The Late Na$^+$ Current - Origin and Pathophysiological Relevance

Antonio Zaza · Marcella Rocchetti

In excitable tissues, voltage-dependent Na$^+$ current (I_{Na}) is best known for supporting autoregenerative depolarization and impulse propagation. Its transient component (I_{NaT}), which is large and terminated within several milliseconds by channel inactivation, fulfils this role. Nevertheless, I_{Na} also includes a smaller sustained component, i.e. one persisting during prolonged membrane depolarization, which contributes to repolarization course. Sustained I_{Na} implies slow or incomplete inactivation of a proportion of the Na$^+$ channels activated during the action potential upstroke. Several mechanisms may underlie this phenomenon and contribute to arrhythmogenesis in different conditions.

Sustained Na$^+$ Currents

Window Currents

Truly steady-state currents may flow through otherwise inactivating channels if membrane potential is maintained in a restricted range ("window"). These currents are commonly referred to as "window currents". The window corresponds to the region of overlap between "activation" and "inactivation" curves, in which the probabilities of channels to be open and not inactivated are both greater than 0 at steady-state. A window is normally present in cardiac myocytes for I_{CaL} and, to a lesser extent for I_{Na} (Fig. 1), but it may be enhanced, or shifted to different potentials, by channel mutations (e.g. the Nav1.5 ΔKPQ mutation) [1].

When, during repolarization, membrane potential moves into the "window", a fraction of channels may recover from inactivation and immediately reactivate. The resulting depolarization supports autoregenerative activation which, albeit concerning a small fraction of total channels, may substantially distort membrane potential course (early afterdepolarizations, EADs) and even trigger propagated activity [2, 3]. The I_{Na} window is narrow and more negative than the plateau phase in normal ventricular myocytes; thus, in the latter I_{CaL} window more likely accounts for EADs generation [4] (Fig. 1). I_{Na} window, somewhat more prominent in normal Purkinje myocytes [5, 6], may be widened in all cell types by Na$^+$ channel mutations.

A further mechanism of I_{Na} enhancement during repolarization is provided by an increase in the rate of channel recovery from inactivation relative to deactivation one. This mechanism, observed in some NaV1.5 mutations, is referred to as "reactivation under non-equilibrium conditions" [7]. This is because the excess Na$^+$ current can be observed only during dynamic changes of membrane potential (non-equilibrium). This is not a proper steady-state component and, notably, it can escape detection by the standard "voltage step" clamp protocols used to assess the functional phenotype of channel mutations.

Late Na$^+$ Current (I_{NaL})

Figure 2a shows that sustained I_{Na}, identified as current sensitive to blockade by tetrodotoxin (TTX), exists also at

A. Zaza · M. Rocchetti
Dipartimento di Biotecnologie e Bioscienze,
Università degli Studi Milano-Bicocca, Milano, Italy

A. Zaza (✉)
Dipartimento di Biotecnologie e Bioscienze,
Università di Milano-Bicocca, Piazza della Scienza 2, room U3-3013,
20126 Milano, Italy
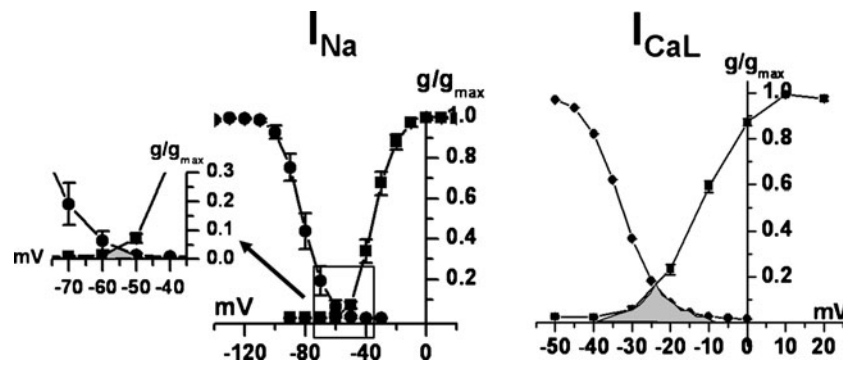e-mail: antonio.zaza@unimib.it

Fig. 1 Comparison of the membrane potential "window" for I_{Na} and I_{CaL} in rat ventricular myocytes. Steady-state activation and inactivation curves for I_{Na} (*left*) and I_{CaL} (*right*). The "window", i.e. the membrane potential range in which activation and inactivation overlap, is filled in grey. I_{Na} window (zoomed in the inset) is smaller and at more negative potentials than I_{CaL} one. (from Rocchetti et al. unpublished)

potentials positive to the I_{Na} window (−65 to −50 mV; shown in in Fig. 1a for the same cell type). This current is provided by a component named "late" Na^+ current (I_{NaL}). I_{NaL}, much smaller than I_{NaT} in normal ventricular myocytes, may be enhanced by more than 3-fold under pathological conditions [8]. Single-channel analysis reveals that the channel gating underlying I_{NaL} is complex and, in addition to late "scattered" openings, it includes a "burst mode" not observed during the transient component of I_{Na} (I_{NaT}) [9, 10]. This led to hypothesize that I_{NaL} might be carried by channel variants other than the one prevailing in cardiac myocytes (NaV1.5), whose expression would be enhanced under pathological conditions (a contribution of NaV1.8 channels to I_{NaL} in mouse and rabbit cardiomyocytes has been indeed reported [11]). However, I_{NaL} with all its gating modes, can be detected by expression of Nav1.5 channels in cell lines (Fig. 2b), where no other Na^+ channel isoforms are present [10, 12]. This proves that I_{NaL} can be carried by the same molecular entity accounting for I_{NaT}; according to this view, I_{NaL} enhancement reflects a gating abnormality. Observations crucial for the development of this interpretation came from single channel analysis of a mutation (ΔKPQ) associated with LQT3 syndrome [13]. The mutant channel had increased probability of burst openings during sustained depolarization, a behaviour interpreted as instability of the inactivated state [1, 13]. The interpretation of I_{NaL} enhancement as a gating abnormality of Nav1.5 channels may hold true also for acquired conditions, as proven in the case of heart failure [14–16]. Nevertheless, we cannot rule out that changes in isoform expression may contribute in some of the diverse conditions in which I_{NaL} enhancement occurs.

