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Review

Intracellular Na^+ regulation in cardiac myocytes

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Abstract

Intracellular $[Na^+]$ ($[Na^+]_i$) is regulated in cardiac myocytes by a balance of Na^+ influx and efflux mechanisms. In the normal cell there is a large steady state electrochemical gradient favoring Na^+ influx. This potential energy is used by numerous transport mechanisms, including Na^+ channels and transporters which couple Na^+ influx to either co- or counter-transport of other ions and solutes. Six sarcolemmal Na^+ influx pathways are discussed in relatively quantitative terms: Na^+ channels, Na^+/Ca^{2+} exchange, Na^+/H^+ exchange, Na^+/Mg^{2+} exchange, Na^+/HCO_3^- cotransport and $Na^+/K^+/2Cl^-$ cotransport. Under normal conditions Na^+/Ca^{2+} exchange and Na^+ channels are the dominant Na^+ influx pathways, but other transporters may become increasingly important during altered conditions (e.g. acidosis or cell volume stress). Mitochondria also exhibit Na^+/Ca^{2+} antiporter and Na^+/H^+ exchange activity that are important in mitochondrial function. These coupled fluxes of Na^+ with Ca^{2+}, H^+ and HCO_3^- make the detailed understanding of $[Na^+]_i$ regulation pivotal to the understanding of both cardiac excitation–contraction coupling and pH regulation. The Na^+/K^+-ATPase is the main route for Na^+ extrusion from cells and $[Na^+]_i$ is a primary regulator under physiological conditions. $[Na^+]_i$ is higher in rat than rabbit ventricular myocytes and the reason appears to be higher Na^+ influx in rat with a consequent rise in Na^+/K^+-ATPase activity (rather than lower Na^+/K^+-ATPase function in rat). This has direct functional consequences. There may also be subcellular $[Na^+]_i$ gradients locally in ventricular myocytes and this may also have important functional implications. Thus, the balance of Na^+ fluxes in heart cells may be complex, but myocyte Na^+ regulation is functionally important and merits focused attention as in this issue.

Keywords: Myocytes; Na/Ca-exchanger; Na/H-exchanger; Na/K-pump; Na-channel

1. General aspects of cardiac Na^+ regulation

Intact cells maintain a large electrochemical $[Na^+]$ gradient across the plasma membrane. While extracellular $[Na^+]$ ($[Na^+]_o$) is ~140 mM, intracellular free $[Na^+]$ ($[Na^+]_i$) is normally 4–16 mM. In excitable cells, the energy stored in the transmembrane Na^+ gradient provides the basis for fast electrical signaling, i.e. propagation of action potentials in neurons and muscles. Many cells utilize the electrochemical Na^+ gradient to couple energetically unfavorable transmembrane solute flow to Na^+ transport (secondary active transport). Examples include Na^+/neurotransmitter, Na^+/glucose and Na^+/amino acids cotransporters as well as various Na^+/ion co- and counter-transporters. The $[Na^+]_i$ also plays an important role in regulating the mitochondrial pH, $[Na^+]$ and $[Ca^{2+}]$, as a substrate for mitochondrial Na^+/H^+ and Na^+/Ca^{2+} exchangers.

Let us consider (Fig. 1A) the free energy of the electrochemical Na^+ gradient $\Delta G_{Na} = RT \ln([Na^+]_i/[Na^+]_o) + zFE_m$, where the first term is the chemical energy while the second is the electrical energy (R is the

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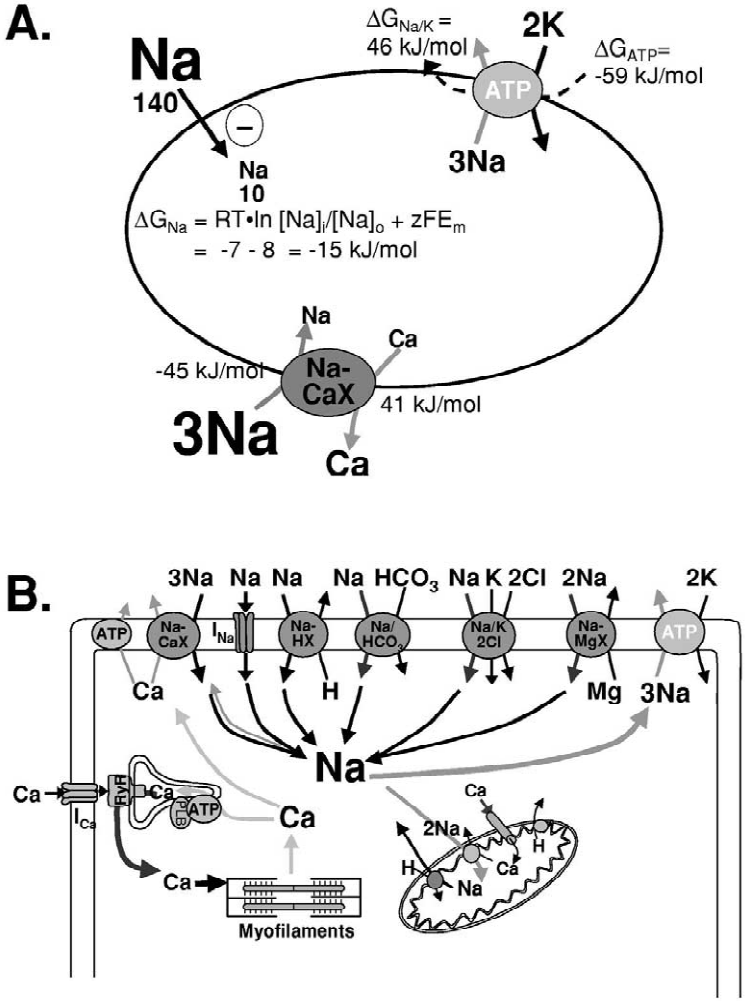

Fig. 1. (A) Schematic illustration of the transmembrane electrochemical Na^+ gradient. (B) Main pathways involved in $[Na]_i$ regulation in cardiac cells.

universal gas constant, T is temperature in °K, z is valence and F is Faraday's constant). For a typical $[Na^+]_i = 10$ mM and $E_m = -80$ mV, $\Delta G_{Na} = -15$ kJ/mol (with the two terms being almost equal, at -7 and -8 kJ/mol, respectively). A negative value for ΔG means that Na entry is energetically favored, in this case that Na^+ ions tend to enter the cell. This ΔG_{Na} is also equivalent to -150 mV in electrical potential terms. The energy to build this potential energy gradient comes from the Na^+/K^+-ATPase, which transports 3 Na^+ out and 2 K^+ in for each ATP hydrolyzed. The transport of 3 mol of Na^+ against its electrochemical gradient requires a free energy of $-3 \cdot \Delta G_{Na} \sim -46$ kJ. The free energy released by the hydrolysis of 1 mol of ATP, ΔG_{ATP}, is -59 kJ. Thus, the Na^+/K^+-ATPase has a remarkable energetic efficiency of ~77%. At resting E_m almost all of the energy used (>95%) goes into the uphill transport of Na^+, because the $[K^+]$ gradient is near its electrochemical equilibrium ($E_K \sim -90$ mV). That is, the uphill chemical $[K^+]$ gradient into the cell is almost exactly balanced by a downhill electrical gradient for the positive K^+ ion. Of course as the cell depolarizes during the cardiac action potential, an increasing fraction of the energy from ATP is used for K^+ pumping. At the peak of the action potential ($E_m \sim +40$ mV) most of the energy goes into pumping K^+ in versus Na^+ out.

This Na^+ energy gradient is used by many processes, including extrusion of Ca^{2+} from the cell via Na^+/Ca^{2+} exchange (NCX). Extrusion of Ca^{2+} is also very uphill energetically, with an electrochemical gradient of ~41 kJ/mol (where the chemical and electrical terms are 26 and 15 kJ/mol, respectively). Thus, it requires the energy obtained from the downhill transport of 3 mol of Na^+ (-45 kJ) to remove each mole of Ca^{2+} (41 kJ), and this agrees with the generally accepted stoichiometry of the NCX (3 Na^+:1 Ca^{2+}). The NCX is relatively unique among the Na^+ exchangers and cotransporters in Fig. 1B in that it may reverse under physiological conditions. In that case Ca^{2+} entry can fuel extrusion of 3 Na^+. This can occur because NCX has a reversal potential (analogous to ion channels) that is in the physiological range of E_m, defined as $E_{Na/Ca} = 3E_{Na} - 2E_{Ca}$ (where E_{Na} and E_{Ca} are the equilibrium E_m for Na^+ and Ca^{2+}). Thus the relative

energies in the Na^+ and NCX gradients can change with conditions and this will be addressed in more detail below (see Na^+/Ca^{2+} exchange section below).

