Regulation of Intracellular and Mitochondrial Sodium in Health and Disease

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Abstract—The transmembrane sodium gradient is essential for both excitability of the cardiac cell and the regulation of the cytoplasmic concentrations of Ca and protons. In addition, movements of Na across the mitochondrial membrane affect matrix protons and calcium. In the first part of the review, we discuss the most important pathways responsible for sarcolemmal and mitochondrial sodium movements. The bulk of the review considers the changes of intracellular Na concentration ([Na⁺]) that occur in disease, specifically, ischemia, reperfusion, and heart failure. We review evidence implicating the increase of intracellular sodium to either increased influx of sodium (via either sodium channels or sodium/hydrogen exchange) or, alternatively, to decreased efflux on the Na/K pump. Although much has been learned about sodium regulation in the heart, there are still many unanswered questions, particularly concerning mitochondrial Na regulation. (Circ Res. 2009;104:292-303.)

Key Words: sodium ■ ischemia/reperfusion ■ heart failure ■ calcium ■ mitochondria ■ ion transport

Intracellular sodium ([Na⁺]) is exquisitely regulated by a series of channels and transporters. The transsarcolemmal Na gradient is a key regulator of the intracellular concentrations of Ca ([Ca²⁺]) and other ions and metabolites. However, [Na⁺] can be dysregulated in cardiac disease, and this dysregulation can contribute to cardiac pathology associated with these diseases. For example, [Na⁺] has been shown to rise during ischemia or simulated ischemia,¹⁻² and this has been shown to contribute to ischemia/reperfusion injury. [Na⁺] has also been suggested to increase in heart failure,⁶⁻⁹ and this has been suggested to contribute to altered Ca regulation, altered contractility, and arrhythmias. As a regulator of [Ca²⁺], [Na⁺] controls contractility, arrhythmogenicity, and energetics. There is also considerable recent interest in the interrelationship between cytosolic and mitochondrial ionic homeostasis and in how mitochondrial concentrations of Na and Ca can regulate mitochondrial function. There is also currently much interest in the beneficial effects of inhibitors of Na channels and carriers. This review focuses on the regulation of [Na⁺], and how this might be altered in diseases such as ischemia and heart failure.

Measurement of Intracellular Na Concentration

It is important to remember that the accuracy of measurements of intracellular sodium concentration depends on the methods used, and this is a particular issue when quantitative data are required. Four techniques have been used to date. 1. The earliest studies used measurements of total Na concentration and radioactive fluxes and corrected for the Na in the extracellular space.¹⁰ This approach has a very limited time resolution. 2. The next approach involved the use of sodium-selective microelectrodes.¹¹ Of all of the available techniques, this is probably the most quantitative but is limited by the need to impale the tissue with 2 microelectrodes (one sodium-selective and the other to measure membrane potential), thus making it next to impossible to use in strongly contracting tissues and whole hearts. 3. ²³Na nuclear magnetic resonance (NMR) can be used to measure intracellular Na as long as a “shift reagent” is used to eliminate the effects of extracellular Na.¹²¹³ This technique has a relative lack of sensitivity; kinetic measurements with a sampling rate of even 1 minute require of the order of a gram of tissue, thus effectively limiting this approach to use on whole hearts. Thus NMR is not a suitable technique for investigating changes of Na, which occur in seconds. 4. The most recently introduced approach is to make use of Na-sensitive fluorescent indicators. The most commonly used is SBFI.¹⁴ This has been used to measure Na in work on single cells⁴ and whole hearts.¹⁵ These indicators can be readily introduced in the membrane-permeant acetoxy methyl ester form, although care must be taken to allow for the fact that some of the indicator will end up in intracellular organelles such as mitochondria. Depending on the circumstances, this can either be a handicap to quantifying cytoplasmic Na concentration or can be used to estimate the mitochondrial Na concentration.⁴ The more recently developed CoroNa series of indicators can be used to selectively measure cytoplasmic (using CoroNa Green) and...
mitochondrial Na (using CoroNa Red).16 However, it is difficult to quantify these fluorescent Na indicators; thus they are useful for measuring rapid changes in Na but are not ideal for obtaining quantitative measurements.

**Regulation of Cytoplasmic Na Under Basal Conditions**

**Sarcolemmal Influx Pathways**

As discussed in a recent review,17 the Na influx into a resting cell occurs by several routes including: Na channels, Na/Ca exchange (NCX), Na/H exchange (NHE), Na/bicarbonate cotransporter, Na/K/2Cl cotransporter, and Na/Mg exchange. The heart is, of course, not normally at rest but, rather, beats regularly. This activity will increase the amount of Na entering via Na channels and also via NCX (as Na enters the cell in exchange for the Ca that enters via the L-type Ca current). In this review, we focus on 3 pathways for Na entry, which appear to be most important in disease: Na channels, NHE, and NCX.

**Na Channels**

The tetrodotoxin (TTX)-sensitive Na channel is activated during the upstroke of the action potential. The degree of opening is governed by both activation (m) and inactivation (h) gates such that depolarization first opens (activates) the channel before closing (inactivation). The major form of the sodium channel is the so-called “cardiac” isoform (Na,v1.5), which is characterized by having a low affinity for the inhibitor TTX. More recent work has, however, identified various “neuronal” isoforms in the heart, in particular Na,v 1.1 and 1.3, which are much more sensitive to TTX.18 The best-recognized role of the sodium current is to produce the fast upstroke of the action potential and therefore to allow propagation of the action potential throughout the heart. However, it has been known for many years that, as well as decreasing the upstroke velocity of the action potential, inhibiting the Na current with TTX shortens the action potential, suggesting that the sodium current plays a role in the plateau of the action potential.19 This is consistent with more recent work showing that mutations in the sodium channel lead to various long QT syndromes.20 The existence of the underlying steady-state or persistent (noninactivating) component of the Na current was first demonstrated in cardiac Purkinje fibers.21 Although this current is very small (≈1%) in comparison to the peak Na current during the upstroke of the action potential, the fact that it is maintained for much longer periods means that it will play a significant role in the total Na influx into the cell. Of particular relevance to the present review is the fact that the persistent Na current is enhanced by hypoxia and may therefore contribute to the increase of [Na+]i observed in ischemia.22 An important question concerns the behavior of the persistent Na current late in ischemia when electric activity has stopped and the membrane potential has depolarized to −50 mV.23 At this potential, the late Na channel will be activated but will only produce Na entry if it is not fully inactivated. It is therefore noteworthy that the persistent Na current shows no sign of inactivation with 1-second duration voltage clamp pulses,24 and it has been suggested that it may therefore contribute to Na entry in hypoxic conditions25 even when the membrane is depolarized. As discussed later, there is therefore considerable excitement in the development of drugs that block the persistent Na current.26 In summary, the 2 major factors expected to affect Na entry into cardiac cells via the Na channel are (1) the frequency of stimulation and (2) the degree of activation of the persistent sodium current.

