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The Role of Cellular Sodium and Calcium Loading in Cardiac Energetics and Arrhythmogenesis: Contribution of the Late Sodium Current

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Introduction

An imbalance between myocardial oxygen supply and demand, as occurs during ischemia, leads to increases of cellular concentrations of sodium and calcium (Figure 8–1).[1] Reperfusion of the ischemic heart may further exacerbate an ischemia-induced loss of ionic homeostasis. Ischemia and ischemic metabolites can increase the influx of sodium into myocytes.[2–7] Concurrent reduction of sodium efflux during ischemia, as a consequence of reductions of cellular ATP and activity of the cell membrane Na^+–K^+-ATPase, allows the intracellular sodium concentration to rise. By virtue of the coupled exchange of sodium and calcium facilitated by the cell membrane Na^+–Ca^{2+} exchanger (NCX), an elevation of the intracellular sodium concentration leads to an increased influx of calcium. An excessive or prolonged increase of the intracellular sodium concentration leads to intracellular calcium ($[Ca^{2+}]_i$) overload. The mechanisms and consequences of sodium and calcium overload are the subject of this review. Evidence supporting a role for the late sodium current (late I_{Na}) as a mechanism of calcium overloading and cardiac dysfunction is emphasized.

Causes of Sodium Overload

The influx of sodium ions through sodium channels is responsible for the upstroke (phase 0) of the cardiac action potential. The magnitude of peak sodium current during phase 0 of an action potential is thus correlated with cardiac excitability and the speed of impulse propagation. Reduction of peak I_{Na} (as in Brugada syndrome) may cause inexcitability and conduction block. However, for every sodium ion that enters a cardiac cell, one must be extruded. Cellular ATP is consumed in this process. Ideally, therefore, I_{Na} should be of large magnitude, but brief. Rapid inactivation of I_{Na} is important for curtailing the influx of sodium into the cell during an action potential, and thus for avoiding sodium overload. An increase of the frequency of excitation of myocytes increases sodium influx and the intracellular concentration of sodium.[8]

The concentration of intracellular sodium is elevated in cardiac myocytes or myocardium exposed to hypoxia, ischemic metabolites, reactive oxygen species, and selected toxins and drugs.[2–7,9–11] It is also increased in myocardium and myocytes isolated from failing hearts[12–14] and from postinfarction remodeled myocardium.[15] Increases of both sodium/hydrogen exchange (NHE) and late I_{Na} [3,5,6,10,16–18] appear to contribute to the rise of the intracellular sodium concentration that is observed during hypoxia, ischemia or simulated ischemia/reperfusion, and heart failure. Inhibitions of either NHE[2,10,11,16,19–21] or late I_{Na}[2,6,16,17,19] in these conditions attenuate the rise in intracellular sodium and are cardioprotective. The sea anemone toxin ATX-II,[22–24] many other natural toxins,[22,25] and agents such as DPI 201–106[26] that increase late I_{Na} by direct actions to interfere with sodium channel inactivation also cause sodium overload that can lead to calcium overload and myocardial electrical and mechanical dysfunction.

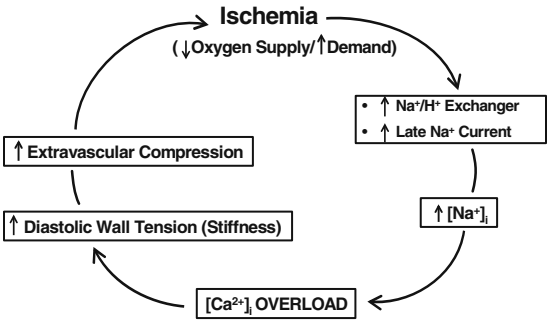

FIGURE 8–1. Positive feedback during ischemia increases the imbalance between myocardial oxygen supply and demand. In this deleterious positive feedback cycle, the $[Na^+]_i$-dependent calcium overload caused by the imbalance between O_2 supply and demand results in a further decrease in O_2 supply and increase in O_2 demand (see text for details).