While Na^+ is analogous to Ca^{2+} in the steep electrochemical gradient favoring ion entry, cells seem to use Na^+ and Ca^{2+} fluxes differently. Ca^{2+} can carry a major depolarizing current in cellular excitation, but in addition free intracellular $[Ca^{2+}]$ ($[Ca^{2+}]_i$) often changes by a factor of ~10 during activation in many cell types (from ~100 nM to ~1 μM, often in less than 100 ms). The large change in $[Ca^{2+}]_i$ allows $[Ca^{2+}]_i$ to function as a remarkably ubiquitous second messenger in regulating processes such as contraction, secretion and transcription. However, while the Na^+ gradient is used directly for electrical signaling via Na^+ current, changes in $[Na^+]_i$ are typically small requiring many seconds to minutes to rise by even 10%. This makes $[Na^+]_i$ itself a poor candidate for the sort of rapid dynamic regulation attributed to $[Ca^{2+}]_i$. However, the high concentration of Na^+ (in the mM range) allows it to readily participate in quantitatively large coupled transport fluxes. This is important for both the transport of ions across membranes of many cell types, but is also important in driving the uphill transport of important cellular substrates such as glucose, amino acids and neurotransmitters.

In the steady-state, $[Na^+]_i$ is determined by the balance between Na^+ influx and efflux. There are several pathways for Na^+ entry into cardiac cells (Table 1 and Fig. 1B). Voltage-gated Na^+ channels are responsible for the upstroke of action potentials. NCX generally uses Na^+ influx to extrude Ca^{2+}, and is the main mechanism for Ca^{2+} extrusion during relaxation. Na^+/H^+ exchange (NHE) extrudes H^+ in exchange for Na^+ and is critical in cellular pH homeostasis. Another important acid extruding transport is the Na^+/HCO_3^- cotransporter (NBC). There is also a $Na^+/K^+/2Cl$ cotransporter (NKCC) important in volume regulation, and a Na^+/Mg^{2+} exchanger (NMgX) that is involved in extruding Mg^{2+}. Na^+ entering the cells through these mechanisms is then extruded by the Na^+/K^+ pump. The pump utilizes energy derived from the hydrolysis of ATP to exchange three internal Na^+ for two external K^+ ions and thus maintains the transmembrane $[Na^+]$ gradient. Intracellular buffering is important in the regulation of many intracellular ions, including Ca^{2+} (100:1), Mg^{2+} (~100:1) and pH (>1000:1). Although data regarding intracellular Na^+ buffering are sparse, we recently measured very low Na^+ buffering capacity (~1.3) in rabbit ventricular myocytes by measuring simultaneously $[Na^+]_i$ and integrated Na^+/K^+ pump current during $[Na^+]_i$ decline upon abrupt pump activation [1]. However, this low global Na^+ buffering capacity does not preclude local subsarcolemmal Na^+ buffering which may exist (see below).

Resting $[Na^+]_i$ in heart cells is in the range of 4–8 mM for most mammalian species [2–7] and much higher (10–15 mM) in rat and mouse [4–9]. This interspecies difference is rather intriguing, as higher resting $[Na^+]_i$ might limit Ca^{2+} extrusion, altering cellular Ca^{2+} balance and also contributes to rest potentiation, seen in rat, mouse and some other cardiac muscle [7]. $[Na^+]_i$ increases during stimulation in a frequency dependent manner. This is because Na^+ influx per unit time is higher due to more frequent activation of Na^+ channels and NCX. Cook et al. [10] showed that there are also regional differences in the regulation of $[Na^+]_i$ in rabbit left ventricle (higher in sub-epicardial than sub-endocardial myocytes) both at rest and during steady-state stimulation at 0.5 Hz. However, the expression of the Na^+/K^+ pump and the pump current density are similar in the two regions [11]. This suggests that regional differences in $[Na^+]_i$ regulation might arise from differences in Na^+ influx.

Because of NCX, NHE and NBC, $[Na^+]_i$ is centrally involved in controlling the $[Ca^{2+}]_i$ and pH_i. Therefore, $[Na^+]_i$ plays an important role in the heart, as both these

Table 1
Na^+ transport in cardiac cells

	Nickname	Transport and stoichiometry	Resting flux rate[a] (mM/min)	Inhibitors
Na extrusion pathways				
Na^+/K^+-ATPase	Na^+ pump, I_{pump}	3 Na^+:2 K^+ exchange	0.77[b]	Cardiac glycosides
Na^+ influx pathways				
Na^+ channel/current	I_{Na}	Uncoupled	0.14	Tetrodotoxin
Na^+/Ca^{2+} exchanger[c]	NCX	3 Na^+:1 Ca^{2+} exchange[d]	0.28	Ni^{2+}
Na^+/H^+ exchanger	NHE	1 Na^+:1 H^+ exchange	0.12	Amiloride, cariporide
Na^+/HCO_3^- cotransporter	NBC	1 Na^+:1 HCO_3^- cotransport[d]	0.12	DIDS, SITS
$Na^+/K^+/2Cl^-$ cotransporter	NKCC	1 Na^+:1 K^+:2 Cl^- cotransport	0.15	Furosemide, bumetanide
Na^+/Mg^{2+} antiporter	NaMgX	2 Na^+/1 Mg^{2+} exchange	0.08	Imipramine

[a] Values are based in part on our measurements in rabbit ventricular myocytes [5] and some extrapolations from guinea-pig data [80,102] (see text for details).

[b] Determined in Ref. [5] for a $[Na^+]_i$ of 5 mM.

[c] Depending on the internal and external Na^+ and Ca^{2+} concentrations and the membrane potential, the Na^+/Ca^{2+} exchanger can also function as a Na^+ extrusion pathway (see text for details).

[d] Stoichiometry is still a matter of debate (see text).

ions are important factors in excitation–contraction coupling (ECC) [12]. An increase in $[Na^+]_i$ would shift the balance of fluxes on the NCX to favor more Ca^{2+} influx and less Ca^{2+} efflux, resulting in larger Ca^{2+} transients and therefore enhanced contractility. This is the likely mechanism of the inotropic effect of cardiac glycosides, which are specific inhibitors of the Na^+/K^+ pump and have been used to enhance cardiac contractility in the treatment of congestive heart failure for more than 200 years. Indeed, relatively small changes of $[Na^+]_i$ can have major effects on contractile force. In cardiac Purkinje fibers, force can double with ~1 mM rise of $[Na^+]_i$ [13]. In ventricular muscle, small increases in $[Na^+]_i$ produce less percentage increase in force, probably because of a ceiling effect. That is, in rabbit ventricle at ~29 °C and 0.5 Hz, control twitches are already ~40% of the maximum myofilament force [14], limiting the extent of further increase.

As Fig. 1B indicates there are many Na^+ entry pathways, but the Na^+/K^+-ATPase is the main Na^+ extrusion pathway. We will first consider Na^+/K^+-ATPase and then Na^+ influx mechanisms.

2. Na^+/K^+ pump

2.1. Na^+/K^+ pump structure, isoforms and expression in the heart

The Na^+/K^+ pump is a member of the P-type ATPase pumps. The reaction mechanism of these ATPases is based on the formation of a phosphorylated intermediate. Accompanying the phosporylation–dephosphorylation process, P-type ATPases bind, occlude and transport ions by cycling between two different conformations, called E1 and E2 [15]. The Na^+/K^+-ATPase has two major subunits: α and β (for a review, see Ref. [16]). The α subunit was first cloned by Shull et al. [17] and Kawakami et al. [18], has a molecular weight of ~110 kDa and contains the binding sites for ATP, Na^+, K^+ and cardiac glycosides (specific inhibitors of the enzyme). The smaller β subunit (~50 kDa) modulates the ATPase activity and is important in the proper membrane insertion of the pump. A third, smaller (~12 kDa) protein (γ subunit) has also been found in various tissues [19] but its physiological function is not yet known. Four α (α_1–α_4) and three β (β_1–β_3) subunits of the Na^+/K^+-ATPase have been identified. The α_1-α_3 isoforms are expressed in a variety of tissues, whereas the α_4 isoform has only been detected in rat testis [20]. Any αβ combination results in a functional pump.

The α_1 isoform is present in the cardiac tissue of all species studied, but there is marked variation of α_2 and α_3 subunit expression among species. All three α-isoforms are present in the human heart [21–23], whereas α_1 and α_3 isoforms are expressed in dog [24,25] and ferret [26]. The α_1 isoform is predominant in rabbit [11] and guinea-pig [23] heart, although small amounts of α_3 [27] and α_2 [28], respectively, have also been reported. In the rat, the α_1 and α_3 isoforms are expressed in fetal and neonatal hearts and the α_3 isoform is replaced by the α_2 isoform early in development [29,30]. Interestingly, the reverse switch (from α_2 to α_3) occurs in hyperthrophied or failing rat heart [31,32]. The β_1 isoform is the only β subunit appreciably expressed in the human heart [22], although a recent report indicated that β_3 is also present [33].

