COMPUTER SIMULATION OF MOLECULAR LEVEL PATHOLOGY IN FAILING HEART

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Abstract

Computer simulation is used to quantitatively explain interactions of cardiac mechanisms on molecular level. Our aim is to help interpret experimental data of cardiac electro-mechanics in case of cardiac failure. While qualitative explanations based on intuition and experience of experimental scientists are frequently offered, calculation often reveals inaccuracies of hypothesizes. In this paper, cardiac intracellular calcium and contraction force are examined under simulated heart failure. The simulations show that two major changes of calcium handling attributed to cardiac failure (SERCA dysfunction and NCX over-expression) are not sufficient to quantitatively explain changes in force generation. Results thus imply that other mechanisms or different conditions play crucial role in cardiac failure. Thus either the model that reflects current understanding to calcium behavior in the heart needs to be refined or further mechanisms must be searched for. Some of them are discussed.

1 Principles (Introduction)

Mathematical Biology. Unquestionably, the complexity of mechanisms of human body functions is enormous. In order to understand disorders of human heart it is necessary to understand at least some of the mechanisms, hopefully the major ones. The most typical approach of a biomedical research is based on experimental work. There, under well defined laboratory conditions, individual molecules and their roles in a concert of the whole cell and body are studied. The advances of such research in terms of our understanding are amazing. However, while the major concepts of body functions are known, it is rather difficult to include all these particular findings in one complex system similar to our body. Instead, particular descriptions are available, some of which may well be contradictory. When trying to interpret new knowledge, intuition and heuristic approach are commonly used. Though such understanding might be right, quantification is almost impossible. Moreover, with thousands of variables any outcomes and interpretation can easily be questioned. The use of mathematics in this field provides a promising tool that could offer more robust results. However, a dramatic problem is essentially incomplete description of biological systems and their enormous complexity that challenges both mathematical science and computation power. A new field of mathematics arises - mathematical biology.

Heart Failure. Cardiovascular related diseases are the major causes of deaths in our population. Consequently, cardiovascular research provides enormous and yet growing numbers of known molecular mechanisms of cardiac excitation-contraction coupling (ECC). It is well known, that in cardiac cells calcium ions (Ca_i^{++}) play a crucial role in regulating contraction. They link electrical activation and mechanical response, i.e. ECC. Increased level of free calcium in the cell triggers the contraction (systole) and subsequent massive removal of Ca_i^{++} causes relaxation (diastole). The changes in concentrations are remarkable - three orders of magnitude during each beat. Thus calcium "cycles" among intracellular space (where it interacts with contractile proteins) and stores.

Ca_i^{++} movements (see Fig. 1) are controlled by transporter proteins. It is frequently reported that in heart failure some of these proteins exhibit altered functions (typically increased or decreased). However, different authors introduce rather different levels of alterations including opposite trends. Of course, such results depend on conditions, HF model, and animal species specific to each experiment. Two major changes are discussed and covered by our model: decreased SERCA activity and variable

activity of NCX typically increased. (Multiple other changes are also reported.) The aim of our work was to asses quantitatively, whether these changes could be major reason of altered Ca_i^{++} kinetics found in heart failure or other mechanisms need to be searched for.

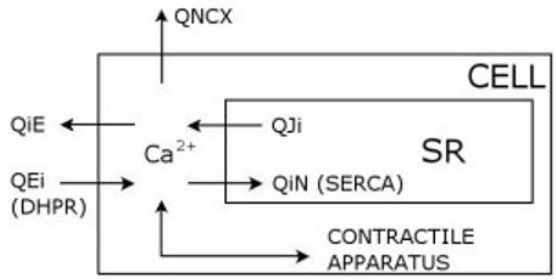


Figure 1: Some Ca_i^{++} transport mechanisms in heart cell

Our compartmental model [1] of excitation-contraction coupling was used and parameters of respective transporters were changed according to experimental data. Simulated results are then compared to experimental findings.

2 Methods

The model [1] comprises of a set of 13 1st-order nonlinear differential equations.