**Na/H Exchange**

NHE uses the energy in the Na gradient to pump H out of the cell. Regulation of NHE has been recently reviewed.27–29 NHE is stimulated by intracellular acidification and it is among the main transporters involved in acid extrusion from the cell.30 It is also important in cell volume regulation and in cytoskeletal reorganization. NHE activity is stimulated by intracellular acidosis via proton binding to an allosteric site in the transporter. However, various agonists can modify the pH at which NHE is activated by phosphorylation. By changing the pH at which NHE is active, hormones can alter cellular pH, and this, in turn, alters cell growth, proliferation, and hypertrophy. Prolonged activation by many of these agonists that activate NHE will cause hypertrophy, and inhibitors of NHE have been shown to attenuate hypertrophy.

**Na/Ca Exchange**

The NCX has been comprehensively reviewed.31 It uses the energy provided by Na ions entering the cell to pump Ca out. The turnover rate of NCX and therefore the Na entry into the cell will depend on the intracellular concentrations of Na and Ca as well as the membrane potential. Because NCX is the major mechanism for pumping Ca out of the cell, then, in the steady state, the Ca efflux through the exchange must equal the Ca influx into the cell which is largely through the L-type Ca current. Therefore any maneuver that increases Ca entry into the cell on the L-type current (eg, β-adrenergic stimulation) will result in increased entry of Na on NCX. Na influx is a linear function of [Ca2+]i,32 and therefore the larger [Ca2+]i, is, the greater Na influx. Of relevance to subsequent considerations in this review of the effects of ischemia, a decrease of intracellular pH decreases the activity of NCX.33 ATP can activate the NCX, an effect that has a millimolar affinity for ATP34 and therefore a decrease of ATP concentration will also decrease NCX activity.

Although (as discussed above) NCX generally operates in the so-called “forward” mode to pump Ca out of the cell, depending on the electrochemical gradient, it can also produce net Ca entry coupled to Na efflux (the reverse mode). Net reverse mode can only occur at potentials positive to the reversal potential of NCX. In turn, the reversal potential depends on the concentration of intracellular Na and Ca. Depolarization of the cell at the start of the action potential can promote reverse NCX. This effect is, however, offset by the increase of [Ca2+]i caused by release from the sarcoplasmic reticulum that biases NCX toward the forward mode.35 It is therefore generally thought that under normal conditions, NCX operates primarily in forward mode. The situation may, however, be different in heart failure, where a combination of increased [Na+]i and decreased Ca release will result in longer periods of reverse mode.36 Of relevance to this review, the increase of [Na+]i observed in ischemia37 might be
expected to increase reverse mode of NCX, although the effects of other factors such as changes of ATP and pH also need to be borne in mind. Finally, it must be noted that if net reverse mode of NCX occurs for any significant period, then there will be net calcium entry. This Ca must be removed from the cytoplasm. In a real steady state this requires Ca transport out of the cell, and it is unclear whether the other sarcolemmal Ca removal, the plasma membrane Ca ATPase (PMCA), has sufficient capacity. In the short term, Ca balance could be maintained by sequestration in mitochondria, although, again, we are unaware of studies that show a quantitative balance between Na entry into the cell on NCX and mitochondrial uptake, although Liu et al have shown that intracellular Na can alter intramitochondrial Ca.

**Other Sarcolemmal Channels**

Although normally thought of as part of the intercellular junctions, connexin (Cx) hemichannels have also been found in the surface membrane and it has been suggested that they may open during metabolic stress, thereby potentially allowing Na entry.

**Na Efflux Pathways**

As mentioned above, whereas NCX can remove Na from the cell under some conditions, it is most likely that the net time-averaged flux of Na through this mechanism is directed into the cell (“forward mode”). This leaves the Na/K pump as the only significant mechanism for pumping Na out of the cell against the electrochemical gradient.

The Na/K ATPase uses the free energy of hydrolysis of ATP to exchange 3 intracellular Na ions for 2 extracellular K, thereby setting the gradients for Na and K across the cell membrane. The details of the structure and regulation of the Na/K ATPase are reviewed elsewhere. Briefly the Na/K ATPase is comprised of α and β subunits. There are 3 α isoforms with different Na affinities and 2 β subunits, although only β1 is expressed in heart. The Na/K ATPase is also regulated by a small phosphoprotein, phospholemman (PLM), in a manner reminiscent of phospholamban regulation of the sarco-/endoplasmic reticulum Ca ATPase (SERCA). PLM, a member of the FXYD family (also known as FXYD-1) is abundant in heart and has been shown to be phosphorylated by adrenergic stimulation. PLM associates with and reduces the Na affinity of the α1 and α2 subunits of the Na/K ATPase. PLM thus reduces the activity of Na/K ATPase by reducing the affinity for Na. Despa et al showed that β-adrenergic stimulation activates the Na/K ATPase in wild-type mice, but not in hearts from mice lacking PLM (PLM-KO). The Na pump activity in wild-type mice following β-adrenergic stimulation was similar to that in PLM-KO hearts. Taken together, these data show that the PLM mediated reduction in Na pump activity is lost when PLM is phosphorylated.

**Regulation of Mitochondrial Na**

**Mitochondrial Na Efflux Mechanism**

Mitochondrial [Na+] is regulated by Na influx and efflux mechanisms. The main Na efflux mechanism is the mitochondrial NHE (see the Figure, A). During electron transport, protons are extruded from the matrix, resulting in a matrix pH that is more alkaline than the cytosol. In isolated mitochondria with the extramitochondrial pH set at ≈7.1, matrix pH is typically measured at around 7.8 (ΔpH ≈0.7). However in many of these studies, mitochondria are maintained in non-physiological buffers, or in the absence of inorganic phosphate (Pi), which would reduce the pH gradient (caused by P/H cotransportor or possibly P/OH antiporter). Matrix pH has been reported to be lower (ΔpH ≈0.04) when measured in a more physiological buffer containing P, at 37°C. In isolated mitochondria, the NHE appears to operate close to equilibrium. In energized mitochondria, which are extruding protons, it is thought that the pH gradient drives the Na gradient. Jung et al found that in respiring mitochondria, the matrix Na was ≈8-fold lower than extramitochondrial Na.

