COMPUTER SIMULATION LENDS NEW INSIGHTS INTO CYANIDE-CAUSED CARDIAC TOXICITY

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ABSTRACT

The development of antidotes against cyanide (CN) poisoning for the protection of the warfighter is still hampered by a lack of detailed understanding of the modulation of CN-affected cellular processes. Advances in high-performance computer technology and algorithms now enable computational models of the cellular processes on the newest high performance scalable computers, for the first time, to furnish many of the sought-after answers and complement animal studies.

A mathematical model of the electrophysiology of cardiac tissue, with initial and boundary conditions based on experimental data from studies using CN as metabolic blockers from the literature, was used to simulate changes in the electrical activity of the heart due to the effect of the presence of CN. Emphasis was on the modulation of ion concentrations in the cells, changes in current magnitudes and the activation of currents that are dormant under normal circumstances.

These calculations show for the first time: (1) disturbance of the energy homeostasis and ion concentrations in cardiac tissue due to CN result in the reversal of the direction from the normal and change in magnitudes of cellular membrane currents. These in turn change the morphology of the action potential and the electrocardiogram. This is the initial step leading to ventricular fibrillation, the usual endpoint in the effect of CN on the heart. (2) CN causes cell swelling and hemorrhaging in cardiac tissue. Cell swelling activates chloride membrane currents affecting homeostasis of the tissue. These effects were shown to be important for the electrical state of the CN-affected tissue and were included for the first time in a model of CN affected cardiac tissue. (3) The calculations reproduced aspects of the changes in an electrocardiogram (ECG) of a subject under the effect of a lethal dose of CN. (4) The obtained results suggest and define the characteristics required of a pharmacological intervention needed to overcome or reverse CN poisoning caused ventricular fibrillation (VF) of vital importance for development of therapeutics for force protection. Primary finding of this research is the importance of the swelling activated chloride current, $I_{Cl,sw}$. Blocking this current, while alleviating ionic overloads in the substrate, stops VF.

1. INTRODUCTION

Cyanide is one of the potent poisons used in warfare since ancient times. Development of effective antidotes for the warfighter is still a work in progress. Many of the CN affected cellular processes can now be mathematically modeled, based on experimental measurements, and the effects of changes in cellular parameters due to the presence of threat agents predicted. Only recently, with scalable computers coming on-line, has computational capability improved to the point where such simulations could be attempted. This paper demonstrates the power of *in silico* (computational) experiments as a cost-effective, ancillary means of advancing the development of threat agent antidotes that is an essential part of force protection. We believe that this new approach will have a major impact on future therapeutic developments. See also Zoltani et al. 2003, 2004a, 2004b, 2004c.

The strategy adopted was to use tissue parameter values in the mathematical simulations available from experimental investigations where CN was used as a metabolic blocking agent. The deviations from the baseline morphology in the calculated action potential, expressing the voltage in the tissue as a function of time, and the mathematically generated electrocardiogram, was related to and correlated with the measured tissue parameters. This gives a direct indication of the effects that have the strongest influence on the deviation of electrical state of the tissue from baseline and are the best candidates for pharmacological intervention in case of CN intoxication.

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
00 DEC 2004	**N/A**	**-**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Computer Simulation Lends New Insights Into Cyanide-Caused Cardiac Toxicity	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **U.S. Army Research Laboratory Computational and Information Sciences Directorate Aberdeen Proving Ground, MD 21005-5066; U.S. Army Medical Research Institute of Chemical Defense Pharmacology Division Aberdeen Proving Ground, MD 21010-5400**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release, distribution unlimited

13. SUPPLEMENTARY NOTES
See also ADM001736, Proceedings for the Army Science Conference (24th) Held on 29 November - 2 December 2005 in Orlando, Florida., The original document contains color images.

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**UU**	**8**	

2. EFFECT OF CYANIDE ON CARDIAC TISSUE

Dose-dependent heterogeneity marks the deposition of CN in cardiac tissue. Histological changes include cell swelling and hemorrhaging. CN has a strong attraction for iron ions and its deposition prevents the transfer of electrons to molecular oxygen. Though there is oxygen available, it can not perform the normoxic function in the adenosine triphosphate (ATP) generation needed by the tissue. The tissue is not able to utilize oxygen 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 catecholamines. The stage is set for changes and deviation from the norm in the electrical activity of the tissue.

One of the first manifestations of the changed electrophysiology is bradycardia that may soon change to Torsade de Pointes and possible culmination in ventricular fibrillation (VF). On the ECG, the P-wave, the atrial depolarization, is eliminated, ST-segment deviation, usually a rise in the slope, becomes noticeable followed by modulation of the T-wave. The changed morphology is expressed in steepening and coalescing of the QRS and the T-waves. A J-wave becomes noticeable. Additional details are given in Katzman et al. 1993, and Wexler et al. 1947.