Different Na^+/K^+-ATPase isoforms have different glycosides and $[Na^+]_i$ sensitivity. In the rat, α_1 is ~100 times more resistant to ouabain than α_2 and α_3. In rabbit, pig, dog and human, the α_1 isoform is much more sensitive to ouabain than in rat (for a review, see Ref. [16]), therefore the differences in glycosides affinity are not as marked. Indeed, it has been recently shown [34] that the affinity of all three human α subunit isoforms for ouabain is similar (~18 nM). Expression of Na^+/K^+-ATPase is higher in ventricle than atrium [22]. In ventricular myocytes, Na^+/K^+ pumps are located in both peripheral sarcolemma and T-tubules [23]. In the rat, the α_1 isoform is preferentially distributed in T-tubules, whereas α_2 and β_1 are homogeneously distributed in the T-tubules and peripheral sarcolemma [23]. The level of expression of different isoforms and/or their cellular localization could have important physiological consequences. James et al. [35] studied the phenotypes of mouse hearts with genetically reduced levels of α_1 or α_2 isoforms. They found that heterozygous α_2 hearts have increased Ca^{2+} transients and contractility whereas the opposite happens for the α_1 heterozygotes. Furthermore, inhibition of the α_2 isoform with ouabain increased the contractility of heterozygous α_1 hearts. These results might indicate a specific role for the α_2 isoform as a regulator of Ca^{2+} in the mouse heart, for example by affecting the function of the Na/Ca exchange. This can happen if the α_2 isoforms are preferentially located in the dyads. This is indeed the case in smooth muscle where the low ouabain-affinity α_1-isoform is ubiquitously distributed, but the higher affinity α_2- and α_3-isoforms are preferentially localized in regions overlying the SR [36].

2.2. Ion transport characteristics of the Na^+/K^+ pump

The Na^+/K^+-ATPase transports three Na^+ ions out and two K^+ ions into the cell using the energy of one ATP molecule, and thus moves out one net charge per cycle. Ion transport by the Na^+/K^+ pump has a functional reversal potential which depends on both intracellular and extracellular $[Na^+]$ and $[K^+]$, as well as the free energy of intracellular ATP hydrolysis (ΔG_{ATP}). Under normal conditions, the reversal potential has been estimated to be about −180 mV [37], such that Na^+/K^+ pump current (I_{pump}) and Na^+ transport are outward over the whole physiological range. The ATP concentration for half-maxi-

mal activation of the cardiac Na^+/K^+ pump is in the range of 80–150 μM [38,39], therefore under control conditions ATP is not rate limiting for the pump (normal ATP levels in cardiac cells are 5–10 mM). However, this can change as [ATP] declines during ischemia or metabolic inhibition, and the simultaneous rise in [ADP] and $[P_i]$ also contribute to a reduced ΔG_{ATP} available for transport. Such a reduction in ΔG_{ATP} would also reduce the $[Na^+]$ and $[K^+]$ gradients that the pump can generate. However, during short-term metabolic inhibition glycolysis may regenerate ATP near the Na^+/K^+-ATPase, making the pump less directly dependent on oxidative phosphorylation [40].

The main physiological regulators of the Na^+/K^+ pump are internal Na^+ and external K^+. The $[Na^+]_i$ for half-maximal pump activation (K_m) in the heart varies widely with the internal and external ionic conditions, in the range of 8–22 mM (for a review, see Ref. [41]). Intracellular K^+ competes with Na^+ for binding to the enzyme at the cytoplasmic surface and results in reduced $[Na^+]_i$-sensitivity of the pump [42]. The activating K_m for extracellular K^+, in the presence of normal external $[Na^+]$, is 1–2 mM [41], therefore the pump is ~70% saturated with respect to external K^+ at a normal concentration of 4 mM. External Na^+ and K^+ compete for common binding sites to the Na^+/K^+-ATPase, therefore the K_m for pump activation by external K^+ is appreciably lower in Na^+-free conditions [43]. The absence of external Na^+ also renders I_{pump} voltage insensitive [43]. With normal Na^+ outside, the E_m-dependence of I_{pump} in ventricular myocytes is sigmoidal in shape with a steep positive slope between −100 and 0 mV and nearly no voltage dependence of I_{pump} at positive potentials [44] (for a review, see Ref. [45]).

As mentioned above, multiple Na^+/K^+-ATPase isoforms exist in cardiac cells from most species, and they might differ functionally because of differential specific intracellular localization, regulation or kinetic characteristics. The $[Na^+]_i$-dependence of the three rat α-subunit isoforms has been studied in transfected cells [16,46]. When transfected into insect cells, the apparent affinity for intracellular Na^+ varies in the order $\alpha_2 > \alpha_1 > \alpha_3$ [16] whereas in HeLa cells the order seems to be $\alpha_1 > \alpha_2 > \alpha_3$ [46]. This difference might be due to the different method used to determine the pump activity (Na^+/K^+-ATPase activity vs. the rate of Na^+ extrusion from intact cells). However, the common point is that α_3 isoform has a much lower Na^+-affinity (K_m~30 mM) than α_1 or α_2, making it less active at physiological $[Na^+]_i$, perhaps serving as a 'safety' mechanism in conditions of Na^+ overload. The α_3 isoform also has a lower $[K^+]_o$ affinity [16].

How much Na^+ is extruded by the Na^+/K^+-ATPase to maintain steady-state $[Na^+]_i$? We measured [5] the $[Na^+]_i$-dependence of the Na^+/K^+ pump-mediated Na^+ efflux in rat and rabbit ventricular myocytes (Fig. 2A). There is little difference in the Na^+/K^+ pump rate for $[Na^+]_i$ below 11 mM. However, Na^+/K^+-ATPase maximum rate (V_{max}) was nearly twice as high in rat and the half-

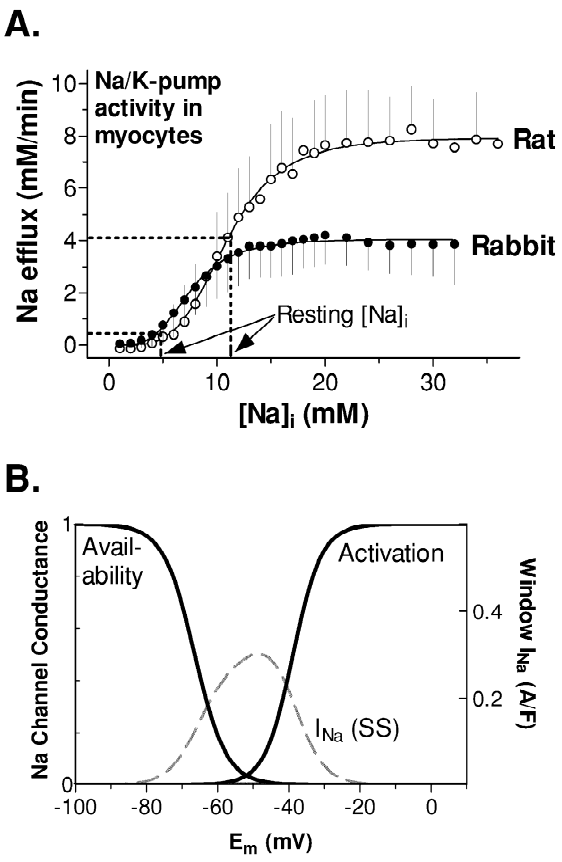

Fig. 2. (A) The $[Na^+]_i$-dependence of Na^+/K^+ pump-mediated Na^+ efflux in rat and rabbit ventricular myocytes. Cells were first Na^+-loaded by inhibiting the Na^+/K^+ pump in the absence of external K^+. Then, the pump was re-activated with 4 mM K^+ and we monitored the time course of $[Na^+]_i$ decline. The rate of $[Na^+]_i$ decline ($-d[Na^+]_i/dt$) was calculated at each $[Na^+]_i$. The solid lines represent the fit with a Hill expression. Replotted from Ref. [5]. (B) Steady-state E_m-dependence of I_{Na} activation, inactivation (or availability) and window current.

activation by Na^+ (K_m(Na) (was higher in rat) 10 vs. 7 mM). The V_{max} values may indicate higher pump protein expression levels in rat versus rabbit. We speculate that this might be a cellular adaptation to a chronically higher $[Na^+]_i$, and there is precedent for such $[Na^+]_i$-dependent upregulation of Na^+/K^+-ATPase expression levels [47]. Fig. 2A indicates that at rest, when $[Na^+]_i$ is 4.5 mM in rabbit and 11.1 mM in rat [5], the Na^+/K^+ pump is only running at ~15% of V_{max} in rabbit ventricle versus ~60% in rat. Therefore rabbit cells might have a greater reserve to cope with an enhanced Na^+ influx brought about by higher stimulation frequency or by pathological conditions. The rate of Na^+ efflux in rat at the higher measured resting $[Na^+]_i$ is higher than the resting Na^+/K^+-pump rate in rabbit ventricular myocytes (Fig. 2A). Since Na^+ efflux must equal Na^+ influx at the steady state, this means that resting Na^+ influx must also be higher in rat than rabbit ventricular myocyte. In the following, we will discuss pathways that contribute to this Na^+ influx.

3. Pathways for Na^+ influx

3.1. Voltage-gated Na^+ channels

Voltage-gated Na^+ channels are activated by depolarization and are responsible for the rapid upstroke of the cardiac action potential (AP). Once threshold depolarization for an AP is reached, the opening of Na^+ channels leads to further depolarization, which activates additional Na^+ channels and depolarization, etc. This positive feedback creates a very rapid depolarization toward the electrochemical potential of Na^+. Depolarization stops, in part because Na^+ channels inactivate, i.e. under sustained depolarization, the amplitude of Na^+ current (I_{Na}) decreases. I_{Na} inactivation effectively switches off Na^+ conductance within a few milliseconds or less (fast inactivation).

