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Control of Cardiac Contraction by Sodium: Promises, Reckonings, and New Beginnings

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Abstract

Several generations of cardiac physiologists have verified that basal cardiac contractility depends strongly on the transsarcolemmal Na gradient, and the underlying molecular mechanisms that link cardiac excitation-contraction coupling (ECC) to the Na gradient have been elucidated in good detail for more than 30 years. In brief, small increases of cytoplasmic Na push cardiac (NCX1) Na/Ca exchangers to increase contractility by increasing the myocyte Ca load. Accordingly, basal cardiac contractility is expected to be physiologically regulated by pathways that modify the cardiac Na gradient and the function of Na transporters. Assuming that this expectation is correct, it remains to be elucidated how in detail signaling pathways affecting the cardiac Na gradient are controlled in response to changing cardiac output requirements. Some puzzle pieces that may facilitate progress are outlined in this short review. Key open issues include (1) whether the concept of local Na gradients is viable, (2) how in detail Na channels, Na transporters and Na/K pumps are regulated by lipids and metabolic processes, (3) the physiological roles of Na/K pump inactivation, and (4) the possibility that key diffusible signaling molecules remain to be discovered.

Graphical abstract

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Keywords

sodium potassium pump; local sodium gradient; glutathionylation; ischemia; cardiac excitation contraction coupling; simulation; sodium gradient; TRP channels

1. Introduction: The promises.

It was discovered shortly after World War II that contraction strength of the heart depends strongly on an apparent antagonism between the extracellular concentrations of Na and Ca [1]. That this antagonism reflects the function of a sarcolemmal Na/Ca exchange system [2] was established twenty years later by Ca flux experiments in two very different cell types, giant nerve axons of squid and crabs [3, 4] and atrial cardiac muscle [5, 6]. The importance of the transmembrane Na gradient *per se* in determining the strength of cardiac contraction was established at nearly the same time [7, 8], and a strong voltage dependence of the apparent Na/Ca antagonism in cardiac muscle was soon documented [9]. Proof that the cardiac Na/Ca exchange system is electrogenic was published in 1980 [10], and Na/Ca exchange currents were described in detail in 1986 in isolated cardiac myocytes [11]. In that same year, it was shown in intact cardiac tissue that the exchanger extrudes substantial amounts of Ca during cardiac relaxation with a depolarizing influence on the late action potential [12, 13]. After its cloning and initial structure-function analysis [2], knockout

studies confirmed that the cardiac Na/Ca exchanger (NCX1) is required for heart glycosides to increase cytoplasmic Ca and thereby exert a positive inotropic action in cardiac myocytes [14]. Therewith, the molecular basis of cardiac ECC control by Na was unequivocally established, and the long-standing expectation was supported that basal contractility would be importantly regulated through regulation of the sarcolemmal Na gradient. The major challenge that remains is to elucidate how (and when) such regulation occurs during the body's responses to changes of cardiac output and downstream consequences of those changes throughout the body.

2. The basics.

Figure 1 illustrates in cartoon form the mechanistic basis of cardiac ECC control by Na and therewith by the regulation of Na influx mechanisms and Na/K pumps. Fig. 1A shows the usual relationships between action potential, cytoplasmic free Ca, and contraction in non-rodent ventricular myocytes. Ca influx begins abruptly with upstroke of the action potential and recedes during the plateau. Ca extrusion via Na/Ca exchange begins slowly during the late plateau and accelerates markedly during repolarization as negative membrane potential favors 3Na/1Ca exchange in the 'forward' mode. During the ECC cycle, Ca influx via Ca channels is balanced by Ca extrusion by Na/Ca exchange, and the Na load caused by the exchange activity likely becomes as large or larger than Na influx needed for action potential upstroke by voltage gated NaV channels. In addition, Na load is incurred by the operation of Na/H exchange and Na conducting channels besides NaVs, including especially TRP-type and HCN nonselective channels, both of which are operative in ventricular myocytes [15, 16].

Fig. 1B illustrates in a rough manner the expected relationships between total Na influx, the cytoplasmic Na concentration, the function of Na/Ca exchange, and the relative magnitude of cardiac contraction. In brief, cardiac physiology occurs entirely within a very narrow range of cytoplasmic Na concentrations, roughly from 5 to 10 mM. Over this range, cytoplasmic Na concentrations increase only about 30% when Na influx increases by 3 fold, and this relationship is with good certainty highly regulated by mechanisms discussed subsequently. A major reason for this dampening is that the Na pump has a steep dependence on the cytoplasmic Na concentration under physiological conditions, the Hill slope sometimes approaching a value of 3. Another reason, discussed subsequently, is that Na/K pumps can inactivate and then reactivate in a Na-dependent manner [17].

Shown in red in Fig. 1B are the extreme high and low cytoplasmic free Ca concentrations that the exchanger can potentially achieve at +30 and -80 mV, respectively. Already at 12 mM cytoplasmic Na, the Na/Ca exchanger will strive to generate nearly a 4 μ M free Ca concentration at positive potentials and will not be able to reduce cytoplasmic free Ca to less than 0.3 μ M. These physical facts constitute an endpoint of Na regulation for cardiac myocytes, and the initiation of Ca overload that results rapidly in cardiac demise. Na influx dare not increase above values that are well 'buffered' by the Na/K pump.

The green curve in Fig. 3B depicts roughly the relative contractility of myocytes over the same range of Na concentrations. This curve will be shifted to the left or right by the

influence of Ca channels and sarcoplasmic reticulum (SR) Ca pumping. Even though Na does not increase in proportion to Na influx, cardiac contraction indeed rises very steeply for two reasons. First, NCX1 Na/Ca exchange function, as both a Ca influx and extrusion mechanism, increases and decreases steeply as cytoplasmic Na increases. Second, cardiac contraction has a steep dependence on the cytoplasmic Ca concentrations, Ca-force relations of the cardiac myofilaments typically have Hill slopes of ~3 in skinned cardiac muscle preparations [18] and have Hill slopes as high as 5 in isolated cardiac myocytes [19, 20]. With this background, it is evident that basal contractility of the heart might be regulated either by regulating Na influx mechanisms or by regulating Na/K pump activity. In the following sections, evidence for regulation via Na influx is first outlined, and then the potential regulation via Na/K pump control is considered in more detail. Given the brevity of this review, the interested reader is referred to recent reviews for more in-depth analysis.

3. Contraction control via regulated Na influx.

The exquisite sensitivity of cardiac contraction to cytoplasmic Na changes has not translated to a simple or clear understanding of how Na transporters and Na/K pumps are regulated. One of many complicating issues is the fact that the mechanisms of Na homeostasis operate differently in different species [21], and likely are regulated differently. Therefore, most of the following generalizations must be weighed against the likelihood that things may be different in different species, rodent versus non-rodent differences being the most confounding problem.

3.1. The response to preload and afterload changes: the Anrep effect.

The ability of the heart to adjust its stroke volume to its filling volume is fundamental for the beat-to-beat regulation of cardiac output. The primary underlying mechanism, known to first-year medical students as the 'Frank-Starling' mechanism, arises mostly from changes of myofilament interactions during stretch which immediately promote cross bridge formation [22]. In addition, however, there are delayed effects of maintained myocyte stretch that increase cardiac contraction over several minutes. Usually called the Anrep effect, this delayed mechanism can be initiated by mechanical stresses induced by increasing either preload (i.e. central venous pressure) or after-load (i.e. increases of arterial pressure) [23, 24]. There is wide agreement that the delayed mechanisms involve an increase of cytoplasmic Ca transients, and there is substantial evidence that autocrine and/or paracrine release of cytokines, primarily angiotensin and endothelin, initiate these effects [25, 26]. Subsequent activation of Na/H exchangers with accumulation cytoplasmic Na is suggested to be the main pathway that leads to increased Ca load and Ca release [27]. In this connection, an increase of cytoplasmic pH might also sensitize the myofilaments to Ca [28]. A parallel activation of Na-permeable ion channels, including multiple mechanosensitive channels [29] and TRP channels, may also contribute to the Na load and promote Ca loading. In this connection, it is suggested that TRP channels [30] may promote Ca loading via reverse Na/Ca exchange in special subsarcolemmal sites where localized Na gradients could occur [31]. Regardless of the details, the great majority of available evidence supports the conclusion that the Anrep effect is a major cardiac regulatory mechanism by which the Na gradient is used to control contraction. Assuming that angiotensin release indeed plays

an important role, the Anrepp effect is a prelude to longer-term signaling in which both locally released angiotensin and circulating angiotensin can modify ion channel expression patterns and may inhibit Na/K pump activity via membrane trafficking mechanisms [32, 33].