On the cellular level, changes in the ion concentrations become important, especially calcium overload of the cell and increase in the extra cellular potassium concentration, $[K^+]_o$. The cell's energy homeostasis, Balaban 2002, is profoundly disturbed, and several compensatory membrane currents are activated and others diminished. Three of the most important ones are the ATP-dependent I_{KATP}, Elliott et al. 1989, the osmotic swelling-activated $I_{Cl,sw}$, and the calcium-dependent $I_{Ca(L)}$. The disequilibrium in the membrane currents caused by the cyanide has grave implications for the cell's electrophysiology. CN-caused cardiac toxicity shares some commonality with ischemia but is different in the level of acidity of the tissue and the nature of some of the activated currents. A number of ancillary effects, including enhanced catecholamine (CA) secretion, the effect of the increase in free Mg^{2+}, and pH changes are not addressed in this paper but we note that CA binds to α and β receptors that affect membrane currents. Additional details are given in Baskin et al. 1997, 2004, Leimdorfer 1950, and Van der Heyden et al. 1985.

3. COMPUTER MODEL

The effect of the presence of CN in the tissue was modeled by changing the tissue parameters to those measured in CN-caused metabolic blockade of cardiac tissue available in the open literature and including the currents activated under these conditions. Two of the more important ones for this model are the activation of I_{KATP} due to decline in the ATP stores and $I_{Cl,sw}$ when the cell volume is modulated. Change in cellular ion concentrations is also an important aspect of CN toxicity. Calcium overload causes the activation of K^+ channels, Ionue, 1998. Rise in $[Na^+]_i$ and $[Ca^{2+}]_i$ enhance the I_{Ks} current that is activated at voltage values much higher than for I_{Kr} . I_{Kr} is reduced by acidification and the presence of external divalent cations, noticeable in CN-affected tissue.

For these numerical simulations cardiac tissue cells of three kinds were considered: epicardial, midmyocardial and endocardial representing the ventricular wall. The important distinction among these cells for these calculations is in the value of the maximum cell conductance. The model of Vandenberg et al., 1997 for osmotic swelling-activated chloride current was modified and incorporated into the simulations. It accounts for the expression of some of the changes in the membrane currents caused by CN-caused lesions, Suzuki, 1968. Cell swelling contributes to the rise of the resting membrane potential and the shortening of the action potential (AP).

For the calculations reported here, a monodomain approach was adopted with fiber orientation (one of the diffusion matrix entries) assumed to be uniform. The propagation of the action potential was based on the following cable equation:

$$\frac{\partial V}{\partial t} = -I_{ion}/C_m + D\left(\frac{\partial^2 V}{\partial x^2} + \frac{\partial^2 V}{\partial y^2}\right)$$

In this equation, V is the membrane voltage and I_{ion} the transmembrane ionic current, mainly made up from the sodium, potassium, calcium and chloride currents, and pumps and exchangers. D is the diffusion constant and C_m the membrane capacitance. Appended are the gating variables of the ionic channels and equations for the change of the ion concentrations. No flux boundary conditions were used. The computational domain consisted of a fiber of 165 cardiac cells incorporating the regional differences of the tissue parameters in the ventricular wall. The signal propagation was described by a reaction diffusion equation. Explicit Euler method is adequate for the solution algorithm although Crank-Nicholson can give more accuracy.

After determining the baseline in these simulations, the approach was to vary the ion channel conductivities thought to be affected in CN-affected tissue. In addition, a series of simulations were performed increasing the extra cellular potassium concentration, the calcium concentration in the cell and the internal sodium level.

Two additional currents, the ATP dependent potassium current and the cell volume dependent chlorine current were activated in the simulation of severely affected tissue. Using the conditions of Table I, first a baseline action potential and then a pseudo-ECG were calculated. The former used the Luo-Rudy model of the cellular processes as described in Ferrero et al., 1996, Shaw et al., 1997. A pulse of 200 mA/cm^2 of 0.5 ms duration initiated the action potential.

Using the formulation of Plonsey et al., 2000, with the pseudo-ECG electrode at 2.0 cm from epicardium, the extra cellular unipolar potential generated by the fiber in the surrounding field was calculated from

$$\Phi_e = \frac{a^2 \sigma_i}{4\sigma_e} \int \left(-\nabla V_m\right) \left[\nabla \frac{1}{r}\right] dx$$

where a is the radius of the fiber, r the distance from the source to the field point, V_m the transmembrane potential and σ_i/σ_e the ratio of the intracellular to the extra cellular conductivities.

The mathematically-generated ECG was calculated on a strip of cardiac tissue made up of three types of cells, endocardial, midmyocardial (M), and epicardial. A distinctive difference among these cells is the maximum value of the channel conductance for the I_{Kr} and the I_{Ks} currents. The conductance ratios for these cells were set at 11:1, 4:1 and 35:1 respectively following experimentally obtained values, Viswanathan et al., 2000.

4. RESULTS

The electrocardiogram and the action potential morphologies are markers of the electrophysiological state of cardiac tissue. The baseline, Figure 1 with the insert showing the epicardial action potential, was the starting point in these simulations. The parametric values of the substrate were modified within the guidelines of Table I, using data from Carmeliet 1999, Antzelevitch et al., 2003, Lukas et al., 1993, Mejia-Alvarez et al., 1992 and Ju et al., 2003 in a series of calculations.

