Cellular Energetic Extension Applied to the Luo-Rudy II Ventricular Cell Model

Sándor M Szilágyi

Petru Maior University, Tîrgu Mureş, Romania

Abstract

The goal of this study is to introduce a new ventricular cell energetic model extension that, in contrast to earlier presented dynamic cell models, allows the simulation of long-term pathological events such as development of hypoxia and anoxia.

We created an energetic ventricular cell model extension that involves the adenosine triphosphate (ATP)-adenosine diphosphate (ADP) cycling mechanism. We chose the well known Luo-Rudy II (LR) ventricular model and extended it with the developed energetic circulation system, so that we can easily validate the obtained results.

The presence of hypoxia reduces the ATP concentration. In LR model a 10%/25%/50%/75% ATP concentration decrease subdued the ionic pumps efficiency by: 4%/14%/32%/55% for calcium pump of the sarcoplasmic reticulum; 3%/11%/26%/45% for external membranous calcium pump; 3%/10%/22%/39% for calcium sodium exchanger; and 2%/8%/18%/33% for sodium potassium exchanger.

The malfunction of the calcium regulation mechanism is the principal danger factor in presence of hypoxia or anoxia and it is the most sensible element during ischemia.

1. Introduction

In the last two decades cardiac mortality rates have declined in most high-income countries, but have increased significantly in many low- and middle-income countries. Despite decades of intensive research, cardiac diseases still remain the biggest cause of deaths worldwide.

The main reason of slow progress represents the partially understood heart functioning. The proper understanding of the cardiac cell functionality is imperial during the long and quite expensive drug development process.

Several cardiac diseases, such as cardiomyopathy, which represent a measurable deterioration of the myocardium's functionality has a long, continuous development process, so these events cannot be properly modeled with fixed parameter cell models. Most cardiac cell models use fixed ionic concentrations and amplification parameters, so the generated activation potential (AP) has a fixed shape.

This problem was partially solved by the development of dynamic cell models, such as Luo-Rudy II (LR) cell model [1-2], that can employ variable ionic concentrations. Several cardiac phenomena can be properly modeled in this way, but most of them have a time- and energy-based development process, so these factors cannot be ignored.

As an example, to explain the development process of hypoxia and anoxia, we must use a time- and energy-based modeling of the cellular functionality [3].

Hypoxia represents an insufficient oxygen level in blood or tissue. In the presence of this pathological condition, despite adequate blood perfusion, the whole organism (generalized hypoxia) or a region of it (tissue hypoxia) suffers from reduced oxygen supply [4].

Hypoxia may be caused by hypoventilation, pulmonary embolism, methemoglobinemia, carbon monoxide poisoning, histotoxic hypoxia, and shunts in the pulmonary circulation.

A partial or total occlusion of a coronary artery leads to myocardial ischemia [5]. In this case a certain region of the heart muscle, which depends on the occluded artery for their supply, begins to suffer metabolic and electrophysiological changes. The rapid fall of oxygen level, which would be necessary to sustain cell's life will develop hypoxia and later anoxia [4]. If the suffering region is too wide, hypoxia may induce sudden cardiac death [6-7].

Cellular energy management cannot be modeled by existing cellular models, so the LR dynamic model was extended with an energetic management component [1-2]. The LR model was selected for extension because obeys the following considerations:

- it can model ventricular cells;
- it has a dynamic characteristic to model metabolic and electrophysiological changes;
- it includes all major ionic currents;

- may have a good robustness;
- demands relatively low computation power.

The main goal of this paper is to present an energetic extension of the LR model that allows studying the development phases of hypoxia and anoxia and other time-dependent cardiac phenomena. The rest of the paper is organized as follows: Section 2 gives a detailed description of the extended LR model and its functioning for normal and pathological cases. Section 3 presents and discusses several aspects of the extended model functionality and the results of simulations carried out using the model. In Section 4, the conclusions are formulated.

2. Methods

2.1. Extended Luo-Rudy II model

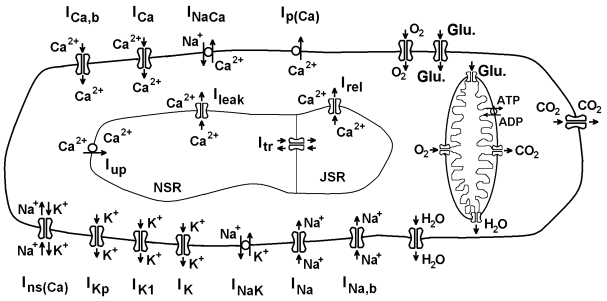

Figure 1. Schematic diagram of the extended Luo-Rudy II model

The LR model has its ionic equation formulated as in the Beeler-Reuter model [8], and is the first dynamic mammalian ventricular cell model that describes the mathematical relation of the ionic currents and determines the shape of the activation potential [1-2]. This model accounts for dynamic changes of ionic concentrations, so it can properly model several pathological cases. The reconstruction model was developed using huge amount of voltage-clamp measurements of the diverse ionic currents, which were performed on guinea pig ventricular cells (GPVC).