An increase of sodium influx through sodium channels, NHE, or other membrane transporters must be matched by an equal increase of sodium extrusion via the Na^+–K^+-ATPase (Na pump). Sodium extrusion rises as the intracellular sodium concentration rises, and equilibrium of influx and efflux is reached at a higher intracellular sodium concentration. Activity of the Na pump is reduced when the cellular content of ATP falls. The fall of cellular ATP content during ischemia/reperfusion is thus associated with a large increase of intracellular sodium, at a time when late I_{Na} and NHE are increased. It is not clear, however, that the activity of the Na^+ pump is reduced or limited in other conditions wherein late I_{Na} is increased. Sodium pump activity was found to be increased in myocytes from failing rabbit hearts.[12] On the other hand, expression of the sodium–potassium ATPase in human myocardium appears to be reduced in heart failure.[27] Regardless, the sodium concentration in myocytes from failing hearts is elevated.[13]

The Late Sodium Current

Late I_{Na} is sodium channel current that persists from 10 to hundreds of milliseconds after the large, brief "spike" of peak I_{Na} that is elicited by depolarization. It may persist throughout the duration of the action potential plateau. Late I_{Na} has no recognized physiological role in the heart, although the relatively large magnitude of this current in Purkinje and M cells may explain the long durations of action potentials in these cells.[28] Random brief openings and long bursts of multiple openings of single sodium channels are responsible for late I_{Na}.[29,30] These openings are normally rare, and late I_{Na} is typically a small current (≈0.1% of peak I_{Na}) in ventricular myocytes. Evidence indicates that channels conducting late and peak I_{Na} are similar; for example, both peak and late I_{Na} are blocked by tetrodotoxin. Late I_{Na} can be distinguished from the sodium "window current"[31] by its presence at voltages outside the range at which window current is observed (which is roughly −40 to −60 mV with a peak at approximately −52 mV);[5] however, when present, the sodium window current contributes to late I_{Na}.[13]

Although late I_{Na} is known to be increased during exposure of myocardial cells to hypoxia and ischemic metabolites,[2,3,5,7,15,32,33] and is increased in myocytes from failing hearts,[14,34] the nature of the modification(s) of the sodium channel that leads to an increase in late I_{Na} during these circumstances is unknown. Reactive oxygen species may be important. Formation of reactive oxygen species is increased during ischemia and greatly increased upon reperfusion of the ischemic myocardium.[5,35,36] Oxidants appear to act either directly on the sodium channel or on a closely associated protein to alter channel inactivation and increase late I_{Na}.[9,36] The ischemic metabolites palmitoylcarnitine,[7] lysophosphatidylcholine,[33] and hydrogen peroxide[37,38] increase late I_{Na} and appear to interfere with sodium channel inactivation. Calcium/calmodulin-dependent kinase II is implicated as a cause of increased late I_{Na}[39] and sodium channel activity can also be modulated by protein kinase A-dependent phosphorylation.[40] In long QT syndrome type 3, an increase of late I_{Na} is caused by sodium channel mutations (most commonly these are single amino acid substitutions in the gene *SCN5A*) that increase the probability that a channel will fail to inactivate properly or will more readily reopen from a closed state.[41–45] Modeling studies suggest that persistent late openings of a relatively small number of sodium channels in LQT3 can significantly increase sodium influx during the cardiac ventricular action potential, and action potential duration.[46]

Consequences of an Increase of Late I_{Na}

Although late I_{Na} is small relative to peak I_{Na}, it persists for the duration of an action potential. It may contribute more to sodium influx than the much larger but briefer peak I_{Na}.[47] Furthermore, late I_{Na} is a depolarizing current during the action potential plateau when total membrane current is relatively small. Thus, small increases of late I_{Na} during the action potential plateau can markedly increase its duration and exacerbate both temporal and spatial dispersions of action potential duration across the ventricular wall.[48] The increases of sodium influx and of action potential duration may be expected to increase the activity of the Na pump and reverse mode NCX, and calcium entry through the L-type calcium channel, respectively. These direct and indirect consequences of late I_{Na} may themselves lead to electrical dysfunction [i.e., increased dispersion of repolarization, early afterdepolarizations (EADs), arrhythmias] and calcium overloading.[49]

