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INVITED REVIEW

Na/K pump regulation of cardiac repolarization: insights from a systems biology approach

Alfonso Bueno-Orovio · Carlos Sánchez · Esther Pueyo · Blanca Rodriguez

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Abstract The sodium-potassium pump is widely recognized as the principal mechanism for active ion transport across the cellular membrane of cardiac tissue, being responsible for the creation and maintenance of the transarcolemmal sodium and potassium gradients, crucial for cardiac cell electrophysiology. Importantly, sodium-potassium pump activity is impaired in a number of major diseased conditions, including ischemia and heart failure. However, its subtle ways of action on cardiac electrophysiology, both directly through its electrogenic nature and indirectly via the regulation of cell homeostasis, make it hard to predict the electrophysiological consequences of reduced sodium-potassium pump activity in cardiac repolarization. In this review, we discuss how recent studies adopting the systems biology approach, through the integration of experimental and modeling methodologies, have identified the sodium-potassium pump as one of the most important ionic mechanisms in regulating key properties of cardiac repolarization and its rate dependence, from subcellular to whole organ levels. These include the role of the pump in the biphasic modulation of cellular repolarization and refractoriness, the rate control of intracellular sodium and calcium dynamics and therefore of the adaptation of repolarization to changes in heart rate, as well as its importance in regulating pro-arrhythmic substrates through modulation of dispersion of repolarization and restitution. Theoretical findings are consistent across a variety of cell types and species including human, and widely in agreement with experimental findings. The novel insights and hypotheses on the role of the pump in cardiac electrophysiology obtained through this integrative approach could eventually lead to novel therapeutic and diagnostic strategies.

Keywords Sodium-potassium pump · Cardiac repolarization · Systems biology · Modeling

A. Bueno-Orovio (✉) · B. Rodriguez
Department of Computer Science, University of Oxford,
Wolfson Building, Parks Road,
Oxford OX1 3QD, UK
e-mail: alfonso.bueno@cs.ox.ac.uk

A. Bueno-Orovio
Oxford Centre for Collaborative Applied Mathematics,
University of Oxford, Oxford, UK

C. Sánchez · E. Pueyo
Communications Technology Group, I3A and IIS,
University of Zaragoza, Zaragoza, Spain

C. Sánchez · E. Pueyo
Biomedical Research Networking Center in Bioengineering,
Biomaterials and Nanomedicine, Zaragoza, Spain

Introduction

The sodium-potassium pump (Na/K pump), also known as Na^+,K^+-ATPase, is the principal mechanism for active ion transport across the membrane of excitable cells [22, 24, 73]. First discovered by the Danish scientist Jens Christian Skou in 1957 in his studies in the crab nerve [71], its ubiquitous importance for basic cell physiology and clinical practice was later recognized by the award of Nobel Prize in Chemistry in 1997.

The Na/K pump is key in the regulation of Na^+ and K^+ transmembrane gradients, transporting three Na^+ out and two K^+ into the cell against their concentration gradients by consuming energy via the hydrolysis of ATP. In cardiac muscle, the creation and maintenance of the transarcolemmal Na^+ and K^+ gradients by the Na/K pump is crucial for a variety of electrophysiological processes including the generation of the resting membrane potential and the initiation and propagation of action potentials, as well as for the regulation of secondary transport processes vital for cell function, like cell volume control or Ca^{2+}

extrusion via the sodium-calcium exchanger (NCX) [41, 60]. In addition, the Na/K pump results in an electrogenic transmembrane current due to the unmatched transposition of three Na^+ and two K^+ ions per ATP unit. It therefore also plays a pivotal role in the regulation of cardiac electrophysiology under physiological and pathological conditions.

In fact, impairment of Na/K pump activity has been shown to take place in a number of diseased conditions, including atrial fibrillation [83], ischemia [20, 21], heart failure [5, 51, 69], hypertension [17, 44, 47], hypo/hyperthyroidism [44, 47], and diabetes [27, 73]. Na/K pump inhibition has also been used for therapeutic action in a number of conditions, like the treatment of atrial fibrillation and heart failure by cardiac glycosides [32, 80]. Its experimental characterization is however challenging, in part due to differences in the magnitude and speed of its associated ion flow compared to other ionic currents [24]. Indeed, ion channels allow for the rapid, passive diffusion of selected ions due to electrical and concentration gradients, so that tens of millions of ions per second can cross the membrane through just one open ion channel. However, ion pumps operate through the entire course of the action potential to slowly, actively transport ions thermodynamically uphill, resulting in a current as small as ~20 aA for a single Na/K molecule [24]. Hence, although it is possible to measure the total current generated by the millions of Na/K pumps located in the entire cell membrane [23, 52, 70], the associated experimental difficulties may have limited the study of the impact of the Na/K pump on cardiac repolarization compared to other ion channel currents. Understanding the therapeutic and pathophysiologic consequences of Na/K pump alterations from the cellular to the whole organ function therefore requires further investigations.