Because of recovery from inactivation upon repolarization takes some time, I_{NaT} availability depends on the interval between excitations. This is true also for I_{NaL}, thus
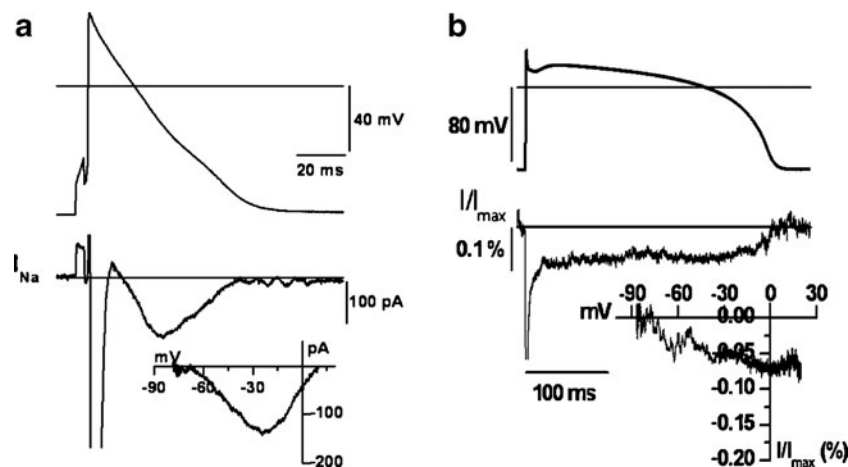

Fig. 2 TTX-sensitive current (*lower panels*, largely representative of I_{Na}) elicited by clamping the membrane with the action potential waveform (*upper panel*) (AP-clamp); the inset in each panel shows the dynamic I/V relation, obtained by plotting current as a function of membrane potential. TTX-sensitive current flowing during repolarization includes all sustained Na^+ components. **a** recording in a rat myocyte, clamped with its own action potential (Rocchetti et al. unpublished); **b** recording in a cell line (HEK239) transfected with NaV1.5 (α and β subunits) and clamped with a human ventricular action potential (from ref [12], modified). I_{NaT} is truncated in all recordings

conferring to this current "reverse" rate-dependency [17], which is partial at physiological rates (approx 50 % reduction from quiescence to 120 b/min) [18].

Mutations prolonging action potential duration (APD) cause enhancement of plateau Na^+ current through one or more of the mechanisms described above. For instance, all the above (widened voltage window, inactivation instability and accelerated recovery) may be operative in ΔKPQ mutants [1], but only non-equilibrium reactivation appears to account for the APD prolonging effect observed with the Il768V mutant [7].

Conditions and Mechanisms of I_{NaL} Enhancement

Although the interest in I_{NaL} pathophysiological role was mostly triggered by identification of Na^+ channel mutations leading to arrhythmogenic QT prolongation [1], secondary I_{NaL} enhancement occurs in association to a surprisingly large number of common disease states (reviewed in [19]), including cardiac hypertrophy/failure and ischemia, not necessarily related to each other in terms of primary pathogenesis. Such a pattern suggests that I_{NaL} enhancement may be a common response to cell stress/dysfunction. Accordingly, in cardiac myocytes, I_{NaL} is enhanced by reactive oxygen species (ROS) [20], whose generation is generally associated to cell distress. The question of whether ischemia can enhance I_{NaL} can be answered only indirectly, because membrane currents can be recorded only in isolated myocytes, which cannot be subjected to ischemia. Nevertheless, I_{NaL} is enhanced by myocyte exposure to hypoxia [21] or ischemic metabolites [22] and I_{NaL} blockade effectively prevents ischemia/reperfusion damage in the intact heart [23].

The search of a common mechanism, mediating I_{NaL} enhancement by cellular stress of heterogeneous etiology, has highlighted the role of Ca^{2+}-calmodulin kinase (CaMKIIδ) activation [24]. This is a Ca^{2+}- and ROS-activated cytosolic enzyme which may, among other targets, phosphorylates NaV1.5 channels. Converging evidence indicates that CaMKIIδ overexpression (or activation) enhances I_{NaL} and generates cardiac abnormalities that are reversed by I_{NaL} blockade [25]. Upstream components of the CaMKII activation pathway, i.e. calmodulin (CaM) and Ca^{2+} itself, have also been shown to enhance I_{NaL} directly, with differences between normal and failing myocytes [26]. While this does not rule out further modes of I_{NaL} enhancement (see ref [27]. for review), CaMKIIδ activation is particularly relevant because its relation with I_{NaL} may set up a vicious feed-back loop (Fig. 3), very likely to contribute to evolution of cell dysfunction and damage in response to stress. Whereas CaMKII inhibition is not available for therapeutic use yet, I_{NaL} blockade may break such a vicious loop [25].