Voltage-gated Na^+ channels are composed of a pore-forming α subunit and auxiliary β subunits. Ten genes encoding α subunits have been identified and nine have been functionally expressed. The α subunit consists of four repeat domains (I–IV), each containing six transmembrane segments (S1–S6) and one membrane reentrant domain, connected by internal and external polypeptides loops [48]. The S4 segments contain four to eight positively charged residues, which serve as voltage sensors. An outward movement of these charges under depolarization underlies the activation of the channel [49]. Inactivation is mediated by the short intracellular loop connecting domains III and IV. One α subunit is associated with one or two auxiliary β subunits, β1, β2 or β3. These auxiliary subunits modulate channel gating, interact with extracellular matrix and play a role as cell adhesion molecules [50]. The primary cardiac α subunit isoform ($Na_v1.5$) is much less sensitive to inhibition by TTX ($K_{0.5}$~2 μM) and is more sensitive to Cd^{2+} block than neuronal or skeletal muscle Na^+ channels.

Fig. 2B shows the E_m-dependence of Na^+ channels activation and availability (or steady-state inactivation). I_{Na} activation and availability curves overlap such that there is almost no steady state availability when E_m is held at values where activation is appreciable. This ensures that activated Na^+ channels inactivate nearly completely whenever activated by depolarization. Na^+ channels require repolarization to recover from inactivation before another AP can occur. This is a main cause of the electrical refractory period. Recovery of I_{Na} requires that the membrane be repolarized to near the diastolic level for a finite period of time (τ~10 ms at −100 mV, 30 ms at −80 mV or 100 ms at −72 mV) before the cell is able to fire another AP. Thus Na^+ channels do not recover very rapidly until repolarization is nearly complete. In this way the long AP contributes to limiting the ability of the cell to respond to an early depolarization. Besides these fast inactivating Na^+ channels, there are indications that a slowly inactivating, persistent Na^+ current ($I_{Na,slow}$) might also be present in cardiac cells [51,52]. This persistent Na^+ current is more sensitive to block by TTX, is activated at more negative potentials and has an amplitude of ~0.5% of the peak transient I_{Na}. It has been shown that $I_{Na,slow}$ has a more prominent contribution to I_{Na} in heart failure and might be partly responsible for the prolongation of the AP [52,53].

As shown in Fig. 2B, there is a very small window at about −52 mV where both channel availability and activation are ~3.5%. This would produce a steady state 'window current' with a conductance of ~0.1% of maximum. Considering a peak I_{Na} of 360 pA/pF and a surface to volume ratio of 6.4 pF/pL_{cyt} [54], this steady current (0.3 A/F) would result in a significant Na^+ influx of 1.4 mM/min. At a resting E_m of −80 mV this window I_{Na} would be 10–30 times smaller, but would be consistent with our measurements of tetrodotoxin (TTX) sensitive resting Na^+ influx in rabbit ventricular myocytes at 23 and 37 °C (0.14–0.18 mM/min [5,55]). This would require a window I_{Na} of ~0.01% of maximum. This and the steepness of the activation and availability curves emphasizes that small shifts in activation or inactivation properties could lead to substantial sustained Na^+ influx via window I_{Na} at relatively negative E_m.

During the cardiac AP upstroke I_{Na} is very large, but very brief. This results in an additional Na^+ influx associated with each AP. Fig. 3C and D shows I_{Na} and the Na^+ influx via Na^+ channels during a typical AP, respectively. Fig. 3D indicates that ~8 μM Na^+ enters the cell via I_{Na} during each AP (6–15 μM is probably a reasonable range). For a cell contracting at 1 Hz, this means that phasic Na^+ channel activation contributes ~0.5 mM/min to the rate of Na^+ influx. This is surprisingly only about a 3-fold increase over the resting I_{Na} influx.

3.2. Na^+/Ca^{2+} exchange

Na^+/Ca^{2+} exchange is the major pathway for Ca^{2+} extrusion from cardiac myocytes. The mammalian NCX forms a multigene family of homologous proteins comprising three isoforms: NCX1, NCX2 and NCX3 (for reviews, see Refs. [56–58]). These isoforms share ~70% amino acid identity in the overall sequences and thus presumably have a very similar molecular structure. NCX1 is the first NCX cloned and is highly expressed in cardiac muscle and brain and to a lesser extent in many other tissues. NCX2 and NCX3 are expressed in a few tissues, such as brain, but their molecular properties and functions have been less well studied.

The cardiac NCX1 consists of 970 amino acids (110 kDa). Approximately half of the NCX1 protein constitutes transmembrane domains, whereas the remaining half (~550 amino acids) forms a large domain exposed on the cytoplasm. The latter domain does not appear to be required for the transport function of NCX1, because a mutant lacking most of it still retains exchange activity

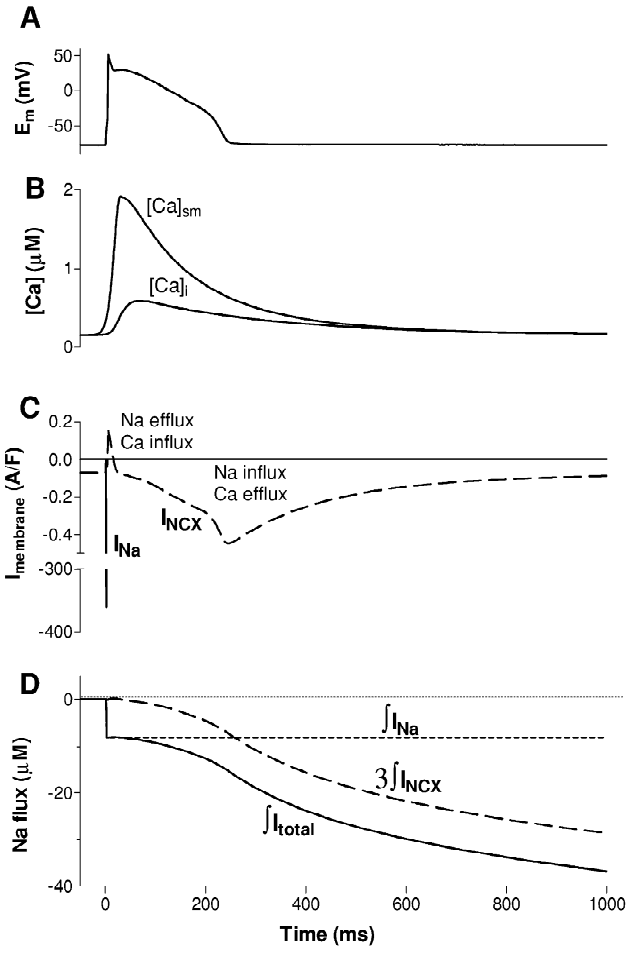

Fig. 3. Calculated I_{Na} and I_{NCX} mediated Na^+ influx in a ventricular myocyte contracting at 1 Hz. (A) Typical action potential for rabbit ventricular myocytes. (B) $[Ca^{2+}]_i$ and $[Ca^{2+}]_{sm}$ transients during an AP. $[Ca^{2+}]_{sm}$ was calculated from the measured $[Ca^{2+}]_i$ transient as described by Weber et al. [72]. (C) Calculated density of Na^+ current via Na^+ channels (I_{Na}) and density of NCX current (I_{NCX}) during an AP using the I_{NCX} equation of Weber et al. [73]. (D) Net Na^+ fluxes via Na^+ channels ($\int I_{Na}$) and NCX ($3\int I_{NCX}$), as well as the total Na^+ flux ($\int I_{total}$) during the AP.

[59]. There were originally thought to be 11 transmembrane domains and a very large cytoplasmic hydrophilic domain [60]. However, new topological data suggest that there are only nine transmembrane spans [61] and a large hydrophilic loop between TMs 5 and 6, with N- and C-termini located on the external and internal sides, respectively. The N-terminal and C-terminal halves of the membrane domain contain two internal repeat sequences of ~40 amino acids, which are designated the α_1 and α_2 repeats. These repeat sequences are conserved in all members of the NCX family as well as in related cation exchangers, and are now thought to be at or near the ion translocation pore. Many details of the NCX1 structure are unknown, including the ion transport pathway, the requirement of the oligomeric protein structure for the function, and changes in the conformation of the exchanger associated with ion transport. The density of NCX1 in the sarcolemma of guinea pig ventricular myocytes has been estimated to be 250–400 NCX/μm^2 [62].

NCX is generally believed to transport three Na^+ ions in exchange for one Ca^{2+} (for a review, see Ref. [56]), thus one net charge is transported per cycle. However, some recent studies indicate a 4:1 [63,64] or, under extremely acidic conditions, a 2:1 stoichiometry [65]. The direction and amount of ion fluxes through NCX depend on the $[Na^+]$ and $[Ca^{2+}]$ on both sides of the membrane, as well as on E_m. If there is more energy in the inward Na^+ gradient (for three Na^+ ions) than in the inward Ca^{2+} gradient (for one Ca^{2+} ion), Ca^{2+} extrusion will be favored. That is: $n(E_{Na} - E_m) > 2(E_{Ca} - E_m)$, where n is the coupling ratio and E_{Ca} and E_{Na} are the thermodynamic equilibrium potentials for Ca^{2+} and Na^+. Then, the E_m value at which the gradients are exactly equal is the reversal potential ($E_{Na/Ca}$) of the I_{NCX}. Hence, $E_{Na/Ca} = (nE_{Na} - 2E_{Ca})/(n-2)$. When E_m is more positive than $E_{Na/Ca}$, outward I_{NCX} is favored. In other words, Ca^{2+} entry/Na^+ exit via the exchanger is favored for $E_m > E_{Na/Ca}$ and Ca^{2+} extrusion/Na^+ entry is favored (inward I_{NCX}) when $E_m < E_{Na/Ca}$. For the normally accepted $n = 3$ and typical values of $E_{Na} = 70$ mV and $E_{Ca} = 125$ mV, $E_{Na/Ca} = -40$ mV. Because the resting membrane potential (~−80 mV) is more negative than $E_{Na/Ca}$, the exchanger will work in Ca^{2+} extrusion/Na^+ influx mode in resting myocytes. During the action potential, NCX briefly works in Ca^{2+} entry/Na^+ exit mode, i.e. $E_m > E_{Na/Ca}$ (see Fig. 3C and discussion below).