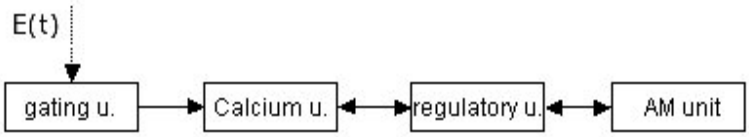


Figure 2: Model scheme – subunits interaction

Model considers of gating, calcium, regulatory and contraction subunits (Fig. 2). Compartmental description of underlying molecular processes is adopted to simulate a) calcium handling, b) contraction control by troponin and c) the reaction kinetics of contractile elements. The contraction model and calcium handling subunit were presented in [1] and [2]. The description of the NCX exchanger that was originally omitted or extremely simplified is included now. In principle, NCX ionic flux is now described after Winslow [3] and further modified as:

$$Q_{NaCa} = \frac{1}{V_c \cdot z \cdot F} \cdot \frac{k_{NaCa}}{1 + k_{sat} \cdot e^{\frac{(r-1) \cdot E \cdot F}{R \cdot T}}} \cdot \left([Na]_i^3 \cdot [Ca]_e \cdot e^{\frac{r \cdot E \cdot F}{R \cdot T}} - [Na]_e^3 \cdot [Ca]_i \cdot e^{\frac{(r-1) \cdot E \cdot F}{R \cdot T}} \right) \quad (1)$$

where A_{cap} is capacitive membrane area, C_{SC} is specific membrane capacity and V_c cellular volume.

Simulation and parameters. The model was implemented in Matlab / Simulink R14. Integration method ode15s (stiff/NDF) was used. As an input, experimentally obtained trace of cardiac action potential was used (fig. 3). [4] Simulation parameters: stimulation frequencies 1, 2 and 3 Hz, simulation duration 300s (to avoid effects of the transient state).

Heart failure model: Chronical congestive failure was simulated according to experimental findings [5] by reducing the activity of SERCA from 100% down to 75, and 50% of normal values. According to some findings reporting NCX activity in failing hearts [6] the NCX activity was varied among 50, 75, 100 and 130% of normal activity. The NCX transporter was also virtually blocked completely (activity set to 0%) so that its (missing) role in ECC could be identified easier.

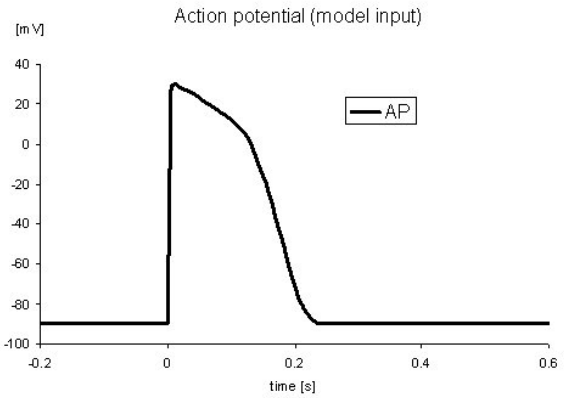


Figure 3: Action potential – model input

3 Results and Discussion

Impaired calcium handling in chronical heart failure (F) was simulated by decreasing SERCA activity to 50% compared to normal. The activity of NCX was varied between 0 and 130%.

Decreased SERCA activity alone resulted in lower Ca_i^{++} maximum (peek) and thus force transient (roughly by 40%) and increased Ca_i^{++} minimum during relaxation phase (compared to non-failing model). This is consistent with many experimental findings ([6], [7]). As a consequence, due to the lower Ca_{MAX} the heart generates less contraction force (necessary for forwarding blood) and increased Ca_{MIN} provides less relaxation (i.e. less filing of the heart by returning blood). Both effects combined mean dramatic reduction of cardiac performance.

