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REVIEW

The late sodium current in heart failure: pathophysiology and clinical relevance

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Abstract

Large and growing body of data suggest that an increased late sodium current ($I_{Na,late}$) can have a significant pathophysiological role in heart failure and other heart diseases. The first goal of this article is to describe how $I_{Na,late}$ functions under physiological circumstances. The second goal is to show the wide range of cellular mechanisms that can increase $I_{Na,late}$ in cardiac disease, and also to describe how the up-regulated $I_{Na,late}$ contributes to the pathophysiology of heart failure. The final section of the article discusses the possible use of $I_{Na,late}$-modifying drugs in heart failure, on the basis of experimental and preclinical data.

Keywords Late sodium current; Heart failure; Sodium channel

Introduction

The cardiac action potential (AP) is the change of membrane voltage caused by temporal and spatial alterations of ionic currents flowing through the cell membrane of cardiac myocytes. Depolarization of the cell membrane causes Na^+ channels to open up for a short period[1,2] producing the early peak sodium current ($I_{Na,early}$). $I_{Na,early}$ is responsible for the upstroke of the cardiac AP. During the AP, these channels can recover from inactivation and reopen, causing a sustained current component, termed $I_{Na,late}$. Although the magnitude of $I_{Na,late}$ is dwarfed by $I_{Na,early}$, it flows throughout the plateau phase of the AP therefore contributing to AP morphology.

Large and continuously growing amount of data suggest that an increased $I_{Na,late}$ can bear pathophysiological role in several acquired heart diseases such as myocardial ischaemia[3] and heart failure.[4,5] An up-regulated $I_{Na,late}$ impairs repolarization and increases intracellular sodium concentration ($[Na^+]_i$) of cardiomyocytes leading to cardiac arrhythmias,[6] contractile dysfunction,[7] and disturbed myocardial energetics.[8]

Electrophysiological identity of the sustained plateau sodium current

Mammalian cells express different isoforms of voltage-dependent sodium channels, having differences in drug sensitivity, kinetics, or unit conductance.

Table 1. Mechanisms contributing to the late sodium current

Single channel activity patterns (burst and late-scattered mode openings)	Maltsev *et al.*[12]
Window current	Zaza *et al.*[28] Wang *et al.*[30] Maltsev *et al.*[32]
Non-equilibrium gating	Clancy *et al.*[35]
Non-cardiac sodium channels	Biet *et al.*[42] Yang *et al.*[43]

The dominant isoform in cardiac tissues is $Na_v1.5$, which is less sensitive to the sodium channel toxin tetrodotoxin (TTX) than other isoforms.[9,10] There are four auxiliary subunits (β_1, β_2, β_3, and β_4) that can associate to the $Na_v1.5$ pore-forming subunit. The larger macromolecular complex also includes attachments to cytoskeleton and scaffolding proteins. All of these molecular connections may modify the kinetics and voltage dependence of the channel.[11]

Mechanisms discussed later in this section (*Table* 1) might coexist and contribute to shaping the profile of sodium current during the AP. Understanding these mechanisms can help to develop new therapeutic interventions targeting $I_{Na,late}$.

Different channel activity patterns may contribute to $I_{Na,late}$

Following depolarization, the majority of closed $Na_v1.5$ channels open rapidly (within a very few milliseconds) then they inactivate in a similarly rapid manner[1,2] producing the $I_{Na,early}$ responsible for the upstroke of the cardiac AP. If the membrane remains depolarized, a small fraction of the channels can reopen. So far, three distinct types of single Na channel activity have been described in human ventricular myocytes: transient mode, burst mode, and late-scattered mode.[12]

Grossly, the transient mode (TM) results in $I_{Na,early}$, whereas the burst mode (BM) and the late-scattered mode (LSM) produce a sustained current component having a magnitude of approximately 0.5–1% of the current peak 50 ms after the upstroke of the depolarizing pulse.[12] This sustained component is referred to as the $I_{Na,late}$. The rapid decline of BM openings leaves LSM the major gating mode shaping the plateau sodium current.

Channel mutations or pathologic conditions can change the contribution of different gating modes to the ensemble current resulting in an increased $I_{Na,late}$.[13–22] The gating modes seem to have different drug sensitivities or affinities[23–25]; therefore, targeted pharmacological modulation of these may exert cardioprotective and anti-arrhythmic effects.[24,26,27]

Window current

There is an overlap between the steady-state activation and inactivation curves of voltage activated sodium channels.[28] This overlap results in a voltage 'window' where channels can recover from inactivation and then they may reopen. If the membrane potential lies within this voltage 'window', a sustained current can be seen. The contribution of this window mechanism to $I_{Na,late}$ under physiological circumstances might be very limited because of two factors. First, there is a big difference between the voltage ranges of the sodium channel 'window' voltage (around −70 mV) and the plateau phase of the AP (usually above 0 mV). Second, the overlap is less than 5% of the maximum current in healthy myocytes.[29–32] Although the window mechanism is unlikely to underlie $I_{Na,late}$ in healthy cardiomyocytes, channel mutations or pathologic regulation can shift either in steady-state activation or inactivation curves therefore altering the voltage window.[33,34] This pathological mechanism contributes to an enhanced $I_{Na,late}$ in several diseases.[33,34]