In this paper, we highlight the contribution of systems biology research, through integration of experimental and theoretical investigations [8, 9, 38], to elucidate the role of the Na/K pump in cardiac electrophysiology from the cellular to the whole organ level, as illustrated in our multiscale perspective for Na/K research presented in Fig. 1. We first describe how insights obtained through experimental and reductionist approaches are integrated in mathematical formulations providing quantitative descriptions of the regulation of Na/K pump activity by ion concentrations, voltage, and signaling pathways such as β-adrenergic stimulation. Then, we focus on how investigations using multiscale cardiac models have identified the Na/K pump as one of the most important ionic mechanisms in regulating cardiac repolarization and its rate dependence, bridging from cellular electrophysiology to clinical electrocardiogram recordings. Finally, we conclude our manuscript by discussing a number of open questions requiring further combined experimental and computational investigations in order to promote our current understanding of the regulation and implications of the Na/K pump activity in cardiac electrophysiology.

Mathematical models of the cardiac Na/K pump current

From the early discoveries of Jens Christian Skou, it was clearly established that both intracellular Na^+ and extracellular K^+ ions are indispensable substrates for the Na/K pump enzymatic activity [71]. In the absence of either Na^+ or K^+ ions, Na/K pumping is precluded, and the steady current through the pump becomes zero. Increasing Na^+ and K^+ concentrations gradually increases Na/K pump activity up to a saturation limit [29, 52, 71]. It was later discovered that the Na/K pump is also strongly influenced by membrane voltage, showing an inward rectification at positive potentials [23, 52].

Presumably, the first theoretical study addressing the mathematical characterization of the Na/K pump current is the historic 1979 study by DiFrancesco et al. [15], where the authors considered a simplified, cleft K^+ dependent definition of the electrogenic pump current. In the development process of this initial modeling effort, two main formulations have become predominant in terms of the Na/K pump dependence on intracellular Na^+ and extracellular K^+ concentrations. The first one goes back in time to the work of DiFrancesco and Noble in their seminal paper from 1985 [16], where Na/K pump electrophysiology, together with NCX dynamics and intracellular Na^+, Ca^{2+}, and K^+ homeostasis, were included for the first time in the biophysical description of a cardiac myocyte. In this formulation, the ionic current associated to the Na/K pump electrogenic nature is given by

$$I_{NaK} = p_{NaK}\left(\frac{[Na^+]_i}{[Na^+]_i + K_{m,Na}}\right)^{\gamma_{Na}}\left(\frac{[K^+]_o}{[K^+]_o + K_{m,K}}\right)^{\gamma_K} f_v$$

where p_{NaK} represents the maximum Na/K pump permeability, $K_{m,Na}$ and $K_{m,K}$ the half affinity constants of Na^+ and K^+, respectively, and γ_{Na} and γ_K the Na^+ and K^+ Hill's coefficients. A slight modification in the definition of the Na^+ and K^+ ionic factors yields the Na/K pump formulation introduced by Luo and Rudy in their 1994 description of the guinea pig ventricular myocyte [43], where

$$I_{NaK} = p_{NaK}\frac{1}{1+\left(\frac{K_{m,Na}}{[Na^+]_i}\right)^{\gamma_{Na}}}\frac{1}{1+\left(\frac{K_{m,K}}{[K^+]_o}\right)^{\gamma_K}} f_v$$

with the same meaning for the above defined quantities. In both formulations, the two ionic factors remain inhibited in the absence of their respective species, gradually increasing for increasing intracellular Na^+ and extracellular K^+ concentrations (Fig. 2a). Small changes in intracellular Na^+ elicit larger

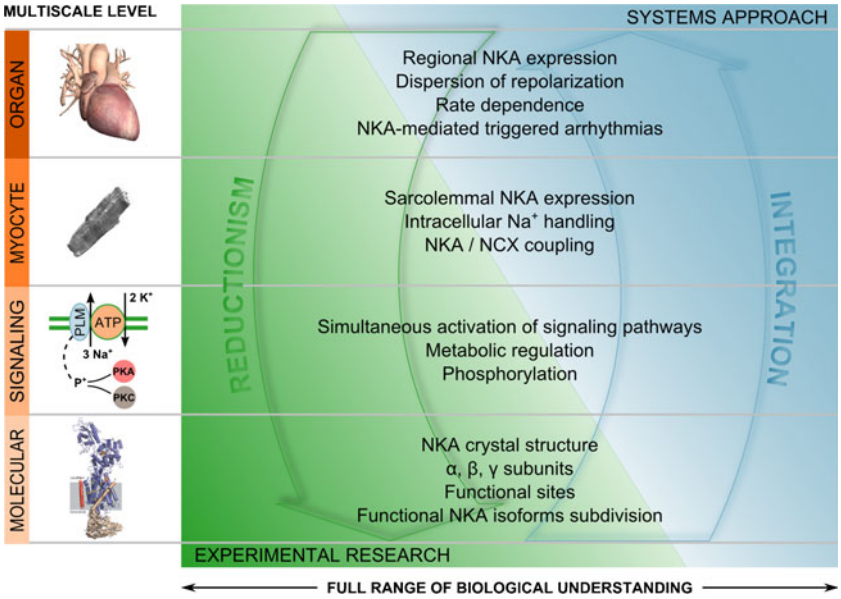

Fig. 1 Multiscale systems approach perspective for Na/K electrophysiology research. Clinical and experimental research may further benefit from this integrative methodology at higher levels of biological integration, in order to promote our current understanding of Na/K function and its multiple ways of action in cardiac electrophysiology. *NKA* Na^+,K^+-ATPase; *NCX* Na^+-Ca^{2+} exchanger

