrophysiological alterations leading to increased rate of "cellular arrhythmias" and (ii) abnormalities of excitation-contraction coupling (ECC) leading to contractile dysfunction. Abnormalities of Na^+ fluxes and regulation of intracellular $[Na^+]$ are likely involved in both. Numerous reports from animal models and human samples agree on a general increase of $[Na^+]_i$ in the presence of HF.[6–8] Increased $[Na^+]_i$ may have important deleterious consequences on arrhytmogenesis, contractility and energetics. We will now discuss the mechanisms involved in Na^+ dysregulation in heart failure and the possible therapeutic role of pharmacological modulation of Na^+ fluxes and homeostasis. A cartoon depicting Na^+ regulation in HF is shown in Figure 2.

Abnormal Ca^{2+} handling in heart failure

In order to understand the implications of abnormal Na^+ homeostasis in HF we have to keep in mind the large Ca^{2+} dysregulation occurring in HF myocytes. Most models of HF, including human, are characterized by decreased SR Ca^{2+}- ATPase (SERCA) expression and upregulation of NCX expression and function (Figure 3). On the other hand, Ca^{2+} current is generally unmodified.[61] Phospholamban (PLB) is the major modulator of SERCA function and its expression is typically unaltered in HF,[62,63] with decreased phosphorylation. The subsequent increase of PLB:SERCA ratio, coupled with the lower PLB phosphorylation, enhances the inhibitory effect of PLB on SERCA, contributing to lower SERCA function in HF. Decreased SERCA activity, by slowing down Ca^{2+} reuptake to the SR, allows more Ca^{2+} to be extruded via the NCX; coupled with the increased NCX expression[64] this results in a net loss of cell Ca^{2+} and contributes to the reduction of SR Ca^{2+} load in HF.[65] Another contributor to the lower SR Ca^{2+} content in HF is the increased diastolic leakage of Ca^{2+} from the SR, which is determined by the hyper-phosphorylation of ryanodine receptors (RyRs) by protein-kinase A (PKA) and/or Ca^{2+}-Calmodulin dependent protein kinase-II (CaMKII).[66] CaMKII activity is increased in HF, and CaMKII-dependent phosphorylation of RyR enhances Ca^{2+} spark frequency and thus spontaneous diastolic SR Ca^{2+} leak,[67] making it one of the leading pathway causing contractile dysfunction and arrhythmogenesis in HF. Enhanced NCX function, combined with the higher probability of spontaneous Ca^{2+} release from the SR, contributes directly to arrhythmogenesis via delayed-afterdepolarizations (DADs). When a large spontaneous Ca^{2+} release event occurs during diastole giving rise to a generalized Ca^{2+} wave, part of the released Ca^{2+} is extruded through the NCX, which generates an inward current that depolarizes the membrane (i.e. a DAD). If large enough, a DAD may reach the threshold for a premature AP, giving rise to a premature activation that can propagate through the

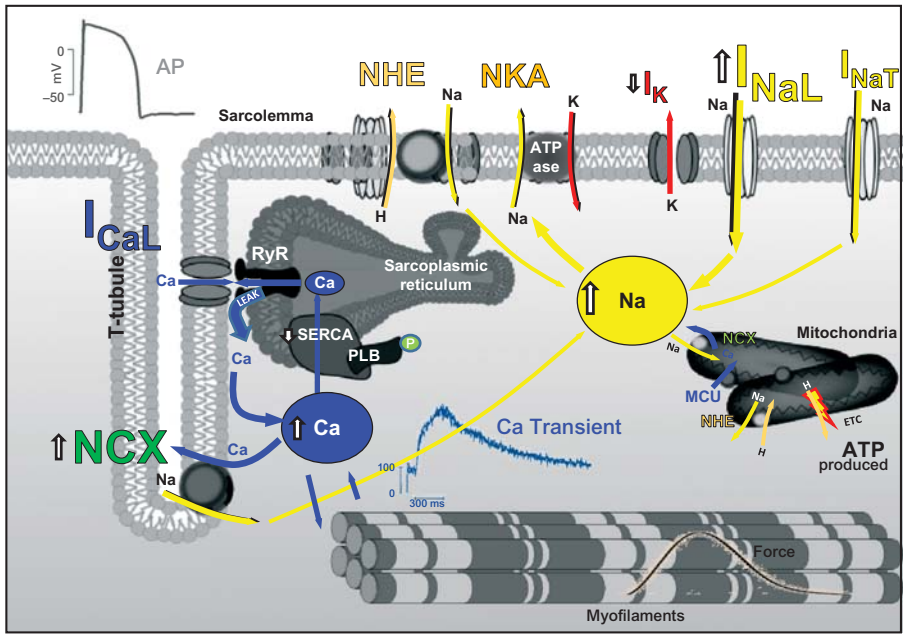

Figure 2. Na^+ homeostasis in heart failure. Cartoon showing the cellular and molecular determinants of abnormal Na^+ homeostasis in HF cardiac cells and their role in determining the dysfunction of Ca^{2+} handling. White thick arrows mark the changes occurring in HF with respect to control cardiomyocytes. The thickness of yellow and blue arrows has been changed to match the relative changes of Na^+ and Ca^{2+} fluxes in HF. Representative AP from reference [18], representative Ca^{2+} transient from Beuckelmann et al.[59] and force twitch from Sossalla et al.[60]

myocardium, triggering sustained arrhythmias. As explained below, abnormal Na^+ homeostasis may further increase the risk of arrhythmias in HF with multiple mechanisms.