Non-equilibrium gating

Membrane potential of cardiac myocytes changes continuously. When sodium channels are functioning in these cells, they are part of this dynamic system. Clancy *et al.*[35] proposed that transition rates between channel states are modulated by the voltage history that the channel experienced before. This means that recovery from inactivation is modulated by the dynamically changing voltage ('non-equilibrium gating'). Experimental data support that $I_{Na,late}$ is larger when evoked with repolarizing voltage ramp or AP shape command compared with conventional square pulses or model simulations.[35–37]

Non-cardiac sodium channels in the heart

Association of electrocardiographic (ECG) abnormalities to epilepsy[38,39] and myotonic disorders[40,41] raised the possibility that mutated non-cardiac sodium channels can cause electrophysiological disturbances in the heart. These non-cardiac sodium channel isoforms ($Na_v1.1$, $Na_v1.2$, $Na_v1.3$, $Na_v1.4$, and $Na_v1.6$) were later identified in cardiac tissue.[17,42–45] Using an elegant approach, Biet *et al.* suggest that the contribution of non-cardiac sodium channels to $I_{Na,late}$ is 44%.[42] Yang *et al.* report that $Na_v1.8$ provide the 38% of $I_{Na,late}$.[43] These experimental data can open a new path of research for isoform specific sodium channel modulators in anti-arrhythmic therapy.

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Physiology of the plateau sodium current

Role of the plateau sodium current in cardiac electrical activity

The plateau phase of cardiac APs is shaped by a delicate balance of tiny inward and outward current fluxes. At the same time, the activation of the repolarizing-delayed rectifier currents is highly sensitive to voltage. Therefore, even small changes of the plateau phase can result in significant alterations of the AP duration.[46]

Contribution of $I_{Na,late}$ to shaping cardiac APs was a subject of debate because of its small density. The substantial AP shortening effect of TTX indicates that the plateau sodium current has an important role in determining AP duration in the conductive system and in ventricular myocytes.[47,48] Recent data applying self-action potential technique show that the plateau sodium current is comparable with the major potassium currents in guinea pig ventricular myocytes.[37] The plateau sodium current carried by both cardiac and non-cardiac Na^+ channels also plays a role in SA node cells.[49,50] $I_{Na,late}$ is an important factor in forming the atrial AP as well.[51,52]

Contribution of $I_{Na,late}$ to the sodium homeostasis of cardiac cells

$[Na^+]_i$ depends on a dynamic equilibrium between Na^+ influx into the cell and Na^+ efflux to the extracellular space. Resting $[Na^+]_i$ is about 4–8 mM in rabbit, guinea pig, and dog ventricular myocytes and higher (9–14 mM) in rat and mouse.[53] Resting $[Na^+]_i$ in human myocytes is believed to be in the 4–10 mM range. Influx of Na^+ occurs through Na^+ channels, Na^+/Ca^{2+} exchanger (NCX), and Na^+/H^+ exchanger (NHE). The Na^+ efflux is mainly mediated by the Na^+/K^+ pump (NKA), but it can also happen through the NCX ('reverse mode') during the first few milliseconds of the AP. To a smaller extent, Na^+/HCO_3^- cotransporter (NBC), Na^+/Mg^{2+} exchanger, and $Na^+/K^+/2Cl^-$ cotransporter can also be involved in the sodium homeostasis of cardiac cells.[53] Additionally, there is a dynamic balance between $[Na^+]_i$ and Na^+ concentration in cellular organelles, such as the mitochondria.

In isolated myocytes, $[Na^+]_i$ increases upon pacing in a frequency-dependent manner, caused by the increased Na^+ influx through Na^+ channels and NCX. Sodium channels are responsible for about one-fourth of the total Na^+ entry into paced cardiac cells.[53] The contribution of the early sodium current peak ($I_{Na,early}$) and the plateau sodium current to the Na^+ entry through Na^+ channels are roughly equal.[28,53–55]

Interactions between the Na^+ and Ca^{2+} homeostasis in cardiomyocytes

Na^+/Ca^{2+} exchanger: the direct link between the Na^+ and Ca^{2+} homeostasis.

The NCX is a secondary active membrane transport mechanism working with a 1 Ca^{2+} to 3 Na^+ stoichiometry.[53,56–59] The function of NCX depends on the net electrochemical gradient of the carried ions and the membrane voltage. Its main role is to remove Ca^{2+} from cardiac myocytes by using the electrochemical gradient of Na^+ ('forward mode'). Although, for a few milliseconds in the beginning of the cardiac AP, it mediates the extrusion of Na^+ from the cell and carries Ca^{2+} into the cytosol ('reverse mode').

$I_{Na,late}$ facilitates Ca^{2+} influx via L-type calcium channels.

As an inward current, $I_{Na,late}$ depolarizes the membrane and therefore lengthens the AP and elevates the plateau phase. The longer time the membrane stays depolarized, the more likely are L-type calcium channels to open or reopen. By using action potential clamp technique, it is well documented that the L-type calcium current is present during the entire plateau.[60–64] It means that a longer AP results in more Ca^{2+} entering into the cytosol.