changes in pump activity [22], reflected by a larger γ_{Na} compared to γ_K in most model parameterizations of the pump. Finally, the f_v term represents the voltage rectification of the Na/K pump, which usually also accounts for the additional modulation of Na/K activity by extracellular Na^+ concentration (Fig. 2b) [43], as experimentally reported [52].

The above formulations for the Na/K pump current have been inherited in most electrophysiological models of sinoatrial, atrial, atrioventricular, Purkinje and ventricular myocytes of a variety of species, as shown in Fig. 3. The DiFrancesco–Noble formulation is present in the only existing model for the atrioventricular node action potential and in the majority of those for the specialized conduction system (sinoatrial node and Purkinje fiber models). On the other hand, the Luo–Rudy formulation has become predominant in mathematical models of atrial and ventricular cardiac myocytes.

Only a minor number of published models make use of independent representations for the Na/K pump electrogenic current. Matsuoka et al. first introduced a Markov chain formulation in order to describe the Post-Albers cycle [45], which models the enzymatic Na/K pump cycle as transitions between different phosphorylated and dephosphorylated states depending on the position of the cation-binding sites across the transarcolemmal domain. Smith and Crampin further extended these active transport ideas, providing a framework to account for thermodynamic principles, as well as for Na/K dependence on intracellular K^+ concentration and ATP and pH sensitivity [72]. Terkildsen et al. later incorporated this Na/K formulation into a computational model of guinea pig ventricular electrophysiology [75]. Other recent computational models also make use of this modeling approach, as the O'Hara et al. description of the human ventricular myocyte [55].

At the signaling level, multiple factors are known to influence the Na/K pump and its tight regulation by the phospholemman (PLM) protein, such as an increased Na/K pump activity under β-adrenergic stimulation due to PLM phosphorylation via protein kinase A (PKA) [4, 12, 13]. A number of theoretical studies have incorporated a phenomenological description of the effects of β-adrenergic stimulation in the Na/K pump current by considering either a 20–30 % increase in the maximum Na/K pump permeability [18, 87] or a 29–35 % decrease in its Na^+ half affinity constant [31, 40]. Latest computational models of the β-adrenergic cascade provide a more detailed characterization of the PKA-dependent phosphorylation of the Na/K current, by considering the fraction of phosphorylated channels in different cellular subcompartments according to their respective concentrations of β-adrenergic receptor agonists [31].

Multiscale studies of Na/K pump regulation of cardiac repolarization

As mentioned earlier, the mathematical descriptions of the cardiac Na/K pump current have been integrated in a variety of models of cardiac cell electrophysiology. Computational studies using the cellular models have allowed quantification of the specific role of the pump in cardiac electrophysiology, by monitoring the evolution of specific ionic properties under a variety of conditions. This has yielded novel insights on the importance of the Na/K pump in regulating cellular repolarization, both through direct effects caused by its electrogenic nature and indirectly through regulation of cell homeostasis. Importantly, these findings have been found to be consistent across species and cell types, including human, rabbit and dog and ventricular and

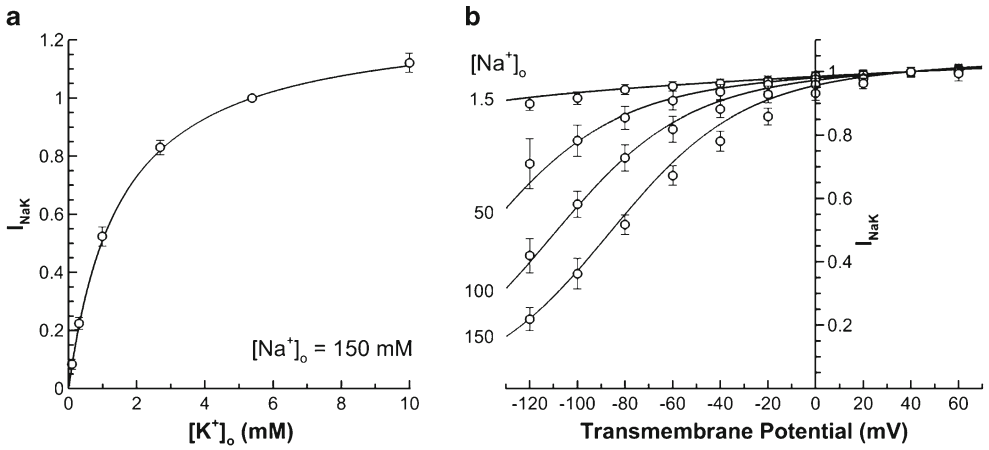

Fig. 2 **a** Influence of extracellular potassium concentration ($[K^+]_o$) in Na/K pump current amplitude, normalized to control runs at 5.4 mM $[K^+]_o$. **b** Voltage dependence of the Na/K pump current for different extracellular sodium concentrations ($[Na^+]_o$) at 5.4 mM $[K^+]_o$, normalized to control runs at 40 mV. Solid lines represent computer model results using model parameters as in [43]. Experimental data (mean ± SD, *open circles*) adapted from [52]