Besides being transport substrates, intracellular Ca^{2+} and Na^+ exert important modulatory effects on NCX activity [57,58]. Binding of intracellular Ca^{2+} to a high affinity site located in the central hydrophilic loop activates the exchanger ($[Ca^{2+}]_i$-dependent activation). It should be appreciated that this allosteric Ca^{2+}-regulatory site is distinct from the catalytic or Ca^{2+}-transport site. While K_m(Ca) values from 22 to 600 nM have been reported for this allosteric regulation by $[Ca^{2+}]_i$, a recent study by Weber et al. [66] found a physiologically relevant K_m(Ca) of 125 nM in intact ferret ventricular myocyte during dynamic $[Ca^{2+}]_i$ changes. This allosteric Ca^{2+}-activation was rapid (within tens of ms) and so it is probably important physiologically during beat-to-beat changes in cellular Ca^{2+}. There is also an inhibitory regulation at high $[Na^+]_i$ [67]. NCX inactivates in a time and $[Na^+]_i$-dependent manner. This $[Na^+]_i$-dependent inactivation could prevent excess Ca^{2+} influx and cellular Ca^{2+} overload under conditions of high $[Na^+]_i$, where net Ca^{2+} influx might be strongly favored. However, the very high $[Na^+]_i$ required to observe Na^+-dependent inactivation of I_{NCX} makes this regulation unlikely to be very important in the physiological modulation of NCX.

NCX exhibits relatively low activity in intact myocytes at rest (partly because $[Ca^{2+}]_i$ is low). Therefore, Na^+ influx via NCX is rather low in quiescent cells. We

measured the rate of Ni-sensitive $[Na^+]_i$ influx upon abrupt Na^+/K^+ pump inhibition in resting rabbit and rat ventricular myocytes. Resting Na^+ influx via NCX was 0.28 mM/min in rabbit ventricular myocyte (Fig. 4A) and 0.64 mM/min in rat. From a functional standpoint, this basal rate of Na^+ influx via NCX would extrude 1.5 and 3.5 μM Ca^{2+}/s in rabbit and rat, respectively. This is on the order of estimates of resting Ca^{2+} leak into ventricular myocytes (0.5–5 μM/s; [68–70]). There is also a sarcolemmal Ca^{2+}-ATPase, and it is reasonable to infer that resting Ca^{2+} extrusion (to match this inward resting Ca^{2+} leak) via NCX and the sarcolemmal Ca^{2+}-ATPase are on the same order of magnitude [70]. Of course NCX uses the $[Na^+]$ electrochemical gradient (rather than ATP) to extrude Ca^{2+}, but the energy still comes from ATP via Na^+/K^+-ATPase.

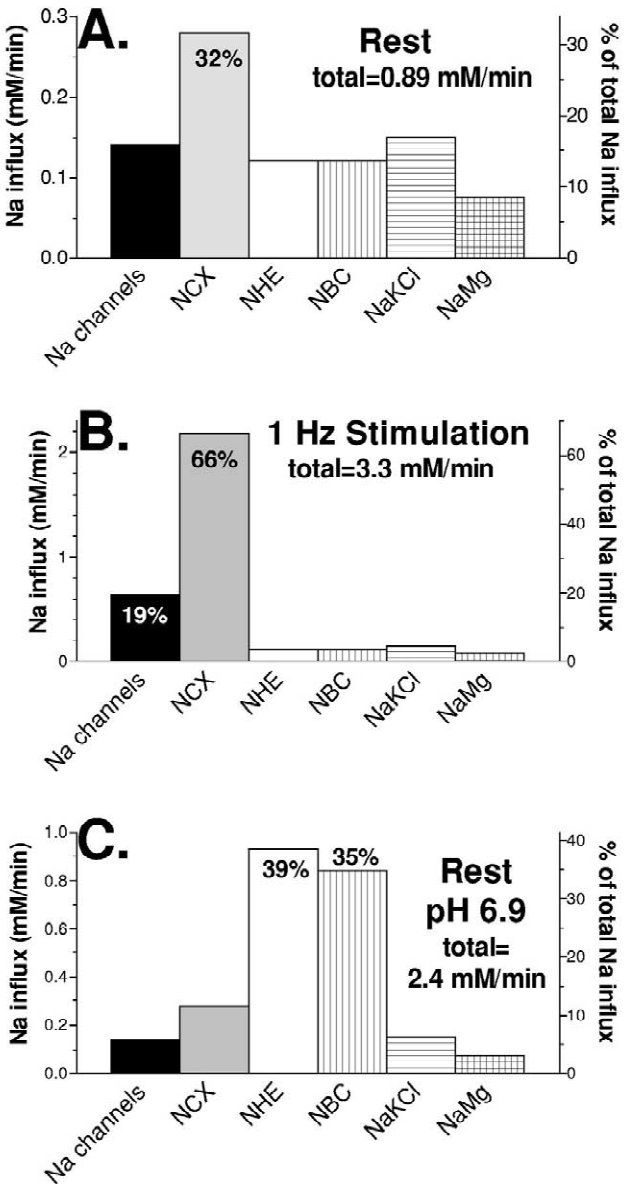

Fig. 4. The rate of Na^+ influx in rabbit ventricular myocytes via different pathways at rest (A), during steady-state stimulation at 1 Hz (B, where only Na^+ current and NCX were increased whereas the values measured in resting myocytes were used for all the other pathways) and in conditions of mild acidosis (C, where only NHE and NBC were increased according to Fig. 5A).

It is not clear why there is higher Na^+ influx via NCX in rat (vs. rabbit) myocytes, especially considering that $[Na^+]_i$ is higher in rat. The density of outward NCX current upon $[Na^+]_o$ removal is higher in rat than rabbit [71], so if that holds for inward NCX current as well that may provide a partial explanation. Ca^{2+} leak into rat myocytes might also be higher (which would require more Na^+ influx via NCX). Resting SR Ca^{2+} spark frequency is also higher in rat versus rabbit [72], and the resulting high local $[Ca^{2+}]_i$ may drive local Ca^{2+} extrusion and Na^+ influx via NCX.

NCX function during the AP is complicated because $[Ca^{2+}]_i$ and E_m change dramatically. This changes the reversal potential, kinetic and allosteric factors. There are also spatial gradients of $[Ca^{2+}]_i$ near the sarcolemma which complicate this picture [73]. Fig. 3 shows I_{NCX} (panel C) and the Na^+ flux via NCX (derived by integrating I_{NCX} and assuming a 3:1 stoichiometry, panel D) expected during an AP. Throughout most of the AP there is net Na^+ influx (Ca^{2+} efflux) via NCX, except for a brief period early in the AP before $[Ca^{2+}]_i$ has risen enough to cause Ca^{2+} extrusion. During each AP and Ca^{2+} transient NCX brings in ~32 μM $[Na^+]_i$. Again, this Na^+ influx is required to extrude the ~10 μM Ca^{2+} which enters the cell via Ca^{2+} current during each AP (on a 3 Na^+:1 Ca^{2+} NCX). For a cell contracting at 1 Hz, this means that NCX contributes ~1.9 mM/min to the rate of Na^+ influx (about seven times higher than the resting Na^+ influx rate via NCX). As can be seen from Fig. 3, this Na^+ entry is more distributed over the course of the AP versus I_{Na}. Figs. 3 and 4 also show that, quantitatively, Na^+ influx via NCX is considerably higher (three to four times) than that which occurs via Na^+ channels.

As mentioned above, NCX is reversible and can also be a Na^+ extrusion mechanism (using the large energy in the Ca^{2+} electrochemical gradient). However, this is unlikely to be a quantitatively important function under normal conditions. This can be appreciated by Fig. 3C where only a very small Na^+ efflux occurs very early during the AP. This situation can change during heart failure where lower Ca^{2+} transient amplitude, higher $[Na^+]_i$ and prolonged AP can all favor greater Na^+ extrusion (and Ca^{2+} influx) via NCX [55]. On the other hand, whatever Ca^{2+} enters via NCX during the AP must also be extruded by NCX during the diastolic interval, making net Na^+ extrusion via NCX negligible. Thus, NCX is probably not very important as a Na^+ extrusion mechanism.

