Dynamic Regulation of Pacemaker Activity by the Na^+-K^+ Pump

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Abstract

The role of Na^+ homeostasis in cardiac pacemaking is not well established. Blocking of the Na^+-K^+ ATPase (NKA) to raise intracellular Na^+ concentration ($[Na^+]_i$) in ventricles, thereby reducing Ca^{2+} removal by the Na^+-Ca^{2+} exchanger (NCX), is widely used to improve cardiac inotropy in patients with congestive heart failure. However, NKA-blocking agents have a narrow therapeutic window, and cardiotoxic effects are common, as excessive Ca^{2+} accumulation is pro-arrhythmic and decreases lusitropy. Here, we updated an existing mathematical model of the mouse sinoatrial node (SAN) myocyte to determine the effects of increasing $[Na^+]_i$ on pacemaker cell function, and test whether high $[Na^+]_i$ levels have disrupting effects similar to those of cardiac glycosides in the ventricle. Model parameter sensitivity analysis revealed that NKA modulation impacts Na^+ and Ca^{2+} homeostasis, as well as several action potential (AP) characteristics. NKA dynamically modulates cell automaticity: upon NKA inhibition SAN firing rate instantaneously increases, due to direct effects on membrane potential (E_m) dynamics, and slowly continues to increase over time, while Na^+ and Ca^{2+} accumulate. Simulations of various degrees of block showed that Na^+ overload can even stop SAN firing. Thus $[Na^+]_i$ plays a fundamental role in the regulation of pacemaker activity.

1. Introduction

Intracellular Na^+ homeostasis is a key regulator of cardiac excitation and contraction [1]. $[Na^+]_i$ elevation is expected to limit Ca^{2+} extrusion via forward mode NCX, and could favor Ca^{2+} entry via reverse mode NCX. Slowing Ca^{2+} extrusion via NCX will tend to elevate diastolic intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and increase sarcoplasmic reticulum (SR) Ca^{2+} content, thus exerting a positive inotropic effect and enhancing contractility. This explains the efficacy of cardiac glycosides in the treatment of congestive heart failure. These compounds promote inotropy by selectively inhibiting NKA and thereby impairing Na^+ extrusion and weakening the NCX Ca^{2+} extrusion gradient. However, they are well known for having undesired arrhythmic consequences [2] by increasing the propensity for spontaneous SR Ca^{2+} release and delayed after-depolarizations. Common symptoms in digitalis-intoxicated patients also include myocardial stiffness, resulting from an excessive increase in $[Ca^{2+}]_i$, which slows myofilaments relaxation.

Administration of cardiac glycosides also decreases heart rate (HR). Their negative chronotropic effect is due to indirect vagomimetic and anti-adrenergic effect of the drug [3]. However, studies in isolated SAN multicellular preparations, devoid of neurohormonal control, reported an increase in firing rate after ouabain administration [3, 4]. This phenomenon, termed "digitalis-induced tachycardia", has been directly associated with the development of supraventricular arrhythmias observed in digitalis-intoxicated patients [3].

Here, we updated a mouse SAN cell computational model [5], constrained to our experimental dataset, to investigate the role of NKA and Na^+ homeostasis in isolated SAN myocyte pacemaker activity. We hypothesize cardiac glycosides "overdose" can have malignant consequences on pacemaking properties, e.g., as described for contractility and E_m instability in ventricular myocytes.

2. Methods

The Kharche et al. model of mouse SAN myocyte [5], implemented in Matlab (Mathworks Inc, Natick, MA), provided the basis for our simulations. Experimental datasets of spontaneous APs and membrane currents used to constrain and validate the model were acquired in amphotericin perforated patch or whole cell recording configurations, respectively, from SAN myocytes isolated from 2-3 months old male C58BL/6J mice as previously described [6, 7]. AP waveform parameters (defined as illustrated in Fig. 1B) were determined for each cell from average waveforms from 5 s recording windows [6, 7].

