[Na\(^+\)]_i handling in the failing human heart

Burkert Pieske\(^a\),*, Steven R. Houser\(^b\)

\(^a\)Abt. Kardiologie und Pneumologie, Zentrum Innere Medizin, Klinik der Georg-August-Universität, Robert-Koch Strasse 40, 37075 Göttingen, Germany
\(^b\)Cardiovascular Research Group, Temple University, Philadelphia, PA, USA

Received 12 November 2002; accepted 4 December 2002

Abstract

Proper contractile function of the heart depends on intact excitation–contraction processes and ion homeostasis of the myocytes. The \(\text{Ca}^{2+}\) ion activates contraction through its binding to troponin C. However, \(\text{Ca}^{2+}\) homeostasis is tightly linked to \(\text{Na}^+\) regulation because the primary mechanism for \(\text{Ca}^{2+}\) efflux in cardiac myocytes is via electrogenic \(\text{Na}^+\)/\(\text{Ca}^{2+}\)-exchange. While altered \(\text{Ca}^{2+}\)-homeostasis has been demonstrated in animal models of heart failure and failing human cardiac tissue, the role of dysfunctional \(\text{Na}^+\) handling processes in altered excitation–contraction coupling remains obscure. Furthermore, altered \(\text{Na}^+\) handling has been implicated in a wide range of cellular processes, such as regulation of membrane potential, pH, and growth. This review will discuss (1) the evidence for altered [\(\text{Na}^+\)], homeostasis in the failing human heart, (2) how alterations in the \(\text{Na}^+\) electrochemical gradient can influence \(\text{Ca}^{2+}\) handling, contractile function, and a number of other cellular processes, and (3) the potential defects in \(\text{Na}^+\) channels and transporters that may underlie altered [\(\text{Na}^+\)], in the failing human heart.

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Keywords: Calcium (cellular); Contractile function; Heart failure; Myocytes; \(\text{Na}^+\)/\(\text{Ca}^{2+}\)-exchanger

1. Introduction

During depolarization, \(\text{Ca}^{2+}\) enters the cell through voltage-dependent \(\text{Ca}^{2+}\)-channels. This \(\text{Ca}^{2+}\)-influx induces opening of the \(\text{Ca}^{2+}\) release channel of the sarcoplasmic reticulum (SR) and release of a larger amount of \(\text{Ca}^{2+}\) stored within the SR into the cytoplasm. Additional \(\text{Ca}^{2+}\) influx may occur through reverse-mode \(\text{Na}^+\)/\(\text{Ca}^{2+}\)-exchange under certain conditions. The combination of \(\text{Ca}^{2+}\) influx and release from the SR abruptly raises systolic [\(\text{Ca}^{2+}\)]. \(\text{Ca}^{2+}\) then binds to troponin C which induces activation of the myofilaments and contraction. Relaxation is initiated by dissociation of \(\text{Ca}^{2+}\) from troponin C, reuptake to the SR through the phospholamban-regulated \(\text{Ca}^{2+}\)-pump (SERCA2a), and trans-sarcolemmal \(\text{Ca}^{2+}\) removal through the \(\text{Na}^+\)/\(\text{Ca}^{2+}\)-exchanger (NCX) in its forward mode [1].

Human heart failure is characterized by defective excitation-coupling processes and dysfunctional \(\text{Ca}^{2+}\) homeostasis [2–4]. Systolic \(\text{Ca}^{2+}\)-concentrations are reduced, diastolic \([\text{Ca}^{2+}]\), is elevated, and the decay time of the \(\text{Ca}^{2+}\)-transient is prolonged. These alterations translate into functional incompetence with reduced systolic shortening, increased diastolic tension, and relaxation abnormalities. One major feature of human heart failure is that these defects become more pronounced with increasing heart rates [3,5]. Nonfailing human myocardium shows a positive force–frequency relationship, but the force–frequency relation is blunted or inverted in the failing human heart, associated with progressive diastolic dysfunction at higher heart rates [6]. These defects are associated with blunted SR \(\text{Ca}^{2+}\)-accumulation [4,7] and were related to altered function and expression of major \(\text{Ca}^{2+}\)-handling proteins (for reviews, see Refs. [8,9]).

Given its critical role in myocyte physiology it is not surprising that \(\text{Ca}^{2+}\) is tightly regulated by a variety of
sarcolemmal and organellar proteins. The critical feature of this review is that Ca$^{2+}$ regulation is closely linked to intracellular Na$^+$ homeostasis because the principal route for Ca$^{2+}$ efflux from myocytes is via the NCX which relies on the energy within the Na$^+$ electrochemical gradient to remove Ca$^{2+}$ from the cytosol (for review, see Ref. [10]).

