

Theoretical Investigation of Ca^{2+} Dynamics in Normal and Hypertensive Vascular Walls

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Abstract

Using a theoretical model of smooth muscle cell from rat mesenteric arterioles, we investigated calcium dynamics in normal and hypertensive vascular wall. The model incorporated a detailed description of plasma membrane, cytosol and intracellular stores. Inhibition of Na^+ - K^+ pump (NaK) simulated the effect of ouabain in salt sensitive hypertension. The inhibition caused intracellular sodium accumulation that compromised or reversed Na^+ - Ca^{2+} exchanger (NCX) and increased resting $[\text{Ca}^{2+}]_i$ and reactivity to norepinephrine (NE). NCX inhibition could compensate the effect of NaK block on the agonist-induced Ca^{2+} plateau, but it further increased resting Ca^{2+} and NE-induced Ca^{2+} transients. These undesirable effects could be avoided by selective inhibition of the reverse mode of NCX. In conclusion, NaK blockade elevated Ca^{2+} levels, in agreement with experimental data, and this effect was reduced by inhibition of NCX reverse mode. The results provide evidence for the beneficial effects of a new class of antihypertensive pharmaceuticals.

1. Introduction

Hypertension represents a major public health problem as it results in increased susceptibility to smooth muscle and endothelium cells dysfunction in vascular walls and cardiovascular risk. Signal transduction events involved in the regulation of vascular tone can be successfully modeled in silico by integrating mechanisms at the cellular level to describe function at a macroscale tissue level. The main determinant of vascular tone and regional blood flow is the intracellular concentration of free calcium ($[\text{Ca}^{2+}]_i$) in smooth muscle cells.

An elaborate network of signaling pathways regulates $[\text{Ca}^{2+}]_i$, and includes both intracellular and extracellular signaling. Mathematical modeling offers a systematic approach for the system analysis and can assist data analysis and designing new experiments. The objective of this work is to investigate the effects of ouabain and inhibition of Na^+ - Ca^{2+} exchange on calcium dynamics

using a detailed mathematical model of smooth muscle cell from rat mesenteric arterioles. Endogenous ouabain is an adrenal cortical hormone that regulates cell Na^+ , and has been implicated in the pathogenesis of some forms of hypertension [1]. Ouabain blocks selectively the Na^+ - K^+ -ATPase, increases intracellular Na^+ concentration, and affects Na^+ gradient-dependent processes, like Na^+ - Ca^{2+} exchange. It has been hypothesized that in salt-sensitive hypertension excessive Na^+ intake promotes the secretion of ouabain, impairs Na^+ - Ca^{2+} exchange, elevates SM calcium, and causes vascular hyperresponsiveness to vasoconstrictors. Inhibition of NCX reverse mode has been reported to have beneficial effect in ouabain-induced hypertension [2].

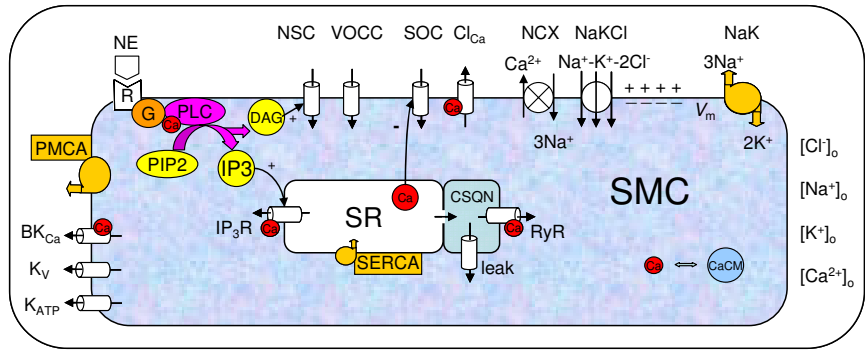
2. Methods

We developed a detailed mathematical model of isolated smooth muscle cell (SMC) from rat mesenteric arterioles (RMA) by combining previous theoretical formulations and experimental data [3]. A schematic diagram of the model is depicted in Fig 1. The model comprises the plasma membrane, the cytosol and the intracellular store. The plasma membrane includes all the major transmembrane currents that have been identified in RMA SMCs, and changes in membrane potential are calculated from:

$$C_m \frac{dV_m}{dt} = -(I_{\text{VOCC}} + I_{\text{Kv}} + I_{\text{BKCa}} + I_{\text{KATP}} + I_{\text{NSC}} + I_{\text{SOC}} + I_{\text{ClCa}} + I_{\text{PMCA}} + I_{\text{NaK}} + I_{\text{NCX}}) \quad (\text{Eq.1})$$

where C_m is membrane capacitance, V_m is membrane potential, I_{VOCC} is a Ca^{2+} current through L-type channels, I_{Kv} and I_{BKCa} are currents through voltage-dependent and Ca^{2+} -activated potassium channels, respectively, I_{KATP} is a current through ATP-dependent K^+ channels, I_{NSC} and I_{SOC} are currents through nonselective and store-operated channels, I_{ClCa} is a chloride current, I_{PMCA} and I_{NaK} are currents through plasma membrane Ca^{2+} -ATPase and Na^+ - K^+ -ATPase, I_{NCX} is a current through Na^+ - Ca^{2+} exchanger. Ionic fluxes through the Na^+ - K^+ -2Cl⁻

Fig.1. Schematic diagram of model components. BK_{Ca} - large conductance Ca²⁺-activated K⁺ channels, K_{ATP} - ATP-sensitive K⁺ channels, K_V - voltage-dependent K⁺ channels, Cl_{Ca} - Ca²⁺-activated Cl⁻ channels, NSC - nonselective cation channels, SOC - store operated channels, VOCC - voltage-operated Ca²⁺ channels, NaK - Na⁺-K⁺-ATPase, PMCA - plasma membrane Ca²⁺-ATPase, NaKCl - Na⁺-K⁺-Cl⁻ cotransporter, NCX - Na⁺-Ca²⁺



exchanger, SR - sarcoplasmic reticulum, IP₃R - IP₃ receptor, RyR - ryanodine receptor, SERCA - SR Ca²⁺-ATPase pumps, CSQN - calsequestrin, CM - calmodulin, R - α₁-adrenoceptor, G - G protein, PLC - phospholipase C.

cotransport system do not exert any influence on the V_m (i.e. process is electroneutral) and therefore are not included in Eq. 1. The Na⁺-K⁺-2Cl⁻ cotransport system is taken into account, however, in the balance of ions in the cytosol.

The intracellular calcium store, mainly representing the sarcoplasmic reticulum, includes uptake and release compartments. The uptake compartment contains IP₃ receptor Ca²⁺ channels (IP₃R), and sarcoplasmic reticulum Ca²⁺-ATPase pumps (SERCA). In the release compartment, Ca²⁺ buffering by calsequestrin (CSQN), ryanodine receptor Ca²⁺ channels (RyR) and a leak current are taken into consideration.

The effect of ouabain was modeled by 20% reduction of the maximum rate of the Na⁺-K⁺ pump. The NCX blockade was simulated by complete inhibition of NCX. The blockade of reverse mode of NCX was simulated by inhibition of NCX only when electrochemical gradients favor Ca²⁺ entry (i.e. reverse mode).

The model was implemented in Fortran 90, and the differential equations were solved with the Gear's backward differentiation formulas (BDF) method for stiff systems using a routine from the IMSL Numerical Library.