3.2 The Bowditch mechanism: frequency inotropy.

It is known since the late 19th century that cardiac contraction can increase markedly with increase of frequency, independent of the action of any hormone [34]. Known as the Bowditch mechanism or frequency inotropy, this effect is very pronounced in most non-rodent hearts [35]. Decades of research revealed that this mechanism has a very complex cellular basis that certainly involves cellular Ca accumulation [36], may in some cases involve a facilitation of Ca influx via Ca channels [37-41], and in some cases clearly involves cytoplasmic Na accumulation related to increased Na influx at higher frequencies [42]. As already noted, both voltage-gated Na channels and Na/Ca exchange operation to extrude Ca support Na accumulation with increase of frequency [42, 43]. This mechanism logically acts against a decrease of cardiac stroke volume as preload decreases and afterload increases with an increase of frequency.

3.3. Long-term cardiac signaling via regulation Na influx through transporters and channels.

As already noted, the regulation of cardiac Na gradients with good certainty plays a major role in cardiac growth responses, i.e. hypertrophy [44], as well as in the regulation of more specific transcriptional 'programs' that rely on Ca signaling [45, 46]. However, it exceeds the scope of this review to consider all of the signaling pathways that can converge to regulate Na/H exchangers [47], TRP channels, HCN channels, and other Na-permeable cardiac ion channels. Suffice it here to point out that Na load caused by all of these mechanisms may be closely connected to signaling that promotes cardiac hypertrophy responses. In the case of NCX1 Na/Ca exchange, most of the Na load is expected to be coupled to the Ca load generated by Ca channels, but it is also possible for exchangers to operate in modes that generate Na 'leaks' [48]. It remains to be seen whether the 'non-stoichiometric' function of NCX1 is physiologically or pathologically important, and the same question applies to numerous other Na transporters, perhaps most notably neurotransmitter transporters [49].

4. Contraction control via regulation of Na/K pumps.

Our understanding of Na/K pump regulation in the heart remains remarkably limited. Alpha subunits of Na/K pumps have a classical clathrin endocytosis motif that can be used to internalize Na/K pumps in dependence on their phosphorylation and possibly ouabain binding [50-52]. Nevertheless, trafficking mechanisms have not been decisively shown to regulate Na/K pumps in physiological circumstances in the heart. One report suggests that angiotensin II promotes extensive endocytosis of Na/K pumps in canine cardiac myocytes and thereby supports generation of transmural gradients of pump activity [32]. Except for this report, it appears that Na/K pumps in cardiac myocytes become internalized primarily in pathological circumstances, such as ischemia and other forms of extreme metabolic stress, by clathrin-independent mechanisms [53, 54].

4.1 Endogenous heart glycosides.

The existence of endogenous heart glycosides [55] could in principle provide a simple regulatory pathway for control of basal contractility. Decreased cardiac output and increased venous volume would in some way promote the release of and/or the synthesis of endogenous heart glycosides and thereby increase basal contractility. Up to now however convincing evidence has not emerged for regulation of cardiac contraction by endogenous glycosides.

4.2. Phospholemman mediated regulation.

The most widely appreciated mechanism of Na/K pump regulation in cardiac myocytes involves the interacting regulatory protein, phospholemman [56]. In analogy to regulation of SERCA Ca pumps by phospholamban, phospholemman is proposed to inhibit Na/K pumps by decreasing the cytoplasmic Na affinity of alpha subunits, and the inhibition can then be relieved by phosphorylation of phospholemman by multiple protein kinases, most clearly by PKA and PKC [57, 58]. In close proximity to its phosphorylation sites, phospholemman is reversibly palmitoylated at two sites, and this palmitoylation seems to be required for the inhibition of Na/K pumps by phospholemman [59]. However, it remains an enigma how the palmitoylation of phospholemman is regulated and what physiological mechanisms might act to alter the phospholemman palmitoylation state. We have found that metabolic stress, specifically reoxygenation after a period of anoxia, promotes phospholemman palmitoylation [54]

The regulation of Na/K pumps by phospholemman was first characterized in ingenious experiments using *Xenopus* oocytes. In these experiments, reversal potentials of Enac Na channel currents were used to calibrate cytoplasmic Na concentrations during Na loading protocols. In this way, the Na dependence of pump currents could be accurately characterized, and it was determined that phosphorylation of phospholemman shifts the apparent affinity of Na/K pumps for Na to somewhat lower Na concentrations [57]. Similar results were obtained using Na-sensitive dyes in intact cardiac myocytes to calibrate Na concentrations and additionally to estimate Na/K pump activity from the rate of change of cytoplasmic Na [60]. However, most labs that have studied Na/K pump regulation via patch clamp do not observe stimulatory effects of protein kinase A activation on pump currents, even when submaximal cytoplasmic Na concentrations are employed [61-64]. After many years of controversy, it now seems certain that stimulatory effects of protein kinase A, if they are pronounced, are lost as a result of dialysis of the cytoplasm in patch clamp, and therefore that a small diffusible molecule is likely required [65]. In the simplest case, the small molecule regulator would be Ca, as proposed by Shattock and colleagues [66]. We cannot support this suggestion up to now, as we have not observed over 20 years effects of cAMP modulation either with or without Ca *and* with or without Ca buffering in murine myocytes [64]. In summary, patch clamp experiments that revealed clear cAMP effects on cardiac Na/K pump currents were nearly always carried out using very high resistance electrodes or using perforated patch methods. Assuming that cAMP kinase indeed activates Na/K pumps, it makes physiological sense that, as the flight-or-fight response subsides, and the heart beats more slowly, basal contractility would be increased by inhibiting Na/K pumps, thereby supporting adequate cardiac output in a resting state at a low frequency of contraction.

4.3 Regulation by glutathionylation and other mechanisms during oxidative stress.

It is known for decades that Na/K pumps usually become inhibited during ischemia and oxidative stress via mechanisms besides nucleotide concentration changes [67, 68]. Much evidence now supports the proposal that this inhibition involves the glutathionylation of Na/K pumps, likely within both beta and alpha subunits, but possibly also in phospholemman [69-71]. Presumably, this regulation of Na/K pumps will prove to be part of a much larger myocyte response to oxidative stress, possibly shutting down many cellular activities so as to reduce the confounding metabolic stress. In this connection, it has been suggested that small molecule inhibitors of Na/K pumps are generated during ischemia [72]. Although their identity remains unknown, the existence of small molecule modulators of Na/K pumps seems increasingly likely, as discussed subsequently.

In addition to *bone fide* regulatory effects, it is certain that cardiac Na/K pumps can be inhibited directly by changes of cell energetics in various forms of metabolic stress. In ischemia, the free energy of ATP hydrolysis can readily fall from 55 kJ/mol to 40 kJ/mol [73]. If myocytes do not depolarize, this change is large enough to not only inhibit Na/K pumps, but to cause reversal of pump activity and transiently support Na loading by reverse pump activity. A major complication in estimating when cellular energetics limit pump activity stems from the possibility that local gradients of nucleotides may exist and that direct nucleotide transfer may occur from enzymes involved in glycolysis to pumps (e.g. [74]). Certainly, we like others find that nucleotides do not diffuse well from the patch pipette tip into myocytes, and we like others find that small fluorophores (e.g. Mag-FURA [75], which might be used to estimate ATP/ADP ratios) equilibrate between the pipette tip and the cytoplasm nearly 10-times slower than expected for free diffusion. Presumably, this reflects strong molecular crowding in myocytes, which remarkably does not affect strongly the diffusion of monovalent ions, as described subsequently. Thus, without knowledge of (potentially) local concentrations of nucleotides, most importantly ADP, it is often difficult to judge whether pump activity changes occur as a result of regulatory processes or as a result of changes of energetics.

