Hypoxia Modeling using Luo-Rudy II Cell Model

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Abstract

This study is aimed to present the development phases of hypoxia and anoxia using the dynamic Luo-Rudy II (LR) ventricular cell model. This task involves the robustness analysis of the selected cell model in low oxygen level circumstances that alter the ionic conductance properties of the cellular membrane and partially or totally inhibit the ionic pump functionality.

We investigated hypoxia and its effect on activation potential, ionic currents, pumps, exchangers, and ionic concentrations involved in the LR model. 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.

The presence of hypoxia has reduced the ionic pump functionality. Decreasing ionic pump power for 2-minute duration by 10%, 25%, 40%, 60%, 75%, respectively, caused a reduction of sodium ionic gradients by 1%, 3%, 7%, 14%, 19%, potassium ionic gradients by 2%, 5%, 10%, 17%, 23%, and calcium ionic gradient by 6%, 14%, 22%, 32%, 46%, respectively.

The calcium regulation mechanism is more sensitive to hypoxia than the potassium regulation, while the sodium regulation is the most robust among the investigated pump functionalities.

1. Introduction

In developed countries, the sudden cardiac death, mostly caused by ventricular fibrillation, represents the principal cause of mortality. Despite decades of intensive research, the mechanisms responsible for ventricular fibrillation are only partially discovered.

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 [1].

In a healthy organism hypoxia may develop during intensive physical exercises, or in the presence of oxygen deficiency in the atmosphere induced by high altitude. In these cases the arterial oxygen level may decrease substantially, however it rarely drops below physiological levels. Mild hypoxia increases heart and respiration rates.

Hypoxia may also be caused by hypoventilation, pulmonary embolism, methemoglobinemia, carbon monoxide poisoning, histotoxic hypoxia and shunts in the pulmonary circulation [2].

A partial or total occlusion of a coronary artery yields to myocardial ischemia [3]. 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 [1]. If the suffering region is too wide, hypoxia may induce sudden cardiac death [2, 4-5].

In order to understand the development phases of hypoxia, it is imperial to investigate the cellular functionality of the heart. A better understanding of the cardiac cell's biochemical properties enabled the development of various computational models. Several cardiac cell models have been published that describe the functionality of various cardiac cell types [6-9], involving the characteristics of the membrane ionic channels and the characteristics of the cellular electrophysiology.

The large variances of events that may induce hypoxia inhibit the possibility to develop a generalized cell model, capable to treat all pathological cases. In order to handle such an inconvenient situation, we selected the Luo-Rudy II model [6-7] that obeys the following considerations:

- it can model ventricular cells;
- it has a dynamic characteristic to model metabolic and electrophysiological changes;
- includes all major ionic currents;
- may have a good robustness;
- demands relatively low computation power.

Our goal in this paper is to study the development phases of hypoxia and anoxia using the dynamic Luo-Rudy II (LR) ventricular cell model. The rest of the paper is organized as follows: Section 2 gives a detailed description of the studied LR model for normal and pathological cases. We outline the effect on the generated

activation potential (AP) of the various parameter alterations. Section 3 presents and discusses several aspects of the model functionality and the results of simulations carried out using the model. In Section 4, the conclusions are formulated.

2. Methods

2.1. Luo-Rudy II model

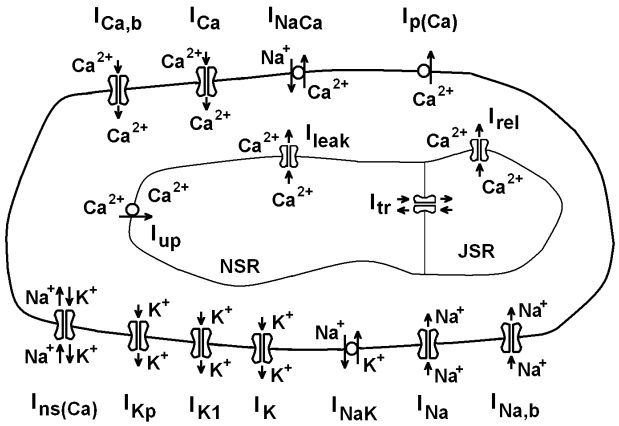

Figure 1. Schematic diagram of the Luo-Rudy II model

The LR model has ionic equation formulated as in the Beeler-Reuter model [10] 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 [6-7]. This model accounts for dynamic changes of ionic concentrations, so several pathological cases can be modeled properly. 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 $[B]$ means 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 1μF/cm^2, and the temperature 37°C.

In 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) or between network SR (NSR) and junction SR (JSR). 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 [7]. 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}.

2.2. Hypoxia and anoxia analysis

Hypoxia induces a mismatch between oxygen supply and demand, and creates altered cell functioning [11]. All ionic currents and the activation potential suffer serious modification [12-13]. 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 [14-15].