3. Results

3.1. Control responses

Figure 2 shows selected control model responses to 0.5 μM NE. Activation of nonselective cation channels by DAG upon NE binding results in large inward sodium current that depolarizes plasma membrane from resting -59 mV to around -43 mV (Fig. 2a). The depolarization increases calcium influx through voltage-operated L-type Ca²⁺ channels (Fig 2b). Increased calcium influx through VOCC channels of the plasma membrane and the IP₃R channels of the SR activated by IP₃ raises cytosolic

calcium concentration and opens ryanodine receptors which leads to further elevation of [Ca²⁺]_i (Fig 2c). Sustained calcium oscillations with period 18s are then generated through the Ca²⁺-induced Ca²⁺-release mechanism (CICR) via RyR. The large Na⁺ influx through NSC significantly increases cytosolic sodium concentration (Fig. 2d, dashed line). Intracellular concentrations of potassium (Fig. 2d, solid line) and chloride (Fig. 2d, dash-dot line) ions remain relatively unaffected. Elevation of [Ca²⁺]_i and [Na⁺]_i increases significantly rates of PMCA (Fig. 2e, solid line) and Na⁺-K⁺-ATPase (Fig. 2d, dashed line). At rest, the Na⁺-Ca²⁺ exchanger works in the forward mode extruding cytosolic calcium. During stimulation with NE, NCX gradually enters the reverse mode (Fig. 2f) due to the sodium accumulation and reduced sodium electrochemical gradient. This causes additional influx of extracellular calcium and elevation of [Ca²⁺]_i.

3.2. Effects of NaK and NCX blockade

Figure 3 shows effect of 20% block of Na⁺-K⁺ pump on cytosolic calcium (solid line) and membrane potential (dotted line) in the presence of 0.6 μM NE. The blockade was applied after steady-state response to NE was reached (after 1000s). The block caused significant calcium elevation from 371 nM to 543 nM, and small membrane depolarization from -45.1 mV to -43.2 mV. Calcium response comprised fast but small component followed by much slower but larger response. The slow response resulted from enhanced Ca²⁺ influx through NCX. Increased rate of NCX reverse mode was caused by additional sodium accumulation following reduced extrusion rate via Na⁺-K⁺ pump. The stoichiometry of the pump is 3Na⁺:2K⁺, thus it generates a net outward repolarizing current and its inhibition produces rapid but small membrane depolarization responsible for the initial fast component of calcium elevation.

Fig. 2. Model responses to $0.5\mu\text{M}$ of NE: (a) membrane potential; (b) Ca^{2+} current through L-type VOCC channels; (c) free cytosolic calcium concentration; (d) cytosolic concentrations of potassium (solid line), sodium (dashed line) and chloride (dash-dot line) ions; (e) currents through plasma membrane Ca^{2+} -ATPase (solid line) and Na^{+} - K^{+} -ATPase (dashed line); (f) current through Na^{+} - Ca^{2+} exchanger.

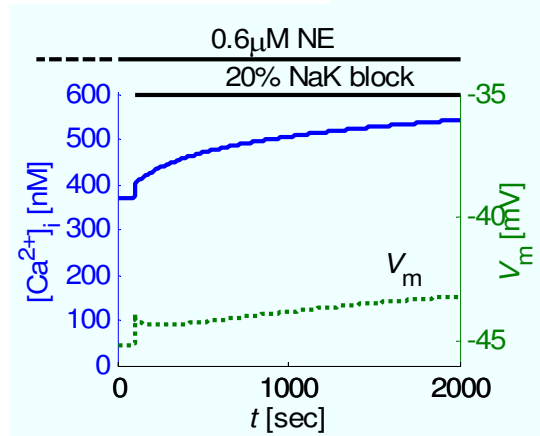
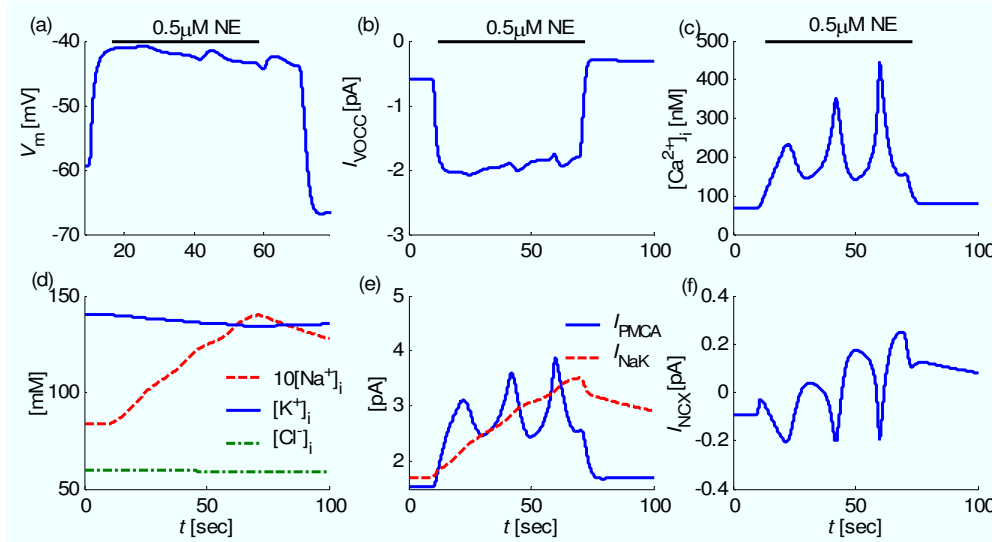