We found marked differences between the simulated AP characteristics and experimental results obtained at physiological temperature (Fig. 1). Thus, we compared the properties of several ionic currents in model vs. experiments, and adjusted the formulation of four

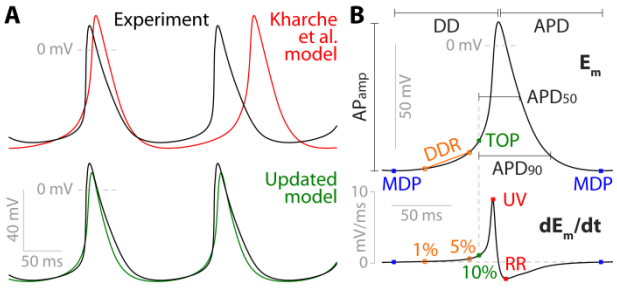
currents to better reproduce our data: namely, we reduced the slope factor of the "funny" current I_f (from 16.3 to 8 mV), negatively shifted (-10 mV) the E_m-dependence of activation and inactivation of L-type Ca^{2+} channel, and decreased its conductance by one third, and increased 2.5 fold the conductances of I_{to} and I_{sus} (transient and steady-state outward K^+ currents).

The above modifications to the Kharche et al. model originated our new baseline model. We used our updated model to create a population of 1000 models following the approach developed by Sobie [8], and performed parameter sensitivity analysis to determine how ionic current and transporters, especially those involved in Na^+ homeostasis, impact steady-state AP characteristics and firing rate. The models in our population were built by perturbing the parameters listed in the legend of Fig. 2 with random scale factors chosen from a log-normal distribution with a median value of 1 and a standard deviation of 0.1. We then simulated ouabain administration (by reducing NKA maximal rate, v_{NKA}) to study the consequence of slow Na^+ accumulation on SAN cell firing rate.

3. Results

The AP waveform and properties in the updated model more closely resemble our experimental results (Fig. 1).

We used a population-based approach to investigate the role of NKA in a condition reflecting the natural intra- and inter-subject variability seen in myocytes. By introducing small perturbations in several key parameters in our baseline model, we generated a group of 1000



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Output	Unit	Kharche	Updated	Experiment
Heart rate (HR)	bpm	283	346	406 ± 20
Upstroke velocity (UV)	mV/ms	9.2	9.1	24.3 ± 4.4
Repolarization rate (RR)	mV/ms	-2.0	-2.4	-2.3 ± 0.2
Maximum diastolic potential (MDP)	mV	-64.5	-60.1	-59.4 ± 1.7
AP amplitude (AP_{amp})	mV	87.1	71.4	73.5 ± 3.2
Take-off potential (TOP)	mV	-46.0	-45.8	-45.9 ± 1.3
AP duration (APD)	ms	119	103	81.9 ± 7.3
APD at 90% repolarization (APD_{90})	ms	74.0	59.8	50.8 ± 2.8
APD at 50% repolarization (APD_{50})	ms	45.6	33.9	35.6 ± 3.3
Diastolic duration (DD)	ms	93.6	70.4	51.1 ± 5.6
Diastolic depolarization rate (DDR)	mV/ms	0.199	0.211	0.079 ± 0.011

Figure 1. Comparison of experimental and simulated APs. A) Representative APs recorded from a mouse SAN cell (black), and simulated APs generated with the Kharche et al. model (red), or our updated model (green). B) Schematic illustration of AP waveform parameters. C) The updated model improves matches to AP waveform parameters. Experimental data are reported as mean ± SE (n=10-14).