To summarize, except for the case of primary (genetic) Na^+ channel defects, I_{NaL} enhancement might be viewed as
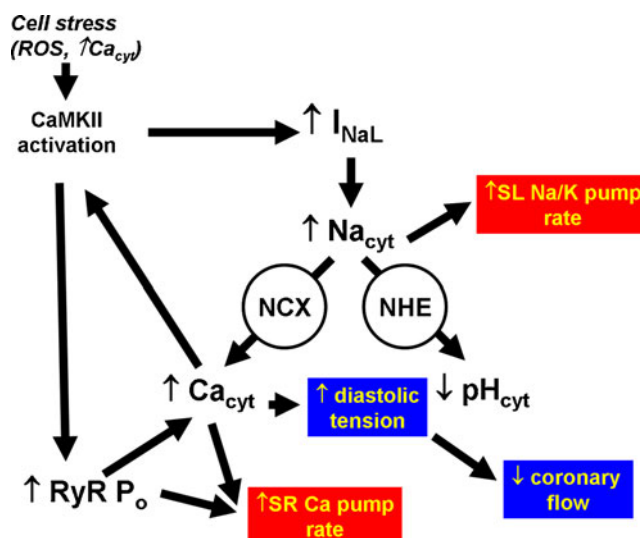

Fig. 3 Network of events linking I_{NaL} enhancements to its pleiotropic effects. Notably, many events are linked in positive feed back loops, likely to amplify and sustain the network once it has been initiated. Experimental observations (see text) suggest that I_{NaL} enhancement and CaMKII activation are key elements in this process. Events in *red boxes* imply increased ATP consumption, those in *blue boxes* reduced oxygen supply

a generic response to cellular stress, that is secondary in origin, but has a pivotal role in mediating functional derangements and disease progression.

I_{NaL} enhancement can also result from abnormalities in proteins other than the Na^+ channel itself, but interacting with it to form macromolecular complexes [28, 29]. For instance, mutations of scaffold and adaptor proteins, as ankyrin-B [30] and caveolin-3 [31] respectively, have been recently identified in LQT3 patients. A detailed review of the factors potentially contributing to I_{NaL} enhancement in heart failure has been provided by Matsev et al. [27].

I_{NaL} Impact on Electrical Activity and Ionic Homeostasis

I_{NaL} directly affects electrical activity (it promotes depolarization or counters repolarization) and provides a route of Na^+ influx. Considering the role of Na^+ gradient in transmembrane transport, the latter may affect cellular solute homeostasis, thus generating a host of indirect consequences of physiological and pathophysiological relevance.

Electrophysiological Effects

I_{NaL} flows during repolarization and may directly affect its course. In normal canine ventricles I_{NaL} is differentially expressed across the wall (M-cells and Purkinje cells > subendocardial cells > subepicardial cells) [17, 32, 33]. Thus, it is conceivably a player in the physiological transmural repolarization gradient and in its rate-dependency in

the dog (APD restitution) [34]. In normal ventricular myocytes, I_{NaL} inhibition by ranolazine (I_{Na} blocker with selectivity for I_{NaL} vs I_{NaT}) causes negligible APD changes. On the other hand, the remarkable effects of I_{Kr} blockade on APD, on its rate-dependency and, most importantly, on repolarization stability are all reversed by ranolazine [18, 35, 36]. These apparently contrasting findings may be reconciled by considering that ranolazine also partially blocks I_{Kr} [37]. Under basal conditions, this may offset the effect of I_{NaL} inhibition on APD; in turn, concomitant I_{NaL} inhibition limits the effect of I_{Kr} inhibition, thus preventing repolarization instability. If this interpretation is correct, we can conclude that I_{NaL} and I_{Kr} are physiologically in balance during normal repolarization; whenever this balance is altered, by either I_{NaL} enhancement or I_{Kr} blockade, repolarization stability is compromised. An extreme example of this condition is advanced heart failure, in which I_{NaL} enhancement and I_{Kr} downregulation coexist and are associated with dramatic repolarization instability [15]. Although the concept of I_{Kr} - I_{NaL} balance is valid in a broad sense, the effects of I_{Kr} blockade and I_{NaL} enhancement on action potential contour are not identical, an observation which may have its counterpart in the differences of clinical presentation between the LQT2 (I_{Kr} deficiency) and LQT3 (I_{NaL} enhancement) syndromes [38].

The direct contribution of I_{NaL} to repolarization course provides a first powerful mechanism linking arrhythmogenesis to I_{NaL} enhancement. Nevertheless, the latter may also facilitate arrhythmias through Ca^{2+} handling abnormalities (see below) and it is difficult to establish which mechanism prevails in a specific condition. The mutual interplay between Ca^{2+} handling and repolarization course [39] may actually make this distinction pointless.

A role of I_{NaL} in arrhythmogenesis is often inferred from the antiarrhythmic effect of its selective blocker ranolazine. While this may be considered legitimate in many cases, there are exceptions due to specificities of drug action. The best example is ranolazine efficacy on atrial arrhythmias, to which mechanisms other than I_{NaL} inhibition may also contribute [40].

Effects on Ionic Homeostasis

I_{Na} represents the main source of Na^+ entry during the cardiac cycle. Abeit I_{NaL} amplitude is normally 1/1000 of that of I_{NaT} [10], I_{NaL} persists throughout repolarization; as a result, I_{NaT} and I_{NaL} may similarly contribute to Na^+ influx during a cardiac cycle [41]. Nevertheless, I_{NaL} inhibition (by TTX or ranolazine) marginally affects Ca^{2+} cycling [42] and contractility [43] in normal hearts, thus suggesting that the attending changes in Na^+ influx are effectively buffered by matching changes in Na^+ extrusion. However, cellular homeostasis can be compromised by the marked increase in Na^+ influx resulting from pathological I_{NaL} enhancement.