To underscore the energetic dilemma posed, murine cardiac myocytes routinely generate 0.5 nA to 1 nA of Na/K pump current when activated by saturating extracellular K. This corresponds to 0.5 mmole of ATP hydrolysis per second per liter cytoplasm, indicating that the majority of cellular ATP would be hydrolyzed in 10 s if synthetic mechanisms were not highly active and/or under rapid feedback control. Surprisingly, our analysis of pump current transients in murine myocytes does not support the idea that ADP accumulates significantly during an extended period of maximal pump activity [17]. Similarly, recent experimental analysis of the equivalent energetic situation in neurons confirms that ATP and ADP levels remain remarkably stable over time when Na/K pumps are activated and deactivated [76], and these authors suggest as explanation that Na/K pumps directly regulate both glycolysis and mitochondrial ATP production. Whether or not this speculation is verified, it is noteworthy that Na/K pumps are suggested to regulate non-canonical forms of cell death by (apparently) direct interactions with signaling proteins on the mitochondrial surface [77].

4.4. Regulation by Ca and lipid signaling.

Being impressed by the small magnitude or absence of protein kinase effects on cardiac Na/K pump currents, both in giant excised patches [78] and in patch clamped myocytes, we began a new search for potentially physiological mechanisms that might regulate cardiac Na/K pumps. After testing many hormones and signaling mechanisms, we found that the most reliable and powerful manipulation to modify pump activity in rodent myocytes was simply to elevate cytoplasmic Ca for a few seconds [64]. In routine experiments this was accomplished by activation of reverse Na/Ca exchange activity (i.e. Ca influx), which would then be followed by a few seconds of spontaneous beating via cycles of Ca release. Similar results were readily obtained via Ca influx through Ca channels when channels were opened with agonists, and strong effects of Ca were also obtained in myocytes from phospholemman knockout mice.

Figure 2A shows a typical example of the protocol employed in mouse myocytes, employing in parallel an electrogenic membrane probe which provides evidence that the effects of Ca occur hand-in-hand with very significant changes of the membrane bilayer. As indicated within Fig. 2A, 7 mM cytoplasmic K was applied repeatedly for 12 s to activate outward Na/K pump current. Between activation cycles, a hydrophobic cation was applied which can cross the membrane and generate an inward current. The cation employed, hexyltriphenylphosphonium (C6-TPP) has a delocalized positive charge that allows it to cross the sarcolemma through the bilayer itself. The transit of C6-TPP across the membrane therefore depends strongly on the ordering and fluidity of the membrane, being enhanced as order decreases. In addition, the C6-TPP current may be enhanced by the presence of negatively charged lipids which would promote its binding to the membrane and/or its transit toward the membrane side with more negative charges.

Three initial episodes of Na/K pump current and C6-TPP current activation show very stable responses. Thereafter, Ca was applied to activate reverse Na/Ca exchange current for 15 s. Subsequent to the episode of Ca influx, peak Na/K pump currents become enhanced by more than two fold and the typical decay of pump current that occurs when cytoplasmic Na is submaximal is strongly reduced. In parallel, the C6-TPP current initially becomes enhanced by 40%, and over the next two minutes declines in parallel with decline of pump current. A subsequent application of Ca then promotes further the decline of the two currents to below baseline, whereby the pump current becomes undetectable. The C6-TPP current declines overall by 4-fold, and Fig. 2B shows the close correlation between the two currents. From these studies, we suggested that a lipid signaling mechanism may generate metabolites that powerfully modify Na/K pump function. This signaling system would appear to have dual dependence on Ca. On the one hand, Ca can promote the generation of a pump activator or suppress generation of an inhibitor to stimulate pump currents. On the other hand, the stimulatory mechanism is labile, allowing only 1 to 3 responses per myocyte. Thereafter, Ca elevations are found to have inhibitory effects that strongly suppress pump activity. Since both the stimulatory and inhibitory effects are reflected in C6-TPP currents, it seems possible that Ca regulates both the generation and break-down of the putative lipid regulator. Up to now, we have eliminated the involvement of a wide range of classical signaling mechanisms in these effects without identifying the regulatory molecule.

5. Local Na gradients.

Many great advances of physiology followed on the realization that Ca signaling can take place in a localized fashion, whereby Ca gradients develop in close vicinities to the pores of Ca-conducting ion channels [79, 80]. In this connection, the microarchitecture of spaces between cell membranes in close approximation favors the generation of local Ca gradients. Furthermore, as a result of extensive Ca buffering, diffusion of Ca through the cytoplasm is remarkably slow, allowing the generation of waves of Ca whose kinetics are important determinants of Ca signaling. In this context it was and remains attractive to envision that Na signaling can occur similarly. Specifically, the idea is highly attractive that local Na signals may generate local crosstalk between Na channels, Na transporters and Ca signaling that relies on Na /Ca exchange [81].

5.1. Failure to confirm the existence of local Na gradients.

One key observation that has been used to support the existence of local Na gradients is that Na/K pump currents decay much more substantially than expected for bulk Na. This was described already in Fig. 2A, and the typical current patterns for saturating Na (110 mM) and subsaturating Na (25 mM) are shown in Fig. 3A. When activated by 7 mM extracellular K, the pump current can be nearly stable with 110 mM Na, whereas it decays with a time constant of 1 to 3 s by more than 50% in the presence of 25 mM Na. Fig. 3B shows the typical Na concentration dependence of peak current and current magnitudes after 10 s of activation in murine myocytes. The decay of current becomes much less pronounced at high Na such that the Na dependence of current shifts to higher Na concentrations. This frequently described result has been widely attributed to depletion of subsarcolemmal Na. Notably, the results shown in Fig. 3B, obtained at 23°C, are very similar to results obtained at 37°C. This was quite a surprising outcome, since pump currents are ~3 fold larger at 37°C and therefore should decay more strongly if decay represents Na depletion.

The logistics of generating a local Na gradient are vastly different than those for Ca, Na concentrations being in the millimolar range and Na concentration changes required to change Ca transport activities also being in the millimolar range. While multiple quantitative suggestions have been made to explain biophysically how local Na gradients in the millimolar range might be generated [82, 83], the problem remains that for gradients to occur Na diffusion must be restricted by several log units from diffusion in free water. Accordingly, water would likely have to be highly structured, as suggested more than 25 years ago by Edward Carmeliet [84]. Although measurements consistent with highly restricted Na diffusion in cardiac myocytes were reported both with the ion microprobe technique [85] and with Na-sensing fluorescent dyes [86], those reports have been contradicted by subsequent studies [87, 88]. Furthermore, one key study suggesting that Na/K pump activity can locally influence Na/Ca exchange activity employed substitution of extracellular Na by Li to 'isolate' Na/Ca exchange currents [89]. Unfortunately, this protocol also activates nearly maximal Na/K pump currents, since Li acts as a K surrogate in Na/K pumps. In another key study, Na/Ca exchange reversal potentials were determined with nickel to assess whether subsarcolemmal Na concentrations change during prolonged activation of Na/K pumps. The outcomes were ambiguous in that reversals changed over

tens of seconds in one set of ionic conditions but not another [90]. Two further complicating issues are that nickel activates confounding currents in cardiac myocytes [17, 91], and the reversal potential of Na/Ca exchange current is not fixed. Rather, it can shift over a substantial range of potentials expected for 3-to-1 up to 4-to-1 exchange [48].