The NCX is an electrogenic transporter that exchanges Ca$^{2+}$ for Na$^+$ in a 1:3 stoichiometry. At negative membrane potentials and normal [Na$^+$], and [Ca$^{2+}$], the NCX functions in the (forward) Na$^+$-in/Ca$^{2+}$-out mode, and reduces diastolic Ca$^{2+}$ levels by extruding Ca$^{2+}$ [11]. During the action potential, and/or when [Na$^+$] is increased, the NCX can function in a ‘reverse mode’, bringing Ca$^{2+}$ into the cell. This Ca$^{2+}$ may promote SR Ca$^{2+}$ loading [12] and also, with the L-type Ca$^{2+}$ current, modulate the release of Ca$^{2+}$ from the SR [13] (but see also Sipido et al. [14] who reported low efficiency of reverse-mode $I_{NaCa}$ in triggering SR Ca$^{2+}$-release). When [Na$^+$] increases, the NCX is biased from forward to reverse mode, resulting in increases in [Ca$^{2+}$], [15]. Therefore, any changes in Na$^+$ regulation in heart failure will directly affect intracellular Ca$^{2+}$ handling. Na$^+$ levels in cardiac myocytes are regulated by a network of ion channels and transporters (Fig. 1), and all of these processes could contribute to abnormal Na$^+$ regulation in the failing heart. However, the possibility of altered [Na$^+$] handling has not been directly analysed in diseased myocardium until recently.

2. [Na$^+$] measurements in cardiac muscle

Various techniques have been used to measure [Na$^+$] in myocardium of animal species, such as Na$^+$-selective microelectrodes [16–19], electron-probe X-ray microanalysis [20], or $^{23}$Na NMR spectroscopy [21]. During the last years, fluorescent Na$^+$-indicators such as sodium-binding benzo[d]furano-isophthalate (SBFI) have been most frequently used [22–25] and allow simultaneous assessment of [Na$^+$] and contractile function. Most of these techniques measure global, bulk cytosolic [Na$^+$], and do not detect subcellular Na$^+$ concentration gradients or organellar trapping. However, it was suggested that small changes in local subcellular [Na$^+$] may suffice to produce significant alterations in Ca$^{2+}$ regulation and contractility. Therefore, novel approaches to measure Na$^+$ with greater accuracy both in terms of concentration gradients and subcellular localisation, such as extended confocal microscopy [26] or two-photon Na$^+$ imaging [27] are likely to provide substantial new information.

3. [Na$^+$] handling in the mammalian heart

Na$^+$ has been measured in cardiomyocytes [23,24,28,29], muscle strips [30,31], and whole hearts [25] from various animals. Several important observations have been made in these studies:

![Fig. 1. [Na$^+$] regulation in myocytes.][1]

[1] Na$^+$ regulation in myocytes. [Na$^+$] influx occurs via sarcolemmal Na$^+$ channels ($I_{Na}$), the forward mode of NCX, and Na$^+$-dependent pH regulators (e.g. NHE and the anion-exchanger (AE)). [Na$^+$] elimination is mediated via the Na$^+$/K$^+$-pump, and possibly via reverse-mode NCX. Elevated [Na$^+$], may increase [Ca$^{2+}$], SR Ca$^{2+}$ content, and SR Ca$^{2+}$ release by its effects on NCX.
(1) \([\text{Na}^+]\), varies in a species-dependent manner: In unpaced myocytes, \([\text{Na}^+]\), varies over a relatively broad range (4–16 mmol/l). This range appears to be species-dependent [13,18,19]. For example, Yao et al. [13] reported \([\text{Na}^+]\), values of 16 mmol/l in mouse myocytes but only 5–8 mmol/l in rabbit myocytes [32]. In general, \([\text{Na}^+]\), appears to be maintained at lower levels in myocytes from large mammals than from smaller mammals (Fig. 2). It is likely that these differences in \([\text{Na}^+]\), regulation are intimately involved in species-related differences in excitation–contraction coupling [15].

(2) \([\text{Na}^+]\), increases with (patho)-physiological stimuli:

(a) \([\text{Na}^+]\), increases with increasing rates of depolarisation: Using \(\text{Na}^+\)-selective microelectrodes, a parallel increase in twitch force and \([\text{Na}^+]\), was observed in guinea-pig, canine, or sheep ventricles [16,17,33]. These authors concluded that an increase in \([\text{Na}^+]\), is an important factor involved in the positive force staircase. A frequency-dependent increase in \([\text{Na}^+]\), has also been shown in the rat heart [25], but this was associated with the typical negative force–frequency relationship of the rat. Therefore, frequency-dependent increases in \([\text{Na}^+]\), may be a general phenomenon, but do not necessarily cause frequency-dependent increases in developed force. Whether or not changes in heart rate produce an increase or decrease in force appears related to action potential (AP) duration [15], the ability of the SR to increase \(\text{Ca}^{2+}\) load [4,34], and restitution characteristics of the SR \(\text{Ca}^{2+}\)-release process [28]. In small mammals (e.g. rats and mice) SR \(\text{Ca}^{2+}\) load and release are near maximum at slow pacing rates, and do not further increase at higher pacing rates even though \([\text{Na}^+]\), (and diastolic \([\text{Ca}^{2+}]\)) rise [34]. In larger mammals including nonfailing human hearts, the SR is not maximally loaded at slow heart rates. As heart rate increases the increased \([\text{Na}^+]\), promotes SR \(\text{Ca}^{2+}\) loading which underlies the positive force–frequency relationship [4,16,17,34].

(b) \([\text{Na}^+]\), and stretch: Stretching cardiac muscle increases \([\text{Na}^+]\), [30,31], followed by a slow increase in \([\text{Ca}^{2+}]\), and force (see also Cingolani et al. and von Lewinski et al. in this Spotlight Issue). The mechanism through which stretch elevates \([\text{Na}^+]\), is under debate, and stretch-activated cation channels [35], \(\text{Na}^+\) current [36] or activation of NHE [30,31,37] have been implicated. However, Hongo et al. [38] observed no stretch-induced changes in \([\text{Na}^+]\), despite the presence of the typical slow increase in force and \([\text{Ca}^{2+}]\), in isolated rat myocytes. In consequence, species differences may exist, and the relevance of stretch for changes in \([\text{Na}^+]\), in human cardiac muscle awaits characterisation.

