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ANIONIC CURRENTS IN HYPOXIA-MEDIATED CARDIAC TOXICITY: A COMPUTER STUDY

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Abstract- Hypoxia-caused modulation of cardiac electrophysiology was modeled by computer simulation. Emphasis was on the effect of activation of anionic channels on the electrical state of the tissue. The model includes implicitly the effect of the presence of reactive oxygen species (ROS) and nitrogen oxide (NO) on myocyte membrane voltage by their contribution to the activation of chloride currents. Three anionic currents were added to the modified Luo-Rudy ionic model of the ventricular action potential used in these calculations. The effect of the activation of the usually dormant currents due to hypoxia results in the modulation of the morphology of the action potential and the ECG. Transition of the ECG to ventricular fibrillation is shown. An important finding reported here is that control of the swelling and protein kinase C (PKC)-activated chloride currents can limit the electrical chaos of pharmacologically-caused hypoxic cardiac toxicity.

Keywords: hypoxia, anionic current, cardiac toxicity, computer model

INTRODUCTION

Anionic currents (8,9,10,12,13) play an important role in the electrophysiology of oxygen deprived tissue. Ischemia as well as pharmacologically-caused hypoxia is characterized by the presence of ROS and NO. ROS, short-lived and extremely reactive, are regulators of cell function by altering the redox state of proteins and ionic channel function. Pharmacological intrusion, such as by cyanide (CN), generates NO that by binding to complex IV of the enzyme cytochrome oxide, changes the mitochondrial calcium flux and diminishes contractility and myocardial energetics. In tandem, ATP content, as measured by nuclear magnetic resonance spectroscopy, drops. NO is responsible for the activation of the CFTR (cystic fibrosis transmembrane conductance regulator) chloride channels on cardiac myocytes that are innervated by nitoxergic neurons that express CFTR and mediate negative inotropy. NO has been shown to inhibit cytochrome oxidase (COX). In addition, NO also affects mitochondrial function and sarcolemmal K_{ATP} when intracellular oxygen level is low, Lamas et al. (15).

Pharmacological intervention by hypoxia-causing substances, such as CN, is manifested in swelling and hemorrhaging of the cells as noted by Suzuki (25). In addition, several usually dormant membrane currents are activated with profound effect on the electrophysiology. CN also activates endogenous protein kinase (PKC) that phosphorylates ion channels and in turn activates the chloride currents $I_{Cl,PKC}$ and $I_{Cl,ATP.}$ The mechanism is subject to debate. Maduh et al. (18) offered "the possibility that rhodanese may be regulated by protein phosphorylation and treatments that alter the phosphorylation state of rhodanese may affect cyanide detoxification via SCN^- formation." Later Maduh et al. (19) observed that PKC inhibitors affected the level of CN toxicity without offering a kinetic mechanism. CN's strong attraction for iron prevents the transfer of electrons to molecular oxygen, in turn negatively affecting the production of adenosine triphosphate (ATP), the energy source of the cell. The decline of the availability of intracellular ATP activates $I_{K,ATP}$ while extracellular ATP, such as released from damaged cells in ischemia, activates $I_{Cl,ATP}$. $I_{Cl,ATP}$ is discussed by Stutts et al. (24) and Matsuura et al. (20). ROS increases permeability of the mitochondrion and thus increase in calcium contents results. Simultaneously calcium overload of the cells is noted with activation of $I_{Cl,Ca}$, (33).

CN has a strong attraction for iron ions in the cell and its attachment prevents the transfer of electrons to molecular oxygen as already noted.

With CN also attached to hemoglobin, the oxygen transporter, the oxygen distribution network is paralyzed. Thus, despite the presence of oxygen in the blood, its interaction with the cells is limited reducing its normoxic function in adenosine triphosphate (ATP) generation and modulation of the energy homeostasis results. Initially glycolysis attempts to replenish the ATP, the energy source, but the replenishment is short lived. Substrate changes include formation of lactic acid and secretion of catecholamines. The stage is set for changes and deviation from the norm in the electrical activity of the tissue.