Modulation of $I_{Na,late}$

The complex modulation of $I_{Na,late}$ by cytosolic Ca^{2+}

The Ca^{2+} underlies excitation–contraction coupling in cardiac myocytes and acts also as a regulator of sarcolemmal transport mechanisms, and other cellular functions. Voltage-gated Na^+ channels are known to be regulated by Ca^{2+}, calmodulin (CaM), and Ca^{2+}–CaM dependent protein kinase II (CaMKII) individually and cooperatively as well.[65–67]

It is widely accepted that signalling through the Ca^{2+}–CaM–CaMKII pathway facilitates the sodium current, especially its late component.[16,65,68]

The Ca^{2+} dependent regulation modifies mainly the inactivation of the sodium channels. Ca^{2+} or CaM binding to the channel causes a ~5 mV shift in the steady-state inactivation (SSI) towards more positive voltages. Although this may not sound important, the proximity of the resting membrane potential and the steepness of the SSI curve make this shift noteworthy. Here, even this small 5 mV change can exert a remarkable effect on the availability of sodium channels. There may be multiple Ca^{2+} and CaM binding locations on the sodium channel allowing for a complex regulation of channel function. Because of this

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complexity, mutations in the Ca^{2+} or CaM sensing regions or pathologic conditions altering the Ca^{2+} sensitivity may lead diverse functional disturbances.

Sodium channels, Ca^{2+}, and calmodulin.

Both Ca^{2+} binding EF hand motifs and CaM binding IQ motifs have already been identified in the $Na_v1.5$ channel structure. In contrast to this, data on direct regulation of $Na_v1.5$ channels by cytosolic Ca^{2+} are controversial. The EF motifs were shown to effectively bind Ca^{2+} ions and that SSI is shifted towards positive voltages when the cytosolic Ca^{2+} content is high.[69] However, conflicting observations from several groups supported that CaM is essential to mediate Ca^{2+} effect, and Ca^{2+} does not regulate sodium channel directly.[70,71] To resolve these contradictory results, Shah *et al.* created a model in which the SSI is modulated by the cooperation between the EF hand and the IQ motifs.[72] Later, Biswas *et al.* have shown that the IQ motif is not essential for the direct Ca^{2+} regulatory effect.[73] They also proposed that CaM-driven regulation is of the cardiac sodium channel is negligible in wild-type channels at physiologically relevant Ca^{2+} concentrations.

The Ca^{2+}/CaM complex forms a bridge between the IQ motif on the C-terminus and the DIII–IV linker region of the sodium channel.[74] This linker region is considered to be the inactivation gate of the sodium channel.[75] When $[Ca^{2+}]_i$ is very low, CaM does not interact with the DIII–IV linker region and inactivation of the channel is not affected.[72,74,76] When Ca^{2+} is elevated, the Ca^{2+}/CaM complex can interact with the DIII–IV linker region, and this configuration shifts the SSI curve to more positive voltages.[74] Besides the direct regulation of sodium channel, CaM also activates CaMKII that also modulates the channel.[77]

Ca^{2+}–calmodulin dependent protein kinase II.

Cardiac cells express the cytoplasmic (δ_C) and nuclear (δ_B) isoforms of CaMKII. Channels located in the sarcolemma are regulated by the cytoplasmic isoform, CaMKIIδ_C.[65,78,79] Active CaMKII phosphorylates sodium channels on three amino acid residues.[80] The role of phosphorylation of these individual sites in channel gating is not completely understood, because of contradictory results. Most results show the SSI curve shifted towards negative voltages[81–83] (but positive shifts have also been reported[84]). The voltage dependence of I_{Na} activation is typically unaltered by CaMKII,[81,82,84,85] but negative shifts have also been seen.[86] Peak $I_{Na,early}$ is typically unaltered (if fully available),[81–83] but increased $I_{Na,early}$ has also been reported.[84] Importantly, there is general agreement among all these studies: that CaMKII increases $I_{Na,late}$. The association between CaMKII activity and an increased $I_{Na,late}$ was confirmed by other authors as well.[68,87]

Modulation by β-adrenergic stimulation and protein kinase C

Stimulating the β-adrenergic pathway under most experimental settings consistently increased early I_{Na}. The effect seems to be mediated both by protein kinase A (PKA) and the stimulatory G protein subunit-α ($G_s\alpha$). PKA-dependent phosphorylation of Na^+ channels facilitates transport from the endoplasmic reticulum to the cell surface,[88] whereas $G_s\alpha$ exerts its effect by enhancing caveolar-associated Na^+ channel availability.[89]

Under protein kinase C (PKC) activation, Na^+ channels are trafficked away from the plasma membrane. Removal of the conserved PKC phosphorylation site S1503 from the channels or inhibition of reactive oxygen species (ROS) production eliminates the PKC-mediated effect, indicating that both channel phosphorylation and ROS are required to decrease the number of channels in the plasma membrane.

It is tempting to conclude that there is a direct correlation between the number of sodium channels in the sarcolemma and the magnitude of $I_{Na,late}$. However, there are no direct observations yet regarding the role of β-adrenergic pathway or PKC in modulating $I_{Na,late}$.