Alterations of late Na^+ current in HF

Increased $[Na^+]_i$ in HF may be due to increased Na^+ influx or decreased Na^+ efflux. Early studies suggested that an excess of Na^+ influx is the major contributor to Na^+ overload and the main source of the increased Na^+ entry is the enhanced "late" or "persistent" Na^+ current.[7,8] As explained before, a component of Na^+ current with slow or incomplete inactivation can be measured in normal human and animal cardiac myocytes.[26] However, acquired and primary cardiac diseases are commonly characterized by abnormally large I_{NaL}. Enhancement of late Na^+ current has been identified in many models of disease and may contribute to their pathogenesis: besides classical LQT3 syndrome, which is due to Na + channel mutations,[68] increase I_{NaL} was also found as a consequence of ankyrin B mutations (LQT_4 syndrome)[69] and caveolin-3 mutations (LQT-CAV_3),[70] suggesting that the altered function of proteins linked to the Na^+ channel can affect its function in a way similar to its own mutations. Moreover, abnormally large I_{NaL} was found in cells from hearts of patients affected by end stage HF,[26,71] in animal models of HF[72–74] and following myocardial infarction.[74] Hypoxia,[75] oxygen free radicals (H_2O_2),[76] ischemic metabolites (like oxidized lipids)[77] and nitric oxide (NO)[78] have been shown to rapidly and directly increase I_{NaL} in experimental conditions, suggesting that peroxydation or nitrosylation of channels may directly alter their inactivation kinetics. Moreover, increased Ca^{2+} -Calmodulin[79] and calmodulin kinase II (CaMKII) activity,[27] both common features of myocardial remodelling in HF, have been shown to increase I_{NaL}. Recent evidence identified CaMKII-mediated phosphorylation of cardiac Na^+ channels at multiple sites is the main regulator of Na^+ channel inactivation.[80] Increased levels of phosphorylation at those sites may therefore be the main responsible for the enhancement of I_{NaL} in HF and other diseases. The effects of oxidative stress on I_{NaL} also seem to be mediated by enhanced CaMKII-mediated phosphorylation due to the intrinsically active oxidized CamKII.[81] From a molecular standpoint, enhanced I_{NaL} in HF is mediated by an increase of bursts and scattered late openings in single NaV1.5 channels,[37] i.e. the same mechanisms found in normal myocytes, although with higher gating rates. All this suggests that in HF, I_{NaL} enhancement is not mediated by expression of different Na^+ channel isoforms other than Nav1.5. In contrast with that,

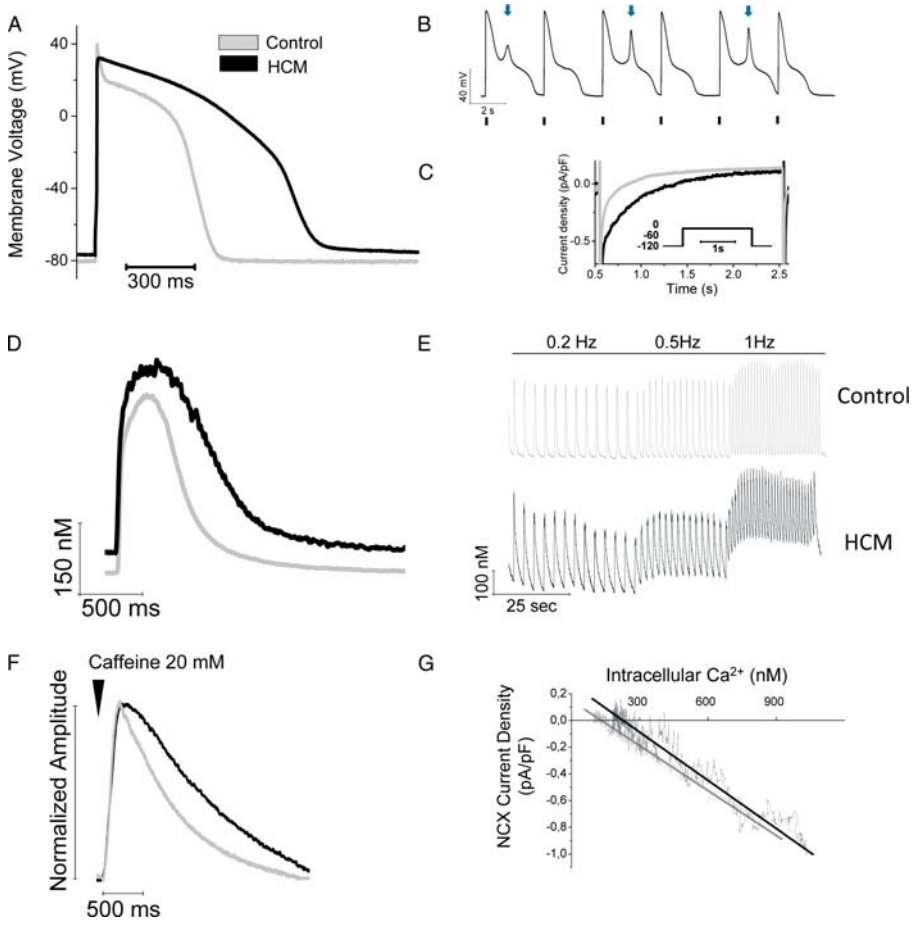

Figure 3. **Electromechanical dysfunction and Na^+ dysregulation in HCM. All panels modified from Coppini et al.[17] (A) Superimposed representative action potentials recorded during stimulation at 0.2 Hz from HCM and control cardiomyocytes. (B) Representative recording from an HCM cardiomyocyte paced at 0.2 Hz, showing EADs. Blue arrows mark EADs. Black lines mark stimuli. (C) Representative I_{NaL} traces from control and HCM cardiomyocytes reciorded during voltage-clamp (voltage protocol in inset). (D) Representative superimposed Ca_i^{2+} transients recorded in current-clamp mode while eliciting action potentials at 0.2 Hz in control and HCM cardiomyocytes. (E) Continuous recordings of Ca_i^{2+} transients elicited at 0.2 Hz, 0.5 Hz and 1 Hz frequency of stimulation, from a control (above) and an HCM (below) cardiomyocyte, highlighting the increased diastolic $[Ca^{2+}]$ upon increase in frequency. (F) Superimposed normalized calcium transients induced by rapid exposure to caffeine, highlighting the slower decay rate in HCM cardiomyocytes and suggesting slower NCX mediated Ca^{2+} extrusion. (G) Current density (NCX current) recorded during the caffeine transient is plotted against $[Ca^{2+}]_i$ during the decay phase of the transient. Each curve is linearly fitted to calculate the $[Ca^{2+}]_i$ level at which NCX current is 0. The representative superimposed traces are derived from the examples in a. In this example, NCX equilibrium is reached when $[Ca^{2+}]_i$ is 141 nM in the control cardiomyocyte and 231 nM in the HCM cardiomyocyte. This suggests an increase of intracellular $[Na^+]$ in HCM cells.**

in a rat model of pressure overload-induced hypertrophy and failure it was shown that the increased I_{NaL} is associated with a large up-regulation of neuronal Na^+ channels (Nav1.1, Nav1.3, and Nav1.6) in the ventricle.[82]