If mitochondrial NHE operates near equilibrium with a stoichiometry of 1 to 1, this 8-fold Na gradient would be in equilibrium with a pH gradient of 0.92, a value slightly higher than typically found in isolated mitochondria. Thus, the Na gradient across the matrix appears to be close to equilibrium with the mitochondrial pH gradient. This is consistent with the observation that the mitochondrial NHE has high activity. However, as mentioned, in situ, the mitochondrial pH gradient is likely to be much lower, and this would reduce the Na gradient across the mitochondria. Thus the Na gradient in situ is thought to be much lower than the 8-fold measured in isolated mitochondria (probably less than a 2-fold Na gradient in cells). Studies in permeabilized cardiac myocytes support the concept that in energized mitochondria, the matrix [Na+] is lower than the cytosolic Na and that metabolic inhibition (which would block proton extrusion from the matrix and dissipate any pH gradient across the mitochondria) results in an increase in matrix [Na+]. However, there are few reliable measurements of matrix Na in intact myocytes, and this is an area that requires additional study.

It is unlikely that mitochondria have any major role in regulating cytosolic [Na+] under basal conditions, but if the mitochondrial pH gradient is increased, this could result in increased Na efflux from the mitochondrion. However, any such increase would likely be transient attributable to the limited Na in the matrix and efflux from the cytosol via the Na/K ATPase. Indeed, in the steady state, there must be no net flux of Na into or out of the mitochondria, and [Na+], will be regulated by the sarcolemma. There are few direct quantitative measurements of matrix [Na+] in situ and none in heart; this is clearly an area that needs additional future studies. Because the Na gradient across the mitochondria has important implications, it will be important to define the levels of matrix [Na+] in vivo.

Mitochondria also contain a K/H exchanger that extrudes K from the matrix in exchange with H. The mitochondrial inner membrane is largely impermeable to K; if K were freely distributed across the mitochondria, given the Δψ of ≈180 mV, matrix K would be ≈100 mol/L. This exchanger is important in regulating mitochondrial volume. Although there is some disagreement, it is generally thought that matrix K is slightly higher than cytosolic K, because of a leak of K into the mitochondria because of the high cytosolic K and the high negative mitochondrial membrane potential.
There are several recent reviews on mitochondrial K regulation and volume regulation.46,47

Mitochondrial Na Influx Mechanism

The mitochondrial Na/Ca exchanger (NCE) appears to be the main Na influx mechanism in cardiac mitochondria. Although there was previously some question regarding whether the NCE was electroneutral or electrogenic, the most recent data in isolated mitochondria agree that the exchanger is electrogenic exchanging 3 Na for 1 Ca.48–52 However, because the stoichiometry of the NCE is an important contributor to the regulation of matrix [Ca], it is critical to have additional data on the stoichiometry either in vivo or under conditions that mimic those observed in vivo. It is also worth noting that, in contrast to the sarcolemmal NCE, the presumed stoichiometry of the NCE has not been verified by direct demonstration of the predicted electrogenic current.

Energized mitochondria have a large inwardly directed membrane potential ($\Delta\psi$), typically in the range $-150$ to $-180$ mV. This large $\Delta\psi$ coupled with an inwardly directed Na gradient, will provide a large driving force for extruding Na from the matrix in exchange for Na entry. Based on typical matrix Ca values measured in myocytes, it appears that NCE is not in equilibrium. If NCE were in electrochemical equilibrium, given typical values for cytoplasmic [Na$^+$] (8 mmol/L), mitochondrial [Na$^+$] (6 mmol/L), and membrane potential ($-160$ mV), it would result in a mitochondrial Ca gradient of $\approx 958$. Thus with a time average [Ca$^{2+}$], of $\approx 300$ nmol/L, matrix [Ca$^{2+}$] would be $\approx 0.3$ nmol/L, a value considerably below the matrix values measured in myocytes ($\approx 100$ nmol/L). Furthermore, a low matrix [Ca$^{2+}$] of $\approx 0.3$ nmol/L would not be consistent with Ca activation of mitochondrial dehydrogenases.53 Schreur et al54 loaded an intact perfused heart with indo-1 and used Mn to quench cytosolic indo-1. They reported that under conditions in which systolic [Ca$^{2+}$] was 673 nmol/L and diastolic [Ca$^{2+}$] was 132 nmol/L, the mitochondrial matrix [Ca$^{2+}$] was measured at 183 nmol/L. There is considerable variation in values reported for matrix Ca, but in general, the values reported for matrix [Ca$^{2+}$] are typically much higher than calculated based on NCE equilibrium.

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Figure. Schematic diagram of sarcolemmal and mitochondrial fluxes. On the sarcolemma, the following transporters and channels are shown (clockwise): Na/K pump; NCX working in Ca efflux (forward) mode; NCX working in Ca influx (reverse) mode; Na channel; Na/H exchange; Ca channel. On the mitochondria, the transporters and channels shown are (clockwise): NCX in Ca entry mode; Na/H exchange; NCX in Ca efflux mode; Ca uniporter; pyruvate transporter; phosphate transporter; F$_1$F$_0$ ATPase; complex I to IV of the respiratory chain. A, Control. [Na$^+$] is $\approx 8$ mmol/L and pH$_i$ $\approx 7.2$. [Ca$^{2+}$], will vary between $\approx 100$ nmol/L in diastole and 1 mmol/L in systole. B, Ischemia. At the sarcolemma (clockwise), the following changes are indicated. [ATP], is decreased, affecting the Na/K pump. Na influx is increased, particularly on the persistent Na channel. Na/H activity is increased. Anaerobic glycolysis produces lactic acid, thereby acidifying the cell. There is also an increase of [Ca$^{2+}$], to $\approx 3$ mmol/L, a decrease of pH$_i$, to 6.0, and an increase of [Na$^+$], to 35 to 40 mmol/L. At the mitochondria (clockwise), changes are: increased Ca entry and Na efflux on NCX, cessation of electron transport and proton efflux, and net ATP synthesis by mitochondria. C, Heart failure. Here, the major sarcolemmal changes compared to control are: an increase in Na channels. The increase of [Na$^+$], to 15 mmol/L will result in more Na entry to mitochondria.