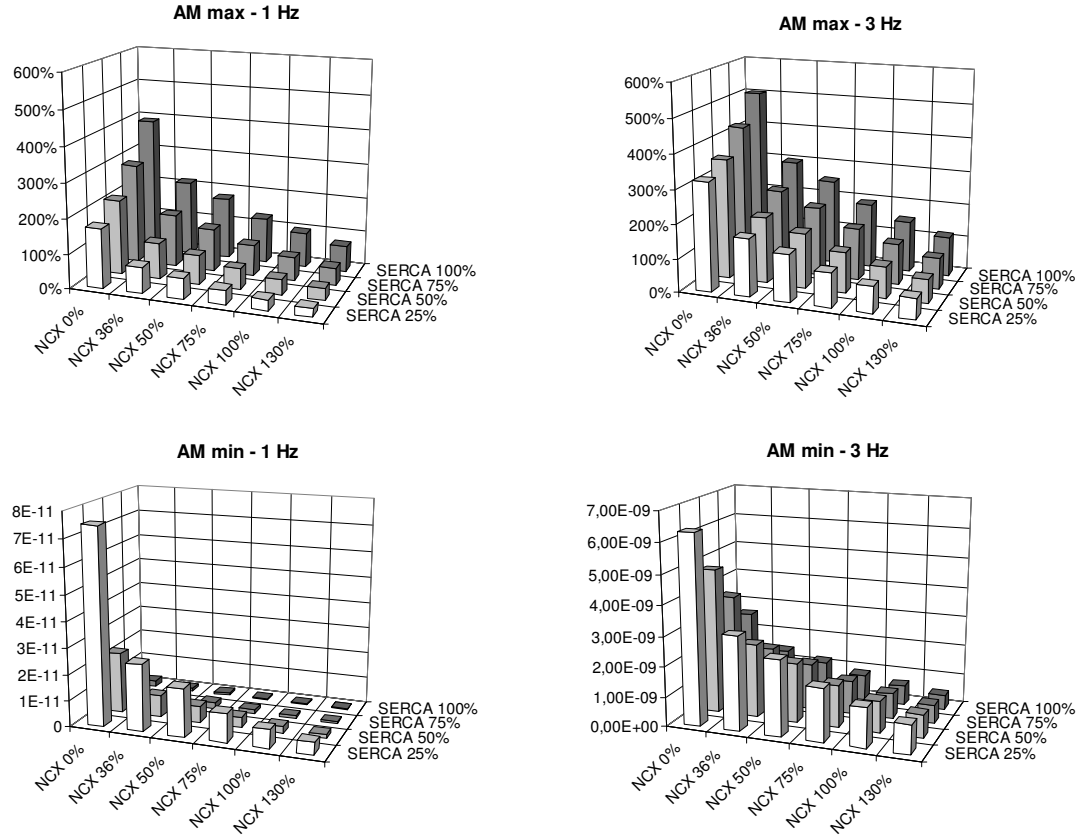


Figure 4: Relative (AM_{MAX}) and absolute (AM_{MIN}) concentrations (mol/l) of AM at 1 and 3Hz in failing heart

Combined effects of varying activities of both transporters (SERCA and NCX) are summarized in Fig. 4 ($AM_{MAX/MIN}$ at 1 and 3Hz). Increasing activity of NCX to 130% of normal almost restores the

relaxation (i.e. cardiac filling) however, at a cost of further decline of maximal force (roughly to 50%) (Fig. 5A). This effect is even more pronounced in high frequencies (3Hz – Fig. 5B). Thus it is unlikely that increase in NCX could be treated as a compensatory mechanism in cardiac failure [6] as sometimes suggested as it fails to restore intracellular calcium available for force generation. [7] However, it is known that NCX has dual mode – calcium removal from the cell (called forward mode) and calcium entry into the cell (reverse mode). Timely switching between the modes would be a perfect solution for the failing heart with SERCA deficiency.

But our simulations revealed only extremely low activity of reverse mode, both under physiological and pathological conditions. By analysis of the equation (1) that describes NCX kinetics it was found that the mode switching (i.e. forward or reverse movement of Ca_i^{++} ions) is determined by the third multiplier

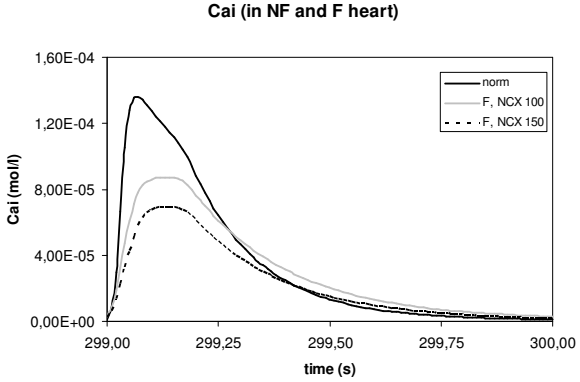
$$[Na]_i^3 \cdot [Ca]_e \cdot e^{\frac{r \cdot E \cdot F}{R \cdot T}} - [Na]_e^3 \cdot [Ca]_i \cdot e^{\frac{(r-1) \cdot E \cdot F}{R \cdot T}}$$

since it determines the parity of the equation. The multiplier can further be simplified by omitting some constants:

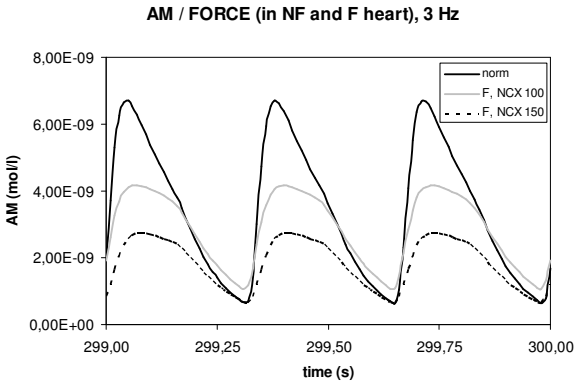
$$[Na]_i^3 \cdot [Ca]_e - [Na]_e^3 \cdot [Ca]_i$$