The changing rate of an ionic concentration is determined by formula:

$$\frac{d[B]}{dt} = -\frac{I_B \cdot A_{Cap}}{V_C \cdot z_B \cdot F},$$

where $[B]$ stands for the ionic concentration, I_B is the sum of all ionic currents carrying ion B, A_{Cap} represents the surface of the capacitive membrane, V_C is the cell's volume, z_B is the ionic valence, and F is the Faraday constant. The membrane capacity was considered $1\mu F/cm^2$, and the temperature 37°C.

In the extended LR model, presented in Figure 1, all included ionic currents may act between the extra- and intracellular region, between intracellular cytoplasm and sarcopasmic reticulum (SR), between network SR (NSR) and junction SR (JSR), and between mitochondria matrix and intracellular cytoplasm. The volume of SR is considered 6% of the whole cell's volume, while JSR represent 8% of the SR's volume. The mitochondria volume fraction is 25%.

The main voltage and time dependent excitatory sodium current is I_{Na} that is active in the depolarization phase. It is responsible for the rapid upstroke of the action potential. The ionic conductance varies with temperature, ionic concentrations and other factors (e.g. drugs). $I_{Na,b}$ represents the linear leakage (LL) sodium current.

There exist three potassium currents: the time-dependent potassium current I_K, the plateau potassium current I_{Kp}, and the time independent I_{K1} current. These currents have an important role in all AP phases except depolarization. $I_{ns(Ca)}$ is a non-specific calcium activated current, which was suspected to conduct the arrhythmogenic transient inward current under calcium overload conditions. Activation gates were hard to identify on GPVC [2]. I_{Ca} represents the time dependent inward calcium current and $I_{Ca,b}$ is formulated as a LL calcium current.

The exterior cell membrane contains three types of ionic pumps. The potassium-calcium ionic exchanger transports three sodium atoms with each calcium atom, generating the I_{NaCa} current that saturates at high negative potentials. $I_{p(Ca)}$ is created by the sarcolemmal calcium ionic pump, which extrudes calcium ions from cytoplasm and maintains a low resting state calcium level. The sodium-potassium exchanger extrudes three sodium ions from the cell in exchange for two potassium ions, thus generating I_{NaK}.

All ionic currents that flow in SR are related to calcium ions whose principal role is the regulation of the cell's contraction and release. The calcium uptake is done by current I_{up}, while the currents I_{leak} and I_{rel} transport calcium to the cytoplasm. The calcium concentration between NSR and JSR is regulated by I_{tr}.

Mitochondria, the rod-shaped organelles that are separated by two membranes from cell's cytoplasm, are the so-called "powerhouses" of cells, converting oxygen, glucose and ADP into ATP, carbon dioxide and water. This conversion is called aerobic respiration, so it is the reason why all animals must breathe oxygen and produce carbon dioxide. Without proper quantity of ATP the cell cannot perform normal metabolic activities. As the energy generator of the cell, mitochondria play an important role in cell physiology and pathology.

In Figure 1, as an extension of the classic LR model, a mitochondrion is observable with its double layer. The outer layer is almost smooth and inhibits large molecules to enter inside, while the inner one has a highly

convoluted shape that greatly increases its surface for better ADP-ATP transfer via the ATP–ADP translocase.

The respiration equation performed in mitochondria is: $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + Energy$, where 36 or 38 (depends on the extra-mitochondrial reducing equivalents) ADP are transformed to ATP. The extension involves eight gates and one transporter that are the necessary additional elements required in the breathing process. Four gates are situated in the cell's membrane and perform the entrance of nutrients (glucose) and oxygen molecule, and expiration of carbon dioxide and water. Other four gates with the same roles are situated in mitochondria's inner surface. In this model extension there exists no ADP-ATP exchanger through cell's membrane, but there is an ADP-ATP exchanger situated in the inner mitochondria surface (the ATP–ADP translocase protein).

2.2. Energetic analysis of hypoxia and chronic cell disease

Hypoxia induces a mismatch between oxygen supply and demand, and creates altered cell functioning [9]. All ionic currents and the activation potential suffer serious modification [10-11]. The simulation of ionic currents in modified environment was performed using the COR cell modeling and development environment that was developed by Dr Alan Garny, and is freely available at the web address http://cor.physiol.ox.ac.uk [12-13].

The ATP deficiency caused by hypoxia drastically reduces cell pump functionality and alters ionic concentrations. In this research we have focused on the effects of hypoxia and chronic cell disease (CCD) caused by improper mitochondria functioning.

All ionic currents, pumps, exchangers, and ionic concentrations involved in the LR model were modified in time during hypoxia, and altered in case of CCD.

In our consideration a healthy ventricular cell stores enough ATP for 20-40 sec normal functioning. In the presence of hypoxia the creation of ATP is obstructed, and all pump functionality is decreased following a logarithmic relation between ATP concentration and pump functionality, so the ventricular cell can still work at relatively low ATP concentrations. We chose the well known Luo-Rudy II (LR) ventricular model and extended it with the developed energetic circulation system, so that we can easily validate the obtained results. To simulate non-pathologic cases we used Na^+ 132-148 mmol/L, K^+ 3.5-5 mmol/L, Ca^{2+} 2 mmol/L extra-cellular, and Na^+ 8-11.4 mmol/L, K^+ 130-175 mmol/L, Ca^{2+} 0.11-0.16 μmol/L intra-cellular ionic concentrations.