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Thus, it would appear that the mitochondrial NCE is not in electrochemical equilibrium. This is likely because of Ca entry via the uniporter and the kinetic properties of the NCE. The maximal activity of the NCE is also low relative to the uniporter (and NHE). Addition of ruthenium red, an inhibitor of the uniporter, leads to lower matrix Ca levels that approach those predicted by NCE equilibrium.5,52 Thus, Ca entry via the uniporter appears to keep NCE from reaching electrochemical equilibrium. It is instructive to look at Figure 6 in Dash and Beard,52 in which modeling shows that in the absence of ruthenium red and the absence of Na (which activates NCE), matrix Ca has a very steep dependence on extramitochondrial Ca. Addition of Mg, which will antagonize the uniporter, markedly reduces the level of matrix Ca at a given extramitochondrial Ca. Denton et al55 found that addition of ruthenium reduced ability of extramitochondrial Ca to activate mitochondrial dehydrogenase, consistent with a lower matrix Ca when the uniporter is inhibited. McCormack et al56 found that the relationship between extramitochondrial \([Ca^{2+}]\) and matrix \([Ca^{2+}]\) is not linear. At low extramitochondrial Ca levels (<400 mmol/L) in the presence of Na and Mg, the matrix \([Ca^{2+}]\) is less than extramitochondrial \([Ca^{2+}]\). However, as extramitochondrial Ca is raised to 0.5 mmol/L, matrix \([Ca^{2+}]\) and extramitochondrial \([Ca^{2+}]\) become equal. These data, which are consistent with recent modeling, may explain the large differences in values reported for matrix \([Ca^{2+}]\). Modeling of matrix \([Ca^{2+}]\) shows that the relationship between cytosolic \([Ca^{2+}]\) and matrix \([Ca^{2+}]\) depends on the rate of NCE relative to the Ca uniporter.5,57

Although outside the scope of this review, the beat-to-beat relationship between cytosolic and matrix Ca has been debated. As cytosolic \([Ca^{2+}]\) rises, matrix \([Ca^{2+}]\) also rises; however, it is debated whether the rise in matrix \([Ca^{2+}]\) integrates the rise in cytosolic \([Ca^{2+}]\), or whether matrix \([Ca^{2+}]\) responds in a beat-to-beat manner (see elsewhere57,58).

As discussed below, with loss of \(\Delta\psi\), which would occur during ischemia or metabolic inhibition, the mitochondrial NCE can reverse and transport Ca into the matrix. Any Na gradient would be dissipated during ischemia or metabolic inhibition because it depends on the pH gradient, which in turn depends on proton extrusion via electron transport.

**Importance of NCE for Regulating Energetics**

Consistent with an important role for NCE in regulating matrix \([Ca^{2+}]\), Cox and Matliah59 have shown that increasing extramitochondrial Na results in a decrease in matrix Ca, measured with fura-2 loaded into the matrix. This Na-dependent decrease in matrix Ca reduced the generation of NADH, consistent with Ca activation of mitochondrial dehydrogenases. With an electrolytic NCE, raising extramitochondrial (or cytosolic) \([Na^{+}]\) would decrease matrix \([Ca^{2+}]\) even in the absence of a Na gradient.

Mitochondria are the site of most of the energy (ATP) production in a cardiac cell. It has been increasingly recognized that matrix ion concentrations, which are modulated by cytosolic ion concentrations, have a major effect in controlling mitochondrial energetics. An increase in matrix \([Ca^{2+}]\) has long been known to activate mitochondrial dehydrogenases (see the Figure, A) and thus regulate the generation of NADH, the initial substrate and source of electrons for the electron transport chain.53,55 Matrix \([Ca^{2+}]\) has also been reported to stimulate the \(F_1F_0\) ATPase, thus stimulating ATP production at multiple sites.60 A number of reviews have recently focused on the role of Ca in regulating mitochondrial energetics.50 Na-dependent regulation of mitochondrial \([Ca^{2+}]\) via NCE could be important in regulating mitochondrial ATP production via activation of mitochondrial dehydrogenases as well as by direct activation of the \(F_1F_0\) ATPase. An increase in matrix \([Ca^{2+}]\) could also activate the mitochondrial Ca-activated K channel (mitoK-Ca). There are also likely to be additional proteins and processes (such as volume regulation and perhaps mitochondrial fission and fusion) that are regulated by matrix \([Ca^{2+}]\) and \([Na^{+}]\). A large increase in matrix \([Ca^{2+}]\) is also reported to activate the mitochondrial transition pore,61 a large conductance channel, leading to cell death by necrosis and/or apoptosis.

In summary, although the Na gradient across the mitochondria has important implications for the matrix \([Ca^{2+}]\), which in turn regulates mitochondrial energetics and cell death, there is still much that we do not understand regarding regulation of mitochondrial \([Na^{+}]\) and \([Ca^{2+}]\). For example, it is important to determine the \(\Delta pH\) in situ mitochondria. Most of the available data regarding mitochondrial parameters such as \(\Delta pH\), \(\Delta\psi\), binding constants for Na and Ca binding to NCE, and \(V_{\text{max}}\) for transporters were obtained in isolated mitochondria, often under conditions that are non-physiological. In spite of its description more than 50 years ago, we still have not identified the Na uniporter at the molecular level, and there is still considerable uncertainty regarding its kinetic parameters.62 There is also uncertainty regarding both the level of matrix \([Na^{+}]\) in situ and the level of matrix \([Ca^{2+}]\) and whether it responds to changes in cytosolic \([Ca^{2+}]\) on a beat-to-beat basis or whether it integrates the changes in cytosolic \([Ca^{2+}]\).57,63 The stoichiometry of NCE is still debated, and the 3:1 stoichiometry needs to be confirmed. Given the importance of mitochondrial Ca in cell energetics and cell death, it will be important to obtain a better understanding of the transport processes that regulate matrix \([Na^{+}]\) and \([Ca^{2+}]\). Present views on the matrix levels of pH, Na, and Ca are provided in the Figure.

**Na Regulation in Disease**

\([Na^{+}]\) is generally reported to be elevated in most models of heart failure and in ischemia/reperfusion.1,2,8,64 Such an increase in \([Na^{+}]\), will have important consequences for contractility, arrhythmogenesis, and energetics. We discuss the mechanisms responsible for altered Na regulation in disease and possible drug targets to ameliorate the altered Na homeostasis.