Reverse mode is active if it is satisfied

$$\left([Na]_i^3 \cdot [Ca]_e - [Na]_e^3 \cdot [Ca]_i\right) > 0 \qquad (2)$$



A



B

Figure 5: A - Intracellular Ca^{++} concentration in NF and F heart (SERCA activity 50%, NCX activity 100% and 150%), at 1 Hz; B - Contractile force in NF and F heart (SERCA activity 50%, NCX activity 100% and 150%), at 3 Hz

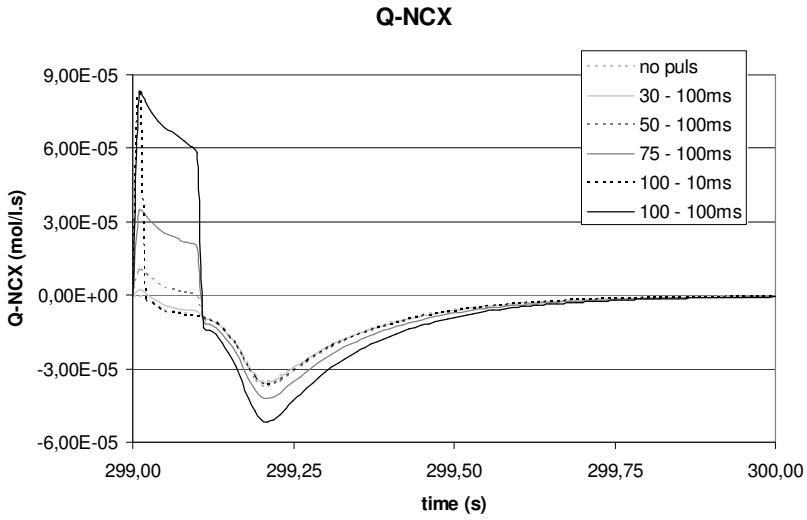


Figure 6: NCX reverse mode ionic flux – experiment results

It can be further assumed that Ca_o and Na_e are rather constant as they reflect concentration of ions in extracellular space which is rather extensive and regulated independently of cardiovascular system. Thus, mathematically, NCX reverse mode can be enhanced if Na_i was increased or Ca_i decreased. Of these two, Ca_i^{++} (intracellular calcium concentration) is calculated within the model by a set of differential equations so there is no simple way how to alter Ca_i other than to refine the model. Instead, effect of changing intracellular concentration of sodium - Na_i was studied. Na_i was experimentally increased to 30, 50, 75 and 100 mM (mmol/l) in the beginning of each beat for 10 or 100 ms (Fig. 6). Though it seems artificial, this transient change can be rationalized. During each beat, concentrations of ions close to the cellular membrane change many fold (for Na and K) or even orders of magnitude (for Ca) and cause "action potential" – a signal for contraction to be triggered. Intra- and extracellular ionic concentrations known from measurements are somehow "averaged" and steady state. It can be assumed that in specific areas close to the activated membrane (action potential) the kinetics of ions (and thus concentrations) can be way different to the rest of the cell. These spaces are usually referred to as "subspaces" (Fig. 8, [8]) – physically not distinctly separated but still distinct. If NCX and sodium channel (responsible for Na influx in early activation) would be in close proximity in the subspace, locally increased Na could make significant NCX reverse mode possible.

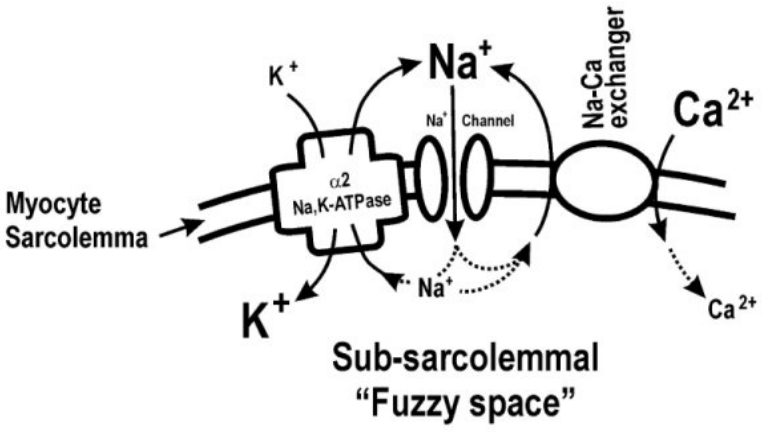


Figure 7: Na^+ subspace/buffer close to NCX exchanger [8]

4 Conclusions

Simulations presented here demonstrate that quantitative description and simulation of experimental biomedical data can provide new insight into mechanisms that are understood just intuitively and can suggest directions of further research. In this case it was shown that if overall excitation-contraction description (and thus model) is correct, further mechanisms that provide enough

calcium for failing cardiac cells need to be searched for. One alternative is increased sodium concentration.

Acknowledgements

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