Na^+ is normally extruded from the cell by the ATP powered Na^+/K^+ pump. Therefore excess Na^+ influx, even when successfully buffered, may increase ATP consumption. Furthermore, if influx exceeds the maximal extrusion rate, Na^+ accumulates in the cytosol, thus partially dissipating its transmembrane gradient. Because the latter energizes many secondary membrane transport mechanisms, most importantly the Na^+/Ca^{2+} exchanger (NCX) and the Na^+/H^+ exchanger (NHE), a pivotal consequence of I_{NaL} enhancement is perturbed homeostasis of intracellular Ca^{2+} and H^+ (Fig. 3).

NCX is the main mechanism of Ca^{2+} extrusion from the cell. Increased cytosolic Na^+ moves its electrochemical equilibrium potential in the negative direction, thus reducing the driving force for its forward operation during diastole and possibly reversing the direction of transport during systole (i.e. Ca^{2+} entry through NCX). The resulting increase in intracellular Ca^{2+} may re-establish NCX driving force, but the system equilibrium is now moved to higher cytosolic Ca^{2+} levels. Under conditions of I_{NaL} enhancement (e.g heart failure) NCX expression may be upregulated [44] and, provided that a driving force still exists, this may increase Ca^{2+} transport rate. However, the effect of this change in sustaining Ca^{2+} extrusion can only be partial, because it vanishes as NCX electrochemical equilibrium is approached. Accordingly, unless maximal Na^+/K^+ pump transport rate is also incremented, an increase in total cellular Ca^{2+} content is a necessary consequence of I_{NaL} enhancement. A further aspect of interest is the distribution of such an increment between subcellular compartments. Stimulation of Ca^{2+} uptake by the sarcoplasmic reticulum (SR) (by SERCA2) is a central component of physiological stimuli meant to increase cell Ca^{2+} content (e.g. by β-adrenergic activation). This ensures that most of the gain concerns SR luminal Ca^{2+}, which results in larger amplitude of Ca^{2+} transient (larger developed force) and lower diastolic Ca^{2+} (accelerated relaxation). This is not the case for I_{NaL} enhancement, in which the Ca^{2+} increment concerns primarily the cytosol, which may only secondarily increase Ca^{2+} in the SR. Although the latter may increase maximal developed force, this is expectedly associated with higher diastolic Ca^{2+}. The consequences of cytosolic Ca^{2+} being persistently elevated are multiple and of pathophysiological relevance.

Opening of SR Ca^{2+} release channels (RyRs) is directly triggered by cytosolic Ca^{2+}, a process facilitated by high Ca^{2+} in the SR lumen [45]. Increased cytosolic Ca^{2+} also activates CaMKII-dependent RyRs phosphorylation, a further mechanism of RyRs facilitation (see below, Fig. 3). It is therefore unsurprising that I_{NaL} enhancement may lead to facilitation of diastolic "Ca^{2+} waves" and the resulting electrical disturbances (delayed afterdepolarizations, DADs) [46]. This represents an important arrhythmogenic mechanism, probably the one prevailing under conditions of altered Ca^{2+} handling (e.g. heart failure).

With persistently elevated cytosolic Ca^{2+}, diastolic function may be hampered by delayed and incomplete sarcomere relaxation. A contribution of I_{NaL} to diastolic dysfunction has been demonstrated in various experimental models of heart failure [25, 42] and ranolazine improved diastolic relaxation in human ischemic heart disease [47]. In addition to its direct hemodynamic impact, diastolic dysfunction may limit coronary flow by extrinsic compression of intramural vessels. Significance of this effect is indirectly demonstrated by the ability of ranolazine to improve myocardial perfusion in the setting of coronary artery disease (likely I_{NaL} enhancement) [48]. Indeed, being ranolazine devoid of significant direct vasodilator effects, the perfusion improvement is likely to result from accelerated diastolic relaxation, a well known factor in modulation of coronary flow [49].

Abnormally elevated cytosolic Ca^{2+} may also activate a number of signalling pathways involved in modulation of cell function and, in the long run, structure and fate. CaMKIIδ activation, is at the same time, a cause (see above) and a consequence [50] of I_{NaL} enhancement. Phosphorylation of SR Ca^{2+} release channels (RyRs) by this kinase increases their open probability [51], ultimately leading to destabilization of the Ca^{2+} store. This accounts for the facilitation of spontaneous Ca^{2+} release events and delayed afterpotentials (DADs, their arrhythmogenic electrical consequence) induced by either I_{NaL} enhancement or CaMKIIδ overexpression [52] and suppressed in both cases by I_{NaL} blockade [25]. Calcineurin, a cytosolic Ca^{2+}-activated phosphatase, regulates translocation to the nucleus of NFAT, a transcriptional regulator centrally involved in hypertrophic remodelling [53]. While there is still no direct evidence for the involvement of this specific pathway in I_{NaL}-induced damage, ranolazine has been shown to affect downstream gene transcription and histomorphometric parameters in heart failure induced by coronary microembolization [54].