atrial tissues [59, 64, 65, 67]. Figs. 4, 5, 6 describe some of the main results of these studies.

Mathematical models of the Na/K pump have allowed dissecting multiscale mechanisms underlying smilingly counterintuitive results. Under the effects of most cardiac glycosides, Na/K current block shortens action potential duration (APD), an action a priori unexpected since the blockade of an outward current should presumably prolong APD (as yet assumed in a number of recent studies on the pump [7, 22]). However, this modulation of action potential repolarization occurs through a biphasic mechanism (Fig. 4a), in which APD is initially notably prolonged after Na/K current reduction, then followed by a progressive shortening below its control value, which is well documented [34, 41, 84]. Understanding the electrophysiological basis of this phenomenon is also of clinical relevance, since the initial prolongation phase has been associated with an increased vulnerability to atrial tachyarrhythmia initiation in patients with paroxysmal supraventricular tachyarrhythmias [30].

Single-cell simulations revealed that the APD shortening caused by Na/K inhibition is due to a cascade-like mechanism initiated by the progressive accumulation of intracellular Na^+ within the membrane cytoplasm [67], in agreement with [41]. The decreased transarcolemmal Na^+ gradient (driving force of

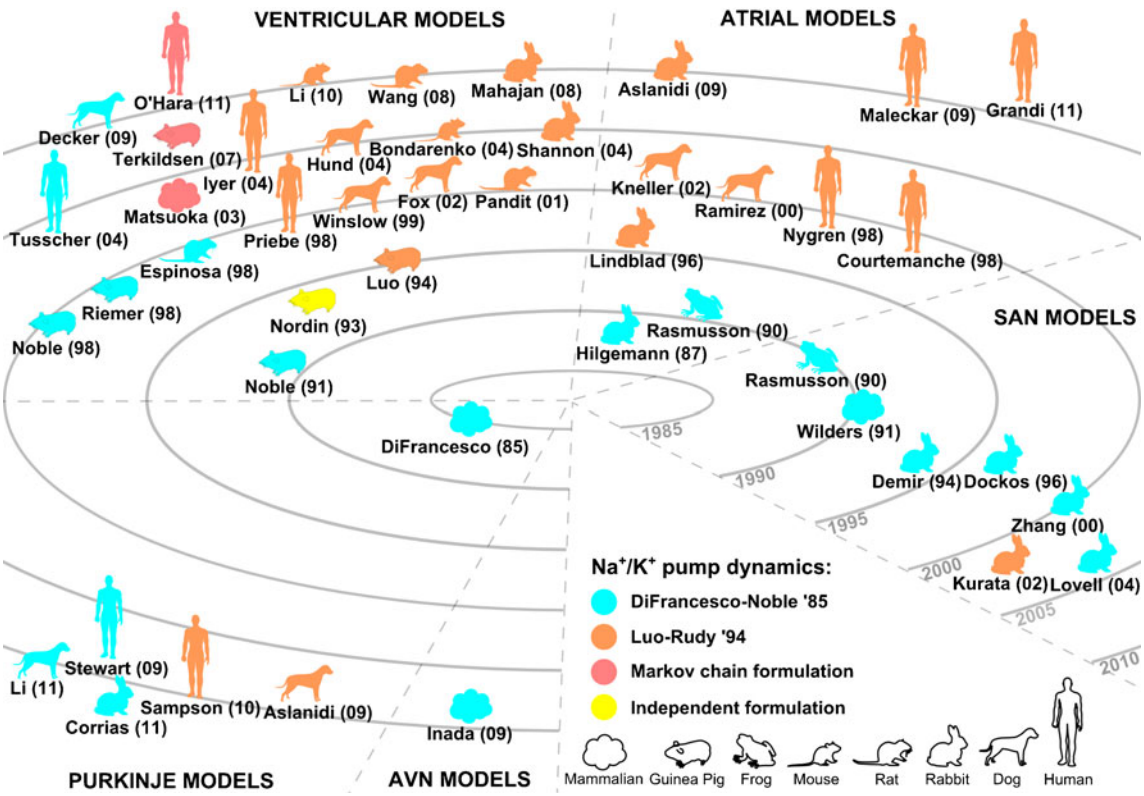

Fig. 3 Inheritance of Na/K pump formulation in a selection of modern models of cardiac electrophysiology. Models are referred by first author's name and year of publication. The complete list of references can be found in [54]