(c) \([\text{Na}^+]\), and hormonal or neuroendocrine activation: Angiotensin II and endothelin-1 have been suggested to increase \([\text{Na}^+]\), (such as in smooth muscle [39,40]), but no direct measurements are available for cardiac tissue. Their effect on \([\text{Na}^+]\), is believed to result from activation of NHE [30,31,41], and possibly, \(\text{Na}^+\) channels [42]. Phenylephrine [43] and insulin-like growth factor-I [44] may also activate NHE but effects on \([\text{Na}^+]\), were not directly assessed. Hyperthyroidism increases \([\text{Na}^+]\), and affects the expression of \(\text{Na}^+\) channels, NCX, and NHE [45]. In contrast, alpha-1 adrenoceptor-stimulation [46] or \(\beta\)-adrenoceptor stimulation [47,48] may reduce \([\text{Na}^+]\),
in cardiac muscle. Treatment with an angiotensin-converting enzyme inhibitor for 8 days significantly reduced $[\text{Na}^+]_i$, in rabbits [49]. Therefore, a number of stimuli, including stretch and hormones, are involved in short-term and long-term regulation of Na$^+$ homeostasis. Given the activation of most of these stimuli in cardiac disease, changes in cellular Na$^+$ handling may be an ubiquitous finding in heart failure.

(3) Small changes in $[\text{Na}^+]_i$, may have large impact on $[\text{Ca}^{2+}]_i$ and contractility: $[\text{Na}^+]_i$, is of paramount importance for contractile function. The relationship between a change in $[\text{Na}^+]_i$, and developed tension is steep [50,51], mainly because small increases in $[\text{Na}^+]_i$, have large impact on Ca$^{2+}$ fluxes through NCX. Bers [15] calculated that an increase in $[\text{Na}^+]_i$, from 9 to 12 mmol/l could shift the reversal potential of NCX ($E_{\text{rev}}$) by $-30 \text{ mV}$. This would tend to increase Ca$^{2+}$ influx via reverse mode NCX during the AP, but also limit Ca$^{2+}$ extrusion via NCX during relaxation and diastole. Force of contraction can double with as little as a 1 mmol/l increase in $[\text{Na}^+]_i$, [50]. The steep dependence of twitch tension (and $[\text{Ca}^{2+}]_i$) on $[\text{Na}^+]_i$, means that maneuvers which produce even small changes in $[\text{Na}^+]_i$, will have significant effects on contraction.

(4) Sodium gradients may exist within myocytes: The general view is that Na$^+$ rapidly diffuses and equilibrates within the cytoplasm. However, several investigators have proposed the existence of a subsarcolemmal space with restricted diffusion properties for Na$^+$ [52–54]. Using electron-probe microscopy, Wendt-Gallitelli [20] described microheterogeneity of subsarcolemmal sodium gradients with $[\text{Na}^+]_i$, amounting to 40 mmol/l within 20 nm of the inner side of the sarcolemmal membrane. This ‘fuzzy-space’ theory suggests that a large increase in subsarcolemmal $[\text{Na}^+]_i$, can be achieved by a quantitatively small Na$^+$ entry. It implies that even if global $[\text{Na}^+]_i$, is unchanged, local changes in subsarcolemmal $[\text{Na}^+]_i$, (that may remain undetected with common techniques) could affect Ca$^{2+}$ handling through NCX. For example, the Na$^+$ influx through forward-mode NCX is too small to affect cytosolic Na$^+$ concentrations [15], but may increase local $[\text{Na}^+]_i$. Terraciano [55] reported that abrupt inhibition of the Na$^+$/K$^+$ pump substantially delays relaxation and the decline of the Ca$^{2+}$-transient as a consequence of local Na$^+$ accumulation with ‘autoinhibition’ of the NCX. The issue of subcellular $[\text{Na}^+]_i$, microdomains clearly deserves further investigation.

3.1. Altered $[\text{Na}^+]_i$, handling in animal models of hypertrophy and heart failure

Elevated cytosolic $[\text{Na}^+]_i$, has been consistently observed in animal models of hypertrophy [56–58]. Gray [19] reported elevated $[\text{Na}^+]_i$, in association with blunted force–frequency behavior in guinea-pigs with myocardial hypertrophy. These studies suggest that increased $[\text{Na}^+]_i$, is an early event in the myocyte response to hemodynamic overload.

The data in heart failure models are less consistent. Slightly reduced $[\text{Na}^+]_i$, was found in rabbits with pacing-induced heart failure [22]. In contrast, Despa et al. [59] recently reported on $[\text{Na}^+]_i$, handling in a rabbit heart failure model induced by a combination of aortic insufficiency and constriction. In this model, myocyte $[\text{Na}^+]_i$, levels were significantly elevated (by $\sim 3 \text{ mM}$) as a result of enhanced Na$^+$ influx through Na$^+$-channels (whereas Na$^+$/K$^+$-pump function was unaltered). Elevated $[\text{Na}^+]_i$, was also observed in a similar rabbit heart failure model, but resulted from enhanced Na$^+$-influx through the sarcolemmal NHE (see Baartscher et al. in this Spotlight Issue). These studies show that elevated $[\text{Na}^+]_i$, is present in most animal models of hypertrophy and failure.