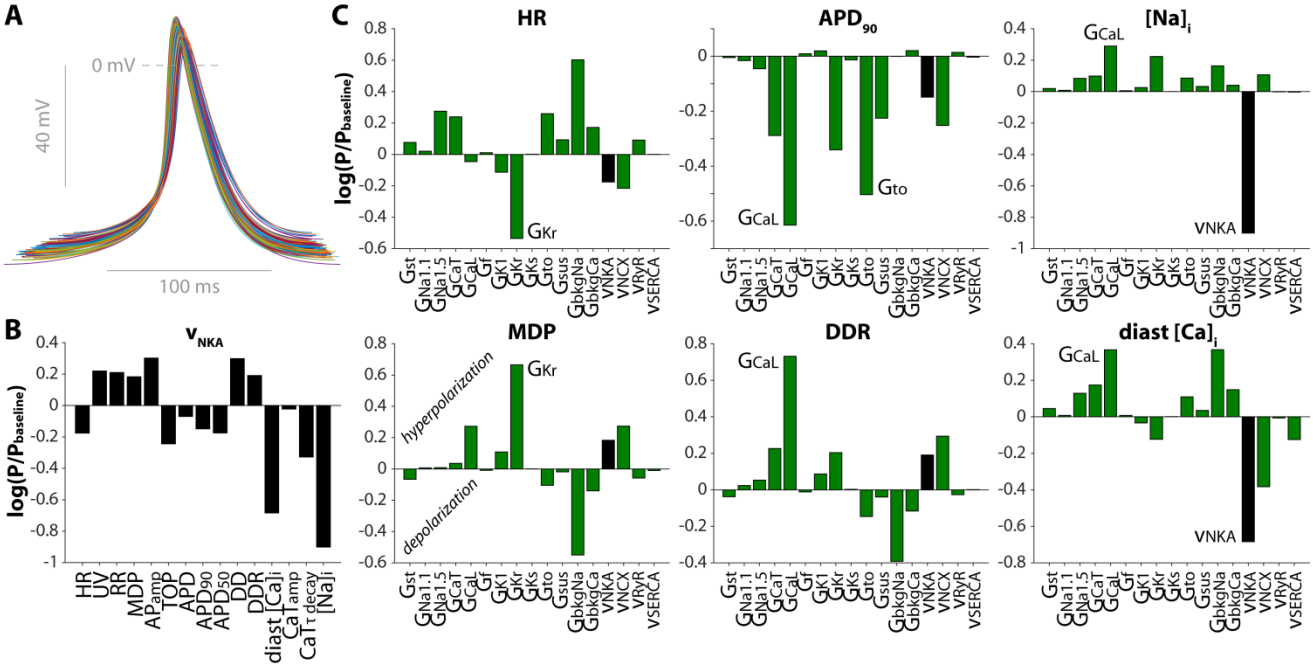

Figure 2. Sensitivity analysis in the population of models. A) APs generated with 100 elements randomly selected in our population. B) Effect of v_{NKA} modulation on all model outputs. C) Effects of modification in conductance of ion currents (I_{st}, $I_{Na1.1}$, $I_{Na1.5}$, I_{CaT}, I_{CaL}, I_f, I_{K1}, I_{Kr}, I_{to}, I_{sus}, I_{bkgNa}, I_{bkgCa}) and maximal rate of ion transport mechanisms (NKA, NCX, RyR, SERCA) on HR, APD_{90}, $[Na^+]_i$, MDP, DDR, and diastolic $[Ca^{2+}]_i$.

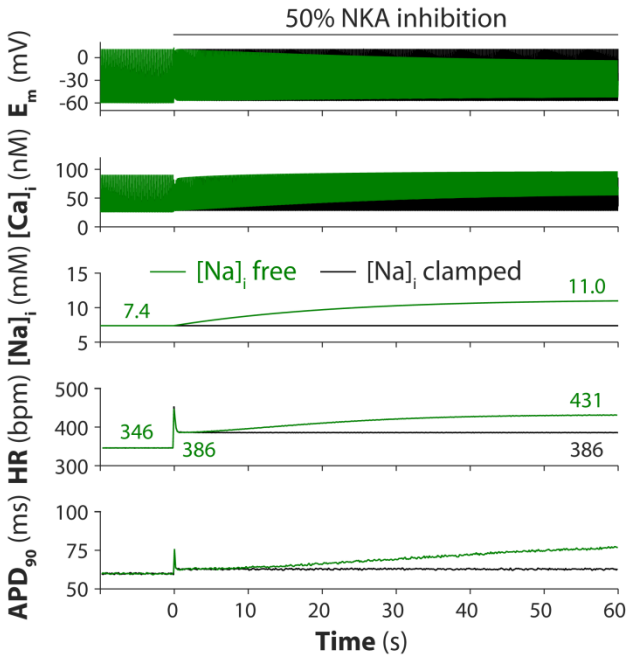

Figure 3. Mild NKA inhibition increases SAN automaticity. Ouabain administration is simulated by blocking NKA by 50% starting at t = 0 s. Time courses of E_m, $[Ca^{2+}]_i$, $[Na^+]_i$, HR and APD_{90} are shown. Results in black are obtained with $[Na^+]_i$ clamped at the initial value.