Oxido-reductive state and cellular metabolites

Cardiac sodium channels are sensitive to the redox state of their environment, and they are also modulated by various metabolites. The presence of hydrogen peroxide and ROS increase $I_{Na,late}$.[90–92] It has been shown that ROS can directly activate CaMKII[93]; therefore, CaMKII can be involved in $I_{Na,late}$ facilitation in the presence of oxygen-free radicals.[94] For a recent review on this topic, see the work of Wagner *et al.*[95]

The sodium channel is known to be modulated by acidosis.[96–99] An increased window current, and a shift of voltage dependency of activation and inactivation towards positive voltages were observed in Xenopus expression system.[97–99] In isolated canine ventricular myocytes, a rightward shift in voltage dependency of activation but not in SSI has been shown resulting in a reduction of $I_{Na,late}$.[96]

Hypoxia has been shown to increase $I_{Na,late}$ by many investigators.[100–105] Following 15 min of hypoxia, Wang *et al.* found increased BM activity that may explain the increased $I_{Na,late}$.

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Intermediary lipid metabolites, such as lysophosphatidylcholine and palmitoylcarnitine, increase $I_{Na,late}$. Lysophosphatidylcholine-treated sodium channels exhibit sustained bursting activity,[106,107] whereas palmitoylcarnitine induces a slowly inactivating sodium current.[108] More recent findings show that polyunsaturated fatty acids, such as docosahexaenoic and eicosapentaenoic acids, reduce both transient and late phase of I_{Na}.[109] The cause of this reduction seemed to be a decreased window voltage range.

Nitric oxide (NO) enhances $I_{Na,late}$.[110] Neural NO synthase (nNOS) is part of a macromolecular complex consisting of $Na_v1.5$, α1-syntrophin, and caveolin-3 among others.[111]

Other mechanisms

Transcriptional regulation. For the human SCN5A gene, *in vitro* promoter/reporter analyses revealed its potential transcriptional regulation.[112,113] However, transcription factors and their binding sites have not been determined yet, and involvement of these designated sequences in *in vivo* SCN5A gene regulation and expression also awaits confirmation.

Glycosylation. In the $Na_v1.5$ protein, there are several amino acid motifs subject to N-glycosylation. Sodium channels in the rat heart have an estimated carbohydrate mass of about 5%.[114] Removing the carbohydrate residues by enzymatic treatment[115] or using naturally sialic-acid deficient channels[116] showed that lack of channel glycosylation causes shifts towards positive voltages in both steady-state activation and inactivation.

Phosphorylation on tyrosine residues. Tyrosine residue Y1495, located in the III–IV linker domain, is the preferred phosphorylation site in human $Na_v1.5$ for the tyrosine kinase Fyn. This tyrosine residue is involved in anchoring of Ca^{2+}/CaM to the $Na_v1.5$ inactivation gate.[117] Phosphorylation on Y1495 shifts SSI towards more positive potentials[118] therefore increasing the window voltage range and thus enhancing $I_{Na,late}$.

Arginine methylation. Arginine residues R513, R526, and R680, located in the linker between domains I and II in $Na_v1.5$, are subject to methylation.[119] The functional relevance of this may be that $Na_v1.5$ mutations R526H and R680H cause Brugada syndrome and Type 3 long-QT syndrome (LQT3), respectively.

Mechanosensitivity. Several voltage-gated ion channels also show gating alterations as a response to mechanical stimuli. Beyder *et al.* investigated the mechanosensitivity of $Na_v1.5$ both in an expression system[120] and in isolated mouse ventricular cells.[121] The latter publication demonstrated that the pressure ramp they applied increased the $Na_v1.5$ late open-channel events by 235%, allowing for the conclusion that mechanical stress enhances $I_{Na,late}$.

Mechanical stress can also activate different signal transduction pathways such as nNOS and CaMKII,[122] which can indirectly enhance $I_{Na,late}$.

Sodium homeostasis and the plateau sodium current in heart failure

The pathophysiology of heart failure (HF) is underlain by a large network of intracellular mechanisms. The exact mechanisms differ depending on aetiology, stage, and comorbidities. Despite the broad pathophysiological spectrum, there are three main cellular features common in all types of HF: (1) alterations in excitation–contraction coupling leading to contractile dysfunction, (2) electrophysiological changes increasing the probability of arrhythmias, and (3) impaired energetics of the cardiomyocytes. Dysregulation of $[Na^+]_i$ homeostasis is likely involved in all of these (*Figure* 1).

$[Na^+]_i$ is increased in heart failure

In myocytes from failing hearts, $[Na^+]_i$ is increased by 2–6 mM compared with non-failing myocytes.[123–126] In a rabbit HF model, induced by pressure-overload and volume-overload, an increased TTX-sensitive Na^+ influx has been shown.[127] This increased influx was present both in resting (!) and in electrically stimulated myocytes. The exact mechanism of the TTX-sensitive influx is not completely clear in resting myocytes. In paced cells, however, it is likely to be caused by an enhanced sustained plateau Na^+ current ($I_{Na,late}$).