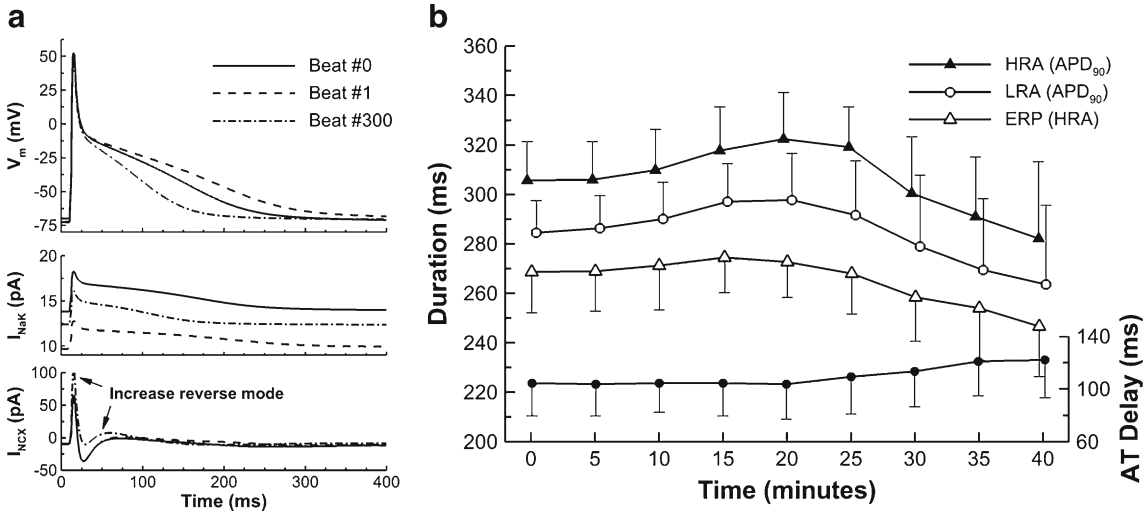

Fig. 4 **a** Computational investigations on the biphasic effect of Na/K block in human atrial action potential duration (APD). Na/K block in steady-state conditions (*beat #0*) leads to the initial prolongation of APD (*beat #1*), then followed by its progressive shortening (*beat #300*). The gradual accumulation of intracellular Na^+ compensates the sudden reduction in Na/K pump current (I_{NaK}), and potentiates the reverse mode of the Na^+-Ca^{2+} exchanger (I_{NCX}), which results in APD shortening. **b** Clinical observations of the biphasic nature of Na/K pump inhibition in human atria (mean ± SD, modified from [30]). APD at 90 % of repolarization (APD_{90}) was monitored at two atrial sites (*HRA* high right atrium, *LRA* low right atrium), exhibiting APD lengthening/shortening after digitalis administration. The in vivo data also shows a similar biphasic behavior in effective refractory period (ERP), and a progressive increase in the activation time (AT) delay between HRA and the His bundle (*filled dots*, right axis of the panel)

the NCX) subsequently leads to a gradual raise of intracellular Ca^{2+}, thus potentiating the outward component (reverse mode) of the exchanger, which contributes to a faster cell repolarization (Fig. 4a). Computer simulations also helped to exclude other possible mechanisms that could have mediated in the biphasic APD response (such as the L-type Ca^{2+} current or an impaired balance of currents between the sarcoplasmic reticulum and the intracellular space). Moreover, tissue simulations further predicted a similar biphasic behavior for effective refractory period (ERP) and a long-term decrease in conduction velocity [67], which were both corroborated by the clinical in vivo data (Fig. 4b) [30]. Finally, the computational study indicated that the above biphasic characteristics of Na/K current block on APD, ERP and conduction velocity were attenuated under conditions of chronic atrial fibrillation [67], for which therapies based on the pharmacological action