One of the manifestations of the changed electrophysiology is bradycardia that may soon change to Torsade de Pointes (TdP) and possible culmination in ventricular fibrillation (VF). On the ECG, the P-wave, indicator of atrial depolarization is eliminated, ST-segment deviation, usually a rise in the slope, becomes noticeable followed by modulation and possible inversion of the T-wave. The changed morphology is also expressed in steepening and coalescing of the QRS and the T-waves. A J-wave becomes noticeable.

Previously, Zoltani et al. (32) reported on the effect of cell swelling activated chloride current on a myocyte's electrophysiology. The current computational study expands that model by including $I_{Cl,PKC}$ and $I_{Cl,ATP}$. The following details the predicted and experimentally validated morphological changes in the AP and the ECG. The current work offers insight in how activated chloride currents affects the AP and the ECG of hypoxia-affected cardiac tissue.

MATERIALS AND METHODS

Movement of anionic ions affect the homeostasis of the electrical state of cardiac tissue. Chloride currents play a role in cellular excitability, cell volume homeostasis, pH and apoptosis. Under hypoxic conditions, several chloride currents have important functions, Duan et al. (8), Ackerman et al. (1), Nilius et al. (21,22). To simulate hypoxic condition in cardiac tissue, CN presence in the tissue was hypothesized. CN induces hypoxic conditions. The analysis incorporated three usually dormant currents, the swelling, the PKC and the ATP associated anionic currents. The current-voltage relationship of the cell membrane was obtained from in vitro experiments and were taken as representative of the trends to be expected.

The simulation was carried out on a two-dimensional piece of tissue encompassing 165 cells in either direction. A stimulus, in form of an electric impulse, enters the tissue all along one edge and proceeds toward the opposite side. As it traverses the tissue, it encounters a region that has been affected by the presence of CN. In this region the usually dormant currents, $I_{Cl,PKC,}$ $I_{Cl,ATP}$, $I_{Cl,sw}$ adjust (activate) for the presence of CN.

Hypoxic tissue displays changes in intracellular osmolarity, in turn activates the swelling-activated anionic current, $I_{Cl,sw}$, (2,3). CN also activates protein kinase C (PKC) that in turn activates a chloride channel current, $I_{Cl,PKC}$, (4, 29). The current also depends on the internal and external chloride concentrations. Damage to cells, such as burst cells, can result in release of ATP to the cytosol leading to another chloride current, $I_{Cl, ATP}$, activation.

$I_{Cl,Ca}$ activates in a voltage-dependent way by calcium binding to the ion channel and initiates repolarization. Permeability of the plasma membrane for chloride is affected by the calcium concentration.

The cell membrane models used in the simulation are based on and extend the Luo-Rudy formulation as used in Cardiowave as discussed earlier in Zoltani et al. (30, 31). The hypothesis adopted for these simulations was that the presence of CN created the conditions necessary for the activation of several, normally inactive, currents that play an important role in the disturbance of the homeostatic electrical conditions.

The swelling-activated anionic current was based on the canine data of Baumgarten et al. (2). .

$$I_{Cl,sw} = 0.08(v + 76.0)\exp((0.0055)*(v + 243.0)),$$

where v is the cell membrane voltage.
The PKC-activated chloride current is based on the guinea-pig experimental findings of Walsh et al. (27) where

$$I_{Cl,PKC} = 0.0202v^2 + 5.606v + 91.9907,$$

where v is the membrane voltage. The experimental findings of Turgeon et al. (26) give a value of PKC concentration of 10 to 40 nmol/L. PKC, Kwan et al. (14), as well as extracellular ATP, Qu (23), induce inhibition of calcium channel currents. In fact, Kwan et al. (14) report that 100 µM of extracellular ATP result in a 50% inhibition of $I_{Ca(L).}$ The effect is species dependent. Liu et al. (17) reported positive inotropic effect of extracellular ATP and an increase in L-type Ca^{2+} channel activity. Our model included a 50% reduction of $I_{Ca(L).}$

Guinea-pig data of Matsuura et al. (20) was used to model the ATP-activated chloride current,

$$I_{Cl,ATP} = 0.000055v^2 + 0.009546v + 0.229524,$$

where v is the membrane voltage.