Cell temperature was set to 37°C and the maximal spontaneous ionic conductance was considered the double of the average value.

3. Results and discussion

Starting from the cell respiration equation, it can be deduced that the ATP production is almost linearly proportional with the quantity of incoming oxygen, because the quantity of ATP produced by anaerobic respiration constitutes only few percent of total ATP.

In pathologic conditions the normal ATP regulation mechanism is seriously disturbed. The CCD may reduce the ATP production as not all mitochondria organelles work properly. In presence of hypoxia the decrement of ATP production has a time-varying property. When the organism is partially deprived from oxygen, the strength of aerobic respiration is seriously decreased, so it is more difficult to produce the necessary ATP quantity [14]. In case of low ATP level the low priority ATP-consuming processes, such as protein- and RNA/DNA-synthesis, are almost entirely stopped, while the high ranked ionic pump functionality is maintained as long as possible [15]. It is also observed that mammalian ventricular cells attempt to prevent calcium overload by hyper-polarizing the SR by opening the ATP-sensitive potassium channels [15].

Unfortunately, in presence of severe hypoxia or anoxia, the mitochondria, in order to maintain its surface potential, turns from being ATP producer to potentially powerful ATP consumer.

The lower quantity of ATP induces reduced ionic pump functionality, so the cell is unable to maintain the proper ionic gradients. Not all ionic gradients are equally reduced, the calcium ionic gradient proved to be more sensitive to reduced ATP level [14].

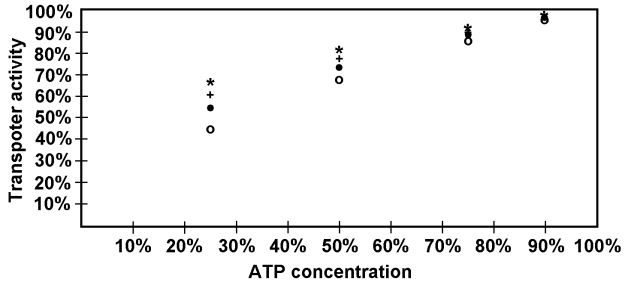

Figure 2. The relation between ATP concentration and ionic transporter functionality for all LR transporters in presence of hypoxia is visualized. The calcium pump of the SR is represented by empty circles (**o**), the external membranous calcium pump by filled circles (●), the calcium sodium exchanger potassium by stars (★) and the sodium potassium exchanger by plus sign (+).

The ventricular cell is the most sensible to sodium gradient changes, it is also highly sensible to potassium gradient modifications, while maintaining the proper calcium gradient is almost as important as the potassium gradient maintenance. By the analysis of LR sensitivity to ionic gradient modifications and ATP regulation process,

it was concluded that the low ATP level has decreased ionic pump functionality.

As presented in Figure 2, the presence of hypoxia reduces the ATP concentration. Table 2 summarizes the In LR model, the ATP concentration decrease subdued the efficiency of ionic pumps, as reflected in Table 1.

Table 1. The decreased ATP concentration caused the following reductions in pump efficiency.

ATP concentration decrease	10%	25%	50%	75%
Calcium pump of the SR	4%	14%	32%	55%
External membranous Calcium pump	3%	11%	26%	45%
Calcium sodium exchanger	3%	10%	22%	39%
Sodium potassium exchanger	2%	8%	18%	33%

Figure 2 shows that in few minutes hypoxia may significantly reduce all ionic pump functionality. The reduced ionic gradients can be tolerated only for a short period, in presence of long-duration hypoxia a self excitation of ventricular cells may occur that develops ventricular fibrillation.

Hypoxia may endanger the patient's life by developing ventricular fibrillation or by a serious damage of ventricular myocardium, caused by cellular necrosis.

4. Conclusion

The malfunction of the calcium regulation mechanism is the principal danger factor in presence of hypoxia or anoxia, and it is the most sensible element during ischemia. Although the altered sodium potassium pump functionality is not considered a dangerous phenomenon, it can disturb calcium regulation, thus indirectly favoring cellular necrosis.

The calcium regulation mechanism is more sensitive to hypoxia than the potassium or sodium regulation.

The new energetic extension of the LR model may throw new light upon the development process of ischemia that can enforce various cardiac rhythm malfunctions such as ventricular fibrillation.

The LR ventricular cell model constitutes a proper tool to simulate various dynamic cell events, such as hypoxia. The ionic gradient modification generated by the improper ionic pump functionality developed in presence of hypoxia may yield dangerous modification in cellular excitation.

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Address for correspondence:

Sándor Miklós Szilágyi
Str. Frantz Liszt nr. 8, 540068 Tîrgu Mureş, Romania
szsandor72@yahoo.com