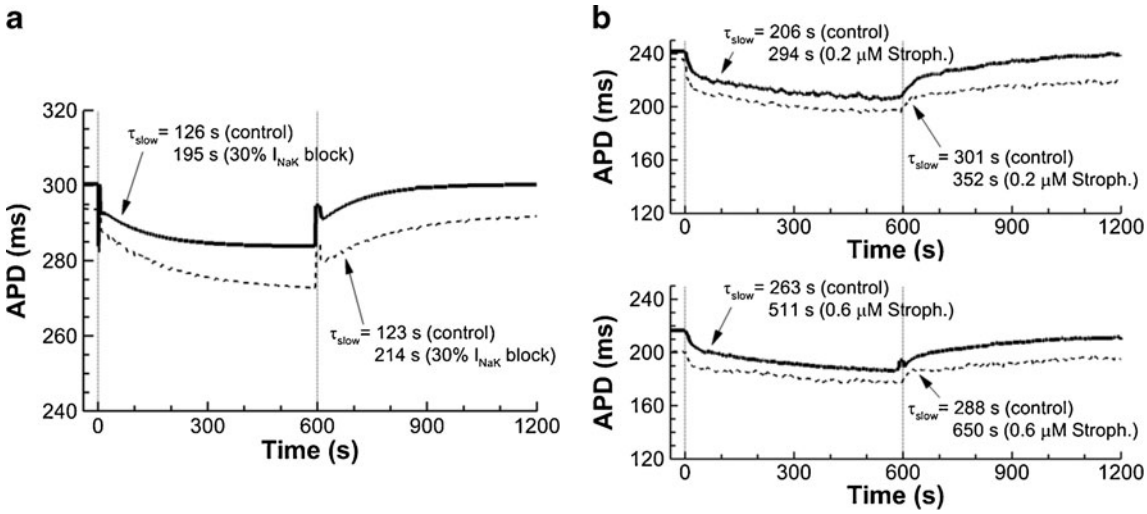

Fig. 5 **a** Effects of Na/K current block in protracting the slow phase of action potential duration (APD) adaptation to sudden changes in pacing rate, in a model of human ventricular electrophysiology. Results are presented for control conditions (thick line) and under partial Na/K current block (dashed line). Changes in pacing rate (1,000 to 600, then to 1,000 ms) are indicated by the dotted vertical lines. **b** Experimental confirmation of the simulation findings in canine ventricular preparations under different concentrations of the Na/K blocker strophanthin (0.2 μM, top; 0.6 μM, bottom). Results adapted from [59]

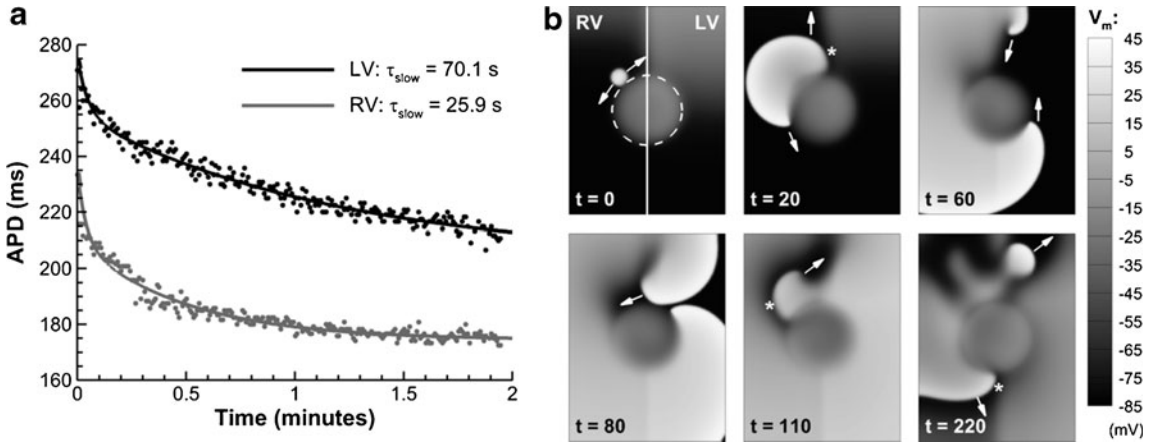

Fig. 6 **a** Clinical evidence of interventricular differences in action potential duration (APD) and APD adaptation in a patient with structurally normal heart. *LV* left ventricle. *RV* right ventricle. **b** Functional consequences of heterogeneous APD adaptation in an idealized model of ischemic heart disease (*dashed area* indicates unexcitable region). Regional differences in APD adaptation generates pronounced patterns of dispersion of repolarization (t=0), which under ectopic activity leads to unidirectional block (t=20, *asterisk*), initiation of reentry (t=60), and sustained fibrillation-like reentrant patterns (t=220). *Color bar* denotes transmembrane potential (mV); times indicated since ectopic stimulation (ms) (modified from [6])

of Na/K block (such as glycosides administration) might still be beneficial.

Simulation studies have also identified the Na/K pump as the main determinant of other important rate-dependent properties of cardiac repolarization, such as the adaptation of APD to changes in heart rate [59, 64, 65]. Fig. 5a shows the characteristics of APD adaptation to sudden modifications in pacing rate in a model of human ventricular electrophysiology, under control conditions and partial block of the Na/K current [59]. Cellular electrophysiological models unveiled that the slow component of the APD adaptation process is predominantly dominated by intracellular Na^+ accumulation [11, 59], and therefore Na/K inhibition significantly lengthens the adaptation time. The key role of the Na/K pump in modulating APD adaptation predicted by the simulations was confirmed experimentally using microelectrode action potential recordings in the presence of the Na/K blocker strophanthin, which at all doses significantly protracted the APD adaptation response, as illustrated in Fig. 5b [59].