Given these dilemmas, we began to question more seriously the local Na hypothesis [64], and a cartoon of our suggested alternative explanation of Na/K pump current decay is shown in Fig. 3C. During the normal pump cycle, Na/K pumps bind 3 Na from the cytoplasmic side (lower left corner of the cycle), phosphorylate themselves, close binding sites, open binding sites to the outside in E2 configuration, release Na to the outside, bind 2 K, occlude the K and dephosphorylate themselves (right side of the cycle), open to the inside and release K, followed by rebinding ATP and Na. Inactivation is suggested to occur from states open the cytoplasmic side (E1 states). When binding sites (I,II and III) are not occupied by Na, the pump adopts configurations that cannot be phosphorylated. Recovery from inactivation likely requires that Na rebinds to inactive configurations. Failure to observe inactivation of Na/K pump currents in excised membrane patches [92] might reflect involvement of diffusible small molecules that interact with ion binding sites and/or phosphorylation sites.

In light of increasing evidence that Na/K pumps may undergo inactivation, we undertook a new study to test whether subsarcolemmal Na concentrations really change in a manner consistent with local Na gradients [17]. In brief, we used veratridine to open voltage gated Na channels and make the sarcolemma of cardiac myocytes permeable to monovalent cations. Using only Na as permeable monovalent cation, we asked whether Na/K pump activity could shift the reversal potential of the Na channel current more than expected for bulk changes of cytoplasmic Na. We found no evidence for a difference between bulk cytoplasmic Na and Na measured via Na channels, and we found that all monovalent ions turn over in standard patch clamp conditions (access resistance, 2.5 to 4 M Ω) with a time constant of 12 to 20 s (see Table 1). In fact, in order to explain the small effects of Na/K pump activity on Na flux through NaV channels, it is essential to assume that Na/K pumps inactivate.

Admittedly, we did not perform one key experiment in that study, namely to directly vary the Na concentration in the pipette tip and observe how quickly subsarcolemmal Na concentrations change, as experienced by cardiac ion channels and transporters. Those experiments have now been carried out. For veratridine-modified Na channels, we find that Na exchanges between the pipette tip and the inner mouth of the NaV channels with at time constant of ~15 s with no evidence of slow exchange components [93]. Results for Na/K pump currents are shown in Fig. 4. In brief, we use microcapillary tubing to change solutions within a few microns of the pipette opening by applying positive pressure to solution reservoirs [78] (see Fig. 4A). In the experiment presented, n-methy-d-glucamine (NMDG) was used as the major monovalent cation on both membrane sides. The pipette tip initially contains 25 mM Na, and the pump current was activated twice as usual with 7 mM K. First, the pump current was activated for just 12 s initially, and subsequently K was applied continuously. Upon perfusion of Na out of the pipette tip, in exchange for NMDG, the pump current decays to slightly below baseline with a time constant of 8.7 s, reintroduction of Na reactivates the pump current with a time constant of 14 s, and pump

current turns off subsequently with a time constant of 4.3 s upon removing Na for a second time from the pipette tip. The fact that the time constants are smaller upon Na removal than for Na application reflects the fact that Na pump current has a steep dependence on cytoplasmic Na. As a result, the time constants for pump current decay are 2 to 3 times smaller than the actual time constants of Na decay at the membrane. With this in mind, we project that the average time constant for Na exchange from the pipette tip to the cytoplasmic surface of Na/K pumps is 15 s, entirely consistent with results for veratridine-modified NaV currents and for experiments using extracellular ion concentration changes in the presence of large sarcolemmal conductances to change cytoplasmic ions. In agreement with others [88], Na diffuses throughout the cytoplasm of myocytes with a diffusion constant that is only about 50% smaller than in free water. In this connection, it was recently described that Na pump currents become enhanced by repetitive activation of voltage-gated Na currents in mouse myocytes [94] as a result of subsarcolemmal Na accumulation. The decay time constant of these effects are about one minute, which is four times longer than expected for complete Na equilibration in myocytes in our experiments.

5.2. Contraction control by Na without local Na gradients.

Given the impression that local Na gradients may not exist, it becomes important to consider implications for our thinking about Na control of cardiac ECC, most importantly how Na influx mechanisms interact functionally with Na/K pumps. This is attempted in Figs. 5 and 6 via very simple simulations that explicitly omit a host of complexities that modify in various ways cardiac ECC. To do so, a simple fixed action potential shape is generated, with voltage oscillating between -80 and $+30$ mV. The upstroke is instantaneous, and it is assumed that each upstroke increases cytoplasmic Na by $10\text{ }\mu\text{mole per liter of cytoplasm}$. This is the minimal amount of Na that can depolarize a small cardiac myocyte with a capacitance of 100 pF by 110 mV . Ca current and Ca release from the sarcoplasmic reticulum are both simulated to take place as conductances that rise and fall as Bateman functions, initiated by the upstroke. Their decay is modified to depend on the 2^{nd} power time, as might be expected for a decay that involves loss of interactions between multiple subunits. The magnitude of SERCA Ca pump activity is simulated to occur in direct dependence on binding of 2 Ca ions, and it is adjusted to relax the Ca transient with a time constant of about 140 ms . Ca buffering is assumed to occur linearly with a 40-to-1 bound-to-free ratio. The contraction is simulated as a two step mechanism that depends on Ca binding with a Hill slope of 2.7. ECC complexities that have been purposely ignored include (1) all details of SR Ca release and Ca channel inactivation by Ca, (2) secondary regulation of Na/Ca exchange by Na and Ca. (3) action potential changes that occur as a result of Na/Ca exchange operation, and (4) nonlinearities of Ca buffering and concentration in the cytoplasm. In this way, it is possible to illustrate in a very straight-forward fashion the influences of Na/K pump activity and Na/K pump regulatory mechanisms.

The immediate activities of Na/K pumps and Na/Ca exchangers are simulated by simple two state models, whereby Na/K pumps are simulated to inactivate slowly into a third state. Background Na conductances and Na/K pump activities are set to generate a resting cytoplasmic Na concentration of 8.2 mM with the average cytoplasmic Na ion turning over to the extracellular space with a time constant of $\sim 130\text{ s}$. The upper panel of Fig. 5A shows

the action potential, Ca transient and contraction waves generated by the model during continuous activity at 1 Hz. The second panel shows the Na/K pump, Na/Ca exchange, and Ca currents generated by the model. The third panel from the top shows the open probability of SR ryanodine Ca release channel activity (RyR_{SR}) and surface membrane CaV channel activity (CaV_{SL}), and the lower panel shows integrals of the SR Ca flux, Ca flux via Ca channels, and Ca flux via Na/Ca exchange. The voltage dependence of Na/K pumps is strong enough to increase pump current by a factor of two during the action potential plateau. As apparent in the bottom panel, Ca influx via Ca channels amounts to about 10% of the peak Ca flux from the sarcoplasmic reticulum via RyR_{SR} channels, and the Na/Ca exchanger extrudes a nearly equivalent amount of Ca by the end of relaxation.

Panels B to D illustrate detail the basic function of this simple ECC model, which is intended to represent roughly human ECC function. From top to bottom in these panels, simulation results are presented for cytoplasmic Na, Na/K pump current, SR Ca concentration, and contraction force. From left to right, the influences presented are (1) doubling of SR Ca uptake rates by SERCA Ca pumps, (2) doubling of the surface membrane Ca conductance, and (3) decreasing the affinity for Na of the number 1 Na binding site of the pump on the cytoplasmic side by two fold. Annotating the results from left to right, a doubling of SERCA pump activity (A) generates as expected a very strong positive inotropic action with SR Ca loading and negligible changes of pump activity or Na. A doubling of Ca influx (B) generates a three-fold increase of contraction force with a greater than three-fold increase of SR Ca load. These effects are accompanied by a one millimolar increase of cytoplasmic Na. It is noteworthy that the amount of Na influx needed to extrude Ca is roughly the same amount needed for upstroke via NaV channels. The third column (C) illustrates the equally large effect of decreasing the affinity of the pump for Na at a single cytoplasmic binding site. The reduction of the Na affinity by a factor of 2 has the same contraction-potentiating effect as a doubling of Ca influx, while cytoplasmic Na increases by only 3 mM. This result underscores that the expression of different pump isoforms with different Na affinities could have rather specific consequences for cardiac ECC in the absence of local Na gradients.