The concentration of the extracellular ATP was taken as ~40nM. Under ischemic conditions, that mirror the presence of CN in the tissue, ATP increases ~10 fold (Ingwall (11).

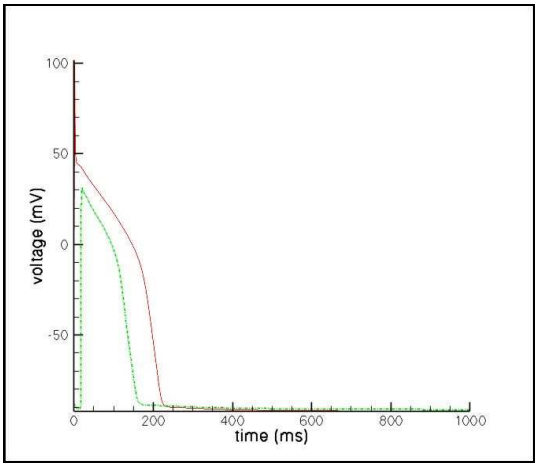
A quantitative tie-in of the amount of NO produced in hypoxia and the change in the membrane currents is at present not possible. The local effect of the enhanced NO concentration are known. Since hypoxia enhances NO production, that in turn inhibits mitochondrial respiration as well as the biosynthesis of cardiolipin that is involved in the production of ATP, the energy balance and processes dependent on it, are affected. Also, NO activates PKC in the heart and inhibits cytochrome oxidase.

The calculations were carried out on Shelton, an IBM p690 SP with 128 CPUs, and JVN, a Linux Networkx cluster with 2048 CPUs, of the Major Shared Resource Center of ARL at APG, MD, using CardioWave. In parallel mode a 32-node calculation required 45 minutes

RESULTS

Cyanide activated anionic currents play a crucial role in hypoxia-caused electrical chaos in cardiac tissue. The present calculations were designed to shed more light on the effect of three anionic currents.

Osmotic changes, that is swelling of the cells, activates $I_{Cl,sw}$. With other anionic currents blocked, $I_{Cl,sw}$ has a profound effect on the electrophysiology of the tissue. The morphology of the action potential of a ventricular myocyte is changed. Notably the heart rate increases (R-R length in the ECG decreases) and the amplitude is lower. The modulation of the electrophysiology caused by 10% activation of the swelling activated anionic current is shown in Fig. 1. The T-wave is inverted, the ST segment is considerably shortened but the QRS of the ECG is not changed significantly.



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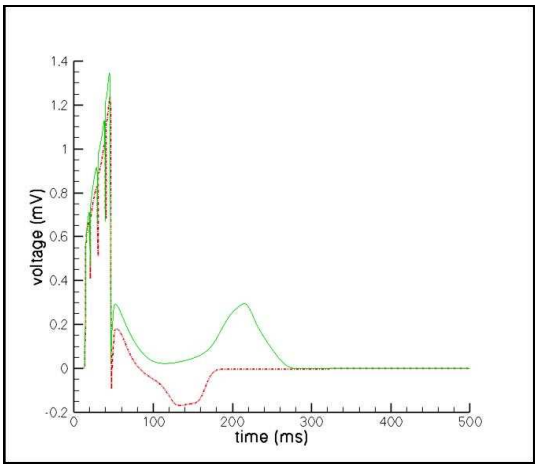
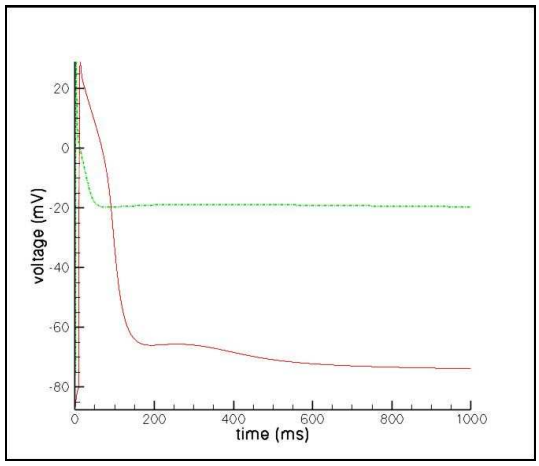


b

Figure 1. (a). The baseline action potential contrasted with the case when 10% of the cell swelling activated chloride channels are active, shown by dot-dashes. The increase in heart rate (shortening R-R) and the decrease in the voltage amplitude are noticeable. **(b).** The simulated baseline ECG and shown by dot-dashes when 10% of the swelling activated anion current is active. The T-wave is inverted, the ST segment is considerably shortened. The QRS of the ECG is not changed significantly.