Much experimental data suggest that late I_{Na} can contribute to myocardial pathology. Sodium influx via late I_{Na} appears to be a major contributor to the rise of intracellular sodium that is observed during ischemia[19] and hypoxia.[3] Myocardial ischemia is known to cause increases of lysophosphatidylcholine, palmitoyl-L-carnitine, and reactive oxygen species (e.g., hydrogen peroxide), and these substances are themselves reported to cause increases of late I_{Na}.[7,33,37,38] Sodium channel blockers (e.g., tetrodotoxin, lidocaine) have been shown to reduce the rise of sodium in rat ventricular myocytes and isolated hearts during hypoxia and ischemia, respectively.[3,5,6,10,11,16,17] This action of sodium channel blockers is associated with an improvement of contractile function and with reduction of the hypoxia/ischemia-induced increase in intracellular calcium concentration.[2,5,6,17,32,50] An increase of late I_{Na} is also arrhythmogenic[14,34,48,51–53] and is the cause of the long QT3 (LQT3) syndrome.[41–45,54] Drug-induced reduction of late I_{Na} is protective against arrhythmias caused by human ether-à-go-go-related gene (hERG) channel blockers[18,51–53,55] and sodium channel mutations that cause LQT3 syndrome.[54,56]

Recent evidence suggests that an increase of the intracellular sodium concentration leads to a decrease of the calcium concentration in the mitochondrion, and a reduction of mitochondrial NADH production.[57] An increase of intracellular sodium accelerates calcium efflux from the mitochondrion via the mitochondrial NCX. It was hypothesized that this may reduce the ability of mitochondria to increase ATP production in response to an increase of ATP consumption, as when heart rate and/or preload are increased, and a relative energy starvation.[57] When isolated guinea pig myocytes were stimulated at 4 Hz in the presence of isoproterenol, mitochondrial calcium concentration and NADH decreased when the intracellular sodium concentration was elevated from 5 to 15 mM.[57]

Sodium-Calcium Exchange

An increase of the intracellular sodium concentration reduces the chemical potential for coupled sodium entry/calcium extrusion via NCX. In a ventricular myocyte, forward exchange (net sodium entry/calcium extrusion) normally exists for all but a few milliseconds following the upstroke of the action potential.[58,59] During this early period of the action potential plateau, both the positive membrane voltage and the elevated subsarcolemmal sodium concentration favor reverse exchange (net sodium extrusion/calcium entry), and the reversal potential of the exchanger is thus positive relative to the membrane potential. If, however, the subsarcolemmal concentration of sodium and/or the membrane voltage during the action potential plateau is abnormally elevated, reverse NCX may persist for a longer time during the action potential plateau. During this time cellular calcium entry via NCX is increased and efflux is decreased. These conditions are expected to increase calcium uptake and loading of the sarcoplasmic reticulum, and thus to increase the magnitude and/or duration of the calcium transient during the next systolic contraction. An elevation of calcium content in the sarcoplasmic reticulum may also trigger calcium release either during repolarization of the action potential or during diastole. This untimely calcium release may cause EADs

and delayed afterdepolarizations (DADs) and aftercontractions.[49,60]

A rise in the intracellular concentration of sodium will lead to an increased exchange of intracellular sodium for extracellular calcium via the "reverse" mode of NCX. There is in general agreement with the fact that the cellular calcium overload that occurs during ischemia and reperfusion is a result of a combination of decreased efflux of calcium ions via the forward mode of NCX and increased influx of calcium ions via the reverse mode of NCX.[1,61–68] A relative increase of activity of NCX in the reverse mode (sodium efflux and calcium influx) is a predictable outcome of both a rise in the intracellular sodium concentration and an increase in duration of the action potential. As noted above, an increase of late I_{Na} causes both an increase of the intracellular sodium concentration and a prolongation of action potential duration, and thus increased activity of NCX in the reverse mode, and calcium influx. Direct evidence in support of the critical role of the reverse mode of NCX in intracellular calcium overload during reperfusion or reoxygenation after ischemia is derived from the observations that inhibitors of NCX,[63,66,67] antisense inhibition of NCX,[62] and knockout of NCX[65] markedly decrease either contractile dysfunction or the rise in intracellular calcium in myocardial cells.

