Na Diffusion Dependent Ca Handling in Rabbit Ventricular Myocytes

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Abstract

Intracellular Na concentration ($[Na]_i$) plays a pivotal role in modulating the electrical and contractile activity of the heart via the Na/Ca and Na/H exchange mechanisms. Several studies showed that important $[Na]_i$ gradients exist close to the cell membrane. Thus, intracellular Na diffusion has to be slow with respect to the rates of Na transport across the sarcolemma. Here we extended the rabbit ventricular action potential and Ca transient model developed in our laboratory to establish $[Na]_i$ gradients that have been inferred directly from experimental data. In our simulations, a dramatic slowing of the Na diffusion coefficient or higher than expected colocalization between Na and Ca transporters is required to predict experimentally derived indirect effects of altered local $[Na]_i$ on Ca handling. The results suggest some directions for further laboratory investigation and modeling.

1. Introduction

Intracellular Na concentration ($[Na]_i$) modulates the electrical and contractile activity of the heart via the Na/Ca and Na/H exchangers (1). The level of $[Na]_i$ is determined by a fine balance between Na influx and efflux. There are several pathways for Na entry into cardiac cells: voltage-gated Na channels are responsible for a transient Na influx initiating the upstroke of action potentials; Na/Ca exchanger (NCX) generally uses Na influx to extrude Ca; Na/H exchange extrudes H in exchange for Na ions. The Na/K pump (NKA) is the major route for Na extrusion. Not only does NKA control $[Na]_i$, but $[Na]_i$ is a main regulator of the pump itself. An increase in $[Na]_i$ shifts the balance of fluxes on the NCX to favor more Ca influx and less Ca efflux, resulting in larger Ca transients and therefore, enhanced contractility. The interplay between Ca handling and $[Na]_i$ may be even more important during heart failure, where NCX is upregulated, and during ischemia/reperfusion where $[Na]_i$ rises during, and contributes to, Ca overload (1). Na regulation can be modulated by Na diffusion, which determines intracellular Na distribution, and by NKA activity, which is affected by local $[Na]_i$.

Several studies have suggested that, in presence of trans-sarcolemmal fluxes, $[Na]_i$ sensed by the membrane transporters might be different from the bulk $[Na]_i$ (2-6). For such $[Na]_i$ gradients to exist, intracellular Na diffusion has to be slow with respect to the rates of Na transport across the sarcolemma.

In this study, the mathematical action potential and Ca handling model developed by our group (7) was extended to establish $[Na]_i$ gradients that have been inferred directly from experimental data (2-4) and was used to theoretically explore the indirect effects of altered local $[Na]_i$ on Ca handling (5-6).

2. Methods

The mathematical model used is described in detail by Shannon et al. (7). Key structural features include four compartments (in series): a junctional cleft, a subsarcolemmal compartment, a bulk cytosolic compartment, and the sarcoplasmic reticulum (SR). Ca released into the junctional cleft diffuses to the subsarcolemmal (SL) compartment then to the bulk cytosol where SR Ca uptake occurs. The action potential was constructed from individual currents found in the normal rabbit ventricular myocyte. Na transporters were simulated as in Shannon et al. (7). To reproduce the influence of NKA on its own function (pump-current sag), the subsarcolemmal $[Na]_{SL}$ or junctional cleft $[Na]_j$ in NKA equations were replaced by $[Na_{pump}]_{SL}$ and $[Na_{pump}]_j$, which are functions of NKA current, representing the dependence of local depletion on NKA current itself:

if $I_{NaK,c} <= I_{NaKthr,c}$

$$\frac{d[Na_{pump}]_c}{dt} = \frac{d[Na]_c}{dt}$$

else if $I_{NaK,c} >= 70\%\ I_{NaKpeak,c}$

$$\frac{d[Na_{pump}]_c}{dt} = \frac{d[Na]_c}{dt} - k_1 I_{NaK}$$

else

$$\frac{d[Na_{pump}]_c}{dt} = \frac{d[Na]_c}{dt} - k_2 I_{NaK}$$

where c is the actual compartment (junctional cleft or

subsarcolemma) and the parameters I_{NaKthr}, k_1 and k_2 are listed in Table 1.

Table 1 – NKA pump current parameters.