The PKC-activated chloride current may be even more important. Fig. 2 shows that when only 10% of the receptors are activated, the action potential disappears shown by the dot-dashed line. Note that the voltage does not return to the resting level. At 0.5% activation, the AP amplitude is lower than at the baseline, the wave length is shortened and the new baseline voltage is now 15 mV higher. Also, at 10% activation of the $I_{Cl,PKC}$ the ECG is eliminated. At the 0.5% level, the QRS width is narrowed, the T-wave inverted in contrast to baseline and the amplitude of the QRS is less. All the other anionic currents were blocked.



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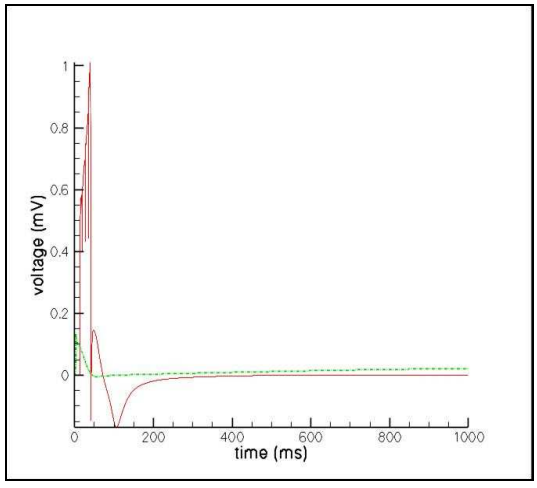

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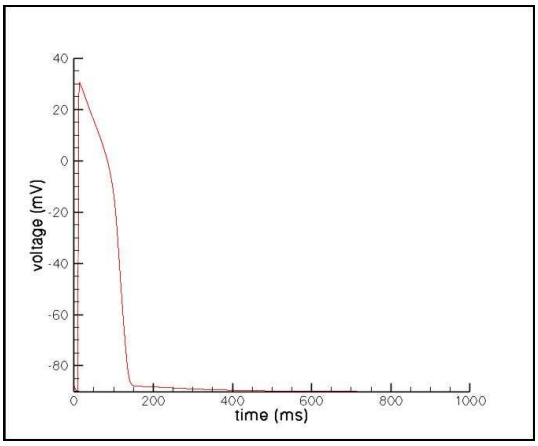
Figure 2. (a). The effect of the PKC activated anionic current. At 10% activation, the action potential disappears shown by the dot-dashed line. Note that the voltage does not return to the starting level. At 0.5% the AP amplitude is lower than at the baseline, the cycle length is shortened and the new baseline voltage is now 15 mV higher. **(b).** At 10% activation of the $I_{Cl,PKC}$ the ECG is eliminated. At the 0.5% level, the QRS width is shortened, the T-wave inverted in contrast to baseline and also the amplitude of the QRS is less. All the other anionic currents were blocked.

The ATP dependent chloride current plays a definite, though much less important role in the hypoxia-induced electrical transformation, see Fig. 3.



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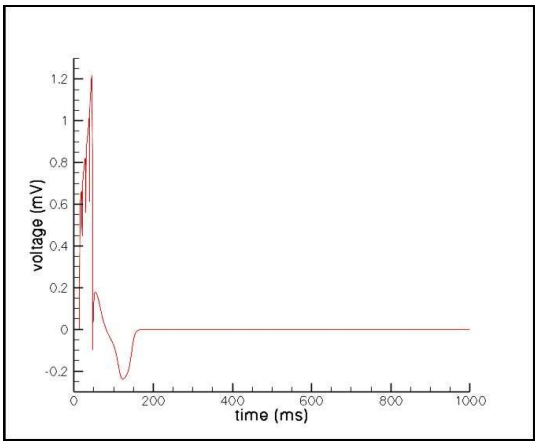