**Ischemia and Reperfusion**

It has been shown using \(^{23}\)Na NMR that \([Na^{+}]\), rises approximately 3- to 4-fold during ischemia (see the Figure, B) to a level in the range of 25 to 40 mmol/L.1,2,65 This increase in \([Na^{+}]\) could be attributable to an increase in Na influx, a decrease in Na extrusion or a combination of both. If the time of ischemia is relatively short (less than 30 minutes in a rabbit or rodent heart), \([Na^{+}]\), recovers quickly during
reperfusion to preischemic levels. The transport mechanisms involved in this rise in \( [Na^+] \), during ischemia and its recovery on reperfusion are discussed below.

**Sarcolemmal Efflux Pathways During Ischemia and Reperfusion**

**Na/K Pump During Ischemia**

The Na/K ATPase extrudes Na from the cell and thereby establishes the inwardly directed Na gradient that provides the driving force for many other exchangers. The effect on \([Na^+]_i\), of a given increase of Na influx will depend on how well the Na/K ATPase can respond. In the steady state, an increase of Na influx must be compensated by an equal increase of Na efflux. This will require an increase of \([Na^+]_o\).

The steeper the dependence of Na/K ATPase rate on \([Na^+]_i\), the smaller the required rise of \([Na^+]_i\). If the Na/K ATPase is partially inhibited, the dependence of rate on \([Na^+]_i\) will be shallower, and therefore a larger increase of \([Na^+]_i\) will be produced by a given increase of Na influx. Because \([Na^+]_i\), steadily rises during ischemia, it is generally assumed that the activity of the Na/K ATPase is reduced during ischemia.66 One problem with a quantitative analysis is that the metabolic effects of ischemia do not develop instantaneously, and therefore effects on ion transport may only develop slowly. However, data suggest that the pump remains active during the first few minutes of ischemia.56,66 The reasons for the eventual inhibition of the Na/K ATPase are not completely clear. Clearly, a fall in ATP will result in inhibition of the Na/K ATPase; however, it has been suggested that the pump becomes inhibited before ATP levels decline to concentrations that would result in inhibition of the Na pump.57 A rise in ADP and P, will also inhibit the pump, although again it is not clear that they rise with the proper time course to account for the inhibition of the pump and the rise in \([Na^+]_i\).

If the fall in bulk ATP is not sufficient to inhibit the pump during the early period of ischemia, what is the mechanism? It is possible that there is a posttranslational modification of the Na/K ATPase or some protein regulating the pump that leads to inhibition. Interestingly, the activity of Na/K ATPase has been reported to be regulated by nitric oxide,68 which can be altered during ischemia. There are also data suggesting that the Na pump might be inhibited by a labile inhibitor generated during ischemia. Fuller et al69 have reported that ischemia produces a labile cytosolic compound which results in inhibition of the Na/K ATPase by a mechanism involving reactive oxygen species. This inhibitor reduces activity of the Na/K ATPase from heart and brain, but not kidney. Interestingly, PLM is reported to be present in heart and brain, but not kidney. In another study, Fuller et al70 suggested that ischemia leads to activation of the Na/KATPase via phosphorylation of PLM, but this activation of the pump is overcome by the inhibitor generated during ischemia. They speculated that if the labile inhibitor is removed rapidly at the start of reperfusion, the activation of the Na/K ATPase would enhance \([Na^+]_i\), recovery following ischemia. Interestingly, Imahashi et al71 find that female mice have less of a rise of \([Na^+]_i\), during ischemia; this appears to be attributable to reduced Na efflux because the differences is eliminated by ouabain. Perhaps female mice have less of the inhibitor (or increased activation of PLM).

Regardless of the mechanism, it appears70 that the activity of the Na/K ATPase during ischemia is significantly reduced such that it cannot keep up with the increased Na influx that occurs. If changes in ATP and its metabolites are insufficient to account for the reduced pump activity during ischemia (see above), then other likely candidates such as posttranslational modifications of the pump itself or regulatory proteins such as PLM should be considered. Identification of the mechanism for the reduced pump activity will provide new drug targets to reduce ischemic injury.

**Na/K Pump During Reperfusion**

After relatively short durations of ischemia, most studies report that reperfusion results in a rapid (within minutes) return to preischemic Na levels.72 This return of \([Na^+]_i\) to baseline levels is mediated primarily by the Na pump, because addition of ouabain blocks the recovery of \([Na^+]_i\), on reperfusion.72 There is some disagreement72,73 regarding whether the Na+ that enters on reperfusion results in a measurable increase in \([Na^+]_i\), or whether it is rapidly extruded via the Na pump and reverse mode NCX resulting in only a slight and very transient spike in \([Na^+]_i\). Most of the \(^{23}Na\) NMR studies find little or no measurable additional rise in \([Na^+]_i\), during reperfusion, unless the Na/K ATPase is inhibited.71,72 Because NMR measurements are signal-averaged over 2 to 5 minutes, it is possible that there could be a transient rise in \([Na^+]_i\), at the very start of ischemia. These data suggest that on reperfusion, the Na/K ATPase is rapidly reactivated and can extrude the increased Na+ that enters. If, as discussed above, the pump is inhibited because of posttranslational modification or the presence of a labile inhibitor, it appears that this inhibition is removed at the start of reperfusion.

**NCX During Ischemia and Reperfusion**

Because NCX is reversible, it can function as both a Na influx and efflux pathway. Na entry via NCX during ischemia is thought to be reduced or inhibited because with the rise in \([Na^+]_i\), the Na gradient falls quickly during ischemia. However, the decrease in the Na gradient that occurs could be attributable, in part, to Na influx via NCX (see the Figure, B). Inhibition of the rise in Na during ischemia (with NHE inhibitors or inhibitors of Na channels) blocks the rise in Ca during ischemia, and this has been taken as evidence that NCX runs in reverse during ischemia.2,74 However, better preservation of the Na gradient would also allow better Ca extrusion via NCX, which would also reduce the rise in Ca during ischemia. In mice lacking NCX1 (NCX-KO), the rise in \([Na^+]_i\), during ischemia was reduced compared to wild-type hearts, which might suggest that NCX functions to produce net Ca efflux and Na influx during ischemia.75 An alternative interpretation is that reduced Ca loading and better preservation of ATP in the NCX-KO hearts leads to enhance Na efflux or less Na entry via NHE (because of less acid generation). Because NCX operates near equilibrium, it may function as a Na influx pathway during early ischemia, until the electrochemical gradient reverses and then its reverse mode will
predominate. Indeed, modeling of NCX fluxes during ischemia are consistent with NCX operating in both directions during ischemia, with reverse mode predominating after the electrochemical gradient reverses. A major role for Ca entry via NCX during ischemia is suggested by studies showing reduced ischemia/reperfusion injury in mice with cardiac specific loss of NCX.