Parameters	SL	junction
I_{NaKthr} [A/F]	0.10	0.05
k_1 [F mM C^{-1}]	1.00	12.5
k_2 [F mM C^{-1}]	0.42	0.42

Na^+ transporters are evenly distributed (11% in junctional membrane and 89% in subsarcolemmal membrane); however, in some simulations here, 50% of Na^+ channels, NKA and NCX were considered to be located in the subsarcolemmal membrane and 50% in the junctional cleft membrane. Na^+ must diffuse from the junction to the bulk compartment through the subsarcolemmal compartment. The Na^+ diffusion coefficients from junctional cleft to SL and from SL to bulk myoplasmic compartment described in Shannon et al. (7) were extracted from the values in aqueous solution reported by Carmeliet (8): $D_{Na(junction-SL)}$ = 1090 and $D_{Na(SL-cytosol)}$ = 1790 $\mu m^2/s$.

3. Results

Cardiac submembrane [Na] transients sensed by NCX. Weber et al. (2) tested whether I_{Na} could increase subsarcolemmal [Na] by using NCX as a biosensor. We simulated their experimental protocol in rabbit ventricular myocytes: with Ca buffered and other currents blocked, Na influx via I_{Na} was controlled by voltage pulses in Fig. 1, A and B. There is almost no Na influx using the voltage pulse in Fig. 1, A. I_{Na} was activated using the voltage pulse in Fig. 1, B and inactivated within 5 ms. The difference of Na influx results in $\Delta[Na]_{sm}$, which drove different NCX currents as seen in Fig. 1, C and reference (2). The D_{Na} coefficient originally set to the value measured in water could not reproduce the experimental findings (Fig. 1, D dashed line). By reducing D_{Na} 200-600 fold as suggested in Despa et al. (3), we reproduce a transient outward ΔI_{NCX} component induced by I_{Na} (Fig. 1, D solid lines). Fitting of these difference currents to single exponentials showed agreement between the experiments and simulations when D_{Na} is reduced 600 fold with respect to $D_{Na,water}$ (simulation τ=16.5 ms vs. experiment τ=16.3 ± 4.6 ms). The predicted changes in junctional and sub-sarcolemmal [Na] are also shown (Fig. 1, E and F). $\Delta[Na]_{SL}$ and $\Delta[Na]_j$ corresponding to peak ΔI_{NCX} are 0.87 and 4 mM (at time of I_{Na} peak values are 1.05 and 4.42 mM respectively), which decayed with time constants of 8 and 17.5 ms respectively.

NKA current sag. Despa and Bers performed simultaneous measurements of NKA pump current (I_{NKA}) and $[Na]_i$ to investigate whether rapid NKA pump activation (after pump blockade) could generate intracellular Na gradients (4). We simulated their

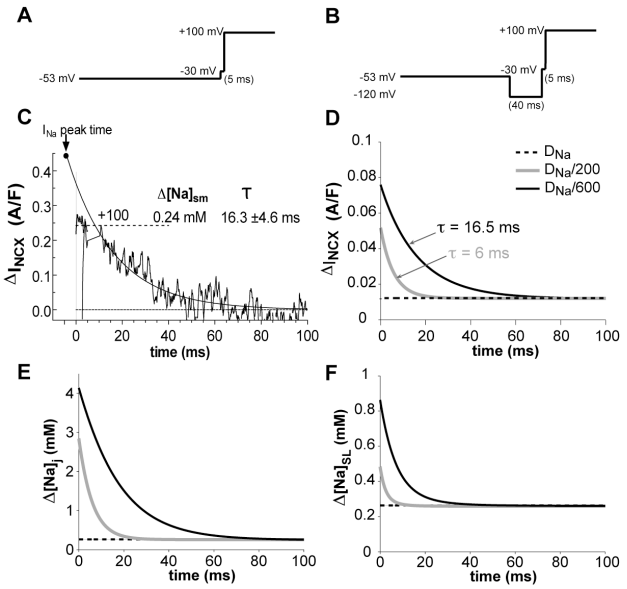

Figure 1 - The difference between the NCX currents measured in the absence (A) and right after I_{Na} (B) where I_{NCX} is measured at 100 mV in experimental data (C) and our model (D) using three different Na diffusion coefficients (D_{Na}, $D_{Na}/200$, and $D_{Na}/600$). The predicted changes in [Na] in the junctional cleft (E) and subsarcolemmal compartment (F) are also shown.