Whatever the reason of I_{NaL} enhancement, this condition is always associated with a prolonged repolarisation causing a remarkable increase in AP duration,[72,83] with increased rate-dependent AP shortening and temporal variability of AP duration.[71,84] AP prolongation leads to reduced repolarisation reserve and therefore increased incidence of early afterdepolarizations (EADs, i.e. premature depolarizations occurring during the plateau phase) and potentially fatal arrhythmias.[85] Previously mentioned cardiac conditions, including HF, are all characterized by increased susceptibility to perturbations of repolarisation (e.g. drugs blocking K^+ currents or electrolyte imbalances) and overall increased risk of arrhythmias. I_{NaL} is an inward current occurring during action potential plateau, when the membrane potential is sensitive to small current changes since the stabilizing effect of I_{K1} is absent. From this stems the marked slowing effect of increased I_{NaL} on the rate of repolarization. A slower

phase 3 repolarization is a strong predictor of arrhythmic risk,[86] likely because it prolongs the time spent in partial refractoriness (the so called "vulnerable window"). The increased rate of EADs in the presence of increased I_{NaL} is likely a direct consequence of the resulting APD prolongation. Indeed, prolonged plateau phase allows calcium current (I_{CaL}) to recover from inactivation and reactivate, thus eliciting the postdepolarization. Prolongation and instability of repolarization in HF is also caused by other electrical abnormalities such as reduction of K^+ currents.[87,88] Due to the reduced repolarizing currents, the effects of I_{NaL} prolongation are more deleterious in HF than in control myocytes.[26] Thus even a minor inhibition of K^+ currents would result in a severe AP prolongation: I_{NaL} thus contributes to the increased susceptibility to AP prolonging agents in HF (i.e. reduced repolarization reserve), contributing to drug-induced pro-arrhythmia. So far we have discussed only the potential deleterious electrophysiological consequences of I_{NaL} augmentation in HF; however, the most severe consequences of the increased I_{NaL} are associated with intracellular Na^+ overload. The total amount of Na^+ ions entering the cell through Na^+ channels is a function of the current amplitude multiplied by its duration. Though the amplitude of I_{NaL} is small, it lasts for a very long time when compared to peak current, leading to an increase in Na^+ influx ranging from 50% to 100% of peak I_{Na} at each activation.[71,89] Thus, I_{NaL} may be the main responsible factor for the increased $[Na^+]_i$ observed in failing myocytes.[7]

Alterations of NCX and NHE in heart failure

In addition to the excess of Na^+ entering the cell via Na^+ channels, increased $[Na^+]_i$ in HF may also rely on enhanced Na^+ entry through the NCX or the NHE. As mentioned above, NCX is upregulated in HF and thus can potentially cause increased Na^+ entry, especially if combined with the increased cytosolic $[Ca^{2+}]$, which is often seen in failing myocytes due to the increased leakage from the SR. However, the contribution of the increased NCX function to the higher total $[Na^+]_i$ in HF appears to be minimal,[6] for two main reasons: (i) the absolute of Na^+ ions entering via the exchanger is relatively low and (ii) the increased forward mode activity of the NCX is counterbalanced by an increased reverse mode (see below), which may become relevant in HF. NHE instead is likely to play a more significant role; Baartscher et al.[9] reported that in a rabbit HF model, the increased $[Na^+]_i$ can be inhibited by blocking the NHE with cariporide. The importance of this mechanisms is confirmed by several reports showing attenuated development of heart failure and related myocardial dysfunction when NHE inhibitors are used.[45,90,91] NHE activity may be increased in HF as a consequence of energetic imbalance leading to intracellular acidosis requiring an increased function of the sarcolemmal proton exchanger.

Alterations of NKA in heart failure

On top of the increased Na^+ influx, decreased Na^+ efflux may contribute to increased $[Na^+]_i$ in HF. Several HF models are characterized by decreased global expression or shift in the isoforms of NKA.[91] Most of the studies however have been done on tissue homogenates and may reflect changes of non-cardiomyocyte protein levels.[92] Only a few studies of NKA function have been performed in HF myocytes, and the results are contradictory: in a model of rat post-myocardial infarction HF a reduced activity was reported,[93] while myocytes from a dog model displayed unaltered maximal NKA pump current.[94] Unaltered pump rate with similar Na^+ affinity was also reported in a rabbit HF model[6] and found that both the maximal Na^+ transport rate and the NKA affinity for internal Na + were not altered, despite the reduced NKA expression in ventricular myocytes.[92] The presence of unaltered function despite less protein count suggests that NKA activity is regulated differently in HF. Indeed, in HF rabbit myocytes expression of phospholemman (PLM) is reduced,[92] determining lower PLM:NKA ratio; combined with the higher PLM phosphorylation, this results into less overall inhibition of NKA by PLM, explaining the maintained pump function. Similar changes of PLM expression and phosphorylation were also observed in human HF.[92]

Effects of increased I_{NaL} and $[Na^+]_i$ on cardiomyocyte function

The previous observations on a number of HF models highlight the limited role of NCX and NHE in determining the increased Na^+ entry and the absence of NKA functional alterations reducing global Na^+ efflux. Therefore the major contributor to the increased $[Na^+]_i$ in HF is the increased Na^+ entry via the late Na^+ current. AS detailed above, $[Na^+]_i$ and $[Ca^{2+}]_i$ within the myocyte are closely interwoven through the NCX, the major Ca^{2+}-extrusion mechanism. $[Na^+]_i$ is notably the main determinant of the overall equilibrium potential of the exchanger (E_{NCX}). An increased $[Na^+]_i$ determines a negative shift of

E_{NCX}, causing less Ca^{2+} extrusion during diastole (decreased forward mode activity) while causing more Ca^{2+} influx (increase reverse-mode operation) during the early phase of the action potential, when membrane voltage is above 0 mV.[17] Thus, I_{NaL} enhancement and the consequent higher $[Na^+]_i$ may cause intracellular Ca^{2+} content to rise substantially due to the combined effect of decreased Ca^{2+} extrusion and increased Ca^{2+} entry. $[Ca^{2+}]_i$ overload due to enhancement of I_{NaL} is very similar to the effects of Na^+/K^+ pump inhibition by digitalis,[95] which leads to increased $[Na^+]_i$ due to reduced Na^+ efflux.

$[Ca^{2+}]_i$ overload brings about a number of short- and long-term changes of myocardial function.[96,97] On the short-term, $[Ca^{2+}]_i$ overload leads to generation of delayed afterdepolarizations (DADs) and the resulting "triggered" arrhythmias (see above). Indeed spontaneous Ca^{2+} release from the SR through RyR channels is facilitated by $[Ca^{2+}]_i$ overload and higher SR Ca^{2+} levels.[98] NCX translates the resulting Ca^{2+} waves into premature depolarizations. In HF, where the RyR is leaky and NCX is overexpressed, the effects of increased $[Na^+]_i$ and the resulting $[Ca^{2+}]_i$ overload in determining arrhythmic events are greatly enhanced.