4. $[\text{Na}^+]_i$, handling in the human heart

4.1. $[\text{Na}^+]_i$, in nonfailing human myocardium

Despite its critical role in the regulation of $[\text{Ca}^{2+}]_i$, and contractility, only few studies on $[\text{Na}^+]_i$, handling in human myocardium are available. Using ion selective microelectrodes, Gray et al. [19] recently reported $[\text{Na}^+]_i$, values of $11.8 \pm 1.4 \text{ mmol/l}$ in control human ventricle. We characterized $[\text{Na}^+]_i$, handling by use of SBFI in ventricular trabeculae from nonfailing human hearts [60]. $[\text{Na}^+]_i$, was determined to be $15.6 \text{ mmol/l}$ at a pacing rate of 0.25 Hz (Fig. 3A). These $[\text{Na}^+]_i$, values in human hearts were higher than those from most previous reports in mammalian myocardium (Fig. 2). Therefore, we performed complementary experiments using a bioassay in myocytes from nonfailing hearts [60]. In these experiments, $[\text{Na}^+]_i$, was determined to be $8.0 \text{ mmol/l}$ at a pacing rate of 0.25 Hz (Fig. 3 B). The $[\text{Na}^+]_i$, levels inferred from the bioassay are more in the range of values reported in other species [29,61]. Taking these three approaches—microelectrodes [19], fluorescent indicator, bioassay [60]—an average $[\text{Na}^+]_i$, of $11.8 \text{ mmol/l}$ can be estimated from the existing data in contracting nonfailing human myocardium. This allows a first comparison to mammalian myocardium (Fig. 2). Though data are still sparse, human myocardium appears to regulate its $[\text{Na}^+]_i$, levels more at the upper end of the species–$[\text{Na}^+]_i$, relationship.

4.2. $[\text{Na}^+]_i$, handling in failing human myocardium

Gray [19] found higher $[\text{Na}^+]_i$, ($14.0–14.3 \text{ mmol/l}$; only two measurements) in hypertrophied (aortic stenosis vs.
control muscles (11.8±1.4 mmol/l), associated with a blunting of the positive force–frequency relation.

We found that [Na\(^+\)] levels were elevated in failing as compared to nonfailing human myocardium (Fig. 3) [60]. [Na\(^+\)] was 15.6±3.2 mmol/l in nonfailing cardiac muscles, but 22.1±2.6 mmol/l in failing muscles (Fig. 3A). We also predicted [Na\(^+\)] by the bioassay technique and confirmed elevated [Na\(^+\)], in failing (12.1 mmol/l) vs. nonfailing (8.0 mmol/l) myocytes (Fig. 3B). Therefore, irrespective of the technique, [Na\(^+\)] was significantly higher in failing as compared to nonfailing human myocardium. This was the first report on elevated [Na\(^+\)] levels in failing human hearts.

4.3. [Na\(^+\)] and force–frequency relation in nonfailing and failing human myocardium

We also assessed the effect of stimulation rate (0.25–3.0 Hz) on [Na\(^+\)], and contractile function. As in other mammalian myocardium [16,17], we observed a rate-dependent increase in [Na\(^+\)], in nonfailing human myocardium (Fig. 4, right), associated with an increase in twitch force (positive force–frequency relationship; Fig. 4, left) and an increase in Ca\(^{2+}\)-transients and SR Ca\(^{2+}\)-content (Fig. 5; data adapted from Refs. [3,4]). [Na\(^+\)], also increased with stimulation frequency in failing myocardium (Fig. 4), and was significantly higher in failing as compared to nonfailing muscles at each pacing rate (Fig. 6, lower panel). However, failing human myocardium was characterized by a rate-dependent decline in twitch force (negative force–frequency), associated with a rate-dependent decline in Ca\(^{2+}\)-transients and blunting of rate-dependent increases in SR Ca\(^{2+}\)-load (Fig. 5).

Besides force–frequency relation (which reflects the amplitude of twitch force generation), nonfailing and failing myocardium also differed with respect to diastolic...
function. Twitch force was similar, but [Na\(^+\)], and diastolic tension were significantly higher in failing vs. nonfailing myocardium at low pacing rates (Fig. 6). At 2.0 Hz, however, developed force was significantly higher in nonfailing as compared to failing myocardium (related to the positive vs. negative force–frequency relationship). While [Na\(^+\)], had increased in both groups, diastolic tension increased only in failing human myocardium (Fig. 6), possibly related to the higher [Na\(^+\)], followed by Ca\(^{2+}\) overload [2]. The principal result from these studies is that [Na\(^+\)], is elevated in failing human myocardium at any physiological stimulation rate.