Contribution of $I_{Na,late}$ to the elevated $[Na^+]_i$ in heart failure

Increased $I_{Na,late}$ was found in cardiomyocytes of patients suffering from end-stage HF[14,32] in animal models of HF[14,17] and following myocardial infarction.[128] There are several pathophysiologic factors in HF that can contribute to the larger $I_{Na,late}$, such as hypoxia,[101,105] oxidative stress (ROS,[90,92] NO[110] mainly by S-nitrosylation of the $Na_v1.5$ channels[111]), ischaemic metabolites (such as oxidized lipids),[107] and mechanical stress.[121] Up-regulation of the Ca^2–calmodulin–CaMKII signalling pathway is also a characteristic feature of HF[129] and has been shown to increase $I_{Na,late}$.[70,81,82,87] Oxidized CaMKII[94] is constitutively active; therefore, the effects of oxidative stress on $I_{Na,late}$ are partly due to the enhanced CaMKII-mediated $Na_v1.5$ phosphorylation. From the perspective of single channel events, enhanced $I_{Na,late}$ in HF is mediated by an increased number of BM and LSM openings in single $Na_V1.5$ channels.[12,130]

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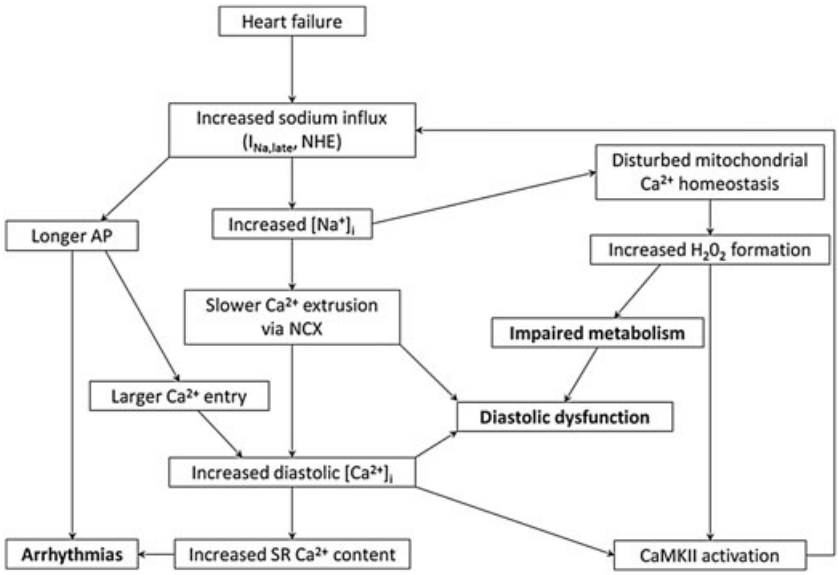

Figure 1. Dysregulation of the sodium homeostasis in heart failure: causes and consequences. AP, action potential; CaMKII, $Ca2^+$–CaM dependent protein kinase II; NCX, sodium–calcium exchanger; NHE, sodium–hydrogen exchanger; SR, sarcoplasmic reticulum.

Sarcolemmal exchange mechanisms in heart failure

Baartscheer *et al.* found that an increased Na^+ influx was mainly mediated by NHE in paced myocytes[131] isolated from failing rabbit hearts. In HF, an increased 'forward mode' activity of the NCX can also promote Na^+ entry because of the elevated diastolic $[Ca^{2+}]_i$ and the up-regulated NCX.[132]

Despite its reduced expression, NKA function does not seem to be altered in HF.[127,131] The more active NKA (compared with its expression level) is likely caused by the reduced relative expression and elevated phosphorylation of phospholemman.[133]

Excitation–contraction coupling and increased $[Na^+]_i$

In HF, the increased $[Na^+]_i$ shifts the NCX mechanism towards the reverse mode, resulting in slower Ca^{2+} removal from cardiac myocytes.[53,134,135] The slowed Ca^{2+} extrusion via NCX and also the reduced sarco-endoplasmic reticulum calcium ATPase (SERCA) function slow down the relaxation of the contractile filaments, therefore causing diastolic dysfunction as demonstrated in several models.[7,136] The slower $[Ca^{2+}]_i$ removal via NCX and the elevated diastolic $[Ca^{2+}]_i$ together will also tend to increase the sarcoplasmic reticulum (SR) Ca^{2+} content (in part limiting the impact of reduced SERCA function).

Arrhythmias and increased $[Na^+]_i$

The failing heart is prone to repolarization abnormalities and therefore arrhythmias. Moreover, an increased $I_{Na,late}$ causes a longer ventricular AP,[37,137] presented as a longer QT interval on the ECG. The longer AP and the increased variability of AP duration[14] increase the probability of early after-depolarizations (EADs), therefore increasing the risk for (fatal) ventricular arrhythmias.[30,138–143] In myocytes overloaded with Ca^{2+}, spontaneous SR Ca^{2+} release can occur,[144] generating delayed after-depolarizations (DADs). This mechanism further contributes to the higher probability of arrhythmias. An enhanced sustained sodium current in atrial cells can also cause atrial fibrillation.[145,146]

CaMKII–$I_{Na,late}$–$[Na^+]_i$–$[Ca^{2+}]_i$–CaMKII feedback

The $[Ca^{2+}]_i$ loading caused by elevated $[Na^+]_i$ in HF may further activate CaMKII (and enhance target phosphorylation), thus creating an arrhythmogenic vicious circle.[80] Yao *et al.* described this feedback where they used both pharmacological (anemone toxin II) and genetic (LQT3 mutation) approaches to increase $I_{Na,late}$ and achieve $[Na^+]_i$ loading.[147] These conditions increased $[Ca^{2+}]_i$ that activated CaMKII and therefore increased CaMKII-dependent phosphorylation of phospholamban (PLB) and ryanodine receptors (RyR) and, as a consequence, generated arrhythmias.