A further consequence of pathological I_{NaL} enhancement is a derangement in cell energy balance. The main ATP-consuming mechanism involved in the control of cellular homeostasis (the Na^+/K^+ and SERCA pumps) are conceivably short-circuited by enhanced sarcolemmal Na^+ influx and increased Ca^{2+} leak from the SR (by RyRs facilitation) (Fig. 3). Therefore, I_{NaL} enhancement is expected to increase ATP consumption by mechanisms not directly involved in force generation, which would translate into decreased mechanical efficiency. This view is supported by the observation that I_{NaL} blockade prevents the drop in myocardial ATP, but not the positive inotropic effect, of ouabain [55]. In addition to improved coronary perfusion, increased myocardial efficiency might partly account for the ability of I_{NaL} blockade to improve exercise capacity of ischemic patients without altering systolic mechanical work [56].

It has been reported that increased cytosolic Na^+ may also jeopardize mitochondrial function (Fig. 4). Concomitant cytosolic Na^+ loading (to 15 mM) impaired Ca^{2+}-triggered
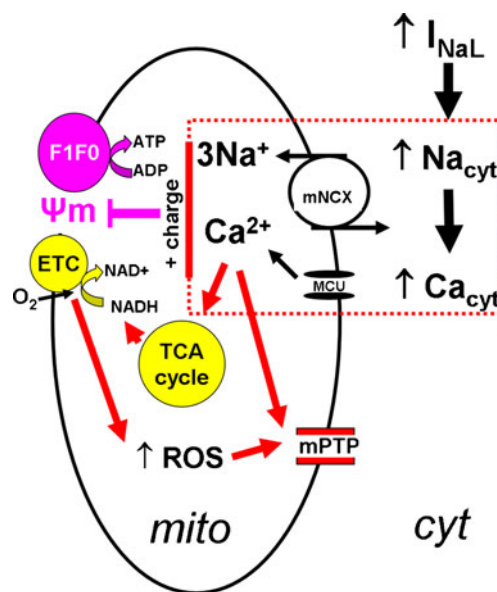

Fig. 4 A plausible sequence of events coupling I_{NaL} enhancement to mitochondrial damage. Functions primarily depressed upon I_{NaL} enhancement are in *purple*, those upregulated to compensate in *yellow*. Elevated cytosolic Ca^{2+} (Ca_{cyt}) loads the mitochondria through the Ca^{2+} uniporter (MCU) and stimulates NADH production by the tricarboxilic acids (TCA) cycle. Excess mitochondrial Ca^{2+} is partially removed by mitochondrial NCX (mNCX), driven by high cytosolic Na^+ [57]. This chain of events (in the *dotted box*) results in positive charge influx, which tends to dissipate the electrical gradient (Ψm) that drives ATP synthesis by the ATP-synthase (F1F0) [64]. The resulting drop in the ATP/ADP ratio is compensated by increasing the electron transport rate (by ETC), fuelled by NADH [57]. In this scheme NADH and ATP levels may be preserved up to a limit [57], but at the cost of increased substrate consumption and ROS production by ETC [43]. Concomitantly elevated mitochondrial Ca^{2+} and ROS facilitate opening of mPTP [43], eventually resulting in loss of membrane selectivity, swelling and disruption of the outer mitochondrial membrane. Release of cyrochrome C and other components of the outer membrane space triggers cell apoptosis [64]

mitochondrial energetic adaptation in patch-clamped myocytes, thereby causing an abnormal drop in NADH (i.e metabolic energy) upon catecholamine challenge [57]. On the other hand, ranolazine pre-treatment failed to modify NADH course during global ischemia/reperfusion in isolated hearts [43]. Notably, in the same preparation, ranolazine opposed ischemia-induced rise in cytosolic and mitochondrial Ca^{2+}; this was associated, as expected, with diminished ROS generation and delayed opening of mitochondrial permeability transition pore (mPTP) [43]. A plausible network of mechanisms in accord with these observations is illustrated in Fig. 4. Altogether, this preliminary evidence suggests that, during ischemia/reperfusion, I_{NaL} inhibition may be more effective in preserving integrity of mitochondria than in improving their function. Although ranolazine effects on energy metabolism can be accounted for by I_{NaL} inhibition, the drug has been also reported to inhibit fatty acid oxidation [58, 59], a further action potentially protecting mitochondria during metabolic stress.

However, this action was observed with drug dosage (60–200 mg/Kg/day) far from that required for the anti-ischemic effect in humans (10–15 mg/Kg/day).

Under normal conditions, cellular H^+ homeostasis may be less sensitive than Ca^{2+} homeostasis to I_{NaL} enhancement, because mechanisms other than NHE contribute to H^+ extrusion. During abrupt reperfusion following acute coronary occlusion, massive H^+ extrusion through NHE contributes to intracellular Na^+ loading, which explains why direct NHE inhibition paradoxically improves Ca^{2+} handling and limits myocardial injury [60]. Nevertheless, the situation might be different in the presence of chronic partial ischemia, when reduced H^+ export by NHE might lead to persistent intracellular acidosis, potentially contributing to impair force development and relaxation. Although awaiting experimental confirmation, this hypothesis is supported by the observation that the increment in intracellular Na^+ caused by Na^+/K^+ pump blockade does produce intracellular acidosis, which can be prevented by concomitant I_{NaL} blockade [55].

Functional Interaction Between I_{NaL} and K^+ Currents

Several observations suggest a complex interplay, with partly unexplained mechanisms, between I_{NaL} and K^+ currents during repolarization. Such an interplay may have consequences on electrophysiology and ionic homeostasis.