During early reperfusion, NCX appears to be primarily a net Na efflux rather than a Na influx pathway. On reperfusion, Na is thought to enter the myocyte via NHE (see below) and the increased \([\text{Na}^+]\) increases Na efflux via the Na/K ATPase and NCX. The reversal of NCX results in Ca loading of the cell. It has been suggested that inhibition of NCX might be a therapeutic target to reduce Ca overload during early ischemia. Several inhibitors (for example, KB-R7943) that are reported to selectively inhibit the “reverse” mode of NCX have been studied. These inhibitors have been recently reviewed. However, both KB-R7943 and SEA400 have been shown to have nonspecific effects, as evidenced by depressing Ca transients in heart tubes that lack NCX. The ability to selectively inhibit NCX in one direction has been challenged on thermodynamic grounds. In brief, at the equilibrium position, forward and reverse modes are equal in magnitude. Selective inhibition of the reverse mode would therefore result in a net forward mode, which is thermodynamically impossible. The measured ability of these inhibitors to inhibit reverse more than forward mode results from the different experimental conditions used to study the two modes. Presumably, the drug binds to a form of the NCX, which exists at a higher concentration during reverse mode. However, it should be noted that these drugs may well block better under the conditions seen during reperfusion, and therefore it is perfectly possible for a drug to block the Ca gain on reperfusion while having little effect on forward mode during normal physiology (because the form of NCX that the drug binds to is less prevalent during normal physiology when it mainly operates in the forward mode).

Sarcolemmal Influx Pathways
There has been much discussion as to the routes by which Na enters the cell during ischemia. The 2 major candidates are NHE and persistent (noninactivating) Na channels.

Na/H Exchange
Studies have shown that addition of NHE inhibitors significantly attenuate the rise in \([\text{Na}^+]\) during ischemia, suggesting a role for NHE in producing increased Na influx. However, the role of NHE in the rise in \([\text{Na}^+]\) during ischemia has been questioned because many of the NHE inhibitors also inhibit persistent Na channels. It is clear that the marked inhibition of the rise in \([\text{Na}^+]\) during ischemia that occurs with amiloride and other nonselective NHE inhibitors is attributable, in part, to inhibition of persistent Na channels. However, this does not preclude a role for NHE. Indeed, recent studies find that more specific NHE inhibitors also reduce the rise in \([\text{Na}^+]\) during ischemia, although the attenuation of the rise in \([\text{Na}^+]\) appears to be less than with nonspecific inhibitors such as amiloride. Further support for a role for NHE comes from studies using mice lacking NHE. These were found to be resistant to ischemia/reperfusion injury compared to wild-type, with better preserved ATP during ischemia and a reduction in the degree of contracture during ischemia.

In contrast to the debate over the mechanism responsible for the rise in \([\text{Na}^+]\) during ischemia, there appears to be agreement that NHE is primarily responsible for the rise in \([\text{Na}^+]\), at the start of reperfusion. During ischemia, intracellular pH falls to \(\approx 6.0\), and the extracellular pH also becomes acidic. On reperfusion, with the normalization of extracellular pH there is now a large outwardly directed proton gradient that increases Na entry via NHE. As mentioned above, the Na that enters is quickly extruded via the Na/K ATPase and the NCX; thus, there is usually little if any measurable rise in \([\text{Na}^+]\), on reperfusion above the levels present at the end of ischemia. Inhibition of NHE on reperfusion results in a slight delay of the recovery of pH, and a slight reduction in the very transient rise in \([\text{Na}^+]\), suggesting that much of the Na that enters via NHE is extruded by the pump. However, the Na that is extruded by the reverse mode of NCX increases \([\text{Ca}^{2+}]\). This increase in \([\text{Ca}^{2+}]\), can have many detrimental effects on cardiac function. It can alter excitation/contraction coupling, contribute to the generation of arrhythmias, activate proteases, and can enter the mitochondria and alter bioenergetics or even activate cell death pathways. Thus, reducing Ca entry via NCX would reduce ischemia/reperfusion injury. A number of strategies has been proposed for reducing Ca entry via NCX, including reducing \([\text{Na}^+]\) entry by inhibiting NHE on reperfusion (or ischemia and reperfusion), inhibition of reverse mode of NCX, and brief acidic reperfusion. Inhibition of the rise in \([\text{Na}^+]\), during ischemia by inhibition of persistent Na channels and/or stimulation of the Na/K ATPase during ischemia and reperfusion may also be beneficial.