The implications of the Na/K pump inactivation mechanism for cardiac ECC are presented in Fig. 6, simulating the mechanism to reproduce reasonably experimental results for Na/K pump currents in patch clamped myocytes under our routine control conditions. Panel A shows the effects of decreasing the inactivation rate by 50 % for 800 s, returning inactivation to normal for 100 s, and then doubling the inactivation rate for 800 s, followed again by returning it to normal. The simulation results shown are from top to bottom, the cytoplasmic Na concentration, the Na/K pump currents, and the contraction force. The decrease of pump inactivation results in an immediate stimulation of Na/K pump current, which subsides as Na decreases back to steady state values, and results in a decrease of contraction force by more than 50%. Cytoplasmic Na decreases by just 1 mM from 8 to 7 mM. Conversely, the doubling of inactivation causes a transient decrease of pump currents, a doubling of contraction force, and an increase of cytoplasmic Na by ~1 mM. In brief, the Na/K pump inactivation mechanism can be expected to profoundly modify cardiac contractility when its activity is modified by signaling mechanisms, Ca elevations being the best documented example to date [64].

Panel B expands upon the effects of Na/K pump inactivation, illustrating how the inactivation process affects the heart's response to an increase of heart rate, an increase of Na influx, and a decrease of Na/K pump activity. In each case, results in the presence of inactivation are presented in color, and results obtained after removing inactivation are shown in gray. Besides removing the inactivation process, pump activity was decreased just enough so that basal cytoplasmic Na was equivalent in the results with and without inactivation. In the presence of Na/K pump inactivation, cytoplasmic Na increases by ~1.5 mM when heart rate is increased from 0.25 to 1 Hz, pump currents increase by nearly three-fold to compensate the increased Na influx, SR Ca doubles, and contraction strength increases by somewhat more than two fold (left column of Fig. 6B). In the absence of inactivation (results colored in gray), Na continues to rise by 3 mM above baseline, pump current changes are similar but decay more slowly after switched back to the low frequency, and the rises of SR Ca and contraction force are nearly twice as large. Results for a doubling of Na influx per upstroke (middle column in Fig. 6B) are qualitatively similar, but substantially smaller in magnitude. The reason for the small magnitude is that the Na influx at upstroke is less than one-half of total Na influx per unit time. A decrease of Na/K pump activity by 50% (right column in Fig. 6B) causes a 1.3 mM increase of cytoplasmic Na, a transient decrease of pump currents, a 66% increase of SR Ca, and a doubling of contraction force. In the absence of inactivation, the increments of Na, Ca and contraction are almost doubled. In short, the inactivation mechanism functionally buffers cytoplasmic Na during changes of Na flux, and its regulation by cell signaling mechanism can allow the heart to strongly modify its response to a change of Na flux.

6. The influence of NCX1 Na/Ca exchange activity per se and its potential regulation.

It would be remiss to ignore the role of NCX1 Na/Ca exchangers in the regulation of cardiac contraction by Na. The idea that Na/Ca exchangers primarily act as 'slaves' to the Na gradient, and i.e. to Na/K pumps and Na channels, has grown in the wake of multiple important experimental outcomes. First, the most selective Na/Ca exchange inhibitors available do not augment cardiac contractility [95-97] or have only very modest stimulatory effects [98]. This is consistent with exchangers effectively buffering cytoplasmic Ca to values that are determined by the Na gradient and membrane potential. Second, the knockout of NCX1 Na/Ca exchangers in mouse ventricular myocardium surprisingly did not result in lethal or even clearly deleterious phenotypes [99]. Third, it is indeed possible to simulate cardiac ECC, especially ECC that depends strongly on intracellular Ca release, such that ECC is overall strongly dependent on the Na gradient but almost impervious to large changes of Na/Ca exchange activity [100]. In short, it is certain that mouse myocytes can make do with sarcolemmal Ca pumps, and functional cardiac ECC can readily be simulated employing surface membrane Ca pumps instead of Na/Ca exchangers to maintain Ca homeostasis [101].

In spite of these impressions, it is far too soon to conclude that Na/Ca exchangers simply enforce the rule of the Na gradient on the heart. While rodent ECC can be simulated so that changes of NCX1 density have only small effects on ECC, this becomes problematic when

Ca influx via Ca channels is significant. As outlined in Figs. 1 and 5, Ca influx by CaV channels constitutes 10% of total ECC Ca in the present simulations. It must be admitted in this context that a reduction of Na/Ca exchange activity in the present model causes a substantial increase of contraction force, and vice versa. Primarily two mechanisms can be evoked to avoid this outcome, which conflicts with data on the effects of exchange inhibitors. First, the secondary activation of Na/Ca exchange activity by Ca [102] can counteract the effect of decreasing NCX1 exchanger density in the sarcolemma. Second, the function of the exchanger as a pure Ca extrusion mechanism in myocytes with strong Ca channel activity, as in Fig. 5A, may be significantly offset by the fact that the exchanger does not operate as a pure 3:1 Na/Ca exchanger [48]. Specifically, the exchanger can generate Ca influx that occurs in an electroneutral fashion as influx of 1 Ca and 1 Na coupled to extrusion of 3 Na, thereby generating a 1Ca-2 Na exchange mode. If this Ca influx mode is relatively strong, the overall function of the exchanger may become neutral with respect to the magnitude of Ca transients and contraction, while still extruding Ca and enforcing the dependence of ECC on the Na gradient. Once again, progress of the field may be limited by our present reliance on rodent models of ECC in which basic functions, e.g. the effects of increasing heart rate as in Fig. 6B, are fundamentally different from the function of non-rodent hearts [103], such as our own.

7. The outlook for fundamental new discoveries.

It is clear that our understanding of how Na is used to control cardiac contraction is far from complete. Most of all, we lack detailed understanding of how signaling pathways, inherent both to the heart itself and originating in other organs, converge to regulate cardiac Na homeostasis, including the secondary inactivation reactions of Na transporters, and thereby regulate basal contractility. There have been setbacks along the way. But there are now encouraging signs that we can fulfill the promises of early studies that revealed the unique sensitivity of cardiac contraction to the transsarcolemmal Na gradient. This short review has highlighted several areas where recent work changes how it seems appropriate to think about Na regulation of the heart. It seems now certain that the heart can modify its regulation by Na, not only by changing the Na gradient but by changing Na/K function to respond differently to Na changes. While not discussed in detail here, secondary regulation of Na/Ca exchangers likely also influences strongly the response of the heart to changes of the Na gradient.

Two major areas of regulation have been left essentially untouched. First, it is increasingly likely that many membrane proteins have signaling functions which arise from their physical interactions with neighboring membrane proteins, and the Na/K pump is long suggested to be a signaling protein in this sense [104, 105]. While the devil remains ominously present in the details, it is likely that pump isoform expression patterns and the localization of Na/K pump isoforms to distinct places in the cardiac myocyte may be tied to pump signaling functions, not only via protein kinases such as SRC kinases, but potentially via physical interactions with NCX1 Na/Ca exchangers [64] and with ion channels. Second, the extensive signaling roles of mitochondria, beyond providing a supply of ATP and controlling the classical apoptosis pathway, continue to become more evident. In this connection, the

control of mitochondrial Ca by mitochondrial Na gradients very likely plays important roles in multiple cardiac pathologies, starting with those associated with diabetes [106].

Beyond these bullet points, there is a clear outlook for discovery of new signaling molecules and possibly signaling systems in unraveling further the function of Na signaling in the heart. The cyclic AMP system appears to regulate Na/K pump activity only in cardiac myocytes that are studied in an intact (or nearly intact) state. This is clearly consistent with loss of a diffusible, small regulatory factor. Ca might be one factor, but it is unlikely to be the sole important factor. Na/K pump inactivation is fundamentally changed and/or lost when pumps are studied in excised patches. This occurs as pump activity increases irreversibly at the onset of experiments in excised patches, consistent with loss of diffusible pump inhibitors [92, 107]. Similarly, the regulatory system underlying large-scale pump stimulation and then inhibition by Ca elevations [64] remains unknown after eliminating a wide range of classical regulatory mechanisms. In summary, we are only at the beginning of understanding how the body regulates cardiac function by modifying the cardiac transsarcolemmal Na gradient and more specifically the function of Na/K pumps. Continued efforts at multiple scientific levels can be expected to provide new insights into well-established signaling pathways that influence cytoplasmic Na, and may well also uncover new biological signaling pathways and signaling molecules.