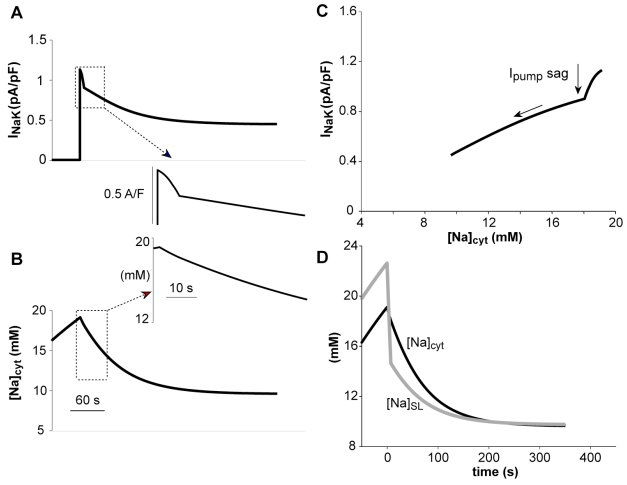

Figure 2 - Modeling results of NKA pump-current sag by changing Na^+ diffusion coefficient ~600 times lower than the value measured in water (8). The plots of NKA current (A), global $[Na]_i$ (B), and NKA current vs. global $[Na]_i$ (C) are the signals upon abrupt reactivation of NKA current after a period of NKA blockade. The fast initial NKA current drop corresponds a small change of global $[Na]_i$ (B, inset), whereas $[Na]_{SL}$ drops significantly within the first few seconds (D).

protocol: in voltage clamped (E_m=-30 mV) rabbit myocytes, NKA is blocked at rest by setting extracellular K concentration, $[K]_o$= 0.01 mM, until the global $[Na]_i$ has raised up to ~18 mM. Then, the pump is reactivated

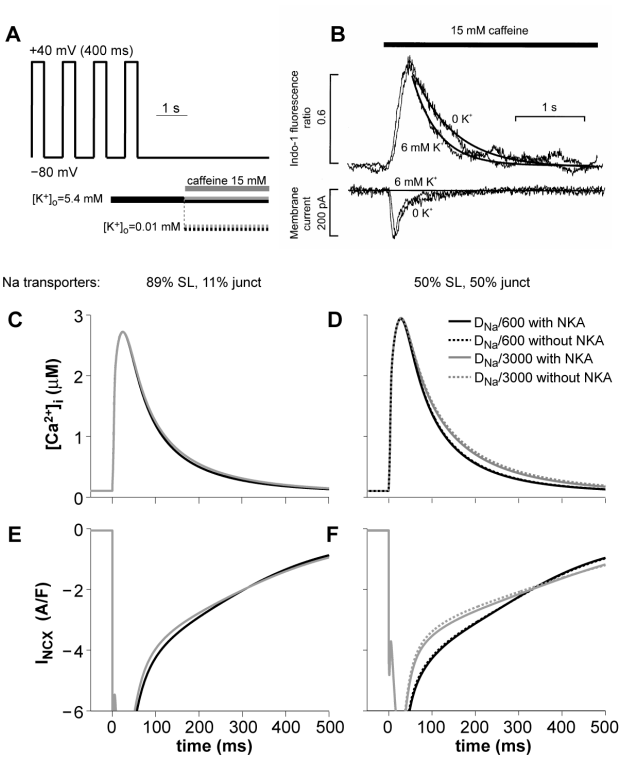

Figure 3 - Caffeine-induced Ca transients before and after NKA pump block. The simulated protocol is depicted in A. Experimental Ca transients and I_{NCX} are shown in B. Model results are shown with D_{Na}/600 and D_{Na}/3000 (left, C and E) and with 50% of Na transporters relocated to the cleft where Ca channels are concentrated (right, D and F).