3.3. Na^+/H^+ exchange

Another Na^+ influx pathway in cardiac cells is the Na^+/H^+ exchanger (NHE), which is important in the

regulation of pH_i and cell volume. NHE functions mainly as a proton extruder in a 1 Na^+:1 H^+ stoichiometry rendering the process electroneutral. There are at least six NHE isoforms thus far identified, with the NHE1–NHE5 apparently restricted to the plasma membrane whereas NHE6 might be the mitochondrial isoform [74] (but see Ref. [75]). NHE1, the so-called housekeeping isoform, is ubiquitously distributed in most tissues and is the primary NHE subtype found in mammalian cardiac cells [76,77]. NHE1 is a 110-kDa glycoprotein and consists of two principal domains: a 500-amino acid transmembrane domain and a 315-amino acid highly hydrophilic carboxyl-terminus cytoplasmic domain (for a review, see Ref. [76]). NHE1 contains 12 membrane-spanning regions that are critical for the maintenance of NHE1 function in terms of proton extrusion. The hydrophilic cytoplasmic region plays an important role in modulation of the exchanger, especially through phosphorylation-dependent reactions [78].

It has recently been suggested that NHE1 is localized primarily in the intercalated disk region of atrial and ventricular myocytes in close proximity to connexin 43, and, to a lesser degree, at the transverse tubular systems [79]. Such NHE1 localization could make NHE especially important in modulating cell-to-cell communication via gap junctions, and local signaling in T-tubules (although there is little functional data in this regard).

NHE activity is extremely sensitive to pH_i. The exchanger is allosterically activated by cytosolic protons, a drop in pH_i below a threshold level promoting the rapid extrusion of acid. Fig. 5A shows the pH_i-dependence of the rate of ion fluxes (Na^+ influx, H^+ efflux) through NHE in guinea-pig ventricular myocytes [80]. NHE is nearly inactive at a physiological pH_i of ~7.2, but it activates quickly when pH_i decreases. NHE activity in the heart seems to be species dependent, with a higher activity (determined as the rate of acid efflux at pH_i 6.9) in rat (2.8 mM/min) versus human (1.1 mM/min) or guinea-pig (0.93 mM/min) ventricular myocytes [80,81]. We also found that at physiological pH_i (and 23 °C) the rate of Na^+ entry via NHE is higher in rat (0.43 mM/min) versus rabbit (0.12 mM/min) ventricle [5]. Although intracellular Na^+ can also affect NHE, it is unlikely to be a major regulator within a physiological range (4–16 mM) of $[Na^+]_i$ [82]. Proton extrusion through NHE is also inhibited by extracellular acidosis [82]. This may be partly a thermodynamic effect (i.e. reducing the $[H^+]$ gradient for proton extrusion), it may also be due to a competition of H^+ with Na^+ for binding to the external transport site of the exchanger [82]. The inhibition of NHE by extracellular acidosis may limit proton extrusion during ischemia, and relief of this inhibition upon reperfusion may contribute to a rapid activation of proton extrusion and Na^+ entry [83]. A consequent gain in $[Na^+]_i$ during reperfusion could also contribute to Ca^{2+} overload, diastolic dysfunction and arrhythmogenesis (due to NCX function). This may be why the NHE inhibitor cariporide (Hoe-642) is a protective agent with respect to reperfusion injury [84,85]. While the events surrounding ischemia/reperfusion are complex and incompletely understood, it seems likely that NHE and NCX are importantly involved.

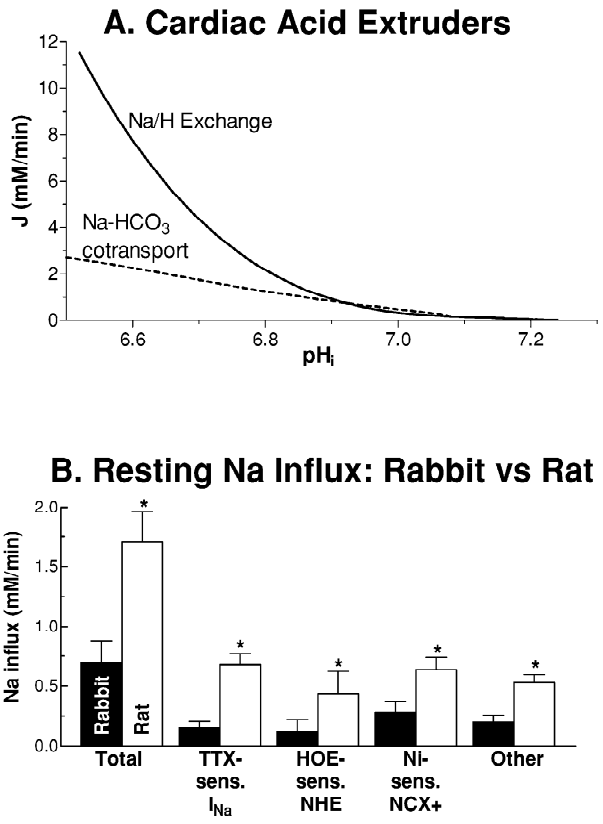

Fig. 5. (A) The pH_i-dependence of Na^+ fluxes through cardiac NHE and NBC, as described in Ref. [80]. At physiological pH (7.0–7.2) the contribution of these transporters to Na^+ influx is comparable (Fig. 2A). (B) The rate of Na^+ influx through different pathways in resting rabbit and rat ventricular myocytes. Na^+ influx was calculated as the initial slope of $[Na^+]_i$ increase following abrupt Na^+/K^+ pump inhibition [5]. TTX-, Hoe-642- and Ni^{2+}-sensitive Na^+ influx was calculated as the difference between the total influx and the influx measured in the presence of 30 μM TTX, 2 μM Hoe-642, or 5 mM Ni^{2+}, respectively.

3.4. Na^+/HCO_3^- cotransporter

Na^+/HCO_3^- cotransport (NBC) also serves as an acid extruder in cardiac myocytes (Na^+ and HCO_3^- influx). NBC was first described in the renal proximal tubule of the salamander [86], where it mediates Na^+ and HCO_3^- efflux across the basolateral membrane. Functionally related cotransporters, which mediate both HCO_3^- influx and efflux, have now been described in many tissues, including the heart (reviewed in Ref. [87]). Cardiac NBC was originally suggested to have a stoichiometry ranging between 1:1 and 1:2 [88] and thus an equilibrium pH_i which, under most conditions, would favor acid extrusion. In guinea-pig ventricular myocytes [88] and sheep Purkinje fibers [89], acid extrusion via NBC appears to be voltage-insensitive, consistent with an electroneutral cotransporter.

Reports in cat papillary muscle [90] and rat ventricular myocytes [91] indicate a strong voltage-sensitivity, consistent with an electrogenic NBC, probably with a stoichiometry of 1 Na^+:2 HCO_3^-. Two classes of electrogenic (NBCe [92] and NBC4 [93]) as well as one electroneutral [94] NBC carrier have been cloned and all three are present in the human heart at the mRNA level. However, the molecular identity of NBCs that mediate acid extrusion in heart cells from different species is not known. NBC is generally inhibited by stilbenes (DIDS and SITS), although the electroneutral isoform NBCn1 cloned from rat aorta is only very weakly inhibited by DIDS [95].

Leem et al. [80] measured the pH_i-dependence of NBC activity in guinea-pig ventricular myocytes and found that NBC activates roughly linearly with decreasing pH_i. Whether or not allosteric stimulation is involved is not known. Fig. 5 shows the pH_i-dependence of Na^+ influx via NBC using the data from Ref. [80] and assuming a 1:1 stoichiometry of the cotransport (as reported for guinea-pig ventricular myocytes). It is notable that for relatively modest acid loads (i.e. to pH_i 6.9), both NHE and NBC are stimulated about equally. Thus, at pH_i values within the normal physiological range, both transporters are about equally important for mediating acid extrusion and they contribute equally to Na^+ influx. For larger intracellular acid challenges, NHE removes more acid than does NBC.

3.5. Other Na^+ influx pathways

3.5.1. $Na^+/K^+/2Cl^-$ cotransporter

The $Na^+/K^+/2Cl^-$ cotransporter (NKCC) was first detected in Ehrlich ascites tumor cells [96]. Since then, the cotransporter has been found in a variety of tissues, including the heart [97–99]. Two NKCC isoforms have been cloned: NKCC1, found in the membrane of a variety of cell types, and NKCC2, identified thus far only in the medullary regions of the kidney [99]. NKCC is inhibited by loop diuretics, such as furosemide and bumetanide. In all tissues investigated, $Na^+/K^+/2Cl^-$ cotransport is electroneutral, with a stoichiometry of 1:1:2 and with an absolute requirement that Na^+, K^+ and Cl^- are all present on the same side of the membrane. In chick cardiac cells, the $[Cl^-]$ and $[Na^+]$ for half-maximal stimulation of the cotransporter are 59 and 40 mM, respectively [97]. Net transport may occur into or out of the cells, the magnitude and direction of this transport being determined by the sum of the chemical potential gradients of the transported ions. In physiological conditions, $Na^+/K^+/2Cl^-$ cotransport is inwardly directed and thus maintains the intracellular $[Cl^-]$ ($[Cl^-]_i$) above its electrochemical equilibrium (~15 mM). Intracellular Cl^- is an important regulator of the cotransporter, where high $[Cl^-]_i$ inhibits ion fluxes in both directions. Gillen and Forbush [100] found a very steep relationship between bumetanide-sensitive ion flux and $[Cl^-]_i$ in HEK-293 cells transfected with human NKCC1. In chick cardiac cells, the $Na^+/K^+/2Cl^-$ cotransporter accounts for 17% of the resting Na^+ influx [97]. $Na^+/K^+/2Cl^-$ cotransport may be important in cell volume regulation [101].