Lazdunski et al proposed more than 20 years ago that inhibition of NHE at the start of reperfusion would be protective. In animal models, addition of NHE inhibitors before ischemia has been shown to reduce ischemia/reperfusion injury. Addition of NHE inhibitors at the start of reperfusion was protective in some but not all studies. In spite of the beneficial effects of NHE inhibitors in preclinical trials, a number of large clinical trials were largely negative. The reason for the failure of the trials has been discussed elsewhere. For the most part, the NHE inhibitors were given long after the start of reperfusion, a time that was not beneficial in the animal studies. It is possible that NHE inhibitors would be useful if given during ischemia or at the very start of reperfusion. A beneficial effect was observed using a post hoc analysis in a study in which the NHE inhibitor cariporide was given to patients undergoing coronary artery bypass. However, a subsequent trial that focused on CABG used a higher dose of cariporide and was stopped because of increased incidence of stroke. In the light of recent data suggesting antianginal effects of ranolazine, it might be interesting to test whether NHE inhibitors have similar effects. It has also been suggested that NHE inhibitors reduce the post ischemic development of hypertrophy.
Na Channel
A role for the persistent Na⁺ channels in the rise in [Na⁺], during ischemia is suggested because inhibitors of these channels such as TTX and lidocaine have been shown to reduce the rise in [Na⁺], during ischemia.²⁴,⁵,⁷³ However, the early NHE inhibitors also blocked persistent Na channels, and some of their ability to reduce ischemic Na⁺, and protect against ischemic injury is attributable to their inhibition of Na channels.⁸⁰ The persistent Na channels have been shown to be active during ischemia but (at least as judged by the lack of effects of TTX) do not appear to contribute during reperfusion.⁷³ Ranolazine, a new “antiischemic” drug that activates pyruvate dehydrogenase¹⁰¹ (in fact, this was its originally described mode of action) has also been shown to inhibit noninactivating Na channels.¹⁰² Ranolazine reduces the frequency and symptoms of angina in patients and allows for better exercise tolerance and has been reported to reduce S-T segment elevation, with no adverse effects on hemodynamics, and also has been reported to be antiarrhythmic.¹⁰³,¹⁰⁴ However, in spite of these beneficial effects, ranolazine did not reduce the primary end point of cardiovascular death, infarction, or recurrent ischemia in the MERLIN trial.¹⁰⁵ The success in treating symptoms of angina with ranolazine might suggest that transient ischemia associated with angina results in an increase in [Na⁺], and that reducing this rise in [Na⁺], is a beneficial strategy. It should be noted that ranolazine also activates pyruvate dehydrogenase which could also contribute to its beneficial effects. However, Wang et al have reported that the beneficial effects of ranolazine are not mediated by inhibition of fatty acid metabolism.¹⁰⁶ The beneficial effects of ranolazine in treating angina might suggest that NHE inhibitors would also be protective in the setting of angina. It is also interesting that although ranolazine reduced symptoms of angina, it did not reduce mortality. These data appear to suggest that reducing the rise in [Na⁺], during transient ischemia, as occurs in angina, has no longer-term beneficial effect in terms of reducing mortality; this may reflect the difficulty to show improvement in mortality on top of current treatment. It would be interesting to see whether ranolazine can alter the development of hypertrophy and/or heart failure, as has been suggested to occur with NHE inhibitors.

Connexin Hemichannels
In addition to the well-characterized role for NHE and persistent Na channels in the rise in [Na⁺], during ischemia, recent data suggest that ischemia can activate a nonselective current through connexin (Cx) hemichannels.³⁷,¹⁰⁷–¹¹⁰ Cx hemichannels come together, one from each cell, to form gap junctions. These hemichannel precursors of gap junctions are permeable to molecules less than approximately Mᵣ 1000. Such a channel would allow influx of Na, Ca, and other ions. John et al¹⁰⁷ reported that Cx hemichannels are opened by metabolic inhibition. The opening of even a small number of these channels can severely disrupt ion homeostasis. It is speculated that opening of these Cx hemichannels might be a step in promoting cell death. The exact role of Cx hemichannels in ion dysfunction during ischemia is not clear, but there are some data suggesting that inhibition of these channels can reduce cell swelling during ischemia. It is also interesting that Cx43 has been shown to localize to the mitochondria with preconditioning.¹¹⁰a The regulation of Cx43 during ischemia is clearly complex and requires further study.

Mitochondrial Transporters
During ischemia, electron transport stops (see the Figure, B) and any mitochondrial pH gradient is likely to be dissipated; this would reduce or dissipate the inwardly directed Na gradient (see the Figure, B). Furthermore, ischemia leads to the loss of membrane potential,¹¹¹ and with a rise in [Ca²⁺], and [Na⁺], during ischemia, the NCE can reverse and transport Ca into the matrix. Assuming a cytosolic [Ca²⁺] of 3000 nmol/L, and little or no Na gradient across the mitochondria, with no Δψ, NCE equilibrium would predict that matrix [Ca²⁺] would be very similar to the cytosolic [Ca²⁺]. With the loss of Δψ, the Ca uniporter would be inhibited and NCE would approach equilibrium. Consistent with a reversal of the mitochondrial NCE during ischemia, Griffith et al¹¹² reported that inhibition of mitochondrial NCE with CGP-37157 during ischemia results in a decrease in matrix [Ca²⁺]. During reperfusion, mitochondrial NCE returns to the preischemic mode of extruding Ca from the matrix. There are some interesting implications regarding reversal of mitochondrial NCE during ischemia. Reversal of NCE would transport Ca from the cytosol to the matrix, thus reducing [Ca²⁺], while increasing matrix Ca.¹¹² The increase in matrix [Ca²⁺] would enhance mitochondrial dehydrogenase,⁵³ thereby increasing NADH, it would also activate F₁F₀ ATPase,⁶⁰ but in the absence of oxygen, there would be little or no electron transport. The increase in both NADH and matrix [Ca²⁺] are factors reported to enhance opening of the mitochondrial permeability transition pore (MPTP),⁶⁵ which is associated with cell death. The reduction in [Ca²⁺], would tend to reduce activation of calcium activated proteases and Ca ATPase, but these protective effects are likely to be offset by the detrimental effects of elevated matrix [Ca²⁺] (ie, activation of MPTP). It is interesting that cardioprotective maneuvers such as diazoxide treatment have been reported to reduce matrix [Ca²⁺] during ischemia.¹¹³ In addition, the antiapoptotic protein Bcl-2 has been reported to reduce activity of the mitochondrial NCE.¹¹⁴ These data suggest that inhibition of mitochondrial NCE during ischemia might be an important therapeutic target.

Heart Failure and Hypertrophy
There have been a number of recent reviews on alterations in [Na⁺], during hypertrophy and heart failure.⁷,⁸,¹¹⁵ We, therefore, focus on the interplay between cytosolic and mitochondrial Na and the effect of altered mitochondrial Na on cell function. Most studies report an increase in [Na⁺], during hypertrophy and heart failure,⁸,⁹,¹¹⁵–¹¹⁸ although not all found an increase.¹¹⁹,¹²⁰ Overall, the data seem to suggest an increase in [Na⁺], in hypertrophy and heart failure in humans. What are the mechanisms that might result in this increase in [Na⁺], and what are the consequences?
Sarcolemmal Influx Pathways

Na Channel

Pogwizd et al\textsuperscript{4} have suggested that the rise in [Na\textsuperscript{+}], in heart failure is attributable to a greater Na influx rather than a reduced Na efflux. The initial rate of Na influx, measured immediately after inhibition of the Na/K ATPase, was found to be \(\approx\)2-fold greater in myocytes from failing hearts compared to control.\textsuperscript{6} Using inhibitors against NHE, persistent Na channel and NCX, Despa et al\textsuperscript{6} concluded that the rise in [Na\textsuperscript{+}] in heart failure is primarily attributable to increased Na influx via persistent Na channels (see the Figure, C) that can be inhibited by TTX, lidocaine, or new drugs such as ranolazine.\textsuperscript{102} This finding might suggest a beneficial effect of ranolazine in reducing the development of hypertrophy and or hypertrophy following myocardial ischemia.