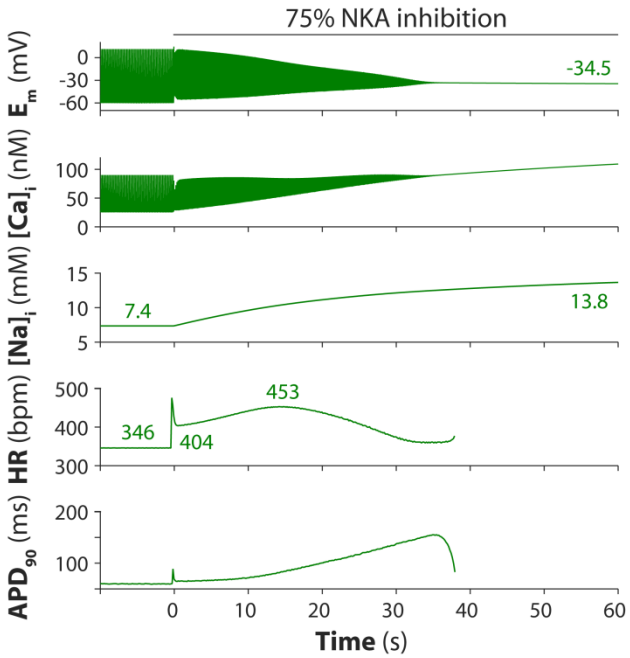

Figure 4. Increased NKA inhibition stops SAN automaticity. 75% NKA block is simulated a t = 0 s. Time courses of E_m, $[Ca^{2+}]_i$, $[Na^+]_i$, HR and APD_{90} are shown.

different models (Fig. 2A). We performed a multivariable linear regression analysis to correlate the changes in the different model outputs (AP properties defined in Fig. 1, and Ca^{2+} and Na^+ homeostasis characteristics) to the changes introduced in model parameters [8]. The result of this analysis is a set of coefficients that can be used to quantify the effect of NKA modulation on each model output (Fig. 2B), and to identify the parameters that mostly influence each output (Fig. 2C).

Our analysis shows that changes in I_{CaL} have the most pronounced impact on APD_{90} and DDR, while I_{Kr} and I_{bkgNa} have dominant (and opposite) effects in the regulation of HR and MDP. The maximal NKA rate, v_{NKA}, plays a major role in regulating diastolic $[Ca^{2+}]_i$. and $[Na^+]_i$ (Fig. 2C). Our model predicts that both concentrations increase when NKA activity is inhibited. Fig. 2B shows that a decrease in v_{NKA} prolongs AP, increases HR, decreases depolarization and repolarization rates, and depolarizes MDP, in agreement with experimental results in rabbit SAN preparations after ouabain administration [4].