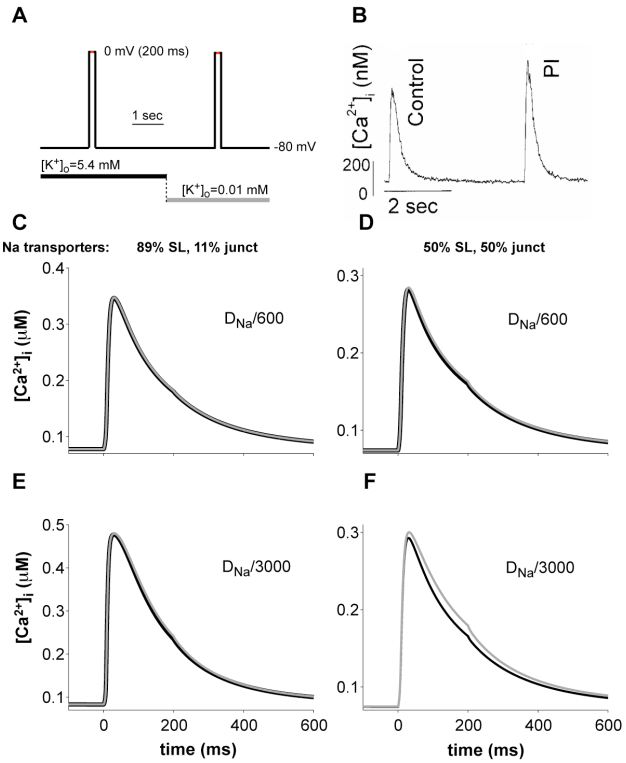

Figure 4 – Effect of NKA pump inhibition on Ca transients. The simulated protocol is depicted in A. Experimental Ca transients are shown in B. Model results are shown with D_{Na}/600 and D_{Na}/3000 (left, C and E) and with 50% of Na transporters relocated to the cleft where Ca channels are concentrated (right, D and F).

for ~5 min (by setting $[K]_o$ to 5.4 mM). Pump reactivation results in a rapid outward shift of in the membrane current, followed by a biphasic decay phase (Fig. 2, A) accompanied by $[Na]_i$ decline (Fig. 2, B). Insets in Fig. 2, A and B, show the first minute of simulation following NKA reactivation: as it has been seen experimentally (4), I_{NKA} initially decays rapidly with little changes in $[Na]_i$ (current sag, Fig. 2, C), followed by a second decay phase accompanied by $[Na]_i$ decline.

Effect of pump inhibition on caffeine-induced Ca transient and NCX decay. The influence of NKA pump activity on the NCX function was also investigated by simulating the protocol implemented by Terracciano (5): NKA is inhibited by removing K from the extracellular solution and caffeine is applied to obtain a rapid increase in cytosolic [Ca] and the functional inhibition of SR Ca uptake. In these conditions, Ca extrusion can be almost entirely attributed to NCX function. The myocytes are paced at 1 Hz with voltage steps (400 ms duration) from -80 mV to +40 mV in K 5.4 mM. Stimulation is stopped and E_m held at -80 mV. After 1 s rest, caffeine is applied for 5 s. The protocol (Fig. 3, A) is repeated and K is removed from the external solution (to inhibit NKA) 400 ms before applying caffeine. Experiments showed that when NKA was rapidly inhibited the cell relaxed more slowly (cell shortening and Ca decline traces), suggesting that NCX-mediated relaxation (Ca extrusion) is NKA dependent (Fig. 3, B). Model results are shown with D_{Na}/600 and D_{Na}/3000 (Fig. 3, C and E) and with 50% of Na transporters relocated to the cleft where Ca channels are concentrated (Fig. 3, D and F). We could not quantitatively reproduce the experimental data (Fig. 3, B); however, with D_{Na}/3000 and 50% relocation of Na transporters we predict a 3% decrease in the relaxation time constant and show that caffeine-induced NCX current is also affected by NKA inhibition (Fig. 3, F).

Effect of pump inhibition on Ca transient in rat ventricular myocytes. Su et al. showed that inhibition of the Na pump causes a significant increase in the Ca transient peak in mouse ventricular myocytes. We mimicked the experiment *in silico* (Fig. 4): after inducing a stable Ca transient (eight pulses at 0.25 Hz, -80 to 0 mV, for 200 s) a test pulse was applied. To assess the effects of abrupt Na pump inhibition, the external K concentration was lowered (to 0.01 mM) for 1.5 s before and continuing during the test pulse (Fig. 4, A). Model results are shown with D_{Na}/600 and D_{Na}/3000 (Fig. 4, C and E) and with 50% of Na transporters relocated to the cleft where Ca channels are concentrated (Fig. 4, D and