Consequences of an Increase of Intracellular Calcium

An increase of the cytosolic concentration of Ca^{2+} is expected to increase the uptake and loading of calcium into the sarcoplasmic reticulum, cell calcium efflux and sodium influx via NCX (forward mode), and calcium binding by and kinase activity of calmodulin, and to enhance I_{Ks},[69] reduce I_{K1},[70] and decrease the chemical potential gradient for calcium influx through the L-type calcium channel.

Mechanical effects of an increase of the cytosolic concentration of Ca^{2+} become apparent during both systole and diastole. Cytosolic calcium overload causes an increased actin/myosin filament interaction and an increase in left ventricular diastolic tension (i.e., "stiffness," a failure to relax normally). As a result, both myocardial contractile work and oxygen consumption, and compression of the vascular space, become abnormally elevated. The result is a reduction of myocardial blood flow during diastole. Consequently, oxygen supply is reduced (especially in the subendocardial region of the left ventricle) while the demand for oxygen to support contraction is further increased. This pattern of cause and effect has the characteristics of a deleterious positive feedback system wherein ischemia leads to further ischemia (Figure 8–1).

Physiological adjustment of calcium uptake and filling of the sarcoplasmic reticulum is a mechanism for rapid regulation of cardiac contractile force in response to changes in heart rate and adrenergic tone.[60] Phosphorylation of phospholamban by cAMP-dependent protein kinase A removes inhibition of Ca^{2+} release channels (ryanodine receptors) and facilitates rapid release of Ca^{2+} in response to calcium influx (i.e., calcium-induced calcium release) after excitation of the myocyte. However, an increase in calcium loading of the sarcoplasmic reticulum also increases the probability of spontaneous calcium release during diastole. An increase of the cytoplasmic concentration of calcium may also trigger spontaneous calcium release from the sarcoplasmic reticulum.[60,71,72] Calcium release during diastole causes an aftercontraction and a transient depolarization of the cell membrane. Some of the released calcium exits the cell via NCX in exchange for extracellular sodium. This coupled exchange of three Na^+ for one Ca^{2+} is electrogenic, and the transient inward current thus produced causes a DAD that may initiate an action potential.[60,73] Thus, DADs may serve as a trigger of arrhythmic activity.[74,75] The spontaneous "unloading" of calcium from the sarcoplasmic reticulum during diastole also causes a relative depletion of the calcium store available for the following systolic contraction, thus reducing contractility.

An increase of the intracellular calcium concentration may cause increased activation of Ca^{2+}/calmodulin-dependent kinase II. Calcium-mediated activation of Ca^{2+}/calmodulin-dependent kinase II is followed by increased phosphorylation of phospholamban, L-type calcium channels, and nitric oxide synthase. The results of increased phosphorylation of these proteins include reduced inhibition by phospholamban of SERCA-mediated

uptake of calcium by the sarcoplasmic reticulum (thus speeding relaxation of contraction), increased I_{Ca}, and increased formation of nitric oxide (NO), respectively. Increased formation of NO may be involved in the induction of late I_{Na}[9] and may mediate enhancement of I_{Ks} by increased intracellular calcium[69] and activation of NCX.[76] Activity of Ca^{2+}/calmodulin-dependent kinase may increase late I_{Na},[39] and inhibition of the enzyme was shown to suppress the NCX-mediated transient inward current.[77]

The Therapeutic Potential of Decreasing Late I_{Na}

Inhibitors of late I_{Na} are reported to reduce the effects of hypoxia or simulated ischemia on isolated, *in vitro* cardiac preparations and the effects of ischemia on animal hearts *in vivo*. Although current inhibitors of late I_{Na} (lidocaine, amiodarone, flecainide, mexiletine, ranolazine, RSD1235, R56865, KC12291, tetrodotoxin, saxitoxin, and n-3 polyunsaturated fatty acids) are only relatively selective inhibitors of this current (versus peak I_{Na} or I_{Kr}, for example), there is substantial evidence that inhibition of late I_{Na} by these compounds is cardioprotective. Inhibitors of late I_{Na} have been shown to attenuate ionic, metabolic, electrical, and mechanical dysfunction in preclinical models of hypoxia, ischemia, heart failure, or sodium overload.[2,5,6,10,16,17,32,50,51,78–82]