We next simulated ouabain administration with the baseline model to evaluate the effect of (a more pronounced) NKA inhibition, and to assess how the expected accumulation of $[Na^+]_i$ over time influences cell automaticity. Upon reduction of v_{NKA} by 50% (Fig. 3), the E_m dynamics are suddenly perturbed. Predicted HR and APD_{90} increase instantaneously, after a transient change (due to the E_m-mediated effect on the other membrane currents) that fades in a few beats, followed by a slower increase that parallels the slow change in $[Na^+]_i$. For HR, the fast and slow responses almost equally contribute to the overall increase (+40 vs. +45 bpm), which reflects the phenomenon of "digitalis-induced tachycardia" [3]. While the instantaneous changes are due to direct effect of NKA (outward current) block on E_m dynamics, the subsequent slow increase is due to the consequences of slow Na^+ rise. In fact, when we repeated the simulation with $[Na^+]_i$ clamped at the initial value, the slow adaptation phase is prevented (black traces in Fig. 4). In this condition, the system reaches a new steady-state in less than 10 s after the block, and the effect on Ca^{2+}, APD, and HR is limited to the fast component mediated by the altered E_m dynamics (i.e., the slow Na^+-mediated effect is absent). When Na^+ and, consequently, Ca^{2+} are free to accumulate, the model predicts progressive depolarization of MDP and reduction of AP amplitude, together with the increase in HR and APD_{90} previously described. These effects are due to the non-linear interplay of ion currents and transporters, whose activity is influenced by and influences Na^+, Ca^{2+} and E_m signals on a beat-to-beat basis. In particular, NCX plays a major role in coupling Na^+ and Ca^{2+} changes, and also influences E_m directly. During the slow response phase to NKA inhibition, the progressive increase in Ca^{2+} load is accompanied by a progressive increase in NCX activity during repolarization. NCX is working in Ca^{2+} extrusion mode, thus the increase in this inward current counteracts the repolarizing currents, prolonging the AP and depolarizing the MDP. The more depolarized diastolic potential

facilitates the following depolarization (e.g., less NCX current is required to reach the threshold), contributing to increase the HR.

When simulating a stronger NKA inhibition (75%, Fig. 4), the model again predicts a fast response followed by gradual slower increases in both HR and APD, paralleling Na^+ and Ca^{2+} accumulation, and decrease of the AP amplitude. Further loading of Na^+ and Ca^{2+} causes HR to start decreasing, until eventually firing activity stops as the E_m stabilizes at about -35 mV. Notably, a very similar time course was experimentally recorded in rabbit SAN preparations by Miyamae and Goto [9]. Our simulations show that the excessive increase in $[Ca^{2+}]_i$ prevents the activation of I_{CaL} (because of the excessive Ca^{2+}-dependent inactivation), which is the current responsible for AP upstroke in SAN myocytes [10].

4. Discussion and conclusions

We performed a computational study to assess the role of NKA and Na^+ homeostasis in the regulation of pacemaker activity in mouse myocytes. By updating the Kharche et al. model of mouse SAN cell [5], we obtained a model that more faithfully recapitulates our experimental dataset (Fig. 1). This updated model was used to quantify the effect of small variations (Fig. 2) and strong inhibition (Figs. 3 and 4) of NKA activity on cell automaticity. Our analysis shows that NKA influences SAN AP waveform properties and firing rate both directly (contributing as outward current) and indirectly, playing a major role in the regulation Na^+ and Ca^{2+} homeostasis. When simulating modest NKA inhibition, we predicted a positive chronotropic effect, a phenomenon of "digitalis-induced tachycardia" [3]. We also showed that the positive chronotropy occurs in two distinct phases: a reduction in the outward NKA current affects E_m dynamics instantaneously; then, reduced Na^+ extrusion causes slow Na^+ accumulation, which modulates Ca^{2+} and E_m signals. We also showed that with stronger NKA inhibition excessive Na^+ and Ca^{2+} accumulation can even stop cell automaticity, as experimentally observed in multicellular SAN preparations [9].

Our analysis confirms that pharmacological NKA inhibition can have opposite consequences in SAN myocytes, depending on the degree of induced Na^+ (and Ca^{2+}) rise, similar to observations in ventricular myocytes [2]. Moderate Na^+ accumulation has a positive inotropic effect in ventricular myocytes and a positive chronotropic effect in SAN cells. Excessive $[Na^+]_i$ increases propensity for arrhythmias and decreases lusitropy in ventricles, and disrupts SAN pacemaker activity, which might contribute to arrhythmia due to ectopic pacemakers.

In conclusion, our study shows that $[Na^+]_i$ plays a crucial role in the regulation of excitability in SAN myocytes and, consequently, of pacemaker activity. Therefore, a detailed characterization of Na^+ handling in isolated SAN myocytes is essential to fully understand cardiac pacemaker function in health and disease.

Acknowledgements

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