4.4. Functional consequences of elevated [Na\(^+\)], in failing human myocardium

Developed force and force–frequency behavior are related to the amplitude of intracellular Ca\(^{2+}\)-transients and SR Ca\(^{2+}\)-content in mammalian and human myocardium (Fig. 5) [3,4,34]. The rate-dependent increases in [Na\(^+\)], are likely to be centrally involved in the positive force–frequency relationship of the normal human heart. Higher [Na\(^+\)], promotes increased [Ca\(^{2+}\)], through its effects on NCX. Nonfailing myocardium with normal SERCA2a activity is able to handle this extra Ca\(^{2+}\) and accumulate it within the SR during diastole, even at high heart rates with short diastolic time intervals. In consequence, SR Ca\(^{2+}\)-stores largely increase with increasing stimulation rates in nonfailing myocardium [4], but diastolic Ca\(^{2+}\) can be maintained at low levels.

In failing human myocardium, Ca\(^{2+}\) handling is altered (for review, see Ref. [9]). Reduced SR Ca\(^{2+}\) uptake, enhanced SR Ca\(^{2+}\) leak, and increased activity of the NCX are contributors to aberrant Ca\(^{2+}\) regulation [9]. An unresolved issue is how peak Ca\(^{2+}\) [3] and the magnitude of contraction [3,62,63] can be similar in nonfailing and failing human myocardium at slow beating rates in the presence of these alterations (see also Fig. 6). Altered
[Na\(^{+}\)], homeostasis may provide an explanation for this observation. The higher level of [Na\(^{+}\)], in failing myocytes alters the thermodynamics of Ca\(^{2+}\) regulation via NCX by reducing the driving force for Ca\(^{2+}\) elimination, and potentially increasing reverse-mode NCX Ca\(^{2+}\) influx and [Ca\(^{2+}\)]. Besides elevated [Na\(^{+}\)], prolonged APD and reduced Ca\(^{2+}\)-transient amplitudes are typical features of failing human myocardium [3,64] and may contribute to reverse-mode NCX function. Using their rabbit heart failure model, Despa et al. [65] calculated that outward $I_{\text{NCX}}$ (i.e. reverse-mode Ca\(^{2+}\) influx) is very brief in control myocardium, but may be prolonged to $\sim 150$ ms with 3 mmol/l higher [Na\(^{+}\)], in failing myocytes. Interestingly, the increase in [Na\(^{+}\)], by $\sim 3$ mmol/l had the largest impact on $I_{\text{NCX}}$, followed by reduced [Ca\(^{2+}\)], and prolonged APD. Consistently, outward $I_{\text{NCX}}$ may occur during the prolonged AP in failing human myocytes [66]. Indeed, we observed substantial reverse-mode NCX Ca\(^{2+}\)-influx, associated with enhanced SR Ca\(^{2+}\)-loading in failing myocytes (Fig. 7).

At slow heart rates (with longer diastolic time) this should allow the depressed SR of the failing myocyte sufficient time to accumulate relatively normal amounts of Ca\(^{2+}\) without substantial elevations in diastolic Ca\(^{2+}\). This may result in comparable basal twitch force at low stimulation rates (Fig. 5). As the pacing frequency increases, the SR of the failing myocyte cannot refill normally [4], even though [Na\(^{+}\)], remains elevated. At these faster rates the excess Na\(^{+}\) in the failing myocyte would favor diastolic Ca\(^{2+}\) overload and increases in diastolic tension (Fig. 6; see also Ref. [6]).

5. Potential mechanisms for elevated [Na\(^{+}\)], in the failing human heart

The mechanisms for elevated [Na\(^{+}\)], in failing human hearts have not yet been identified. Na\(^{+}\) homeostasis is regulated by a network of ion channels and transporters (Fig. 1), and alterations in gene expression or function of these proteins have been described. In addition, pathophysiological stimuli such as wall stress or neuroendocrine activity modulate Na\(^{+}\) handling. Since the latter factors are controlled under in vitro conditions, differences in Na\(^{+}\) handling between nonfailing and failing myocardium may even be underestimated in the experimental setting.

5.1. Na\(^{+}\) influx in the failing human heart

5.1.1. Voltage-gated Na\(^{+}\) channels

A major pathway for Na\(^{+}\) entry is the voltage-gated Na\(^{+}\) channel. Na\(^{+}\) channels are responsible for a large, brief inward $I_{\text{Na}}$ which causes the rising phase of the action potential (see Bers et al. in this Spotlight Issue). Normally, Na\(^{+}\) channels inactivate quickly and completely, and rarely reopen [67]. However, under certain conditions reopening of Na\(^{+}\) channels can produce a persistent Na\(^{+}\)-influx. Bers [15] calculated that a ‘window’ $I_{\text{Na}}$ exists at a resting membrane potential of $-80$ mV which is responsible for about 30% of the Na\(^{+}\)-channel mediated Na\(^{+}\)-influx.

Besides the transient Na\(^{+}\) current ($I_{\text{Na,t}}$), a late, slowly inactivating Na\(^{+}\) current ($I_{\text{Na,L}}$) has been observed in mammalian hearts [68]. Maltsev [69] described a late $I_{\text{Na,L}}$ with ultraslow inactivation kinetics in human myocardium which contributed to AP duration. This $I_{\text{Na,L}}$ tended to be larger in myocytes from hearts with dilated cardiomyopathy (but not ischemic cardiomyopathy) as compared to nonfailing hearts [69]. The magnitude of $I_{\text{Na,L}}$...
relative to \( I_{na,1} \) was also increased in myocytes from the midmyocardial portion of failing human hearts [70]. Enhanced \( I_{na,1} \) has also been observed in a canine heart failure model [71]. Therefore, this slowly inactivating \( Na^+ \) current could contribute to elevated \([Na^+]_i\), and AP prolongation in the failing human heart.