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Cellular energetics and increased $[Na^+]_i$

There is a dynamic balance between intracellular and mitochondrial Na^+ and Ca^{2+} concentrations, regulated by the mitochondrial NCX. Mitochondrial Ca^{2+} regulates oxidative phosphorylation and therefore production of both adenosine triphosphate (ATP) and ROS.[148] Elevated $[Na^+]_i$ in HF impairs mitochondrial Ca^{2+} accumulation at high pacing rates, therefore decreasing NADH/NAD^+ redox potential. This will increase H_2O_2 formation in myocytes from failing hearts, negatively affecting cardiac metabolism during rapid pacing.[8] The oxidative stress caused by enhanced H_2O_2 production will activate $I_{Na,late}$ (both directly and through CaMKII activation[93]) therefore further increasing $[Na^+]_i$.[94] This also suggests that ROS production, such as elevated $[Na^+]_i$, can be a cause and also a result of CaMKII activation.

$I_{Na,late}$ as a therapeutic target

Most $Na_v1.5$ inhibitors affect $I_{Na,early}$ and $I_{Na,late}$ in the same fashion, reducing excitability and impulse propagation together with the plateau sodium current. Ranolazine differs from the 'classic' inhibitors, because it is selective to $I_{Na,late}$ (IC_{50} values are around 6 μM for $I_{Na,late}$[149,150] and 244 μM for $I_{Na,early}$[150] in canine ventricular myocytes), even though the molecular mechanism of the selectivity is still not clear. At clinically relevant concentrations, ranolazine also inhibits other ionic currents, such as I_{Kr} (about 40% reduction at 6 μM) and $I_{Ca,L}$ (about 25% reduction at 6 μM).[149] This means that application of ranolazine and inhibiting $I_{Na,late}$ are not identical concepts. When $I_{Na,late}$ inhibition is achieved by ranolazine, its effects on other ion channels always have to be kept in mind. Ranolazine also inhibits fatty acid oxidation[151]; however, this effect can be observed only at concentrations much higher than the therapeutic plasma levels.

Although in the vast majority of experimental and clinical studies that ranolazine is used, there are two recently developed selective $I_{Na,late}$ inhibitors: F15845 and GS-458967. These compounds are under experimental investigation, with promising early results as potential anti-ischaemic[152] and anti-arrhythmic[153,154] drug candidates.

The role of $I_{Na,late}$ inhibitors in atrial and ventricular arrhythmias are beyond the scope of this work. For recent reviews on these topics, see the work of Antzelevitch *et al.*,[6] Maier *et al.*,[3] and Burashnikov *et al.*[52]

Experimental pathophysiology studies

Myocardial ischaemia.

Ranolazine decreases elevated $[Na^+]_i$ caused by simulated myocardial ischaemia[155] and ischaemic metabolites such as ROS.[92] F15845 also reduces $[Na^+]_i$ overload during no-flow ischaemia in isolated mouse hearts.[156]

Because of the reduction of $[Na^+]_i$ loading, Ca^{2+} elimination through NCX is normalized. This may also improve myocardial diastolic function,[90,157] therefore reducing diastolic wall tension. The reduced wall tension improves perfusion in the left ventricular (LV) wall, restoring the myocardial oxygen supply, and this could account for the anti-ischaemic effect of $I_{Na,late}$ inhibitors. This was confirmed in a clinical study including 20 patients with coronary artery disease.[158] Short-term ranolazine treatment reduced the size of reversible perfusion defects [evaluated by single-photon emission computed tomography (SPECT) imaging] and increased the average exercise time during treadmill exercise testing.

In the presence of lower $[Ca^{2+}]_i$, myofilaments will hydrolyze less ATP therefore the myocardial oxygen demand is expected to decrease. This was confirmed in isolated perfused rabbit hearts subjected to ischaemia and reperfusion.[159] Ranolazine reduced the increase in tissue $[Ca^{2+}]_i$ content and largely inhibited the decrease in ATP content. In the presence of ranolazine, the improved perfusion and the lower oxygen consumption together restore the myocardial demand–supply balance.

Myocardial function in non-failing experimental models.

In several non-HF animal models, ranolazine reversed diastolic dysfunction (at least partially) during ischaemia and reperfusion,[159,160] in the presence of ischaemic metabolites such as ROS[92] or palmitoylcarnitine,[161] in induced hypertension[162] and in spontaneously hypertensive rats.[163] Interestingly, Lovelock *et al.*[162] suggest that ranolazine can directly modulate myofilament calcium sensitivity besides the well-established ion channel effects.

Heart failure.