Ca^{2+} overload may also contribute to the slower repolarization in HF and thus affect the higher rate of early-afterdepolarizations (EADs). Indeed increased sub-sarcolemmal Ca^{2+} during the action potential plateau may reduce Ca^{2+}-dependent K^+ repolarizing currents (such as I_{K1}),[99] prolonging repolarization. In cardiac tissue, most EADs are preceded by abnormal Ca^{2+} transients, highlighting the role of $[Ca^{2+}]_i$ overload in the generation of arrhythmias related to prolonged AP.[100]

Sustained $[Ca^{2+}]_i$ overload during diastole directly impairs the relaxation rate of the myocardium, determining diastolic dysfunction in HF and cardiac hypertrophy. Increased I_{NaL} and $[Na^+]_i$ may significantly contribute to diastolic dysfunction in cardiac diseases with different mechanisms:

1) Increased AP duration renders Ca^{2+} release longer and prolongs contraction: inhomogeneous prolongation of APD will increase variability of twitch duration in the ventricle, leading to uncoordinated relaxation.
2) Increased diastolic $[Ca^{2+}]_i$ leads to slower and incomplete myofilament inactivation and relaxation; this phenomenon is extremely more pronounced at high pacing rates, due to a more marked cytosolic Na^+ and Ca^{2+} overload. At high pacing rates, the extreme $[Ca^{2+}]_i$ overload extending throughout the entire diastolic period may severely impair relaxation, effectively provoking contractures.[101]
3) Spontaneous Ca^{2+} releases induced by $[Ca^{2+}]_i$ overload generate diastolic after-contractions that further impair and delay relaxation.[102]

A further consequence of the impaired diastolic function determined by enhancement of I_{NaL} and $[Na^+]_i$ overload is a reduction of myocardial blood flow perfusion. Due extrinsic compression of blood vessels by the contracting muscle,[103] myocardial coronary flow is limited during systole, and reaches a maximum during the initial relaxation phase. In HF, the early diastolic phase (isovolumic relaxation) is slower, due to the delayed decay of cytosolic Ca^{2+} transient, myofilament force and wall stress. Increased I_{NaL} may play a significant role in that phenomenon. Indeed, experimental I_{NaL} enhancement by ATX-II determines reduced coronary blood flow[83] and pharmacological block of I_{NaL} ameliorates perfusion.[101]

The increase of $[Na^+]_i$ and the resulting $[Ca^{2+}]_i$ overload may also affect cardiomyocyte energetics. As detailed above, mitochondrial Ca^{2+} is essential to maintain cell energy balance. In presence of high cytosolic $[Na^+]$, mitochondrial NCX increases Ca^{2+} extrusion from the mitochondrial matrix, lowering mitochondrial $[Ca^{2+}]$. This results in reduced ATP production, thus creating a mismatch between ATP consumption and production.[104] Additionally, high $[Na^+]_i$ decreases the extrusion of H^+ through the NHE; thus, I_{NaL} enhancement may also lead to cellular acidosis in HF, with severe effects on cell metabolism.[105]

Finally, chronically increased $[Ca^{2+}]_i$ due to increased I_{NaL} and $[Na^+]_i$ leads to activation of numerous intracellular signaling pathways: increased activity of PKC, CaMKII and calcineurin not only acutely affects Ca^{2+} homeostasis and membrane currents but may also activate transcriptional factors, ultimately leading to hypertrophic response and accelerating cellular remodelling.[106,107] Hyper-activation of CaMKII can in turn further increases I_{NaL} (by phosphorylating Na^+ channels) and $[Ca^{2+}]_i$ (by increasing SR Ca^{2+} leakage following RyR phosphorylation),[67] creating a vicious circle. In HF and

hypertrophy, increased CaMKII activity has a pivotal role in supporting a positive feedback loop between I_{NaL} and $[Ca^{2+}]_i$ overload.[27]

In conclusion, increased I_{NaL} not only directly affects cell electrophysiology but also alters Ca^{2+} homeostasis with potential consequences for contractile function, cell metabolism and altered cell signaling leading to hypertrophic response.

NA^+ REGULATION IN HYPERTROPHIC CARDIOMYOPATHY

Hypertrophic cardiomyopathy (HCM) is the most common genetic heart disease,[108,109] with a prevalence in the general population of 1:500, representing a leading cause of sudden cardiac death in the young[109] and a prevalent cause of heart failure and stroke.[110] Increased arrhythmogenesis and diastolic dysfunction are the two main pathophysiological features of this disease.[111]