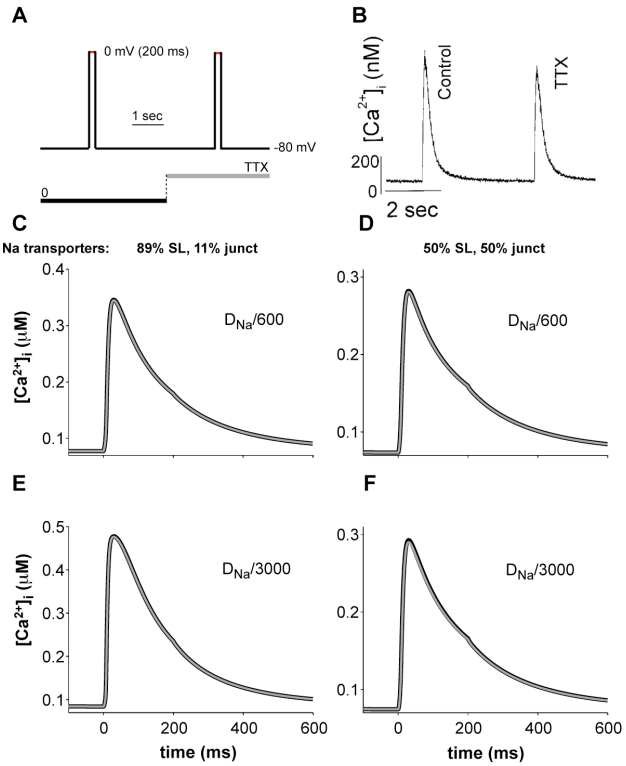

Figure 5 – Effect of I_{Na} inhibition on Ca transients. The simulated protocol is depicted in A. Experimental Ca transients are shown in B. Model results are shown with D_{Na}/600 and D_{Na}/3000 (left, C and E) and with 50% of Na transporters relocated to the cleft where Ca channels are concentrated (right, D and F).

F). With D_{Na}/3000 and 50% relocation of Na transporters, 1.5 s pump inhibition caused a 3% increase in $[Ca]_i$ peak (Fig. 4, F) vs. the 15% increase experimentally observed (Fig. 4, B).

Effect of Na current inhibition on Ca transient in rat ventricular myocytes. Abrupt blockade of I_{Na} (by TTX) resulted on the other hand in a significant decrease in the Ca transient peak in mouse ventricular myocytes. We simulated the protocol depicted in Fig. 5, A, and after inducing a stable Ca transient (eight pulses at 0.25 Hz, -80 to 0 mV, for 200 s) applied a test pulse. The Na current was abruptly inhibited 1.5 s before and during the test pulse. Simulation results are shown with D_{Na}/600 and D_{Na}/3000 (Fig. 5, C and E) and with 50% of Na transporters relocated to the cleft where Ca channels are concentrated (Fig. 5, D and F). A little reduction in $[Ca]_i$ peak (1.8 % vs. 9% seen experimentally) is observed only with D_{Na}/3000 and 50% relocation of Na transporters.

4. Discussion and conclusions

We challenged our model to reproduce a number of experimental observations on the effects of local Na on Na transporters and co-localization between Na and Ca transporters. In order to simulate the NKA pump current sag, D_{Na} has to be dramatically decreased (not shown) to ~15000 times lower than that measured in water (e.g. ~7000 times lower than in muscle cytoplasm (9)). However, such low diffusion coefficient is difficult to imagine in a physical sense. We here formulate an alternative interpretation for pump-current sag, suggesting that such behaviour might be due to local depletion of $[Na]_i$ near NKA protein, i.e., the Na concentration NKA could sense is very local [Na], instead of $[Na]_{sm}$ (Fig. 2). This hypothesis would need to be addressed by further experimental work. As for the effects of local Na concentration on Ca handling, we concluded that by slowing D_{Na} (2 orders of magnitude to for the effects of I_{Na} on I_{NCX} and 3-4 for the effects of NKA inhibition on $[Ca]_i$) and simulating co-localization of Na and Ca transporters in the junction the model better approximates the experimental results. These needed changes may indicate physiologically relevant cellular phenomena which are yet to be accounted for.

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