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Appendix.

Description of the simulation model.

The simulations were performed in Matlab.

Action potential.

The contraction frequency is 1 Hz in all simulations. The action potential is simulated with an instantaneous upstroke from -85 to $+30$ mV and a fixed falling exponential waveform with a steep dependence time after upstroke, t_{cyc} .

$$E_m = -85 + 115 \times e^{-(t_{cyc} / 150)^5} \quad (1)$$

The cytoplasmic Na concentration is incremented by $10 \mu\text{M}$ at each upstroke. With a mixing volume of 10 pL , this amount of Na influx will depolarize 115 pF of membrane by 110 mV .

Ca conductances.

The Ca conductance of the sarcolemma (G_{ca}) and of the SR (G_{SRCa}) are simulated as Bateman functions with maximal conductances of G_{camax} and G_{SRmax} . The inactivation components of the functions are squared with respect to time to generate delayed functions with steep descent.

$$G_{Ca} = G_{Camax} \times (1 - e^{-tcyc / 2}) \times e^{-(tcyc / 70)^2} \quad (2)$$

$$G_{SRCa} = G_{SRmax} \times (1 - e^{-tcyc / 4}) \times e^{-(tcyc / 80)^2} \quad (3)$$

Parmeter Settings.

$$G_{camax}=200 \text{ (pA)} \quad G_{SRmax}=10000 \text{ (pA/mM)}$$

Na/K pump activity.

The Na/K pumps are simulated as binding on both membrane sides 2 Na or 2 K in one set of binding sites, and 1 Na at a separate site. Voltage dependence occurs primarily at the single Na binding site open to the outside with weaker voltage dependence at the other sites and on cytoplasmic Na binding. All ion dependencies from the literature and standard current-voltage relations are recreated reasonably, and the affinities and rate constants are consistent with an energy of ATP hydrolysis of 54 kJ/mole.

In the following routine, F_{no} is the fraction of pumps in the E2 state (open to the outside) with the single Na site occupied, N_o is the extracellular Na concentration, and K_{no3} is the dissociation constant for the single selective Na site. D_o is a temporary variable, K_{no1} and K_{no2} are the dissociation constants for Na of the dual ion binding sites, K_{ko1} and K_{ko2} are the dissociation constants for K of the dual sites. F_{k2o} and F_{n2o} are the fractional occupation of the dual sites by K and Na, respectively. F_{ni} , N_i , K_{ni2} , K_{ni1} , K_{ni2} , K_{ki1} , K_{ki2} , F_{k2i} and F_{n2i} are the equivalent variables for cytoplasmic ion binding in E₁ states. F_{adp} , F_{atp} and F_{pi} are the fractional occupation of ADP, ATP and inorganic phosphate (P_i) binding sites with dissociation constants, K_{adp} , K_{adp} and K_{pi} . Phosphorylation is treated as an instantaneous reaction, and K_1 is the outward translocation rate of phosphorylated E₁ states with 3 Na occluded. K_2 is the inward translocation rate of E₂ states with 3 Na bound, K_3 is the inward translocation of E₂ states with 2 K bound which also corresponds to the dephosphorylation rate. And K_4 is the rate of translocation of E₁ states with two bound K, a bound phosphate and an empty single Na site. E_2 and E_1 are the fractions of pumps in E₂ and E₁ states. F_{act} is the fraction of pumps that are active (i.e. not inactivated), where the inactivation rate is K_{inact} and the recovery rate is K_{rec} . I_{pump} is the pump current in pA using K_{pump} as a scaling variable.

$$F_{no} = N_o / (N_o + K_{no3} \times e^{Em / 26 \times 0.9}) \quad (4)$$

$$D_o = 1 + N_o / K_{no1} \times (1 + N_o / K_{no2}) + K_o / K_{ko1} \times (1 + K_o / K_{ko2}) \quad (5)$$

$$F_{k2o} = K_o \times K_o / K_{ko1} / K_{ko2} / D_o \quad (6)$$

$$F_{n2o} = N_o \times N_o / K_{no1} / K_{no2} / D_o \quad (7)$$

$$F_{ni} = N_i / (N_i + K_{ni3}) \quad (8)$$

$$D_i = 1 + N_i / K_{ni1} \times (1 + N_i / K_{ni2}) + K_i / K_{ki1} \times (1 + K_i / K_{ki2}) \quad (9)$$

$$F_{k2i} = K_i \times K_i / K_{ki1} / K_{ki2} / D_i \quad (10)$$

$$F_{n2i} = N_i \times N_i / K_{ni1} / K_{ni2} / D_i \quad (11)$$

$$F_{adp} = ADP / (ADP + K_{adp}) \quad (12)$$

$$F_{atp} = ATP / K_{atp} / (1 + ATP / K_{atp} + P_i / K_{pi}) \quad (13)$$

$$F_{pi} = P_i / K_{pi} / (1 + ATP / K_{atp} + P_i / K_{pi}) \quad (14)$$

$$K_1 = F_{ni} \times F_{n2i} \times F_{atp} \times (1 - F_{adp}) \times e^{(Em / 55 \times 0.1)} \times 2 \quad (15)$$

$$K_2 = F_{no} \times F_{n2o} \times (F_{adp} + K_{fail}) \times e^{(-Em / 55 \times 0.1)} \times 2 \quad (16)$$

$$K_3 = F_{k2o} \times (1 - F_{no}) \quad (17)$$

$$K_4 = F_{k2i} \times F_{pi} \times (1 - F_{ni}) \quad (18)$$

$$E_2 = (K_1 + K_4) / (K_1 + K_2 + K_3 + K_4) \quad (19)$$

$$E_1 = 1 - E_2 \quad (20)$$

$$Pump = E_1 \times K_1 - E_2 \times K_2 \quad (21)$$

$$F_{act} = F_{n2i} \times F_{ni} \times K_{rec} / (F_{n2i} \times F_{ni} \times K_{rec} + E_1 \times F_{act} \times (1 - F_{n2i}) \times K_{inact}) \quad (22)$$

$$I_{pump} = Pump \times F_{act} \times K_{pump} \quad (23)$$

Parameter settings.

$N_o=140$	$K_o=7$	$K_i=120$	$ADP=0.05$	$P_i=0.3$	$ATP=6$
$K_{no3}=900$	$K_{no1}=10$	$K_{no2}=10$	$K_{ko1}=0.3$	$K_{ko2}=0.2$	$K_{mi3}=4$
$K_{ni1}=6$	$K_{mi2}=6$	$K_{ki1}=20$	$K_{ki2}=30$	(mM)	
$K_{inact}=0.8$	$K_{rec}=3$	(S^{-1})	$K_{fail}=0.3$		

Note: K_{fail} is a probability that Na-loaded E_2 sites undergo a 'failed' backward step to the E_1 state in the absence of cytoplasmic ADP. In the presence of extracellular K such events are negligible.

$K_{pump}=600$ or 2500 , without and with inactivation, respectively. (**pA**)

Na/Ca exchanger activity.

The simplest possible consecutive Na/Ca exchange model was employed with symmetrical ion binding affinities and with voltage dependence divided equally between Na translocation and Ca translocation. Ca binding on both membrane sides takes place competitively with the consecutive binding of 3 Na ions. Current-voltage relations and ion dependencies of NCX1 are only approximate, but they are adequate for the purpose of these simulations.