HCM is associated with a complex electrophysiological cardiomyocyte remodelling involving multiple changes in transmembrane currents ultimately leading to susceptibility to ventricular arrhythmias. We have recently characterized the electrophysiological, Ca^{2+} handling and contractile abnormalities occurring in human HCM myocardium as concurrent determinants of diastolic dysfunction and arrhythmias in this disease.[17] We used isolated myocytes and intact trabeculae from myocardial specimens from the interventricular septum of HCM patients undergoing surgical myectomy, compared with non-hypertrophic surgical patients. Results of patch clamp studies showed that the action potential duration (APD) recorded at various frequencies of stimulation was markedly prolonged in cardiomyocytes from patients with HCM (HCM cardiomyocytes) compared to controls (Figure 3A). Prolonged APD was associated with prolonged QTc in patients from the HCM group (average QTc = 470 ms). Of note, a recent large-scale study on HCM patients reported that QT prolongation is a common feature in patients with this disease[112] and may be a predictor of clinical outcome. Prolonged APD led to increased risk of early after depolarisations (EADs),[85] which were 6-fold more frequent in HCM cardiomyocytes than in control cells (Figure 3B). Interestingly, the degree of APD prolongation was associated with the frequency of ventricular arrhythmias in patients: patients with severely prolonged APD had a higher frequency of documented non-sustained ventricular tachycardia at 24 h ECG.[17] APD prolongation in HCM cardiomyocytes is determined by an imbalance between inward and outward transmembrane currents: repolarizing K^+ currents were reduced and L-Type Ca^{2+} current was increased. Importantly, we also observed that the amplitude of I_{NaL} in HCM cardiomyocytes was larger compared to control patients, likely contributing to APD prolongation (Figure 3C). APD and ion current abnormalities determined significant alterations of intracellular Ca^{2+} handling in HCM cardiomyocytes (Figure 3D). The amplitude of Ca_i^{2+} transients were similar in HCM and in control cardiomyocytes. Conversely, the kinetics of Ca_i^{2+} transient, were significantly longer and intracellular diastolic Ca^{2+} concentration ($[Ca^{2+}]_i$) was markedly higher in HCM compared to control (Figure 3E). Interestingly, $[Ca^{2+}]_i$ overload was accompanied by an increased rate of spontaneous Ca^{2+} releases from the SR and DADs in HCM cardiomyocytes, thus contributing to arrhythmogenesis. Additionally, we found a slightly increased SR Ca^{2+} content in HCM cardiomyocytes while the rate of Ca^{2+} extrusion via NCX was reduced (Figure 3F). In keeping with this observation, we measured a negative shift of the reversal potential of NCX (E_{NCX}) that suggests increased $[Na^+]_i$ (Figure 3G). Indeed, we reported an increased $[Na^+]_i$ in HCM myocytes, directly assessed by virtue of fluorescence measurements with Asante Natrium Green 2 Na^+-selective dye. Based on this data, we suggested that in HCM enhanced I_{NaL}-mediated Na^+ influx leads to a sustained increase in $[Na^+]_i$, providing the driving force for an increased rate of Ca^{2+} entry trough NCX, which is also overexpressed. However, increased $[Na^+]_i$ also impairs forward-mode NCX function, leading to the observed reduction of Ca^{2+} extrusion rate during caffeine exposure and likely contributing to slow down Ca^{2+} transient decay and increase diastolic $[Ca^{2+}]_i$, in combination with SERCA down regulation. The observed abnormalities of excitation-contraction coupling resulted in slower relaxation and increased diastolic tension in intact trabeculae isolated from the same samples. Interestingly, increased activity of CaMKII appears to underlie several of the observed changes in HCM cardiomyocytes: increased phosphorylation of Na^+ channels determined enhanced I_{NaL},[27,80] while increased RyR phosphorylation caused SR Ca^{2+} leak and DADs.[66] We concluded that, among the several ion channels and EC-coupling protein changes, increase of I_{NaL} and $[Na^+]_i$ overload are major contributors to electro-mechanical cardiomyocyte dysfunction, which may be strongly associated with the increased arrhythmogenesis and diastolic dysfunction observed in HCM patients. Na^+ dysregulation in HCM is described in the cartoon in Figure 4.

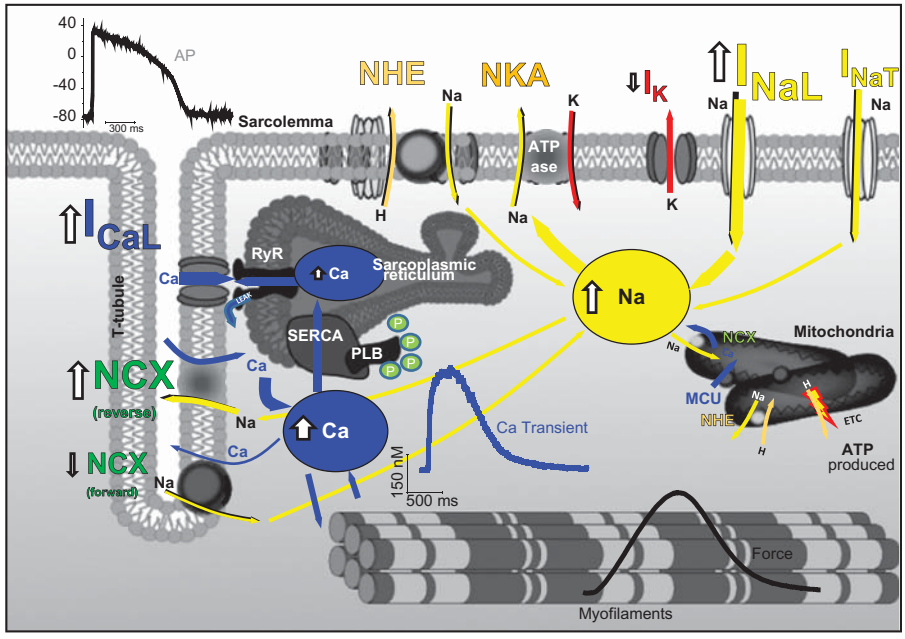

Figure 4. Na^+ homeostasis in hypertrophic cardiomyopathy. Cartoon showing the cellular and molecular determinants of abnormal Na^+ homeostasis in cardiac cells from HCM patients and their role in determining the dysfunction of Ca^{2+} handling and contraction. White thick arrows mark the changes occurring in HCM with respect to control cardiomyocytes. The thickness of yellow and blue arrows has been changed to match the relative changes of Na^+ and Ca^{2+} fluxes in HCM vs. control cells. Representative traces from Coppini et al.[17]

ROLE OF PHARMACOLOGICAL I_{NAL} INHIBITION IN CARDIAC DISEASES

Recent work highlighted the emerging role of I_{NaL} in the pathogenesis of HF, hypertrophic cardiomyopathy and other cardiac diseases, making I_{NaL} an interesting pharmacological target for disease management. We have previously highlighted the severe consequences of I_{NaL} enhancement in terms of arrhythmogenesis and contractile dysfunction. I_{NaL} inhibition may therefore improve both electrical and mechanical function of cardiomyocytes in many disease states. Therapeutic I_{NaL} inhibition has been attempted in a number of conditions in which I_{NaL} is abnormally enhanced, including LQT3, acute ischemia, experimental oxidative stress and heart failure[72,101,113–116]: in all these, I_{NaL} block determined shortening of APD, reduced temporal and spatial variability of repolarization and abolished EAD occurrence, highlighting a clear antiarrhythmic effect. Since I_{NaL} is carried by cardiac Na^+ channels, all drugs that interact with NaV1.5 channels affect I_{NaL}. Indeed late Na^+ current is inhibited by all classical Na^+ channel blockers, comprising not only experimental agents such as Cd^{2+}, Tetrodotoxin (TTX) and Saxitoxin (STX),[26,71] but also clinically employed drugs. These include: (i) flecainide,[117,118] which proved effective in shortening APD and reducing EADs in models of Long QT Type 3 (LQT3) syndrome induced by NaV1.5 mutations; (ii) mexiletine,[113,119] which demonstrated a clear ability to prevent the occurrence of arrhythmias induced by experimental APD prolongation and reduced dispersion of repolarization in LQT3[120]; (iii) lidocaine[121] and quinidine,[122] which effectively blocked genetically or experimentally enhanced I_{NaL}. However, all of these drugs exert an effective I_{NaL} block only at relatively high concentrations; at such concentrations, classical Na^+ channel blockers also strongly inhibit peak Na^+ current with great effects on myocardial excitability and conduction velocity. Under these conditions, the potential anti-arrhythmic effects of I_{NaL} block may be overcome by the pro-arrhythmic consequences of the slower conduction.[122] Among classical Na^+ channel blockers, flecainide displays the highest selectivity for the late component of Na^+ current[117]; however, the elevated pro-arrhythmic risk associated with flecainide use in structural heart diseases[123,124] prevents its clinical use in conditions where I_{NaL} is enhanced (heart failure, cardiomyopathies). Indeed flecainide, which inhibits peak Na^+ current other than I_{NaL}, causes significant conduction delay[125] and has been associated with increased mortality in post-myocardial infarction patients[126]: conduction slowing (induced by I_{Na} peak block) combined with reduced refractoriness (due to shorter APD via I_{NaL} block) favors reentry circuits.[127]