Hypoxia in few minutes raises serious ATP deficiency that reduces cell pump functionality and alters ionic concentrations. In this research we have focused on the effects of short hypoxia, considering time varying ionic concentrations, conductance and pump functionality.

All ionic currents, pumps, exchangers, and ionic concentrations involved in the LR model were modeled in time during hypoxia development process.

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. In the presence of acute hypoxia all internal-external ionic concentration gradients were reduced by up to 3 times. We considered that the aggravated external membranous ionic pump functionality was decreased by up to 10 times and the SR Ca^{2+} pump activity was reduced by up to 5 times. 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

The presence of hypoxia has reduced the ionic pump functionality. Decreasing ionic pump power for 2-minute duration by 10%, 25%, 40%, 60%, 75%, respectively, caused a reduction of sodium ionic gradients by 1%, 3%, 7%, 14%, 19%, potassium ionic gradients by 2%, 5%, 10%, 17%, 23%, and calcium ionic gradient by 6%, 14%, 22%, 32%, 46%, respectively.

In case of 5-minute duration, the same ionic pump power reduction led to ionic gradient fall by 2%, 5%, 11%, 26%, 52% (Na^+), 4%, 9%, 17%, 41%, 68% (K^+), and 11%, 19%, 34%, 58%, 75%, respectively (Ca^{2+}).

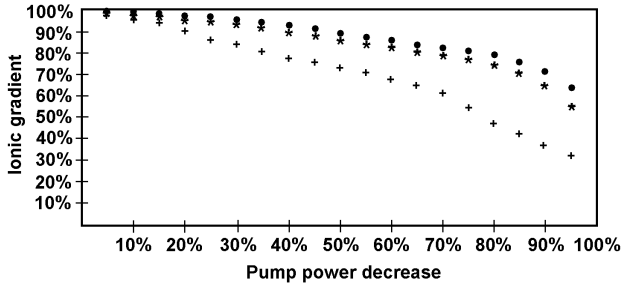

Figure 2. The relation between lower ionic gradients and reduced pump functionality for 2-minute hypoxia duration. Sodium ion is represented by circles (●), potassium by stars (★) and calcium by plus sign (+).

Hypoxia produced a low oxygen level that rendered more difficult to produce the necessary ATP quantity [16]. The lower quantity of ATP induced reduced ionic pump functionality, so the cell was unable to maintain the proper ionic gradients. Not all ionic gradients were equally reduced, the calcium ionic gradient proved to be more sensitive to reduced ATP level. The relation between lower ionic gradients and reduced pump functionality for 2-minute hypoxia duration is visualized in Figure 2, the effects of a 5-minute hypoxia duration can be seen in Figure 3.

Figures 2 and 3 shows that hypoxia induces an extremely low pump functionality that drastically reduces ionic gradients, so the cell can maintain its proper functioning only for few minutes. A relatively low pump power decrease may be supported by the cell for a long period of time without long-term consequences.

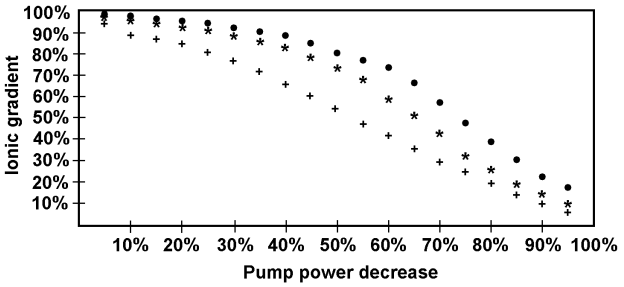

Figure 3. The relation between lower ionic gradients and reduced pump functionality for 5-minute hypoxia duration. Sodium ion is represented by circles (●), potassium by stars (★) and calcium by plus sign (+).

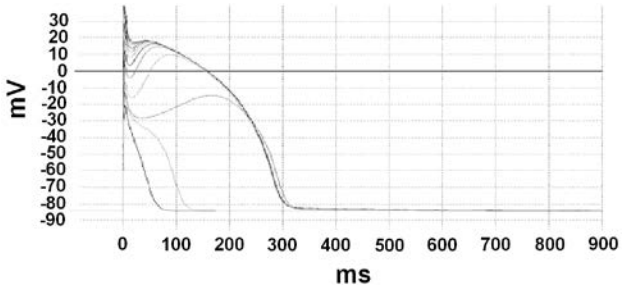

Figure 4. The generated activation potential in case of modified maximal sodium ion conductance. The conductance was lowered up to 10 times.

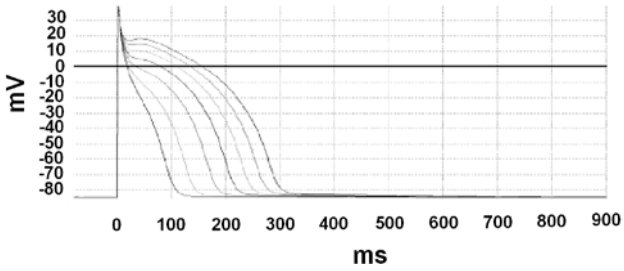

Figure 5. The generated activation potential in case of modified maximal calcium ion conductance. The conductance was lowered by up to 60%.