Fig. 3. Effect of 20% block of Na^{+} - K^{+} pump on cytosolic calcium (solid line) and membrane potential (dotted line) in the presence of $0.6\mu\text{M}$ NE.

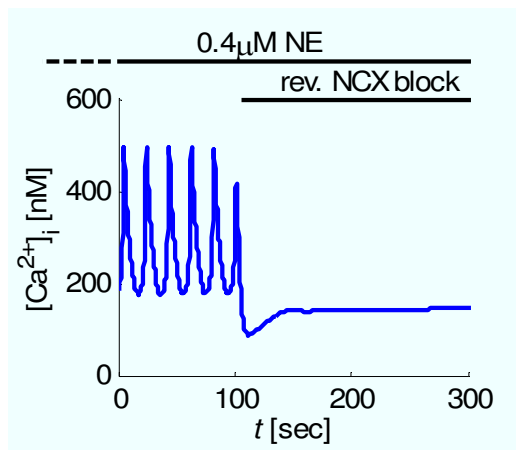


Fig. 4. Inhibition of the NCX reverse mode in continuous presence of NE significantly reduced $[\text{Ca}^{2+}]_i$.

Figure 4 shows effect of inhibition of the NCX reverse mode on $[\text{Ca}^{2+}]_i$ after a steady-state response to $0.4\mu\text{M}$ NE was reached. Because the exchanger worked in the reverse mode during the NE stimulation, the inhibition rapidly reduced cytosolic calcium levels. The remaining Ca^{2+} elevation above resting level is attributed to Ca^{2+} influx through VOCC. Since sodium fluxes *via* NCX are small compared to currents through NSC channels, the blockade had little effect on $[\text{Na}^{+}]_i$ and V_m .

Figure 5 compares initial calcium responses to $0.5\mu\text{M}$ NE under control conditions (dotted line), 20% NaK block (solid line), 20% NaK block and NCX reverse mode inhibition (dashed line), and 20% NaK block and total NCX inhibition (dash-dot line). The NaK pump blockade significantly increased amplitude of the initial calcium transient and this effect was even more amplified by simultaneous total NCX inhibition.

Figure 6 summarizes effects of the different blocking scenarios on the resting $[\text{Ca}^{2+}]_i$, amplitude of the initial Ca^{2+} peak, and mean Ca^{2+} during plateau induced by $0.5\mu\text{M}$ NE. The NaK block elevated resting $[\text{Ca}^{2+}]_i$ by increasing $[\text{Na}^{+}]_i$ and reducing Ca^{2+} extrusion *via* NCX. Simultaneous inhibition of NCX reverse mode did not affect Ca^{2+} , indicating that at rest NCX remained in the forward mode. Consequently, complete inhibition of NCX raised resting calcium. Elevated resting Ca^{2+} raises calcium content in the internal stores, allowing larger calcium release and calcium peak immediately after stimulation (calcium amplification by SR). NaK block increased also plateau calcium levels in presence of NE, as already shown in Fig. 3 for $0.6\mu\text{M}$ NE. This effect on plateau could be compensated by inhibiting NCX reverse mode as well as total NCX inhibition.