In ventricular myocytes from dogs and humans with chronic heart failure, wherein late I_{Na} is augmented,[14] the duration of the action potential (APD) is prolonged[34,82] and EADs are common.[83] The sodium channel blockers tetrodotoxin, saxitoxin, and lidocaine were shown to shorten the APD and suppress EADs in ventricular myocytes from failing hearts.[34,84] Ranolazine is reported to inhibit late I_{Na} in ventricular myocytes from dogs with chronic heart failure with a potency of 6.4 μM and to shorten APD and suppress EADs in these myocytes at concentrations of 5 and 10 μM.[85]

Dispersion of ventricular repolarization and beat-to-beat variability of APD (also referred to as instability of APD) are frequently observed in myocytes from failing dog hearts, in ischemic preparations, and in myocardial tissue exposed to either ATX-II or drugs and ionic conditions that prolong the QT interval. An increased dispersion of repolarization is associated with electrical (T-wave) and mechanical alternans and proarrhythmia,[86] and is predictive of torsades de pointes ventricular tachyarrhythmia.[87] The role of late I_{Na} in increasing beat-to-beat variability of APD and the suppression of this variability by tetrodotoxin, saxitoxin, and lidocaine have been reported.[34,84,88] Ranolazine (5 and 10 μM) reduces the variability of APD in single ventricular myocytes from dogs with heart failure[85] and in myocytes exposed to ATX-II.[52] Thus, inhibition of late I_{Na} with sodium channel blockers suppresses arrhythmogenic abnormalities of ventricular repolarization (i.e., EADs and increased dispersion of ventricular repolarization) that are associated with abnormal intracellular sodium and calcium homeostasis and with the occurrence of ventricular tachycardias.[53,79,89,90]

Selective inhibitors of late I_{Na} have therapeutic potential in the treatment of cardiac disease. Inhibitors of cardiac late I_{Na} are expected to be safe and effective because late I_{Na} is both significant and common in pathological settings such as ischemic heart failure, but not in healthy myocardium where its inhibition is presumably without consequence. Ranolazine was recently approved for the treatment of chronic stable angina[91] with the rationale that reduction of late I_{Na} should reduce Na-induced calcium overload, improve diastolic relaxation, and reduce ischemia (see Figure 8–1). Ranolazine appears to be the most selective inhibitor of the late sodium current in current clinical practice.[81] It binds to the local anesthetic binding site of the sodium channel and selectively reduces late relative to peak sodium current.[92] It does not reduce heart rate, cardiac output, or blood pressure, and is not a vasodilator. Ranolazine does not appear to be arrhythmogenic.[53,90,93] However, ranolazine and other inhibitors of late I_{Na} may decrease arrhythmic activity caused by blockers of I_{Kr}.[18,52,53,55,90,94] Further studies are needed to investigate the antiarrhythmic effects of blockers of the late sodium current.[48,94]

In summary, sodium and sodium-induced calcium overloading are characteristic of ischemia and contribute to electrical and mechanical dysfunction. An increase of the late sodium current is proposed as a mechanism causing sodium overloading during hypoxia/reperfusion and heart

failure, and in patients with gain-of-function *SCN5A* mutations and possibly in other cardiac diseases. Evidence suggests that late I_{Na} is increased by ischemia, hypoxia, and reperfusion of ischemic myocardium. It appears that the timing of the increase of late I_{Na} precedes electrical and mechanical dysfunction, but more experiments are needed to determine the timing of ionic and functional events in ischemia. Enhancers of late I_{Na}, such as ATX-II, H_2O_2, and ischemic metabolites, and *SCN5A* mutations cause functional effects that mimic many but not all (e.g., ischemia-induced activation of $I_{K,ATP}$ and shortening of APD are not a result of increased late I_{Na}) of the effects of ischemia/reperfusion and hypoxia. Blockers of late I_{Na} reduce mechanical and electrical dysfunction caused by ischemia/reperfusion and hypoxia. Selective inhibition of late I_{Na} has not been demonstrated to be detrimental to normal cardiac function, but further studies of late I_{Na} in noncardiac tissues are needed to identify the physiological and/or pathological roles of late I_{Na} in these tissues. The therapeutic potential of selective inhibitors of late I_{Na} is under investigation.

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