These mechanistic investigations on the time course of APD adaptation have also allowed providing a cellular and ionic basis to important clinical findings at the body-surface electrocardiogram, such as those suggesting QT-rate adaptation as a risk-stratification biomarker of arrhythmic mortality in patients with ischemic heart disease [26, 58]. Indeed, the simulation study in [59] showed that: (1) QT-rate adaptation in the electrocardiogram can be explained by APD adaptation at the cellular level; (2) the Na/K pump current is the main ionic determinant of APD and QT-rate adaptation; and (3) inhibition of the Na/K pump activity results in abnormalities in Na^+ and Ca^{2+} homeostasis, which may increase arrhythmic risk through alterations in ERP, also leading to an increased likelihood of early- and delayed-afterdepolarizations [28, 59, 84].

In this regard, a recent study combining in vivo electrophysiological recordings in human and computer simulations has shed further light into additional contributions of heterogeneous APD adaptation in modulating the pro-arrhythmic substrate [6]. Electrophysiological measurements simultaneously acquired from the left and right ventricles of patients with structurally normal hearts revealed significant interventricular differences in APD and APD adaptation in the in vivo human ventricles (Fig. 6a). The simulation study then expanded the information obtained from the electrophysiological recordings to show that interventricular differences in APD adaptation may increase dispersion of repolarization and potentiate arrhythmogenesis, promoting unidirectional block and reentry (Fig. 6b) [6]. Due to the strong link between APD adaptation and the Na/K pump activity identified in previous studies, the interventricular differences in APD adaptation could be likely due to regional differences in Na/K expression, as reported by Wang et al. in the non-failing human heart [79]. Similar interventricular differences in Na/K expression and activity have been reported in other species, such as the rat ventricles [39].

Simulation studies have also suggested a role for the Na/K pump in regulating other major properties of cardiac repolarization, such as APD restitution. This magnitude relates the variation observed in APD after a modification in pacing rate, either in steady-state conditions (dynamic APD restitution) or in the beat immediately after the change in pacing rate (S_1–S_2 APD restitution). When plotted against pacing cycle length or diastolic interval, a steep slope of the APD restitution curve is considered as an arrhythmic-risk biomarker related to inducibility of reentry and the transition

from sustained arrhythmias to fibrillation [53, 81, 86]. Computational investigations using ventricular and atrial cell models in human, rabbit, and dog have shown that Na/K current block notably flattens restitution curves [59, 64, 65, 67], therefore decreasing life-threatening risk during reentrant behavior. The above findings were found to be qualitatively consistent across species and cell types despite the use of cellular models with different Na/K formulations and parameterizations, even though quantitative differences exist. In this regard, the in vivo human atrial study in [30] demonstrated, during the phase of action potential shortening after digoxin administration, a narrowing in the gap between the termination of effective refractoriness and the completion of action potential repolarization, coinciding with a diminished vulnerability to tachyarrhythmias. However, experimental data on APD restitution during pharmacological Na/K block are scarce and limited, and further experimental research is needed in order to validate these results.

Finally, a systems biology approach has enabled dissecting the specific contribution of the Na/K pump in a number of pathophysiological conditions, difficult to determine experimentally. Computational investigations on ischemia-induced electrophysiological changes driven by impaired cellular metabolism showed that the balance between inactivation and activation of the Na/K pump, in companionship with the activation of the ATP-sensitive K^+ current, underlies the time course of extracellular K^+ accumulation during myocardial ischemia, which predisposes the heart to the development of reentry and lethal ventricular arrhythmias [63, 75]. The analysis of the different Na^+ flux pathways further suggested that the reduced Na/K flux during acute myocardial ischemia plays the largest role in exacerbated Na^+ overload during reperfusion, and therefore in the possible development of reperfusion injury [61]. Accordingly, ischemic preconditioning via increased Na/K pump activity has been experimentally reported to prevent ischemic/reperfusion injury and to improve myocytes survival during an ischemic attack [2, 49]. Mechanistic investigations using computational models have also evidenced that intracellular Na^+ accumulation in heart failure is mainly driven by the electrophysiological remodeling of the Na/K pump current, consequently contributing to the deregulation of intracellular Ca^{2+} homeostasis in the failing cardiac myocytes [57, 76]. Computational studies have also been used to investigate the role of cardiac mechanoelectric feedback as an additional factor to arrhythmogenesis in Ca^{2+} overloaded cardiomyocytes under conditions of Na/K pump inhibition [37].

Discussion and perspectives

The ubiquitous importance of the Na/K pump at all levels of cellular physiology is nowadays well accepted and established. In cardiac muscle, its electrophysiological action has been traditionally linked to determining a number of basic properties crucial for the generation and propagation of cardiac electrical activity, such as the regulation of subsarcolemmal Na^+ concentrations or the control of the resting membrane potential. However, the results analyzed in this review highlight a number of additional contributions of the pump for cardiac repolarization, due to both its electrogenic nature and homeostasis regulatory action. These include the modulation of APD, APD adaptation to changes in heart rate, dispersion of repolarization, and rate dependence of intracellular Na^+ and Ca^{2+} dynamics.