The impact on repolarization of blocking endogenous I_{NaL} in the presence of K^+ current inhibition (I_{Kr}, I_{to} or I_{K1}) [36] may look disproportionately large to the small magnitude of the inward current removed by I_{NaL} block. Although ranolazine, often used as the I_{NaL} blocker, shares the channel binding domain on HERG channels (carrying I_{Kr}) with E-4031, it cannot competitively displace E-4031 [36]. Moreover, ranolazine effects are largely mimicked by TTX, which does not bind to HERG channels. Therefore, this disproportion cannot be attributed to ancillary ranolazine properties and remains largely unexplained.

A further relevant aspect of I_{NaL} blockade is its ability to interfere with the intrinsic reverse rate-dependency of APD and of its amplification by K^+ channel blockers [18]. Because I_{NaL} decreases at faster rates (see above), I_{NaL} block shortens APD more at slower ones, thus dampening the intrinsic reverse rate-dependency of repolarization [18]. Rate-dependency of I_{NaL} may also account for the ability of I_{NaL} block to flatten "APD restitution" curves, an action strongly correlated with antifibrillatory effect [34].

Recent work on olfactory neurons highlights a structurally organized relationship between Na^+ channels and the Na^+-activated K^+ current (I_{KNa}) [61], whereby the latter is specifically activated by Na^+ influx through I_{NaL}. I_{KNa} activation may oppose I_{NaL}-induced membrane depolarization but, by doing this, it would increases the driving force for Na^+ influx. Thus, coupling to I_{KNa} might partially dissociate the effects of I_{NaL} enhancement on membrane potential from those on intracellular ionic homeostasis. I_{KNa} is expressed in (guinea-pig) ventricular myocytes [62] and contributes to regulate APD [63]. However, its structural association with cardiac I_{Na} channels and functional interaction with I_{NaL} enhancement has not been investigated thus far.

Conclusions

Although of small magnitude, endogenous I_{NaL} contributes in setting repolarization course of normal myocytes, being in a delicate balance with K^+ currents. Disruption of this balance may strongly affect repolarization and its stability. Furthermore, I_{NaL} enhancement, occurring in many pathological conditions as a general response to cell stress, can profoundly alter intracellular ionic homeostasis with consequences on contractile function, electrical stability and cell fate. Accordingly, I_{NaL} represents a therapeutic target with an expectedly pleiotropic effect, which is being gradually unveiled by experimental and clinical studies.

References

1. Wang DW, Yazawa K, George Jr AL, Bennett PB. Characterization of human cardiac Na+ channel mutations in the congenital long QT syndrome. Proc Natl Acad Sci U S A. 1996;93:13200–5.
2. January CT, Riddle JM. Early afterdepolarizations: mechanism of induction and block. A role for L-type Ca2+ current. Circ Res. 1989;64:977–90.
3. Zeng J, Rudy Y. Early afterdepolarizations in cardiac myocytes: mechanism and rate dependence. Biophys J. 1995;68:949–64.
4. Hirano Y, Moscucci A, January CT. Direct measurement of L-type Ca2+ window current in heart cells. Circ Res. 1992;70:445–55.
5. Attwell D, Cohen IS, Eisner DA, Ohba M, Ojeda C. The steady state TTX-sensitive ("window") sodium current in cardiac Purkinje fibres. Pflügers Arch. 1979;379:137–42.
6. Colatsky TJ. Mechanism of action of lidocaine and quinidine on action potential duration in rabbit cardiac Purkinje fibers. Effects on steady state Na+ currents? Circ Res. 1982;50:17–27.
7. Clancy CE, Tateyama M, Liu H, Wehrens XH, Kass RS. Non-equilibrium gating in cardiac Na+ channels: an original mechanism of arrhythmia. Circulation. 2003;107:2233–7.
8. Berecki G, Zegers JG, Bhuiyan ZA, Verkerk AO, Wilders R, van Ginneken AC. Long-QT syndrome-related sodium channel mutations probed by the dynamic action potential clamp technique. J Physiol. 2006;570:237–50.
9. Patlak JB, Ortiz M. Slow currents through single sodium channels of the adult rat heart. J Gen Physiol. 1985;86:89–104.
10. Maltsev VA, Undrovinas AI. A multi-modal composition of the late Na+ current in human ventricular cardiomyocytes. Cardiovasc Res. 2006;69:116–27.

11. Yang T, Atack TC, Stroud DM, Zhang W, Hall L, Roden DM. Blocking Scn10a channels in heart reduces late sodium current and is antiarrhythmic. Circ Res. 2012;111:322–32.

12. Marangoni S, Di Resta C, Rocchetti M, Barile L, Rizzetto R, Summa A, et al. A Brugada syndrome mutation (p.S216L) and its modulation by p.H558R polymorphism: standard and dynamic characterization. Cardiovasc Res. 2011;91:606–16.

13. Bennett PB, Yazawa K, Makita N, George ALJ. Molecular mechanism for an inherited cardiac arrhythmia. Nature. 1995;376:683–5.

14. Undrovinas AI, Maltsev VA, Kyle JW, Silverman N, Sabbah HN. Gating of the late Na+ channel in normal and failing human myocardium. J Mol Cell Cardiol. 2002;34:1477–89.

15. Maltsev VA, Sabbah HN, Higgins RS, Silverman N, Lesch M, Undrovinas AI. Novel, ultraslow inactivating sodium current in human ventricular cardiomyocytes. Circulation. 1998;98:2545–52.

16. Maltsev VA, Silverman N, Sabbah HN, Undrovinas AI. Chronic heart failure slows late sodium current in human and canine ventricular myocytes: implications for repolarization variability. Eur J Heart Fail. 2007;9:219–27.