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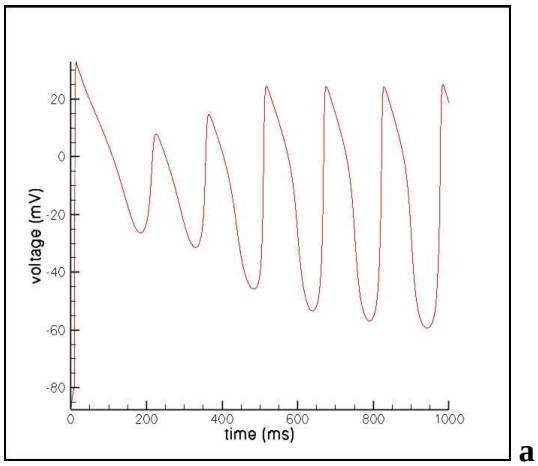
Figure 3. (a). The action potential when only the $I_{Cl,ATP}$ anionic current is active. The amplitude is only half of the baseline and the cycle length is shortened by one third. **(b).** The ECG in this case shows inversion of the T-wave, shortened ST and narrowed QRS.

When all the currents are active, the homeostasis of the electrical state needed for proper functioning is totally destroyed. At even 1% activation, when all three of the discussed anionic currents are present, total electrical chaos can ensue as shown in Fig. 4. Even at 10% activation of $I_{CL,PKC,}$ with other anionic currents blocked, the initial rise of the voltage is shifted left and the voltage fails to return to the starting value, the repolarization does not take place. At 50% activation, not shown here, a second "repolarization" bump is generated, further disturbing the normal electrophysiological events possibly signaling the onset of tachycardia.



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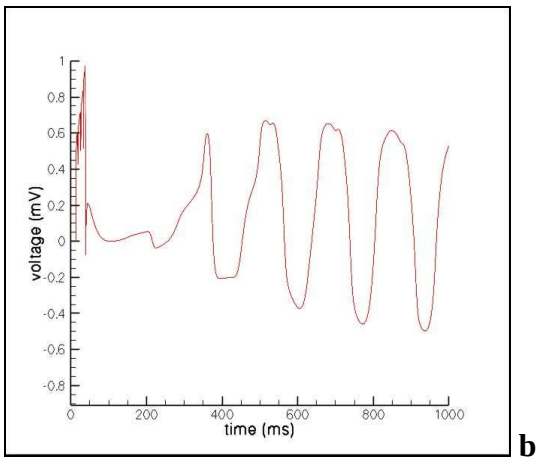

b

Figure 4. (a). The three anionic currents, $I_{Cl,sw}$, $I_{Cl,ATP,}$ $I_{Cl,PKC}$ at 1% activation with the $I_{Cl(L)}$ at 50% of the baseline magnitude. The action potential does not return to baseline, the amplitude is seriously diminished and an oscillating behavior ensues. **(b).** The transition of the ECG to ventricular fibrillation when all three of the anionic currents are activated to 1% is shown.

ECG devolves into irregular oscillations, reminiscent of aspects of ventricular fibrillation when all three anionic, i.e. chloride currents are activated to 1%.

The computer simulation reproduces what has been experimentally surmised and adds new insights. The effect of CN on the ECG on man was reported by Wexler et al. (28), Leimdorfer (16) on cats and monkeys and Cope (5) on dogs. After a brief period of bradycardia, changes in the ST and QRS and modulation with possible disappearance of the T-wave were noted. De Busk et al. (6), in the case of attempted suicide with CN noted progressive ST segment shortening and origin of the T-wave high on the R-wave, analogously to Wexler et al. (28). This paper shows for the first time the adverse effect of the CN-activated PKC current on the electrophysiology of the heart.

Fig. 5 shows for the first time the calculated transition to VF when the three ionic currents are activated. It shows the transition of the ECG to ventricular fibrillation with $I_{Cl,sw}$, $I_{Cl,ATP}$ at 0.1% and $I_{Cl,PKC}$ at 0.01% activation. When the $I_{Cl,PKC}$ activation is raised to 0.05%, the ECG disappears, demonstrated by the horizontal trace.