Gaining of these novel insights has been only possible through the use of a systems biology approach that allows for a unified integration of experimental and computational investigations, bridging from the ionic to the whole organ levels. Mathematical formulations of the Na/K provide quantitative descriptions of its regulation by ion concentrations, voltage and signaling pathways, thus granting the assessment of the specific role of the pump under a variety of conditions. The integration of additional existing knowledge in this modeling framework may therefore help us to further advance our still limited understanding of the impact of the Na/K pump in cardiac electrophysiology.

As a case in point, much is known at present in terms of the crystal structure of the Na/K pump and its conformal composition of α and β subunits (and an additional γ subunit in other tissues, such as kidney and brain) [50]. However, although four α and three β subunit isoforms have been found, only α_1, α_2, and β_1 are expressed at significant levels in cardiac myocytes [22, 46]. In cardiac myocytes, a differential expression of Na/K isoforms in the sarcolemmal membrane, with α_2 subunits found more predominantly at the t-tubules, has been reported [3, 74]. Correspondingly, recent experimental studies have also indicated that the α_2 isoform may have a greater impact on intracellular Ca^{2+} handling than α_1 pumps [13], the latter being responsible for maintaining a separate Na^+ pool [14]. The voltage rectification of the α_2 subunit was also found to be more strongly influenced by extracellular Na^+ and K^+ [10, 33]. These facts suggest the idea of a possible subdivision of the cardiac Na/K pump current in computational models of cardiac electrophysiology into two separate components (as it was once made for the rapid and slow contributions of the K^+ rectifier current). This, together with the definition of appropriate Na^+ subcompartments in the cellular models, might help to further elucidate the mechanisms of intracellular Na^+ handling and Na/K-NCX coupling, and of those responsible of Na/K pump-mediated triggered arrhythmias driven by intracellular Ca^{2+} overload, such as delayed-afterdepolarizations due to cardiac glycosides intoxication [36, 80, 84].

At the signaling level, a significant effort is being developed in order to incorporate adrenergic Na/K pump

regulation into cellular models of cardiac electrophysiology, such as PLM phosphorylation via PKA [31]. On the other hand, additional processes such as PLM phosphorylation by protein kinase C [4, 12] still remain computationally unexplored. To render an even more complicated picture, further signaling pathways such as nitric oxide stimulation of the Na/K pump [82], the role of oxidant stress on reducing the pump current [70], Na/K inhibition by PLM palmitoylation [77], increased secretion of atrial natriuretic peptides by endogenous Na/K pump blockers [42], or Na/K regulation by acetylcholine, insulin, or hormones [27], are nowadays also well established. As noted by Fuller et al., "the consequences of the simultaneous activation of all the signaling pathways […] are hard to predict, and the balance between them will undoubtedly vary between health and disease" [22]. The use of electrophysiological models incorporating metabolic regulation and up-to-date knowledge on the complete adrenergic stimulation cascade might therefore provide newer insights into how the concurrent activation of these regulatory agents influences Na/K pump activity and cellular electrophysiology, in particular under conditions of impaired metabolic supply, such as myocardial ischemia. Simulation studies using whole organ models could also be useful in order to determine how dispersion of repolarization and rate-dependent properties are regulated at the organ level by Na/K function.

As mentioned throughout this review, the main pharmacological blockers of the Na/K pump are cardiac glycosides. Safe administration of these drugs has been regarded as a difficult task for their narrow safety margin and severe side effects [80]. The most potent inotropic agents, such as ouabain, digoxin, or digitalis, are in general associated with lower toxic-to-therapeutic ratios [36, 66]. Other cardiac glycosides, such as dihydroouabain or hydrochloride compounds, have been however reported as having similar digoxin-like cardiotonic effects with larger safety margins [1, 62, 66]. Whereas additional Ca^{2+} buffering and signal transduction pathways may underlie the observed differences in inotropy for these agents, Na/K pump inhibition appears to be the single mechanism regulating toxicity [80]. The comparison of experimental results is somewhat challenging, due to differences in experimental conditions, tissue types, and animal species, and new experimental settings to optimize the study of inotropic agents on cardiac contractility and spontaneous rhythmic contractions are being developed [48, 85]. Several steroid-like compounds containing a core structure similar to cardiac glycosides have also been found in many Chinese herbs used to promote blood circulation, such as ginsenosides or Danshen, although their side effects are expected to be much less severe because of their lower Na/K pump affinity [78]. Importantly, additional antiarrhythmic drugs with multichannel mode of action, such as amiodarone, are also known to possess a Na/K pump inhibitory effect. Amiodarone is known to be more efficient in maintaining sinus rhythm than some of its derivatives which do not affect the pump activity, such as dronedarone [19, 56]. Na/K pump inhibition could hence be an important factor in explaining the antiarrhythmic differences in action of these drugs. Therefore, novel theoretical findings will also be key to direct further experimental research by providing novel hypotheses on the role of the Na/K pump in cardiac electromechanical activity and arrhythmogenesis, as well as in the development of possible new pharmacological treatments targeting this current [25, 35, 68].

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