Sodium channelopathies may contribute to altered \( Na^+ \) handling in distinct disease entities. Inherited mutations in SCN5A, the gene encoding the human cardiac sodium channel, have been linked to congenital forms of the long QT syndrome (‘gain of function’ of the \( Na^+ \) channel) [72]. Mutations in the SCN5A gene have not been associated with heart failure, but the concept of channelopathies is not restricted to genetic disorders: \( Na^+ \) channels in cells isolated from the epicardial border zone of infarcted canine hearts reveal striking gating defects, including slowed recovery of inactivation [73].

The quantitative importance of \( Na^+ \) influx through \( Na^+ \) channels (relative to other \( Na^+ \) transporters, such as NHE and NCX) is unknown. From data in rabbit and guinea pig, Bers [15] calculated that despite the large peak \( I_{na} \) (>1 nA/pF), the amount of \( Na^+ \) which enters the cell is only \( \approx 15 \) \( \mu M \) per beat because the current is so brief. This is less than the 30 \( \mu M \) \( Na^+ \) influx per beat via NCX [65]. However, Bers [15] derived that if only 0.5% of \( I_{na} \) would persist throughout the AP this would easily double the amount of \( Na^+ \) entry through \( Na^+ \) channels. In addition, the potential existence of microdomains (see above) would greatly increase the functional relevance of abnormal \( Na^+ \) channel gating [52]. Therefore, tight control of gating processes of \( Na^+ \) channels are of major importance for proper \( Na^+ \) handling, but alterations in \( Na^+ \) currents are poorly understood in the failing human heart.

5.1.2. \( Na^+ /Ca^{2+} \)-exchange

Since NCX is the major trans-sarcolemmal \( Ca^{2+} \) elimina- tion mechanism, \( Na^+ \) may enter the myocytes via NCX in quantitatively relevant amounts. Despa et al. [65] calculated that in isolated rabbit myocytes stimulated at 1 Hz, excitation-dependent \( Na^+ \) influx totals 37 \( \mu mol/l \) per beat, the larger part (30 \( \mu mol/l \)) resulting from forward-mode NCX. Indeed, under conditions of impaired \( Na^+ \)-pump function \( Na^+ \) may locally accumulate and inhibit NCX forward mode [55].

The NCX can also work in its reverse mode, i.e. 3 \( Na^+ \) ions are extruded for influx of 1 \( Ca^{2+} \) ion. Leblanc and Hume [74] demonstrated that inward movements of \( Na^+ \) ions may determine excitation–contraction processes. Their results suggest that \( Na^+ \) influx through \( Na^+ \) channels occurs in a restricted space, causes the local \([Na^+]_i\), to increase sufficiently to activate the NCX reverse mode, thereby inducing \( Ca^{2+} \) influx supplementary to the \( Ca^{2+} \) influx through the \( Ca^{2+} \) channels. However, Sipido et al. [14] reported reverse-mode NCX to be less efficient in triggering SR \( Ca^{2+} \) release, and this was also the case in rabbit myocytes [65].

Recent work suggests that the failing human heart is characterized by increased trans-sarcolemmal \( Ca^{2+} \) cycling [4,75], suggesting a greater role for NCX. Indeed, a number of studies demonstrated increased NCX expression and activity in failing human hearts (for review, see Refs. [8,9]), but contradictory results exist [76], and large interindividual variability was reported [6]. Expression levels of the exchanger are controlled by sympathetic tone [77] and were normalized in patients on beta-blocker therapy [78]. Upregulation of NCX would magnify the functional impact of altered \([Na^+]_i\), in the failing heart, since forward-, but also reverse-mode function (see Fig. 7) could be enhanced. Indeed, the reverse-mode NCX current increased with increasing pipette \( Na^+ \) in isolated mouse myocytes [79], and in transgenic mice overexpressing the NCX [13,79]. Therefore, we suggest that elevated \([Na^+]_i\), in concert with reduced \( Ca^{2+} \) transients and prolonged APs favors reverse-mode \( Ca^{2+} \) influx during depolarisation and re- duces forward-mode \( Ca^{2+} \) elimination. Since transport direction at given \( Na^+ \) and \( Ca^{2+} \) gradients and membrane potential are not affected by expression levels, NCX overexpression may increase both \( Ca^{2+} \) influx through reverse-mode, but also \( Ca^{2+} \) elimination through forward mode function.

5.1.3. \( Na^+ /H^+ \)-exchange

The sarcolemmal NHE extrudes \( H^+ \) in a 1:1-stoichiometry for \( Na^+ \) influx. In consequence, increased activity or expression of NHE may be involved in elevated \( Na^+ \) levels, but the quantitative relevance of this mechanism for \( Na^+ \) influx in heart failure is controversial. The NHE activity is regulated by intracellular \( H^+ \) concentrations and a number of physiological stimuli, such as neurohormones and stretch. These mechanisms increase NHE activity by increasing its \( pH \) sensitivity, and this is followed by elevated \([Na^+]_i\), [30,31] (but see also Cingolani et al. in this Spotlight Issue). Consistently, endothelin-1 and stretch activate NHE in isolated human myocardium [80,81].