Similar beneficial effects of ranolazine were observed in HF animal models too. In a murine CaMKII overexpressing model,[7] ranolazine normalized the increased diastolic tension in papillary muscles. In a sequential intracoronary embolization-induced canine HF model, acute administration of ranolazine increased LV mechanical efficiency, without affecting myocardial oxygen consumption or free fatty acid uptake.[164] In the same model, ranolazine increased LV ejection fraction (EF) and stroke volume and decreased the elevated end-diastolic pressure.[165] Furthermore, these effects were not seen in control animals.

Long-term ranolazine treatment has also been tested in this canine model. Ranolazine was applied for 3 months, alone, and also in combination with metoprolol or enalapril.[157] Treatment with ranolazine increased LV EF, improved LV end-systolic volume and end-diastolic volume relative to placebo, and prevented progressive LV enlargement. Ranolazine also attenuated LV remodelling at the cellular level by reducing fibrosis and cardiomyocyte hypertrophy, and normalizing protein expression levels of key calcium homeostasis proteins (NCX, 'GATA' motif binding transcription

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factor 4, SERCA). Combining ranolazine with either metoprolol or enalapril improved LV function and prevented LV remodelling even further than ranolazine alone.

Human experimental data are also available. Trabeculae isolated from failing human hearts showed a frequency-dependent increase in diastolic tension.[90] Ranolazine reduced the diastolic tension of these muscle strips. In hypertrophic cardiomyopathy (HCM), Coppini *et al.* found prolonged AP, occurrence of EADs and DADs, impaired $[Ca^{2+}]_i$ handling, and increased diastolic $[Ca^{2+}]_i$ in cardiomyocytes.[4] Ranolazine partially reversed these HCM-related abnormalities in cellular function. They also reported an increased autophosphorylation level of CaMKII and enhanced phosphorylation of its downstream targets, such as L-type calcium channel, RyR, PLB, and $Na_v1.5$. These findings of Coppini *et al.* raise the question again: how a mutant sarcomeric protein causes such a complex remodelling process involving CaMKII and myriads of ion channel and calcium handling proteins.

Clinical studies

Myocardial ischaemia.

Among $I_{Na,late}$ blockers, clinical studies have been conducted only with ranolazine so far. The first Phase 2 clinical trials with this agent were conducted in the early 1990s with favourable results.[166,167] Ranolazine was approved by the Food and Drug Administration as an anti-anginal medication in 2006 following the outcome of the MARISA,[168] CARISA,[169] and ERICA[170] clinical trials. In these trials, ranolazine in monotherapy or in combination with other drugs increased exercise duration, lengthened exercise time until angina or until significant ST-depression, and reduced the number of angina episodes as well as nitroglycerin consumption. Ranolazine administration also resulted in a significantly longer exercise time when compared with atenolol.[171] This finding is likely underlain by the fact that ranolazine does not decrease the rate-pressure product, an unfavourable haemodynamic effect caused by many anti-ischaemic drug.

The MERLIN-TIMI-36 trial investigated the effect of clinical outcome and safety of ranolazine therapy in more than 6500 patients with non-ST-elevation acute coronary syndrome (ACS).[172] Ranolazine did not significantly reduce cardiovascular death or myocardial infarction compared with standard therapy; however, a significant reduction in recurrent ischaemia[172] and significantly lower incidence of arrhythmias[173] were reported. These results did not support the role of ranolazine in the short-term management of ACS but demonstrated the safety and efficacy of ranolazine as an anti-anginal agent. Recently, a new Phase 3 trial (RIVER-PCI)[174] started to evaluate the efficacy of ranolazine in patients with a history of chronic angina and incomplete revascularization after percutaneous coronary intervention.

Ranolazine improves myocardial function in non-heart failure patients.

In a small study involving 15 patients with previous transmural myocardial infarction, LV hemodynamic and angiographic data were obtained and analysed before and after intravenous infusion of ranolazine.[175] In these patients, ranolazine improved diastolic function of the *non-infarcted* ischaemic myocardial segments. In a study by Moss *et al.*,[176] LQT3 patients received ranolazine intravenously, which shortened the LV isovolumetric relaxation and accelerated the early filling phase.

In patients with stable angina, an echocardiographic study investigated the effect of oral ranolazine.[177] The patients had normal ejection fraction and slightly impaired diastolic function at the time of enrollment. After 2 months of treatment, ranolazine decreased isovolumetric contraction and relaxation time, and increased ejection time. In patients with coronary artery disease, an exercise or vasodilator stress test combined with SPECT imaging showed that ranolazine improved systolic and diastolic LV synchrony after 4 weeks of treatment.[178]

Heart failure.

Ischaemic heart disease is the most frequent cause of HF, and anti-ischaemic drugs can have potentially beneficial effects in HF as well. On the basis of these, testing the efficacy of ranolazine in HF patients seems to be a logical choice. Despite the large and growing volume of promising experimental data and favourable results in non-HF patients, only a few clinical studies have been investigated the effect of ranolazine in HF.