In the following routine, K_{em} , D_o and D_i are temporary variables. C_o , C_i , N_o and N_i are the extracellular and intracellular Ca and Na concentrations, K_{nx} is the dissociation constant of all Na binding sites, and K_{cx} is the dissociation constant for Ca on both membrane sides. F_{co} , F_{3no} , F_{ci} and F_{3ni} are the fractions of outward and inward facing exchangers with binding sites occupied by Ca or 3 Na. E_1 and E_2 are the fractions of exchangers open to the cytoplasmic and extracellular sides. I_{ncx} is the calculated exchange current in pA using K_{ncx} as a scaling factor.

$$D_o = C_o / K_{cx} + 1 + N_o / K_{nx} \times (1 + N_o / K_{nx} \times (1 + N_o / K_{nx})) \quad (24)$$

$$D_i = C_i / K_{cx} + 1 + N_i / K_{nx} \times (1 + N_i / K_{nx} \times (1 + N_i / K_{nx})) \quad (25)$$

$$F_{co} = C_o / K_{cx} / D_o \quad (26)$$

$$F_{3no} = N_o^3 / K_{nx}^3 / D_o \quad (27)$$

$$F_{ci} = C_i / K_{cx} / D_i \quad (28)$$

$$F_{3ni} = N_i^3 / K_{nx}^3 / D_i \quad (29)$$

$$K_{em} = e^{0.25 \times E_m / 26} \quad (30)$$

$$E_2 = (F_{ci} / K_{em} + F_{3ni} \times K_{em}) / (F_{ci} / K_{em} + F_{3ni} \times K_{em} + F_{co} \times K_{em} + F_{3no} / K_{em}) \quad (31)$$

$$E_1 = 1 - E_2 \quad (32)$$

$$I_{ncx} = (F_{co} \times K_{em} \times E_2 - F_{ci} / K_{em} \times E_1) \times K_{ncx} \quad (33)$$

Parameter Settings.

$C_o=1.5$	$K_{nx}=20$	$K_{cx}=0.05$ (mM)
$K_{ncx}=2600$	(pA)	

Background Na influx.

A background Na influx is calculated in pA (I_{na}) as a single file channel pathway with a Na dissociation constant, K_D , of 100 mM. With $K_{em} = e^{E_m / 26.0}$

$$I_{na} = -G_{na} \times E_m \times (N_o - N_i \times K_{em}) / ((K_n + N_o + N_i) \times (K_{em} - 1.0)) \quad (34)$$

Parameter Settings.

$G_{na}=0.05$ (pA/mV)

Cytoplasmic Na concentration changes are calculated in mM/s from the three Na fluxes simulated, background Na conductance (I_{na}), Na/K pumps (I_{pump}) and Na/Ca exchange (I_{ncx}). Simulating in milliseconds, and assuming a cytoplasmic volume of 10 pL, pA of monovalent ion flux converts to mM/s with a conversion factor of 10^{-6} .

$$dN_i / dt = (-I_{na} - I_{pump} - I_{ncx} \times 3) \times 10^{-6} \quad (35)$$

Ca current.

The Ca current is calculated in pA from the Ca conductance, simulated in equation 2, and assuming a single file pathway with a Ca dissociation constant, K_{Ca} , of 10 mM. With $K_{em} = e^2 \times E_m / 26.0$

$$I_{Ca} = -G_{Ca} \times E_m \times (C_o - C_i \times K_{em}) / ((K_{Ca} + C_o + C_i) \times (K_{em} - 1.0)) \quad (36)$$

Ca uptake and release by the SR.

The simulations assume that Ca uptake by the SR is proportional to a Hill equation with a slope of 2 and K_{50} of 2 μ M (0.002 mM), and in addition to the Ca conductance activate upon upstroke the possibility of a continuous SR Ca leak is included. In the following routine, F_{sr} is the fractional occupation of SR Ca pumps by cytoplasmic Ca, K_{50} is the half-maximal free Ca for SR Ca uptake, K_{srleak} is a rate constant for a continuous SR leak, and Ca_{SR} is the SR Ca concentration. dCa_{SR}/dt is the Ca flux between the cytoplasm and the SR in pA units. Ca flux causes 5-fold greater Ca concentration changes in the SR than in the cytoplasm, and the cytoplasmic total to free Ca ratio is fixed at 40:1. Assuming 10 pL volume of the cytoplasm, pA convert to mM/s with a factor of 10^5 . C_{tot} is the total cytoplasmic Ca concentration in mM and C_i is the free concentration.

$$F_{sr} = C_i^2 / (C_i^2 + K_{50}^2) \quad (37)$$

$$dCa_{SR} / dt = (F_{sr} \times K_{srup} - Ca_{SR} \times K_{srleak} - Ca_{SR} \times G_{SRCa}) \times 5 \times 10^{-6} \quad (38)$$

$$dC_{tot} / dt = (-dCa_{SR} / dt + Incx - I_{Ca} / 2) \times 10^{-6} \quad (39)$$

$$C_i = C_{tot} / 40 \quad (40)$$

Parameter Settings

$$K_{50}=0.002 \text{ (mM)} \quad K_{srleak}=0.0001 \text{ (pA/mM)} \quad K_{srup}=7000 \text{ (pA)}$$

Contraction force.

Contraction force develops in a two-step mechanism to approach a peak value given by a simple Hill equation with a slope of 2.7 and a K_{50} of 2.7 μ M. F_{myo} is the relative developed force, and R_1 and R_2 are first and second rate constants, respectively.

$$dF_1 / dt = ((C_i \times 1000)^{2.7} / ((C_i \times 1000)^{2.7} + 1.6^{2.7}) - F_1) \times R_1 \quad (41)$$

$$dF_{myo} / dt = (F_1 - F_{myo}) \times R_2 \quad (42)$$

Parameter settings.

$$R_1=0.02 \quad R_2=0.06 \text{ (s}^{-1}\text{)}$$

Initial ion concentrations (mM)

$$C_{tot}=0.001 \quad N_i=8.25 \quad Ca_{SR}=0.35 \text{ (mM)}$$

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Highlights

- Molecular mechanisms that control cardiac contraction via the Na gradient are well established, but it is not well established how these mechanisms are physiologically regulated.
- Cardiac Na/K pumps may be regulated by small diffusible molecules that remain to be identified.
- Ca appears to regulate cardiac Na/K pumps via lipid signaling.
- The existence of local Na gradients in cardiac myocytes is questionable.
- The existence of local ATP and ADP gradients may be more viable than local Na gradients.
- Na/K pump inactivation and translational control of Na/K pump isoform expression provide many flexible possibilities to regulate basal contractility of the heart.

Figure 1.

The physical basis of cardiac contraction control by Na. **A.** Typical ventricular action potential in a non-rodent heart, cytoplasmic Ca transient and contraction waveform. Ca influx occurs primarily by L-type Ca channels during the early action potential, while Ca extrusion occurs primarily during action potential repolarization and the early diastolic period via Na/Ca exchange. Na/Ca exchange may contribute a small Ca influx before reversing to extrude Ca. In myocytes with strong Ca influx, the Na load from Ca extrusion by Na/Ca exchange is likely as large or larger than the load generated via NaV sodium channels. Na extrusion via Na/K pumps balances the Na influx mechanisms in steady state with the mean Na pump current amounting to 25 to 40 pA, equivalent to an overall Na flux of 8 to 15 mmol/Liter cell water per minute. **B.** Dependence of cardiac cytoplasmic Na, Ca and contraction on total Na influx. In the physiological range of cytoplasmic Na, a 3-fold increase of Na influx results in only a 30 to 40% increase of Na, owing to steep dependence of Na/K pump activity on cytoplasmic Na under physiological conditions and its autoregulation by inactivation, described subsequently. The peak Ca concentration that Na/Ca exchange can potentially achieve at +30 mV rises steeply from <0.3 μM at 4 mM Na to 2.5 μM at 12 mM Na. The lowest free cytoplasmic free Ca that can be achieved in diastole ranges from 20 nM at 5 mM Na to 0.3 μM at 12 mM. Accordingly, Na/Ca exchangers overload myocytes with Ca when Na increases above 12 mM.

Figure 2.