However, there are few reports on expression and activity of NHE in heart failure. Contractile dysfunction and lower \( Ca^{2+} \) concentrations in rat diabetic cardiomyopathy were related to depressed activity of NHE and lower \([Na^+]_i\), levels [82,83]. Increased activity of NHE was associated with enhanced NHE-mediated \( Na^+ \) influx and elevated \([Na^+]_i\), in a rabbit heart failure model (see Baartscher et al. in this Spotlight Issue).

There is only one publication that directly addresses NHE function in failing human myocardium. In myocytes isolated from failing human hearts, the activity of NHE was significantly higher than in myocytes from nonfailing hearts, but protein expression levels were unchanged [84]. Therefore, intrinsic and stimulated NHE activity may be increased in human heart failure and contribute to elevated \([Na^+]_i\).
5.2. Na\(^+\) efflux in the failing human heart: the Na\(^+\)/K\(^+\) pump

The sodium pump is, besides minor contributions of reverse-mode NCX, the main mechanism by which Na\(^+\) is eliminated from the cytosol. Changes in the abundance, isoform expression pattern, and function of the sodium pump may be involved in elevated [Na\(^+\)]\(_{i}\) in the failing human heart. This topic is presented in detail in this Spotlight Issue by Schwinger et al. and will only be discussed here briefly.

The sodium pump transports Na\(^+\) out and K\(^+\) into the cell in a 3:2 stoichiometry against respective concentration gradients (it is therefore electrogenic and produces a repolarising current). Cardiac glycosides inhibit enzymatic activity and transport, thereby increasing [Na\(^+\)]\(_{i}\), reducing NCX forward mode and favoring NCX reverse mode.

A number of studies have investigated the potential role of altered expression patterns of sodium pumps in heart failure. Earlier investigations have led to contradictory results of whether or not the sodium pump is downregulated in human heart failure [89, 90]. Recent data indicate more consistently that both expression and activity of sodium pumps are significantly reduced, and isoform shifts of pump molecules occur [76].

The mechanisms involved in isoform shift and reduced expression levels of the Na pump remain unclear. Elevated [Na\(^+\)]\(_{i}\), directly increased Na\(^+\)/K\(^+\)-ATPase \(\alpha\) and \(\beta\) isoform expression [91, 92]. Consistently, glycoside stimulation (as well as phenylephrine and endothelin-1) increased expression levels of the sodium pump [93], even in the absence of [Ca\(^{2+}\)]\(_{i}\) [91]. Therefore, other, yet undefined stimuli may be involved in reduced gene expression of the sodium pump despite elevated [Na\(^+\)]\(_{i}\). It was speculated that a systemic factor contributes, since Na\(^+\)/K\(^+\)-ATPase expression is also reduced in skeletal muscle in heart failure [94].

No data are available that relate expression or activity of the sodium pump to [Na\(^+\)]\(_{i}\), in the human heart. However, studies in guinea-pig heart demonstrate that atrial muscle, compared with ventricular muscle, has less than half the Na\(^+\)/K\(^+\)-ATPase activity, but 3 mmol/l higher [Na\(^+\)]\(_{i}\) [95]. Therefore, a reduced Na\(^+\) elimination capacity through downregulated Na\(^+\)/K\(^+\)-ATPase may contribute to increased [Na\(^+\)]\(_{i}\) in the failing human heart.

However, the contribution of altered sodium pump function for elevated [Na\(^+\)]\(_{i}\), in failing human myocardium has not been assessed. Most of the above mentioned expression studies were performed in tissue homogenates and could also reflect changes in nonmyocytes. Moreover, these measurements cannot differentiate between functional and inactive pumps. Despa [59] demonstrated that \(V_{\text{max}}\) (and \(K_{\text{m}}\)) of the Na\(^+\)/K\(^+\)-ATPase as well as pump-mediated Na\(^+\) efflux were even higher in failing as compared to nonfailing rabbit myocytes. Therefore, reduced Na\(^+\) efflux cannot explain higher [Na\(^+\)]\(_{i}\) in this heart failure model, where elevated [Na\(^+\)]\(_{i}\), was related to a 2-fold higher Na\(^+\) influx rate. Higher resting sodium pump current (~2-fold) has also been reported for dogs with chronic atrioventricular block [58]. Therefore, the mechanism(s) for elevated [Na\(^+\)]\(_{i}\), in failing human myocardium clearly await further characterisation.

6. Clinical consequences of elevated [Na\(^+\)]\(_{i}\), in heart failure

Elevated [Na\(^+\)]\(_{i}\) in myocardial hypertrophy and heart failure probably occurs as a consequence of altered function and expression of a variety of Na\(^+\) handling proteins (see above). Furthermore, a number of pathophysiological stimuli associated with heart failure may aggravate [Na\(^+\)]\(_{i}\) overload, such as tachycardia, increased wall stress, neuro-endocrine stimulation, ischemia or acidosis. In the following, we will briefly discuss potential clinical consequences of elevated [Na\(^+\)]\(_{i}\), under these conditions.

6.1. Elevated [Na\(^+\)]\(_{i}\), and contractile function

As discussed in the previous sections, elevated [Na\(^+\)]\(_{i}\), may maintain contractile force at low heart rates, but further impair diastolic function at higher heart rates. [Na\(^+\)]\(_{i}\) overload, followed by [Ca\(^{2+}\)]\(_{i}\) overload, may be one reason for the detrimental effects of tachycardia in heart failure patients.