3.5.2. Na^+/Mg^{2+} antiporter

The concentration of free Mg^{2+} in the cytoplasm ($[Mg^{2+}]_i$, 0.5–1 mM) is several hundred times lower than expected if Mg^{2+} ions were at electrochemical equilibrium. Since Mg^{2+} is a permeant ion across the plasmalemma, it must be constantly extruded from the cells. The main mechanism that removes Mg^{2+} from the cells is the Na^+/Mg^{2+} antiporter. However, its presence in the heart is controversial. Some studies reported the functional presence of Na^+/Mg^{2+} exchange in guinea-pig and ferret ventricle [102] whereas others found no evidence for it in guinea-pig [103] and chicken heart [104]. Such studies are generally based on changes in $[Mg^{2+}]_i$ when $[Na^+]_o$ is varied. However, Mg^{2+} is heavily buffered in cells and its membrane permeability is rather low, therefore the changes in $[Mg^{2+}]_i$ are minimal. Also, changes in $[Na^+]_o$ affect $[Ca^{2+}]_i$ and pH_i and these in turn will affect intracellular Mg^{2+} buffering. Thus, the changes in $[Mg^{2+}]_i$ in such protocols might not be mediated solely by the Na^+/Mg^{2+} exchanger. Tashiro and Konishi [105] used rat ventricular myocytes rendered Mg^{2+}-permeable with ionomycin in high $[Mg^{2+}]_o$ conditions to facilitate passive Mg^{2+} influx. The rate of $[Mg^{2+}]_i$ rise was significantly smaller in the presence of 140 mM external Na^+ than in its absence. Washout of ionomycin and lowering extracellular Mg^{2+} caused rapid decline of $[Mg^{2+}]_i$ when $[Na^+]_o$ is 140 mM. This Mg^{2+} efflux was completely inhibited by withdrawal of extracellular Na^+ and was largely attenuated by imipramine, a known inhibitor of Na^+/Mg^{2+} exchange. At 140 mM external Na^+, the rate of Mg^{2+} transport through the exchanger was 25–50 μM/min. Mg^{2+} transport depended on $[Na^+]_o$ according to a Hill-type curve, with the mid-point at ~80 mM and a Hill coefficient of 2. This suggests that the exchanger has a stoichiometry of 2 Na^+:1 Mg^{2+} and therefore is electroneutral. A more complicated stoichiometry (2 Na^++2 K^++2 Cl^-:1 Mg^{2+}) has been proposed for the exchanger in squid giant axons and barnacle muscle cells [106].

There may also be other Na^+ flux pathways that we have not discussed (e.g. background Na^+ channels), but we feel that these are likely to be minor quantitatively. We can account for most of the measured Na^+ fluxes by the mechanisms mentioned above (Fig. 4).

4. Balance of Na^+ influx and efflux

4.1. Quiescence

At steady state, Na^+ influx and efflux must be equal. Fig. 4A shows the rate of resting Na^+ influx through the

pathways discussed above. The values are based in part on our measurements in rabbit ventricular myocytes [5] and some extrapolations from guinea-pig data [80,102] and correspond to mammalian species with lower resting $[Na^+]_i$ (i.e. other than rat and mouse). Adding the contribution of each pathway, the total Na^+ influx in resting myocytes is 0.88 mM/min. Na^+ influx pathways unaccounted for in Fig. 4A might increase this rate by ~0.1 mM/min [5]. Fig. 2A indicates that the Na^+/K^+ pump will extrude this amount of Na^+ if resting $[Na^+]_i$ is 5.3 mM (5.5 mM if the additional 0.1-mM/min Na^+influx is considered). So, this principle of flux balance is also met with these quantitative resting Na^+ flux estimates.

4.2. Why is resting $[Na^+]_i$ higher in rat versus rabbit?

Now we can revisit the question of why resting $[Na^+]_i$ in rat is higher than in rabbit ventricular myocytes. In reference to Fig. 2A we concluded that resting Na^+/K^+-ATPase-mediated Na^+ efflux was higher in rat than rabbit and we inferred that this must be matched by higher Na^+ influx. We evaluated resting Na^+ influx by abrupt Na^+/K^+-ATPase block and measurement of the initial rate of rise of $[Na^+]_i$ [5]. Indeed, as Fig. 5B shows, we found the resting Na^+ influx to be 2.4 times higher in rat versus rabbit ventricular myocytes. Several Na^+ influx pathways appear to contribute to different extents. For example, TTX-sensitive Na^+ influx was almost five times higher in rat, while the Ni-sensitive Na^+ influx (presumably mainly NCX) was only about two times higher in rat. These results confirm that the likely reason $[Na^+]_i$ is higher in rat is because resting Na^+ influx is higher. This would stimulate higher Na^+/K^+-ATPase-mediated Na^+ efflux (and possibly Na^+/K^+-ATPase expression) until a new steady state balance is achieved at higher levels of both Na^+ influx and efflux. This scenario might also hold for other species where $[Na^+]_i$ is high (where mouse is like rat) and where $[Na^+]_i$ is low (where guinea-pig is like rabbit).

4.3. Stimulation

As described above Na^+ influx is increased in contracting myocytes, at least because of increased Na^+ entry via I_{Na} and NCX (Fig. 3). Fig. 4B shows estimates of Na^+ influx in myocytes contracting at 1 Hz. The total Na^+ influx in contracting cells is increased by ~3.7-fold (vs. rest) to ~3.5 mM/min. We only increased I_{Na} and NCX (by ~3 and 7-fold as discussed above), but certainly cannot exclude the possibility of increased Na^+ influx by other pathways (e.g. NHE and NBC). This higher Na^+ influx would require a matching increase in Na^+ efflux via the Na^+/K^+ pump and this would occur at ~11 mM $[Na^+]_i$ (Fig. 2A). This is higher than the $[Na^+]_i$ we measure in rabbit at 1-Hz stimulation (~8 mM). Part of this difference could reflect restricted subsarcolemmal diffusion of Na^+ (see below). It will be valuable for additional quantitative data to be measured for these estimates to be further refined. In conclusion, while the various Na^+ influx pathways differ little at rest with only NCX being much larger than the others (and only by ~2-fold), Na^+ channels and NCX are likely to become much more dominant during normal electrical and contractile activity (in this example providing 85% of the total Na^+ influx).

4.4. Acidosis

Acidosis is a major consequence of myocardial ischemia and contributes to the ischemic decline in force. The major negative inotropic effect is mainly due to intracellular versus extracellular acidosis and is caused in large part by decreased myofilament Ca^{2+} sensitivity. Ca^{2+} transient amplitude during abrupt acidosis can be initially increased, unchanged or decreased [107–109]. However, in almost all reports there is then a progressive increase in twitch $\Delta[Ca^{2+}]_i$ and this causes a partial recovery of contractions. Acidosis can also gradually increase diastolic $[Ca^{2+}]_i$ [110].

Low pH_i stimulates proton extrusion via NHE and NBC (Fig. 5A), especially when pH_o is relatively normal. Thus, resting Na^+ influx is enhanced during acidosis. Fig. 4C shows estimations of the rate of resting Na^+ influx through various pathways in the case of a mild acidosis (pH_i 6.9). With respect to Fig. 4A only NHE and NBC were increased as predicted from Fig. 5A. Of course there may also be inhibition of other Na^+ influx pathways as well (e.g. NCX and I_{Na}). Our expectation is that NHE and NBC increase greatly and in this case account for 75% of resting Na^+ influx. Furthermore, the Na^+/K^+ pump is partially inhibited at low pH_i [111]. These two factors lead to an increase in $[Na^+]_i$ [112], which may contribute to the slow recovery of contractility via a shift in NCX and increase in $[Ca^{2+}]_i$. NCX is also inhibited by low pH_i [113]. This may limit the ability of NCX to extrude Ca^{2+} (especially at high $[Na^+]_i$). As with cardiac glycosides, this may also contribute to increased SR Ca^{2+} content and larger Ca^{2+} transients seen during acidosis, but could also lead to Ca^{2+} overload and consequent arrhythmias.

During severe hypoxia (associated with ischemia) there is a large rise in $[Na^+]_i$ which can reach 40 mM in prolonged anoxia. Eigel and Hadley [114] evaluated the cellular basis of the Na^+ gain. The main mechanisms of net Na^+ gain during anoxia were TTX sensitive Na^+ channels and Hoe-642-sensitive Na^+/H^+ exchange. TTX and Hoe-642 each blocked ~50% of the Na^+ gain during simple anoxia. For anoxia plus extracellular acidosis the Na^+ gain was blocked by TTX, but not by Hoe-642. However, when anoxia or acidosis (pH 6.85) was coupled with high $[K^+]_o$ (10 mM) to simulate ischemia, the Na^+ gain was suppressed almost completely by Hoe-642, but not by TTX. It is unclear why Na^+ channel influx would

occur in the cases with normal $[K^+]_o$ (while cells are quiescent), but anoxia and ischemic metabolites have been shown to produce persistent Na^+ channel opening in ventricular myocytes [115,116]. Since depolarization prevented the TTX-sensitive Na^+ gain, it is possible that anoxia allows a tiny window I_{Na} at resting E_m, but inactivation prevails upon depolarization in high $[K^+]_o$. Thus, during ischemia $[Na^+]_i$ rise may be mediated by Na^+/H^+ exchange and Na^+ channel entry [117].