Pharmacological intervention can improve, in fact under certain circumstances remedy, the electrical chaos. Fig. 6 shows the case when the swelling-activated anionic current is blocked and only the PKC-activated current is present. Note that the T-wave inversion is no longer present, but heart rate is increase and the resting voltage, upper horizontal trace, exceeds 0.25 mV.

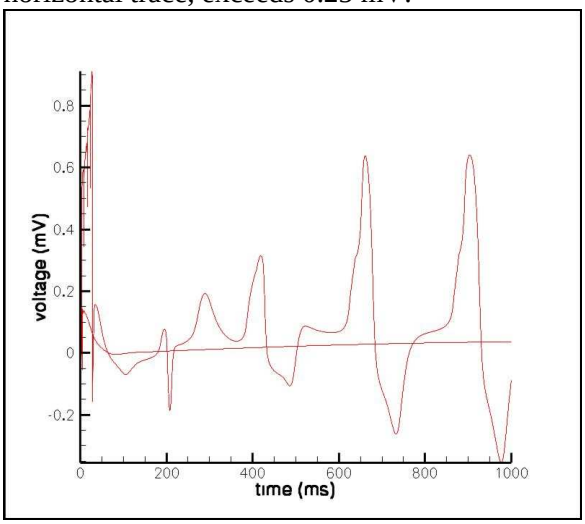

Figure 5. The transition of the ECG to ventricular fibrillation with $I_{Cl,sw}$, $I_{Cl,ATP}$ at 0.1% and $I_{Cl,PKC}$ at 0.01% activation. When the $I_{Cl,PKC}$ activation is raised to 0.05%, the ECG disappears, shown by the horizontal trace.

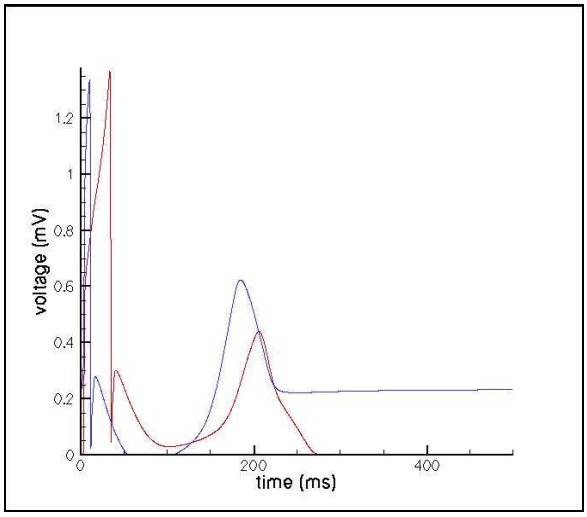

Figure 6. The transition of the ECG to ventricular fibrillation with $I_{Cl,sw}$, $I_{Cl,ATP}$ at 0.1% and $I_{Cl,PKC}$ at 0.01% activation. When the $I_{Cl,PKC}$ activation is raised to 0.05%, the ECG disappears, shown by the horizontal trace.

DISCUSSION

CN-caused hypoxia and tissue anisotropy has an important influence on the electrophysiology of the cardiac tissue. The calculations were intended to shed more light on the importance of anionic currents in hypoxia of cardiac tissue. PKC activation of a small number of available receptors interferes with the homeostatic electrical state of the tissue. Of equal importance are swelling-activated chloride currents. $I_{Cl,ATP}$ contributes, but is of lesser importance in determining anion current influenced outcomes. Our model predicts the modulation in the AP due to the CN activation of $I_{Cl,sw}$, $I_{Cl,PKC}$ and $I_{Cl,ATP}$. The results should be taken as indicator of the expected trends only, since experimental data from several animal models were used in the model. The results reemphasize the importance of the role of chloride currents in the electrical derangement caused by CN in the tissue. *In silico* simulations offer insights into the cellular disparagement under these circumstances.

Control of anionic currents have to play a major role in the pharmacological counter-measures of hypoxia-caused electrical chaos in cardiac tissue.

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