17. Zygmunt AC, Eddlestone GT, Thomas GP, Nesterenko VV, Antzelevitch C. Larger late sodium conductance in M cells contributes to electrical heterogeneity in canine ventricle. Am J Physiol Heart Circ Physiol. 2001;281:H689–97.

18. Wu L, Ma J, Li H, Wang C, Grandi E, Zhang P, et al. Late sodium current contributes to the reverse rate-dependent effect of IKr inhibition on ventricular repolarization. Circulation. 2011;123:1713–20.

19. Zaza A, Belardinelli L, Shryock JC. Pathophysiology and pharmacology of the cardiac "late sodium current". Pharmacol Ther. 2008;119:326–39.

20. Ward CA, Giles WR. Ionic mechanism of the effects of hydrogen peroxide in rat ventricular myocytes. J Physiol. 1997;500 (Pt 3):631–42.

21. Ju YK, Saint DA, Gage PW. Hypoxia increases persistent sodium current in rat ventricular myocytes. J Physiol. 1996;497(Pt 2):337–47.

22. Wu J, Corr PB. Palmitoyl carnitine modifies sodium currents and induces transient inward current in ventricular myocytes. Am J Physiol. 1994;266:H1034–46.

23. Wang P, Fraser H, Lloyd SG, McVeigh JJ, Belardinelli L, Chatham JC. A comparison between ranolazine and CVT-4325, a novel inhibitor of fatty acid oxidation, on cardiac metabolism and left ventricular function in rat isolated perfused heart during ischemia and reperfusion. J Pharmacol Exp Ther. 2007;321:213–20.

24. Wagner S, Dybkova N, Rasenack EC, Jacobshagen C, Fabritz L, Kirchhof P, et al. Ca/calmodulin-dependent protein kinase II regulates cardiac Na channels. J Clin Invest. 2006;116:3127–38.

25. Sossalla S, Maurer U, Schotola H, Hartmann N, Didie M, Zimmermann WH, et al. Diastolic dysfunction and arrhythmias caused by overexpression of CaMKIIdelta(C) can be reversed by inhibition of late Na (+) current. Basic Res Cardiol. 2011;106:263–72.

26. Maltsev VA, Reznikov V, Undrovinas NA, Sabbah HN, Undrovinas A. Modulation of late sodium current by Ca2+, calmodulin, and CaMKII in normal and failing dog cardiomyocytes: similarities and differences. Am J Physiol Heart Circ Physiol. 2008;294:H1597–608.

27. Maltsev VA, Undrovinas A. Late sodium current in failing heart: friend or foe? Prog Biophys Mol Biol. 2008;96:421–51.

28. Abriel H, Kass RS. Regulation of the voltage-gated cardiac sodium channel Nav1.5 by interacting proteins. Trends Cardiovasc Med. 2005;15:35–40.

29. Meadows LS, Isom LL. Sodium channels as macromolecular complexes: implications for inherited arrhythmia syndromes. Cardiovasc Res. 2005;67:448–58.

30. Mohler PJ, Schott JJ, Gramolini AO, Dilly KW, Guatimosim S, duBell WH, et al. Ankyrin-B mutation causes type 4 long-QT cardiac arrhythmia and sudden cardiac death. Nature. 2003;421:634–9.

31. Vatta M, Ackerman MJ, Ye B, Makielski JC, Ughanze EE, Taylor EW, et al. Mutant caveolin-3 induces persistent late sodium current and is associated with long-QT syndrome. Circulation. 2006;114:2104–12.

32. Gintant GA, Datyner NB, Cohen IS. Slow inactivation of a tetrodotoxin-sensitive current in canine cardiac Purkinje fibers. Biophys J. 1984;45:509–12.

33. Vassalle M, Bocchi L, Du F. A slowly inactivating sodium current (INa2) in the plateau range in canine cardiac Purkinje single cells. Exp Physiol. 2007;92:161–73.

34. Morita N, Lee JH, Xie Y, Sovari A, Qu Z, Weiss JN, et al. Suppression of re-entrant and multifocal ventricular fibrillation by the late sodium current blocker ranolazine. J Am Coll Cardiol. 2011;57:366–75.

35. Wu L, Shryock JC, Song Y, Li Y, Antzelevitch C, Belardinelli L. Antiarrhythmic effects of ranolazine in a guinea pig in vitro model of long-QT syndrome. J Pharmacol Exp Ther. 2004;310:599–605.

36. Wu L, Rajamani S, Li H, January CT, Shryock JC, Belardinelli L. Reduction of repolarization reserve unmasks the proarrhythmic role of endogenous late Na(+) current in the heart. Am J Physiol Heart Circ Physiol. 2009;297:H1048–57.

37. Antzelevitch C, Belardinelli L, Zygmunt AC, Burashnikov A, Di Diego JM, Fish JM, et al. Electrophysiological effects of ranolazine, a novel antianginal agent with antiarrhythmic properties. Circulation. 2004;110:904–10.

38. Studenik CR, Zhou Z, January CT. Differences in action potential and early afterdepolarization properties in LQT2 and LQT3 models of long QT syndrome. Br J Pharmacol. 2001;132:85–92.

39. Wilson LD, Wan X, Rosenbaum DS. Cellular alternans: a mechanism linking calcium cycling proteins to cardiac arrhythmogenesis. Ann N Y Acad Sci. 2006;1080:216–34.

40. Antzelevitch C, Burashnikov A, Sicouri S, Belardinelli L. Electrophysiological basis for the antiarrhythmic actions of ranolazine. Heart Rhythm. 2011;8:1281–90.