Na/H Exchange

Baartscheer et al\textsuperscript{8} also have reported an increase in Na influx in heart failure that can be inhibited by cariporide (an NHE inhibitor), suggesting a role for increased Na entry by NHE in heart failure (see the Figure, C). Consistent with this finding, a number of studies have shown that NHE inhibitors can block or attenuate the development of heart failure.\textsuperscript{29,121,122} The debate about whether NHE or Na channels are responsible for the increase in Na during hypertrophy is somewhat similar to the arguments about the rise in [Na\textsuperscript{+}] during ischemia. Additional studies are needed to resolve the question, but it is possible that both contribute and that their relative contribution depends on the model.

Other Na Influx Pathways

Despa et al\textsuperscript{6} suggest that NCX does not contribute to the rise in [Na\textsuperscript{+}] during hypertrophy. The contribution of other Na influx pathways, such as Cx hemichannels, transient receptor potential channels, and Na-bicarbonate transporters, to the increase in Na during hypertrophy and heart failure has not been studied in detail.

Sarcolemmal Na Efflux Pathways

Na/K Pump

There are data suggesting both decreased expression and altered expression of different isoforms of the Na/K ATPase in some models of heart failure. As discussed above, the Na/K ATPase contains \(\alpha\) and \(\beta\) subunits.\textsuperscript{39} Alterations in \(\alpha\) isoforms have been reported in hypertrophy and heart failure, although there is no consistent pattern. Studies examining activity of Na/K ATPase in heart failure have also been conflicting. Studies report a decrease in Na affinity with no change in \(V_{\text{max}}\),\textsuperscript{123} a decrease in \(V_{\text{max}}\) and no change in Na affinity,\textsuperscript{124} and no change in either \(V_{\text{max}}\) or Na affinity.\textsuperscript{8} Alterations in phosphorylation of PLM could also alter Na/K ATPase activity. Bossuyt et al\textsuperscript{125} reported that in heart failure, PLM expression is decreased to a greater extent than the Na/K ATPase and that PLM is more phosphorylated in heart failure. Taken together, these observations would suggest less PLM mediated inhibition of the Na pump in heart failure.

Thus, changes in heart failure and hypertrophy include a decrease in expression of the Na/K ATPase with no consistent change in activity and a decrease in PLM concomitant with an increase in phosphorylation of PLM. It has been suggested that the changes in PLM might offset the decrease in expression, thereby accounting for the lack of difference in activity. At present, the results are somewhat discrepant and additional studies are needed.

Na/Ca Exchange

There are data suggesting an increase in NCX levels and/or activity with hypertrophy and heart failure.\textsuperscript{115,126,127} It has been proposed that this increase in NCX might help remove Ca from the cell and compensate in part for decreased SERCA, which occurs in heart failure. Offset against this is the fact that the rise in [Na\textsuperscript{+}], in hypertrophy and heart failure will reduce the driving force for Ca extrusion via NCX (see the Figure, C) and thus contribute to the increase in diastolic Ca observed in heart failure. The overall effect will depend on the relative changes of NCX expression and [Na\textsuperscript{+}].

Mitochondrial Transporters

As discussed above, the increase in [Na\textsuperscript{+}], during hypertrophy and heart failure is likely attributable to increased Na entry across the plasma membrane, and the mitochondrial NCX does not contribute to this rise in [Na\textsuperscript{+}]. However, the rise in [Na\textsuperscript{+}], during heart failure has been suggested to lower mitochondrial [Ca\textsuperscript{2+}], resulting from an increased Na gradient across the mitochondria and therefore a greater driving force for Ca efflux from the mitochondria via mitochondrial NCX (see the Figure, C).\textsuperscript{38} A number of studies have shown that increasing cytosolic (or extramitochondrial) [Na\textsuperscript{+}] results in a decrease in matrix [Ca\textsuperscript{2+}].\textsuperscript{38,59} However, there is also an increase in diastolic Ca with hypertrophy that will require Ca extrusion against a larger gradient. An increase in [Ca\textsuperscript{2+}], will also increase uptake by the Ca uniporter. Furthermore, with an electrogenic NCE,\textsuperscript{49,52} the mitochondrial membrane potential becomes a factor, and it might change during heart failure. A decrease in mitochondrial membrane potential would tend to offset the stimulation of the NCE that would occur with an increase in cytosolic Na. Another factor is the mitochondrial pH gradient, which apparently sets the Na gradient, and, if the matrix pH is altered during heart failure, this could alter the Na gradient. Thus, it is difficult to predict a priori what effect heart failure will have on mitochondrial [Na\textsuperscript{+}] and [Ca\textsuperscript{2+}]. In spite of these concerns, the data of Liu et al\textsuperscript{38} suggest that the increase in [Na\textsuperscript{+}], that occurs in heart failure can alter mitochondrial [Ca\textsuperscript{2+}] and mitochondrial energetics. They showed that an increase in [Na\textsuperscript{+}], reduced mitochondrial [Ca\textsuperscript{2+}] and increased oxidation of mitochondrial NADH. They further showed that myocytes from failing hearts had a higher [Na\textsuperscript{+}], (16.8 versus 5.2 mmol/L in control), and net oxidation of NADH occurred with pacing. Treatment of failing myocytes with the mitochondrial NCE inhibitor CGP-37157 blocked the oxidation of NADH that occurred when failing myocytes were paced. These data show that inhibition of mitochondrial NCE increases the production of NADH, which, as discussed above, occurs via Ca-activated NADH-linked dehydrogenases. Thus, this reduction in mitochondrial [Ca\textsuperscript{2+}], secondary to elevated

Conclusions

Although much work has been carried out on the fluxes of sodium across both the surface membrane and mitochondrial membranes, it is clear that a complete understanding of sodium regulation, particularly in diseases, still eludes us. Future studies will need to elucidate the regulation of mitochondrial ion transporters and how they interact with plasma membrane transporters to regulate ions and metabolism in heart during physiology and disease.

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