Elevated [Na\(^+\)]\(_{i}\) also helps to explain the increased sensitivity of the failing human heart to cardiac glycosides or Na\(^+\) channel activators. In fact, Na\(^+\) channel agonists such as DPI 201–106 induced diastolic dysfunction, diastolic Ca\(^{2+}\) overload, and aftercontractions in isolated human myocardium. Due to their large spectrum of side effects, Na\(^+\) channel activators are currently not used in the treatment of heart failure (for review, see Ref. [96]).

5.2. Na\(^+\)–HCO\(^{-}\)\(_3\) cotransport

It is assumed that the electrogenic Na\(^+\)–HCO\(^{-}\)\(_3\)-co-transporter (NBC) operates with a stoichiometry of 1 Na\(^+\) ion coupled to 2 HCO\(^{-}\)\(_3\) ions. In guinea-pig ventricular myocytes, this symport activity accounts for 40–50% of total acid extrusion [85]. Depolarisation of the cell membrane increases the driving force for electrogenic transport systems. Accordingly, Camilioni de Hurtado et al. [86] observed a NBC-mediated increase in pH\(_i\) upon increases in stimulation rate in cat papillary muscle. Therefore, increased Na\(^+\) influx through NBC may contribute to the rise in [Na\(^+\)]\(_{i}\), with higher stimulation rates. Recently, Khandoudi et al. [87] reported an increased expression of NBC in failing human myocardium. The exact role of NBC for Na\(^+\) homeostasis in the human heart is unknown, but it is evident that this cotransport may substantially increase [Na\(^+\)]\(_{i}\), under certain conditions (for review, see Ref. [88]).
6.2. Elevated \([\text{Na}^+]\) and arrhythmias

A major consequence of elevated \([\text{Na}^+]\), is an accompanying rise of \([\text{Ca}^{2+}]\). If this induces SR \(\text{Ca}^{2+}\) overload, episodes of spontaneous SR \(\text{Ca}^{2+}\) release (and contractions) in isolated rat myocytes. This release can activate \(I_{\text{Na/Ca}}\) to depolarise the cell and trigger arrhythmogenic DADs [98]. Support for the idea of pro-arrhythmogenic actions of elevated \([\text{Na}^+]\) also comes from studies that have used drugs that inhibit \([\text{Na}^+]\) overload (for review, see Ref. [99]).

6.3. Elevated \([\text{Na}^+]\) and myocardial remodeling and growth

\(\text{Na}^+\) is intimately linked to pathophysiological stimuli involved in cardiac growth and hypertrophy (Fig. 8). A number of signal transduction mechanisms involved in \(\text{Na}^+\) regulation, such as the NHE or the \(\text{Na}^+ / \text{K}^+\)-ATPase, have been implicated in regulation of gene expression, growth, and remodeling.

Elevated \([\text{Na}^+]\) was consistently observed in animal models of hypertrophy [56–58] and in human hypertrophied myocardium [19]. Sodium ions are important mediators of cell hypertrophy [100,101], and \(\text{Na}^+\) exerts hypertrophic effects in isolated cardiac myocytes [100]. \(\text{Na}^+\) ions directly increase gene expression of the \(\alpha\)-isoform genes [90]. In addition, \(\text{Na}^+/\text{K}^+\)-ATPase inhibition by cardiac glycosides induces hypertrophy in rat myocytes [102]. Importantly, the \([\text{Na}^+]\) dependent increase in \([\text{Ca}^{2+}]\), may exert growth-promoting effects through activation of calcineurin and calcium-dependent transcription factors.

The most recent support related to growth-promoting effects of \([\text{Na}^+]\), may arise from pharmacological blockade of NHE. Long-term NHE stimulation is related to myocardial growth, remodeling, and failure, and these features can be prevented in various animal models by NHE blockers such as cariporide [103–105]. The exact mechanism how NHE inhibition prevents remodeling is unknown, but preventing \(\text{Na}^+\) (and \(\text{Ca}^{2+}\)) overload may be involved. However, whether NHE inhibition reduces \([\text{Na}^+]\), and prevents hypertrophy and heart failure has not been tested in humans.

7. Conclusion

\(\text{Na}^+\) handling in cardiomyocytes involves the delicate balance of a number of ion channels and transporters, and most of these mechanisms are altered in heart failure. In consequence, intracellular \(\text{Na}^+\) homeostasis is shifted towards higher \([\text{Na}^+]\), in mammalian models of hypertrophy and failure, and in the failing human heart. However, \([\text{Na}^+]\), itself is critically involved in a number of physiological processes, such as intracellular \(\text{Ca}^{2+}\) handling, contraction–relaxation processes, pH regulation, energy metabolism, and cell growth. Functionally, the higher \([\text{Na}^+]\), in the failing human heart may maintain
normal systolic [Ca\(^{2+}\)] and contractions at low heart rates, but induce cytosolic Ca\(^{2+}\) overload with impaired relaxation and diastolic dysfunction at higher heart rates. The exact mechanisms responsible for shifting [Na\(^{+}\)], to a higher equilibrium remain to be identified. Even more importantly, we need to explore the role of altered Na\(^{+}\) handling in hypertrophy and for initiation and progression of heart failure.

Acknowledgements

This work was supported by DFG grant Pi 414/1-1.

References