41. Makielski JC, Farley AL. Na(+) current in human ventricle: implications for sodium loading and homeostasis. J Cardiovasc Electrophysiol. 2006;17 Suppl 1:S15–20.

42. Undrovinas NA, Maltsev VA, Belardinelli L, Sabbah HN, Undrovinas A. Late sodium current contributes to diastolic cell Ca2+ accumulation in chronic heart failure. J Physiol Sci. 2010;60:245–57.

43. Aldakkak M, Camara AK, Heisner JS, Yang M, Stowe DF. Ranolazine reduces Ca2+ overload and oxidative stress and improves mitochondrial integrity to protect against ischemia reperfusion injury in isolated hearts. Pharmacol Res. 2011;64:381–92.

44. Bers DM, Despa S, Bossuyt J. Regulation of Ca2+ and Na+ in normal and failing cardiac myocytes. Ann N Y Acad Sci. 2006;1080:165–77.

45. Gyorke S, Gyorke I, Lukyanenko V, Terentyev D, Viatchenko-Karpinski S, Wiesner TF. Regulation of sarcoplasmic reticulum calcium release by luminal calcium in cardiac muscle. Front Biosci. 2002;7:d1454–63.

46. Fredj S, Lindegger N, Sampson KJ, Carmeliet P, Kass RS. Altered Na+ channels promote pause-induced spontaneous diastolic activity in long QT syndrome type 3 myocytes. Circ Res. 2006;99:1225–32.

47. Figueredo VM, Pressman GS, Romero-Corral A, Murdock E, Holderbach P, Morris DL. Improvement in left ventricular systolic and diastolic performance during ranolazine treatment in patients with stable angina. J Cardiovasc Pharmacol Ther. 2010.

48. Venkataraman R, Belardinelli L, Blackburn B, Heo J, Iskandrian AE. A study of the effects of ranolazine using automated quantitative analysis of serial myocardial perfusion images. JACC Cardiovasc Imaging. 2009;2:1301–9.

49. Duncker DJ, Bache RJ. Regulation of coronary blood flow during exercise. Physiol Rev. 2008;88:1009–86.

50. Yao L, Fan P, Jiang Z, Viatchenko-Karpinski S, Wu Y, Kornyeyev D, et al. Nav1.5-dependent persistent Na+ influx activates CaMKII in rat ventricular myocytes and N1325S mice. Am J Physiol Cell Physiol. 2011;301:C577–86.

51. Maier LS, Bers DM. Role of Ca2+/calmodulin-dependent protein kinase (CaMK) in excitation-contraction coupling in the heart. Cardiovasc Res. 2007;73:631–40.

52. Maier LS, Zhang T, Chen L, DeSantiago J, Brown JH, Bers DM. Transgenic CaMKIIdeltaC overexpression uniquely alters cardiac myocyte Ca2+ handling: reduced SR Ca2+ load and activated SR Ca2+ release. Circ Res. 2003;92:904–11.

53. Heineke J, Molkentin JD. Regulation of cardiac hypertrophy by intracellular signalling pathways. Nat Rev Mol Cell Biol. 2006;7:589–600.

54. Rastogi S, Sharov VG, Mishra S, Gupta RC, Blackburn B, Belardinelli L, et al. Ranolazine combined with enalapril or metoprolol prevents progressive LV dysfunction and remodeling in dogs with moderate heart failure. Am J Physiol Heart Circ Physiol. 2008;295:H2149–55.

55. Hoyer K, Song Y, Wang D, Phan D, Balschi J, Ingwall JS, et al. Reducing the late sodium current improves cardiac function during sodium pump inhibition by ouabain. J Pharmacol Exp Ther. 2011;337:513–23.

56. Stone PH, Chaitman BR, Stocke K, Sano J, DeVault A, Koch GG. The anti-ischemic mechanism of action of ranolazine in stable ischemic heart disease. J Am Coll Cardiol. 2010;56:934–42.

57. Maack C, Cortassa S, Aon MA, Ganesan AN, Liu T, O'Rourke B. Elevated cytosolic Na+ decreases mitochondrial Ca2+ uptake during excitation-contraction coupling and impairs energetic adaptation in cardiac myocytes. Circ Res. 2006;99:172–82.

58. Abdalla S, Fu X, Elzahwy SS, Klaetschke K, Streichert T, Quitterer U. Up-regulation of the cardiac lipid metabolism at the onset of heart failure. Cardiovasc Hematol Agents Med Chem. 2011;9:190–206.

59. Fang YH, Piao L, Hong Z, Toth PT, Marsboom G, Bache-Wiig P, et al. Therapeutic inhibition of fatty acid oxidation in right ventricular hypertrophy: exploiting Randle's cycle. J Mol Med (Berl). 2012;90:31–43.

60. Vaughan-Jones RD, Spitzer KW, Swietach P. Intracellular pH regulation in heart. J Mol Cell Cardiol. 2009;46:318–31.

61. Hage TA, Salkoff L. Sodium-activated potassium channels are functionally coupled to persistent sodium currents. J Neurosci. 2012;32:2714–21.

62. Luk HN, Carmeliet E. Na(+)-activated K+ current in cardiac cells: rectification, open probability, block and role in digitalis toxicity. Pflugers Arch. 1990;416:766–8.

63. Rodrigo GC, Chapman RA. A sodium-activated potassium current in intact ventricular myocytes isolated from the guinea-pig heart. Exp Physiol. 1990;75:839–42.

64. Murgia M, Giorgi C, Pinton P, Rizzuto R. Controlling metabolism and cell death: at the heart of mitochondrial calcium signalling. J Mol Cell Cardiol. 2009;46:781–8.