Amiodarone was shown to have I_{Na} blockade capacity as an ancillary effect. Interestingly, amiodarone effectively blocked I_{NaL} in cardiomyocytes from human failing hearts at concentrations as low as 5 μM, i.e. within the therapeutic range of the drug; amiodarone produced almost no effect on the transient Na^+ current at these concentrations.[128] These results suggest that selective block of I_{NaL} over peak current may mediate part of the anti-arrhythmic effects of amiodarone and contribute to limit its pro-arrhythmic toxicity, making it a relatively safe drug also in the presence of structural heart disease.[129] However, amiodarone is far from being a selective agent, in that exerts a potent blockade of repolarizing K^+ currents (mainly I_{Kr}), with potential pro-arrhythmic consequences,[130,131] especially when assumed in association with other QT prolonging drugs. As detailed above, in presence of severely increased I_{NaL}, the APD prolonging effect of K^+ current blockers is enhanced, raising the risk of proarrhythmic effects.[132,133] From all these observations stems the need of a drug that blocks I_{NaL} with high selectivity.

Developed in the 1990s as a cardiac specific partial fatty acid oxidation inhibitor, ranolazine was first experimented as a possible anti-anginal compound, with relatively good results. Ranolazine was found to be safe in humans in doses up to 2 grams/day, and it was effective in reducing symptoms in two clinical trials on chronic stable angina patients.[134,135] It rapidly became clear that the anti-anginal and anti-ischemic effects of ranolazine were not due to the supposed metabolic effects (which are negligible at therapeutic concentrations), but rather to its selective blockade of the late sodium current. Its electrophysiological effects were in fact described in 2004[136] Ranolazine exerts an inhibitory effect on I_{Kr} and a slight I_{CaL} block[136]; but the main effect was a potent inhibition of I_{NaL} with small or no effect on peak I_{Na} within the therapeutic concentration range. Inhibition of I_{Kr} is generally considered pro-arrhythmic. However, in a large scale trial on non ST-elevation myocardial infarction patients, ranolazine significantly reduced the incidence of ventricular tachycardia[137] and was not associated with QRS prolongation or increased mortality in these patients.[138] Recent work showed that ranolazine inhibits triggered activity and its propagation in pulmonary veins, the main source of atrial ectopies, and reduces afterdepolarizations in atrial myocardium from surgical patients,[139] suggesting that ranolazine may also play a role in the prevention of atrial fibrillation.[140] These observations support the view that I_{NaL} inhibition by ranolazine may be antiarrhythmic, even in the presence of a limited concomitant block of I_{Kr}. I_{NaL}, whose density is highly variable among different layers and regions of the ventricle, strongly contributes to spatial differences in repolarization. I_{NaL} inhibition is therefore associated with a reduced transmural dispersion of repolarization,[136] contributing to its anti-arrhythmic effects. Ranolazine also inhibits other cardiac currents, including I_{CaL}, I_{Ks}, I_{K1}, I_{to} and I_{NCX}.[136] However, except for I_{CaL}, the concentrations of ranolazine required to inhibit these other ion currents are at least 10-times higher than those required to block I_{NaL}. Therefore, only inhibition of I_{NaL}, I_{CaL} and I_{Kr} may contribute to ranolazine effects at therapeutic concentrations. Recently, a newly developed I_{NaL} inhibitor (GS-458967) was shown to inhibit late current with a higher selectivity as compared with ranolazine, even at 10-times lower concentrations; GS-458967 effectively reduced experimentally induced cellular arrhythmias (EADs and DADs).[141]

We have previously highlighted the multiple deleterious effects of increased I_{NaL} on cardiomyocyte function. I_{NaL} inhibition, via reduction of Na^+ influx, antagonizes $[Na^+]_i$ elevation, thus preventing $[Ca^{2+}]_i$ overload secondary to $[Na^+]_i$ overload. The increased $[Ca^{2+}]_i$ in HF and other disease settings can be viewed as a compensatory mechanism for contractile dysfunction. However, it appears that the detrimental consequences of increased $[Ca^{2+}]_i$ (i.e. increased SR Ca^{2+} leakage, increased diastolic tension) prevail over the advantages. Indeed, ranolazine abolished the occurrence of spontaneous after-contractions and reduced diastolic tension without decreasing contractility in myocytes and intact trabeculae from human and rat failing hearts.[60,115] Reduction of $[Ca^{2+}]_i$ should improve diastolic function but also impair systolic contraction. Instead, in diseased myocytes ranolazine only reduced diastolic $[Ca^{2+}]_i$ without affecting neither the $[Ca^{2+}]_i$ transient amplitude nor SR Ca^{2+} content,[60] explaining the global amelioration of the contractile profile seen in intact muscle, especially at higher stimulation rates. The reason why ranolazine is able to differently modulate diastolic $[Ca^{2+}]_i$ and SR $Ca^{2+}$is the concomitant reduction of Ca^{2+} leak from the SR.[142] When the lower $[Na^+]_i$ leads to decreased $[Ca^{2+}]_i$ the resulting reduction of leak may improve the ability of the SR to reuptake Ca^{2+}, thus preserving integrity of Ca^{2+} store.