Facilitation of murine cardiac Na/K pump currents by Ca elevations that cause cycles of spontaneous Ca release from the SR is accompanied by parallel changes of the physical state of the sarcolemma. The extracellular solution contains 135 mM n-methyl-d-glucamine (NMDG) and 7 mM Na as monovalent cations. The cytoplasmic solution contains 25 mM Na and 100 mM K. *Reproduced from Fig. 10 eLIFE [64] published under the Creative Commons Attribution 4.0 International Public License (CC BY 4.0; <https://creativecommons.org/licenses/by/4.0/>).* **A.** Na/K pump currents in a mouse myocyte are activated for 12 s by application of 7 mM extracellular K. Currents decay by ~70% with a time constant of ~2 s. Between pump activations, 20 μM C6-TPP is applied for 8 s. The resulting inward current depends on physical properties of the sarcolemma that include ‘ordering’ and fluidity. Activation of reverse Na/Ca exchange current (i.e. Ca influx) results in a long-term activation of Na/K pump currents whereby the fractional decay of pump current decreases from 70% to 20% and peak currents are more than doubled. This stimulatory effect of Ca and its decay occur in parallel with sarcolemma changes that enhance and suppress the permeability of a hydrophobic cation, hexyltriphenylphosphonium (C6-TPP). C6-TPP current increases in parallel, and the two currents decrease in parallel subsequently over 2 min. Thereafter, a second Ca elevation fully suppresses pump current and further decreases C6-TPP current. **B.** The magnitudes of Na/K pump currents and C6-TPP currents show a strong correlation ($r = -0.979$).

Figure 3.

Local Na depletion versus Na/K pump inactivation. *Modified from Fig. 2, eLIFE [64].* **A.** Na/K pump currents with different cytoplasmic Na concentrations. With saturating (110 mM) cytoplasmic Na, the pump current shows very little decay during application of extracellular K (7 mM) for 20 s. With lower cytoplasmic Na concentrations, the pump current decays in a multiphasic fashion. A fast component of decay (1 to 3 s time constant) likely reflects an intrinsic inactivation mechanism, while slower components of decay (15 to 60 s time constant) likely correspond to a decrease of bulk cytoplasmic Na. **B.** Plot of peak pump current (black) and current after 10 s (red) at 23°C. While magnitudes of these currents are only about 30% of currents at 37°C, the early decay components are somewhat slower but of similar magnitude to results at 37°C. This outcome supports other evidence that the early current decay components do not depend on Na depletion. **C.** Hypothetical mechanism of Na/K pump inactivation. During the normal pump cycle, Na/K pumps bind 3 Na from the cytoplasmic side (lower left corner of the cycle), phosphorylate themselves, close binding sites, open binding sites to the outside in E2 configuration, release Na to the outside, bind 2 K, occlude the K and dephosphorylate themselves (right side of the cycle), open to the inside and release K, followed by rebinding ATP and Na. Inactivation is suggested to occur from states open the cytoplasmic side (E1 states). When binding sites (I, II and III) are not occupied by Na, the pump adopts configurations that cannot be phosphorylated. Recovery from inactivation likely requires that Na rebinds to inactive configurations. Irreversible up-regulation of Na/K pump currents in giant excised patches and subsequent failure to observe inactivation of Na/K pump currents in excised membrane patches [92] may reflect an involvement of diffusible small molecules that interact with ion binding sites and/or phosphorylation sites.

Figure 4.

Subsarcolemmal cytoplasmic Na in patch clamped murine myocytes exchanges with the patch pipette tip with a time constant of 15 s. **A.** Diagram of pipette perfusion method in which a microcapillary is inserted to within 10 μm of the cell opening. Positive pressure is used to force solution from reservoirs into the pipette tip, thereby effectively changing the solution composition at the cell opening within 1 to 2 s. **B.** Time course of subsarcolemmal Na changes sensed by Na/K pump. The pipette contains 25 mM Na at the onset of the experiment. Na/K pump currents are activated by applying 7 mM K using NMDG as the second extracellular and cytoplasmic monovalent cation (115 mM in and 140 mM out). Pump current is activated by applying 7 mM K on the outside. Pump current declines with a time constant of 8.7 s upon perfusing Na out of the pipette tip, pump current is reactivated with a time constant of 14 s when Na is reintroduced into the pipette tip, and pump current declines with a time constant of 4.3 seconds upon the second removal of Na. There is no evidence for any slow components of Na exchange. The time course of current decline is likely artificially accelerated by the steep Na concentration dependence of Na/K pumps. Assuming a Hill slope of 2.6, the time constants for decreasing Na would in fact be 23 and 11 s, respectively, while the ascending time constant of 14 s, may be somewhat overestimated as a result of the nonlinearity. Therewith, the best estimate for the time constant of Na turnover to the pipette, an average of these three estimates, is 15 s.

Figure 5.

Simple simulation of cardiac myocyte Na and Ca homeostasis. Heart rate is 1 Hz.

A. Top panel. The (fixed) action potential waveform (blue), cytoplasmic free Ca transient (red), and contraction (black) are simulated as outlined in the text and Appendix. **Second panel.** Na/K pump current (I_{pump}), Na/Ca exchange (I_{ncx}), and Ca current waveforms. The Ca current waveform is fixed. **Third panel.** Time courses of the Ca channel conductance (CaV_{SL}) and SR ryanodine channel (RyR_{SR}) openings. **Fourth panel.** Integrals of Ca flux across the SR membrane, the surface membrane Ca current (I_{Ca}), and the Na/Ca exchange current (I_{NCX}). **B.** Simulated effects of increasing SERCA SR Ca pump activity by two fold for 800 s. **C.** Simulated effects of increasing surface membrane Ca channel activity by two fold for 800 s. **D.** Simulated effects of decreasing the affinity of the first Na binding site of Na/K pumps on the cytoplasmic side by 50% for 800 s. From top to bottom, the simulated results presented are cytoplasmic Na concentration, Na/K pump current, SR Ca concentration, and developed force as a percentage of maximal force.

Figure 6.

Regulation of cardiac ECC by inactivation of Na/K pumps. Inactivation occurs when cytoplasmic binding sites are not fully occupied, and recovery is promoted by the full occupancy of inactivated pump binding sites by Na. Heart rate is 1 Hz unless indicated otherwise. **A.** Effect of changing the inactivation rate by a factor of 2. Cytoplasmic Na falls by ~1 mM and contraction force by 50% when inactivation is decreased by 50%. Conversely, cytoplasmic Na increases by 1 mM and contraction force by 100% when inactivation rate is doubled. Steady state pump currents (blue) are almost unchanged. **B.** From left to right the simulations show effects of increasing heart rate from 0.25 to 1 Hz, doubling Na influx upon upstroke, and decreasing Na/K pump rate by 50%. The colored traces in foreground are results with inactivation. All gray-colored traces are simulations without inactivation in which the Na/K pump density was adjusted to generate the same basal Na concentration as with inactivation. In all cases, the presence of inactivation 'buffers' cytoplasmic Na so that Na concentration changes are dampened in comparison to simulations in the absence of inactivation.

Table 1.

Time constants with which cytoplasmic monovalent ions turn over to the patch pipette in mouse myocytes patch clamped with pipettes that generate 2.5 to 4 MΩ resistances using standard cytoplasmic solutions as described. In brief, all monovalent ions turn over similarly with no clear evidence of any diffusion restrictions.

Ion	Turnover Manipulation	Observed Current	Time Constant	Reference
Na	Pipette perfusion	Na/K pump	15 s	This article
Na	Pipette perfusion	Veratridine Na currents	16 s	[93]
Na	Extracellular Na	Veratridine Na currents	17-22 s	[17]
Na	Extracellular Na	Nystatin Na currents	17-22 s	[17]
K	Extracellular K	Veratridine KK currents	9-13 s	[17]
K	Extracellular K	Nystatin K currents	18-22 s	[17]
Li	Extracellular Li	Veratridine Li currents	9-13 s	[17]
Cs	Extracellular Cs	Nystatin Cs currents	15-21 s	[17]
Cl	Extracellular Cl	Nystatin Cl currents	10-21 s	[17]