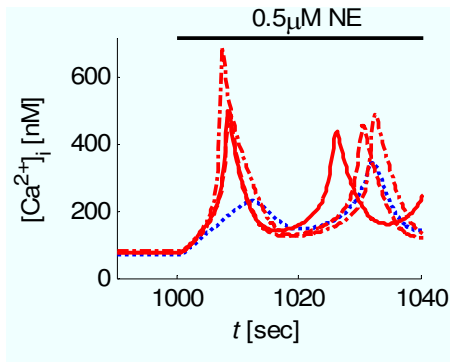


Fig. 5. Transient calcium responses to 0.5 μ M NE under control conditions (dotted line), 20% NaK block (solid line), 20% NaK block and NCX reverse mode inhibition (dashed line), and 20% NaK block and total NCX inhibition (dash-dot line).

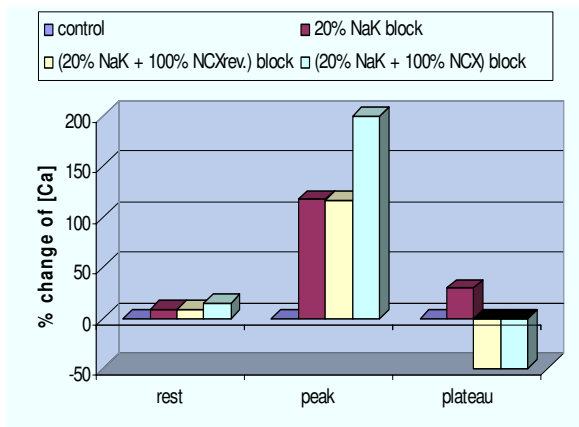


Fig. 6. Effects of different blocking scenarios on resting $[Ca^{2+}]_i$, amplitude of the initial Ca^{2+} peak, and mean Ca^{2+} during plateau phase induced by 0.5 μ M NE.

4. Discussion and conclusions

First we investigated model responses to NE stimulation under control conditions (Fig. 2). A characteristic feature of the model is generation of calcium oscillations similar to that observed experimentally in isolated rat mesenteric arterioles [4]. The model advances previous theoretical formulation by incorporating a description of intracellular sodium balance that affects Ca^{2+} fluxes via Na^+-Ca^{2+} exchange. Block of the Na^+-K^+ pump in continuous presence of NE (Fig. 3) increased $[Ca^{2+}]_i$, in agreement with data from [4]. The model suggests that this is mainly due to increased Ca^{2+} influx via NCX, following sodium accumulation, and not because of membrane depolarization. This is in lines with the general scheme proposed for the effect of ouabain [1]. Inhibition of NCX in presence of NE (Fig. 4) reduced calcium, consistently

with data from [4] and [6]. In the model, this effect is explained by NCX working in the reverse mode during exposure to NE. An integrated endothelium – SMC model will examine in the future the relative contribution of other proposed mechanisms [4]. The model also validates Blaustein’s proposition [5] for calcium amplification in the SR in the presence of ouabain (Fig. 5) that results in increased transient hyperresponsiveness to NE. Inhibition of the NCX reverse mode can partially compensate the effects of ouabain (Fig. 6), in agreement with recent experimental data [2]. Interestingly, the model suggests that a total NCX inhibition is less desirable. Thus, results support the beneficial effects of a new class of antihypertensive pharmaceuticals that will selectively block reverse mode of NCX in salt sensitive hypertension.

In conclusion, compromised Na^+-K^+ pump elevated calcium levels both in absence and presence of stimulation, in agreement with experimental data. These effects can be partially compensated by inhibition of the NCX reverse mode.

Acknowledgements

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