I_{NaL} inhibition has marked cardioprotective effects in conditions of myocardial ischemia, when increased activity of the NHE leads to increased Na^+ influx.[143] Inhibition of I_{NaL} may improve intracellular pH homeostasis by allowing a maintained rate of proton extrusion via the NHE. Finally,

recent observations indicate that I_{NaL} inhibition, via amelioration of relaxation and reduction of end-diastolic pressure, may significantly enhance coronary blood flow, especially in ischemic conditions.[83]

In keeping with the favorable mechanical consequences of I_{NaL} inhibition, ranolazine, unlike other anti-ischemic drugs, does not decrease the "double product" of arterial pressure and heart rate which is a valuable measurement of cardiac overall mechanical work.[144] In conclusion, recent evidence support the assumption that ranolazine markedly improves the contractile efficiency of diseased myocardium and increases coronary blood flow, independently of changes in cardiac work.

I_{NAL} INHIBITION REVERSES ELECTRO-MECHANICAL DYSFUNCTION IN HYPERTROPHIC CARDIOMYOPATHY

Among the several changes identified in cellular electrophysiology and Ca^{2+} handling in our recent study on human HCM cardiomyocytes,[17] we found that the increased late sodium current is amenable for a targeted correction by virtue of the selective blocker ranolazine. Since I_{NaL} is potentially involved

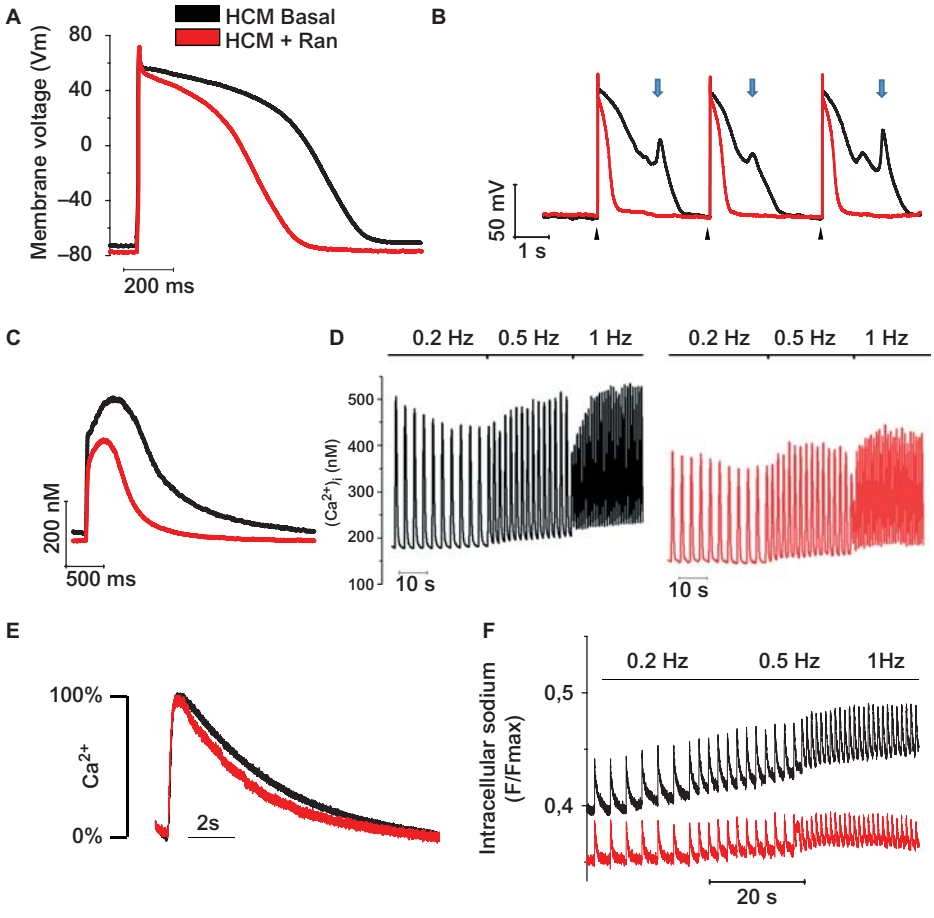

Figure 5. Late sodium current inhibition reverses electro-mechanical dysfunction in HCM. All panels are modified from Coppini et al.[17] (A) Action potentials at 0.2 Hz from an HCM cardiomyocyte before (Basal) and after exposure to 10 μmol/L ranolazine (Ran). (B) Representative trace from a HCM cardiomyocyte in the absence of ranolazine showing multiple early after depolarizations (EADs), marked by blue arrows. A trace from the same cardiomyocyte in the presence of ranolazine is shown superimposed in red: the drug abolishes EADs. Black arrowheads mark stimuli. (C) Superimposed representative Ca_i^{2+} transients at 0.2 Hz from an HCM cardiomyocyte (same as A) at baseline and with ranolazine. (D) Trains of Ca_i^{2+} transients elicited at 0.2, 0.5, and 1 Hz in an HCM myocyte before (left) and following (right) exposure to ranolazine, highlighting reduction of diastolic $[Ca^{2+}]_i$. (E) Superimposed caffeine-induced calcium transients in the absence and presence of Ran in a HCM cardiomyocyte, highlighting the faster time constant (τ) of caffeine-induced Ca_i^{2+} transient decay, suggesting higher rate of NCX-mediated Ca^{2+} extrusion. (F) Effects of ranolazine on intracellular Na^+. Representative traces of Asante Natrium Green florescence, recorded at stimulation rates of 0.2 Hz, 0.5 Hz and 1 Hz in the absence (black) and presence (red) of 10 μM Ran.

both in determining APD prolongation and Ca^{2+} handling abnormalities leading to abnormal relaxation, I_{NaL} inhibition is expected to positively affect both. In control cardiomyocytes, ranolazine (10 μM) did not significantly affect the APD and the effects of ranolazine on the amplitude and kinetics of Ca_i^{2+} transients were negligible. On the contrary, ranolazine significantly reduced APD in HCM cardiomyocytes (Figure 5A). Consistently, the occurrence of EADs in HCM cardiomyocytes was approximately halved by the application of ranolazine (Figure 5B). We found that ranolazine 10 μM markedly blocked I_{NaL} in HCM myocytes, confirming that the described effects of the drug are consistent with specific I_{NaL} inhibition. In HCM cardiomyocytes, the APD shortening by ranolazine was paralleled by accelerated rise and decay time of Ca_i^{2+} transients, associated with no significant decrease of Ca_i^{2+} transient amplitude (Figure 5C). Ranolazine significantly reduced intracellular diastolic $[Ca^{2+}]_i$ in HCM myocytes and attenuated the increase in diastolic $[Ca^{2+}]_i$ following increase in stimulation rate (Figure 5D). In agreement with these results, we found that ranolazine slightly reduced SR Ca^{2+}-load in HCM myocytes and significantly accelerated caffeine-induced Ca_i^{2+} transients, suggesting an increased rate of Ca^{2+}-extrusion through the NCX upon exposure to the drug (Figure 5E). The beneficial consequences of I_{NaL} inhibition on Ca^{2+} handing in HCM cardiomyocytes were mediated by a marked reduction of $[Na^+]_i$, as confirmed by direct assessment using Na^+ -selective fluorescent indicators (Figure 5F). Reduced $[Na^+]_i$ shifted E_{NCX} back to positive levels, potentiating forward mode operation of the NCX.