A subgroup analysis of the MERLIN-TIMI 36 trial shows that patients with higher than 80 pg/mL brain natriuretic peptide (BNP) levels ranolazine significantly reduced the primary end point (21% relative risk reduction (RRR) compared with placebo, $P = 0.009$).[179] This was mainly due to a decrease in recurrent ischaemia (22% RRR, $P = 0.036$); however, similar trends were observed with regard to myocardial infarction (21% RRR, $P = 0.1$) and cardiovascular death (13% RRR, $P = 0.39$). It is worth to mention that ranolazine had no effect in individuals with BNP levels lower than 80 pg/mL. However, ranolazine therapy did not significantly reduce BNP levels or the incidence of new or worsening HF. There were about 800 HF patients involved in the MERLIN-TIMI 36 trial, so maybe, a trial with greater statistical power concentrating on HF patients can prove the usefulness of ranolazine in HF.

The RALI-DHF trial was a prospective, randomized, double-blind, placebo-controlled study involving 20 patients with HF with preserved ejection fraction (HFpEF, 'diastolic heart failure').[180] This small study was focusing on haemodynamic parameters, exercise testing, and echocardiographic evaluation. After 30 min of acute ranolazine administration slightly improved haemodynamic parameters (e.g. pulmonary

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capillary wedge pressure), but there was no improvement in relaxation parameters. Ranolazine had no effect on exercise testing parameters, on echocardiographic data, or on laboratory findings including NT-proBNP. The RALI-DHF study has several limitations. Its small sample size, considering the heterogeneity of HFpEF, is not suitable to provide a solid base for Phase 3 studies. In addition, echocardiographic evaluation after only 2 weeks of oral therapy was too early and may have prevented the investigators from identifying potential longer term effects of ranolazine in HFpEF.[181] Furthermore, short-term hemodynamic parameters and long-term clinical endpoints do not correlate, which further complicates the interpretation of the RALI-DHF study.

There is an ongoing multi-centre, double-blind, placebo-controlled Phase 2 clinical study, RESTYLE-HCM (EudraCT Number: 2011-004507-20) investigating the efficacy of oral ranolazine in patients with symptomatic HCM. The study evaluates ranolazine with respect to exercise capacity, diastolic function, symptomatic status, and proBNP levels together with drug safety assessment.

On the basis of the evidence earlier, ranolazine may have therapeutic role in the treatment of systolic and diastolic HF in the future; however, further clinical investigations are necessary for this.[5,182]

Conclusions

The $I_{Na,late}$ plays an important pathophysiological role in myocardial ischaemia and HF. Several cellular mechanisms involved in these myocardial diseases can increase $I_{Na,late}$. An up-regulated $I_{Na,late}$ prolongs the cardiac AP and increases intracellular sodium concentration causing Ca^{2+} overload of cardiomyocytes. These factors together can lead to cardiac arrhythmias, contractile dysfunction, and impaired energetics of the heart.

Ranolazine is the only approved pharmacological agent targeting $I_{Na,late}$ although its effects on other ionic currents are also significant. On the basis of large clinical studies, ranolazine has been an effective and safe anti-anginal medication since 2006, and more recent evidence also shows its beneficial effects in atrial and ventricular arrhythmias. Experimental results also demonstrate that $I_{Na,late}$ inhibitors, especially ranolazine, exert favourable effects on myocardial diastolic (and to a smaller extent, systolic) function. So far, there are no evidence-based drugs to treat HFpEF patients, which is a very important therapeutic niche to fill. Therefore, further clinical trials are warranted to evaluate the efficacy and safety of $I_{Na,late}$ inhibition in patients with HFpEF.

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Declaration of interest statement

None declared.

List of abbreviations*

$[Ca^{2+}]_i$: intracellular calcium concentration
$[Na^+]_i$: intracellular sodium concentration
ACS: acute coronary syndrome
AP: action potential
BM: burst mode gating
BNP: brain natriuretic peptide
CaM: calmodulin
CaMKII: Ca^{2+}–CaM dependent protein kinase II
DAD: delayed after-depolarization
EAD: early after-depolarization
ECG: electrocardiography; electrocardiographic
EF: ejection fraction
$G_s\alpha$: stimulatory G protein subunit-α
HCM: hypertrophic cardiomyopathy
HF: heart failure
HFpEF: heart failure with preserved ejection fraction
IC_{50}: half inhibitory concentration
$I_{Ca,L}$: L-type calcium current
I_{Kr}: rapid component of the delayed rectifier potassium current
$I_{Na,early}$: early (peak) sodium current
$I_{Na,late}$: late sodium current
LQT3: Type 3 long-QT syndrome
LSM: late-scattered mode gating
LV: left ventricle; left ventricular
$Na_v1.5$: voltage-gated sodium channel 1.5
NBC: sodium bicarbonate cotransporter
NCX: sodium–calcium exchanger
NHE: sodium–hydrogen exchanger
NKA: sodium–potassium ATPase
nNOS: neural nitric oxide synthase
NO: nitric oxide
PKA: protein kinase A
PKC: protein kinase C
PLB: phospholamban
ROS: reactive oxygen species
RRR: relative risk reduction
RyR: ryanodine receptor
SCNA5: gene encoding the α-subunit of the voltage-gated sodium channel 1.5
SERCA: sarcoplasmic reticulum calcium ATPase
SPECT: single-photon emission computed tomography
SR: sarcoplasmic reticulum
SSI: steady-state inactivation
TM: transient mode gating
TTX: tetrodotoxin
*Abbreviations of clinical trials are not included

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