The LR model contains more than twenty voltage-related constants that in our consideration may depend on external and internal conditions. The effect of all modified parameters on the shape of AP cannot be presented in this paper, so we selected two of them to demonstrate the importance of these parameters.

The modification of the sodium and calcium ion conductance of the cell membrane has a crucial role in the AP (see Figure 4 and Figure 5). We can observe a massive decrease of the rapid sodium spike during depolarization. The 10 times lowered sodium conductance may happen in case of hypoxia and can generate improper heart functionality.

The situation is more dangerous in case of lowered calcium ion conductance. As observed in Figure 5, the conductivity decrease of about 2 times has serous impact on AP shape and cell excitation activity. The low inner calcium concentration of the cells generates a high calcium gradient. If this voltage difference is lowered due to improper ion channel activity, all further cell activity may be inhibited and the cell dies.

Even in case of relatively small duration of hypoxia, the improper cell activity may generate uncoordinated cell excitation and contraction that may cause tachycardia or deadly ventricular fibrillation.

4. Conclusion

The calcium regulation mechanism is more sensitive to hypoxia than the potassium regulation, while the sodium regulation is the most robust among the investigated pump functionalities.

The LR ventricular cell model constitutes a proper tool to simulate various dynamic cell events, such as hypoxia. The ion conductance modification generated by the improper functionality of the ion channels in case of hypoxia may yield dangerous modification in AP function.

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References

[1] Gaur N, Rudy Y, Hool L. Contributions of ion channel currents to ventricular action potential changes and induction of early afterdepolarizations during acute hypoxia. Circ Res 2009; 105: 1196–203.

[2] Fink M, Niederer SA, Cherry EM, Fenton FH, Koivumäki JT, Seemann G, Thul R, Zhang H, Sachse FB, Beard D, Crampin EJ, Smith NP. Cardiac cell modelling: observations from the heart of the cardiac physiome project. Prog Biophys Mol Bio 2011; 104(1-3):2-21.

[3] Shaw RM, Rudy Y. Electrophysiologic effects of acute myocardial ischemia: a theoretical study of altered cell excitability and action potential duration. Cardiovasc Res 1997; 35(2):256-72.

[4] Trenor B, Romero L, Cardona K, Gomis J, Saiz J, Ferrero JM(Jr.). Multiscale modeling of myocardial electrical activity: from cell to organ. In: Gargiulo GD, McEwan A. Applied Biomedical Engineering. InTech, 2011:337-60.

[5] Szilágyi SM, Szilágyi L, Benyó Z. A patient specific electro-mechanical model of the heart. Comput Meth Prog Bio 2011; 101(2):183–200.

[6] Luo CH, Rudy Y. A dynamic model of the cardiac ventricular action potential I. Simulations of ionic currents and concentration changes. Circ Res 1994; 74:1071–96.

[7] Luo CH, Rudy Y. A dynamic model of the cardiac ventricular action potential. II. Afterdepolarizations, triggered activity, and potentiation. Circ Res 1994; 74:1097–113.

[8] ten Tusscher KHWJ, Bernus O, Hren R, Panfilov AV. Comparison of electrophysiological models for human ventricular cells and tissues. Prog Biophys Mol Bio 2006; 90(1–3):326–345.

[9] ten Tusscher KHWJ, Panfilov AV. Modelling of the ventricular conduction system. Prog Biophys Mol Bio 2008; 96(1–3):152–70.

[10] Beeler GW, Reuter H. Reconstruction of the action potential of ventricular myocardial fibres. J Physiol 1977; 268:177-210.

[11] Hool LC. Acute hypoxia differentially regulates K(+) channels. Implications with respect to cardiac arrhythmia. Eur Biophys J 2005; 34:369–376.

[12] Bernus O, Wilders R, Zemlin CW, Verschelde H, Panfilov AV. A computationally efficient electro-physiological model of human ventricular cells. Am J Physiol 2002; 282(6):H2296–308.

[13] Bueno-Orovio A, Cherry EM, Fenton FH. Minimal model for human ventricular action potentials in tissue. J Theor Biol 2008; 253(3):544-60.

[14] Garny A, Kohl P, Noble D. Cellular Open Resource (COR): a public CellML based environment for modelling biological function. Int J Bifurcat Chaos 2003; 13(12):3579-90.

[15] Garny A, Noble D, Hunter PJ, Kohl P. Cellular Open Resource (COR): current status and future directions. Philos T R Soc A 2009; 367:1885-906.

[16] Venkatesh N, Lamp ST, Weiss JN. Sulfonylureas, ATP-sensitive Kq channels, and cellular Kq loss during hypoxia, ischemia, and metabolic inhibition in mammalian ventricle. Circ Res 1991; 69:623–37.

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