The beneficial effect of ranolazine was finally investigated in intact tissue measuring isometric twitch tension from intact ventricular trabeculae.[17] Ranolazine, speeded up the kinetics of force development and shortened the overall twitch duration. Further, it significantly diminished the rise of diastolic tension upon increase in stimulation frequency, without significantly reducing peak isometric (active) force. According to our results, by reducing $[Na^+]_i$ overload, and thus diastolic $[Ca^{2+}]_i$, ranolazine has the potential to improve diastolic function, a cause of symptoms in most HCM patients, and to indirectly improve myocardial perfusion, thus impacting on functional limitation and quality of life. Taken together these results highlight the great potential of I_{NaL} inhibition for the reduction of arrhythmias and diastolic dysfunction in HCM patients.

In the long term, the effect on calcium homeostasis is expected to down regulate a number of remodelling pathways that strictly depend on myocyte driven signalling: this may in turn bring benefit to extracellular matrix remodelling, reducing fibroblasts growth and collagen production in

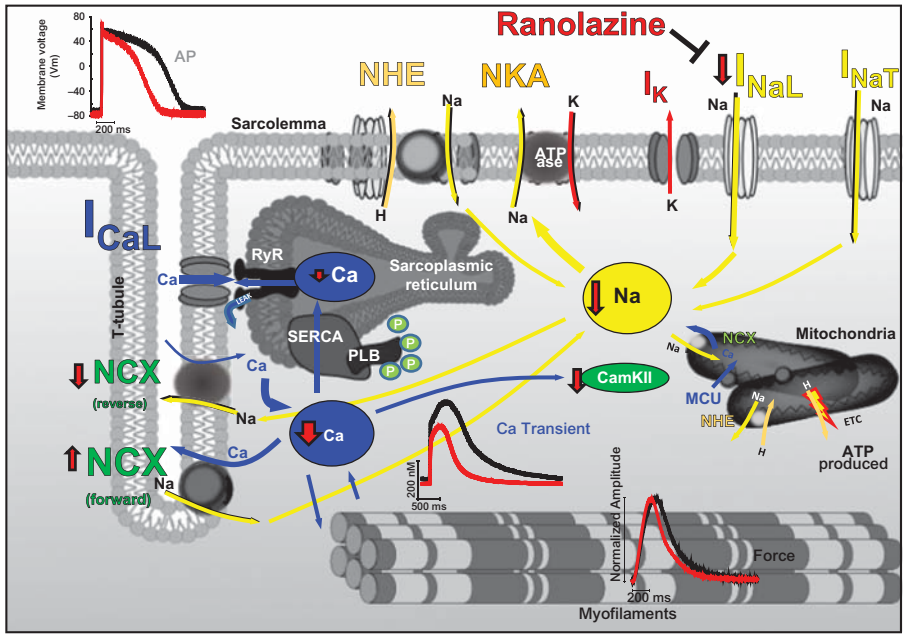

Figure 6. Effects of I_{NaL} inhibition in HCM. Cartoon showing the cellular and molecular determinants of the effects of I_{NaL} inhibition in HCM cardiac cells and their role in determining the ameliorated Ca^{2+} handling and contraction in presence of the ranolazine. Red thick arrows mark the changes occurring in HCM in the presence of ranolazine with respect to the baseline condition. Representative traces from Coppini et al.[17] Baseline traces are black and the corresponding traces in the presence of ranolazine are red.

hypertrophied myocardium. In particular, reduction of intracellular Ca^{2+} overload, may lead to sustained lower CaMKII activity, eventually affecting the functional and structural remodelling of HCM myocardium, with possible implications for disease progression.[145] In keeping with this observation, ranolazine administration has been shown to reduce the degree of myocyte hypertrophy and interstitial fibrosis in experimental hypertrophy models.[146] Thus, late sodium current inhibition in HCM may be capable of preventing disease progression towards the end-stage phase, by acting upon one of its most critical determinants – diastolic dysfunction, myocardial ischemia and fibrosis. In principle, late sodium current inhibition is the ideal therapeutic strategy for HCM patients, with a wide range of positive actions, which may have a critical impact on acute symptoms as well as on the natural history of the disease. The effects of ranolazine on HCM cardiomyocytes and the role of CaMKII are summarized in Figure 6.

CONCLUSIONS

The numerous ion transport pathways involved in the regulation of $[Na^+]_i$ are critical in mediating both contractile and electrical activity of cardiac myocytes. $[Na^+]_i$ regulation and $[Ca^{2+}]_i$ homeostasis are highly interdependent, and the delicate dynamic interplay of $[Ca^{2+}]_i$ and $[Na^+]_i$ is deeply impaired in pathophysiological situations such as heart failure and hypertrophic cardiomyopathy, contributing to contractile dysfunction and arrhythmogenesis. The progressive clarification of the involvement of I_{NaL} in the pathogenesis of Na^+ and Ca^{2+} overload has rendered this current an appealing pharmacological target. The most significant advantage of I_{NaL} inhibition is its potential to simultaneously target the consequences of disease-related functional changes (e.g., arrhythmias and contractile dysfunction) as well as the mechanisms underlying its progression (e.g., myocardial remodelling, ischemia). The potential therapeutic value of I_{NaL} inhibition discussed in this review, in particular in heart failure and hypertrophic cardiomyopathy, still awaits confirmation from clinical trials. Whereas I_{NaL} inhibitors have a proven efficacy in patients with ischemic heart disease, confirmed by clinical trials, the most attractive clinical applications of I_{NaL} inhibition, heart failure and hypertrophy, remain largely unexplored and deserve further pre-clinical and clinical investigation.

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