5. Mitochondrial Na^+ transport

Fig. 1B shows mitochondrial Na^+ movements. $[Na^+]_i$ can importantly regulate mitochondrial $[Ca^{2+}]$, because mitochondrial Ca^{2+} extrusion occurs mainly via a Na^+/Ca^{2+} antiporter, which may be electroneutral (2 Na^+:1 Ca^{2+}), but might also be >2:1 [118–120]. The $[Na^+]_i$ dependence of this Na^+/Ca^{2+} antiporter is sigmoidal with half-maximal activity at ~5–8 mM Na^+, making this system sensitive to physiological $[Na^+]_i$ changes [118,121]. While variations in bulk cytoplasmic $[Na^+]$ during the cardiac cycle are probably insufficient to cause rapid release of mitochondrial Ca^{2+}, large changes in $[Na^+]$ can induce substantial mitochondrial Ca^{2+} release in vitro [118]. The V_{max} value for Ca^{2+} extrusion (and Na^+ entry) via mitochondrial Na^+/Ca^{2+} exchange in the heart is ~15 nmol Ca/mg protein per min [122] or ~0.9 mM/min. This gives the mitochondrial Na^+/Ca^{2+} antiport comparable flux capacity to some sarcolemmal Na^+ transporters.

Na^+ entering mitochondria through the Na^+/Ca^{2+} antiporter is then extruded via a Na^+/H^+ exchange system, driven by the electrochemical gradient of protons (which is created by the electron transport chain). The mitochondrial Na^+/H^+ exchanger is reversible and seems to be symmetrical in its interaction with Na^+ [123]. The K_m for Na^+ transport is ~26 mM at an intramitochondrial pH of 8.0 [124] and decreases with more alkaline mitochondrial pH. Matrix protons compete with Na^+ for binding to a common site and thus inhibit the exchanger with a mid-point of ~30 nM (pH~7.5) [125]. The mitochondrial Na^+/H^+ antiport shares many properties with the sarcolemmal NHE, however it differs in some aspects such as the sensitivity to amiloride analogues and relative lack of regulation [123].

Mitochondrial $[Na^+]$ ($[Na^+]_m$) appears to be lower than $[Na^+]_i$ [8,126]. $[Na^+]_m$ increases linearly with $[Na^+]_i$ and the $[Na^+]$ gradient across the mitochondrial inner membrane depends on the energetic state of the mitochondria [126]. In isolated mitochondria, the Na^+ gradient was equal to the electrochemical H^+ gradient over a wide range of external $[Na^+]$ and changed rapidly in response to changes in matrix pH [126], suggesting that Na^+ distribution across the inner mitochondrial membrane is effectively determined by the equilibrium of the Na^+/H^+ antiporter. The quantitative role of mitochondria in overall $[Na^+]_i$ regulation is unclear. While in heart, mitochondria occupy ~35% of the total cell volume it should be kept in mind that mitochondria are a totally intracellular compartment and as such are a limited source or sink. However, since $[Na^+]_m$ rises with $[Na^+]_i$, Na^+ entry into mitochondria may serve as a buffer for $[Na^+]_i$, and could limit changes in $[Na^+]_i$ during pathophysiological conditions.

6. Intracellular Na^+ gradients

Several studies have provided evidence for the presence of a $[Na^+]$ gradient between the subsarcolemmal space ($[Na^+]_{sm}$) and bulk cytosol in arterial smooth muscle [127] and cardiac myocytes [128–132]. The existence of such intracellular $[Na^+]$ gradients implies restricted diffusion with respect to transport rate. Wendt-Gallitelli et al. [133] used electron probe microanalysis to measure $[Na^+]$ in a volume within 20 nm of the inner side of the sarcolemma of guinea-pig ventricular myocytes. $[Na^+]_{sm}$ increased up to 40 mM during stimulation of the cells, with no change in bulk $[Na^+]_i$. This steep subsarcolemmal $[Na^+]$ gradient dissipated, but only within minutes of the end of stimulation. This is surprisingly slow dissipation of such a $[Na^+]$ gradient, and this method measures total rather than free $[Na^+]$. This could reflect increased binding of Na^+ in a subsarcolemmal space, which could in turn alter Na^+ diffusion.

A transient peak in the Na^+/K^+ pump current has been observed in voltage-clamped ventricular myocytes, when the Na^+/K^+ pump was abruptly reactivated after a period of pump blockade [1,128,131]. I_{pump} then decayed over a few minutes to a steady-state level. This I_{pump} sag might be the result of lowering $[Na^+]_{sm}$ because of the pump activity (even when global $[Na^+]_i$ was presumed or measured to be little changed). Peak I_{pump} also increased in response to a train of depolarizations prior to Na^+/K^+ pump reactivation [134], suggesting that activation of Na^+ channels increases $[Na^+]_{sm}$. Lipp and Niggli [135] have also observed that I_{Na} can activate Ca^{2+} influx via NCX, inferring that I_{Na} raised $[Na^+]_{sm}$. While some preliminary data imply that I_{Na} may not modify local $[Na^+]_{sm}$ sufficiently to alter NCX or Na^+/K^+-ATPase [136,137], the whole issue of $[Na^+]_i$ gradients requires further study.

Su et al. [134] showed that abrupt Na^+/K^+-ATPase inhibition in mouse ventricular myocytes increases the efficacy of a given I_{Ca} to trigger SR Ca^{2+} release (Fig. 6). This was interpreted as a prevention of Na^+ extrusion in the SR junctional cleft allowing local cleft $[Na^+]_{sm}$ to rise and favor Ca^{2+} entry via NCX. Abrupt Na^+/K^+ pump inhibition was also shown to slow the decline of caffeine-induced Ca^{2+} transients and I_{NCX} [132]. Goldhaber et al. [138] also found that abrupt removal of $[Na^+]_o$ caused an increase in resting Ca^{2+} spark frequency, and they attributed this to a blockade of tonic Ca^{2+} removal from the junctional cleft by Na^+/Ca^{2+} exchange. All of these data

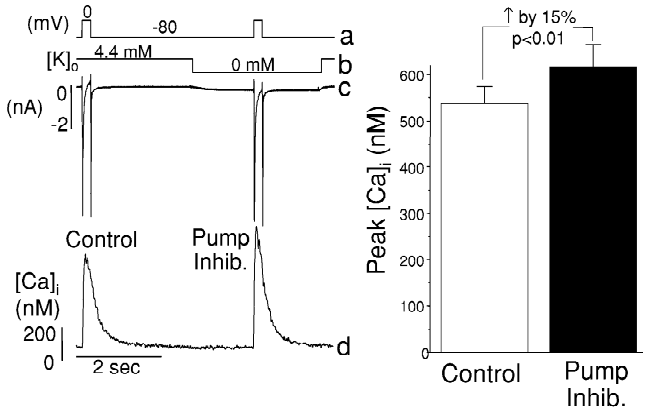

Fig. 6. Effects of abrupt inhibition of the Na^+/K^+ pump (Pump Inhib.) on $[Ca^{2+}]_i$ transients and membrane currents. The left panel shows the last of a series of eight voltage-clamp steps (−80 to 0 mV) to induce a stable $[Ca^{2+}]_i$ transient magnitude (a), membrane currents (c, including I_{Na} and I_{Ca}) and $[Ca^{2+}]_i$ transients (d). Abrupt Pump Inhib. (by $[K^+]_o$ removal using a rapid solution switcher, b) for 1.5 s immediately before and during the test pulse was evidenced by the inward shift in holding current (c). With Pump Inhib. peak $[Ca^{2+}]_i$ was significantly higher (d and bar graph). Results are mean±S.E.M. of 16 cells. Pipette $[Na^+]$ was 15 mM. (From Su et al. [134])

suggest that local or junctional microdomain $[Na^+]_{sm}$ and $[Ca^{2+}]_i$ may be controlled by local Na^+/K^+-ATPase, Na^+ channels and NCX, such that, when the system is not at steady-state, they differ significantly from bulk $[Na^+]_i$ and $[Ca^{2+}]_i$. Thus, a normally functioning Na^+/K^+-ATPase may maintain $[Na^+]_i$ and $[Ca^{2+}]_i$ in the junctional cleft at lower values than bulk cytosolic, and this may limit CICR. These issues, however, merit further study and quantitative characterization. Some of these results are difficult to reconcile with expected ion diffusional characteristics in cells or are counterbalanced by other results. For example, ultrastructural studies also indicate that Na^+ channels, NCX and SR Ca^{2+} release channels in rat ventricular myocytes are not co-localized with each other [139]. On the other hand, the Na^+/K^+ pump and NCX are co-localized in smooth muscle [140]. It appears that there is much still to learn about cellular Na^+ regulation. We hope that this integrative overview of cardiac Na^+ regulation helps to provide an initial semi-quantitative framework for future work and subsequent articles in this focused issue.

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