Figure 2 shows the effect of CN intoxication on the AP of ventricular tissue. Notable are the shortened cycle length (CL), a sign of tachycardia, the lowering of the wave amplitude and the rise of the resting potential. Figure 3 shows the effect on the ECG, whose morphology has been significantly altered. A T-wave as a separate entity is no longer in evidence, the shape of the QRS portion has been altered and the peak has been halved. With the chloride current activated, a further deviation of the shape of the wave from the norm, Figure 4, occurs.

The calculations reproduced aspects of the changes in an electrocardiogram (ECG) of a subject under the effect of a lethal dose of CN, Figure 5 from Wexler et al., 1947. The change in the morphology is reproduced as shown in Figure 6.

A series of simulations to determine the effect of the individual parametric changes are summarized in Figure 6 concentrating on the effect of intracellular calcium overload, reduced availability of ATP and high external potassium concentration. The red trace shows the effect of a 50% overload of internal sodium. The blue curve represents potassium and calcium overload. The green ECG trace shows the effect of the activation of the chloride channel under ion concentration overload conditions. The reversal from normal direction of the membrane currents was noted in the data.

Disturbance of the energy homeostasis and ion concentrations in cardiac tissue due to CN result in the reversal of the direction from the normal and change in magnitudes of cellular membrane currents. These in turn change the morphology of the action potential and the electrocardiogram. This is the initial step leading to ventricular fibrillation, the usual endpoint in the effect of CN on the heart. Several of the membrane currents reverse direction under these tissue conditions. The negative trending T-wave in the ECG indicates pathological behavior, abnormal repolarization of the ventricle.

CN causes cell swelling and hemorrhaging in cardiac tissue. Cell swelling activates chloride membrane currents affecting homeostasis of the tissue. These effects were shown to be important for the electrical state of the CN-affected tissue and were included for the first time in a model of CN affected cardiac tissue. Pharmacological intervention can reverse effect of CN intoxication as shown in Figures 7-8. Figure 9 shows that in addition to the restoration of ion concentrations of the cardiac tissue, blocking the swelling-activated chloride current, $I_{Cl,sw}$ is needed to terminate VF. There are several drugs that block $I_{Cl,sw}$.

5. DISCUSSION

Exposure to CN has immediate consequences on the electrophysiology of the heart. This computational study focused on several of the determining factors characterizing the morphology of the action potential and the ECG.

CN changes the energy homeostasis, ion concentrations and causes the activation of several membrane currents. These include I_{KATP}, $I_{Cl,sw}$ and the throttling of $I_{Ca(L)}$. Their inclusion into the model

presented is vital for the simulation of the CN-caused ECG modulation. Considerable refinements in the results were possible by inclusion into the model of the swelling-activated chloride current. The simulation was able to approximate changes observed in the ECG of a subject under CN toxicity. The calculations showed the rise in the resting voltage and demonstrated how it could be restored to baseline. Means of recapturing the width of the AP post CN modulation, important for preventing arrhythmia, was also illustrated. These approaches also allow modification of the ST segment and J-wave modulations caused by CN. The ST changes seen in the experimental data was clearly reproduced in the model, Figure 6. The QRS widening and modulation of the wave is also seen in the calculational results, for example Figure 3. Parametric changes, as shown in Figures 7-8 can change the morphology of the AP and the electrophysiology of the tissue. The vital step, shown here for the first time is the blocking of the swelling-activated current, shown in Figure 9.

In silico techniques are powerful ancillary tools for the understanding of the modulation of cellular processes by threat agents. The results of this study narrow the search on the requirements on the means of pharmacological intervention to counter the effect of cyanide-caused cardiac toxicity. Of special importance may be the restoration of ATP, potassium and calcium concentrations in the ventricular cells, increase of the level of $I_{Ca(L)}$ and modulating of $I_{Cl,sw}$.

ACKNOWLEDGMENTS

It is a pleasure to thank Dr. John Pormann of Duke University for his advice in the use of Cardiowave. Special thanks go to the U.S. Army Research Laboratory Major Shared Resource Center (MSRC) at Aberdeen Proving Ground, MD for the use of its high performance computer assets, including the SP-4.

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Table I

	Baseline	CN-affected Cell	Ischemia
Model Quantity	(mM)	(mM)	(mM)
$[Na^+]_i$	10.0	Incr. ~2.5 x	10 - 20
$[Na^+]_o$	145.0	134.0	140.0
$[K^+]_i$	150.0	125.0	125.0 (acidic env.)
$[K^+]_o$	4.0	10.0	4 -16
$[Ca^{2+}]_i$	0.0003	Incr. >3 x	0.0003 – 0.0009
$[Ca^{2+}]_o$	1.8	2.0	~2.0
[ATP]	5.0	3.0	3.0 <

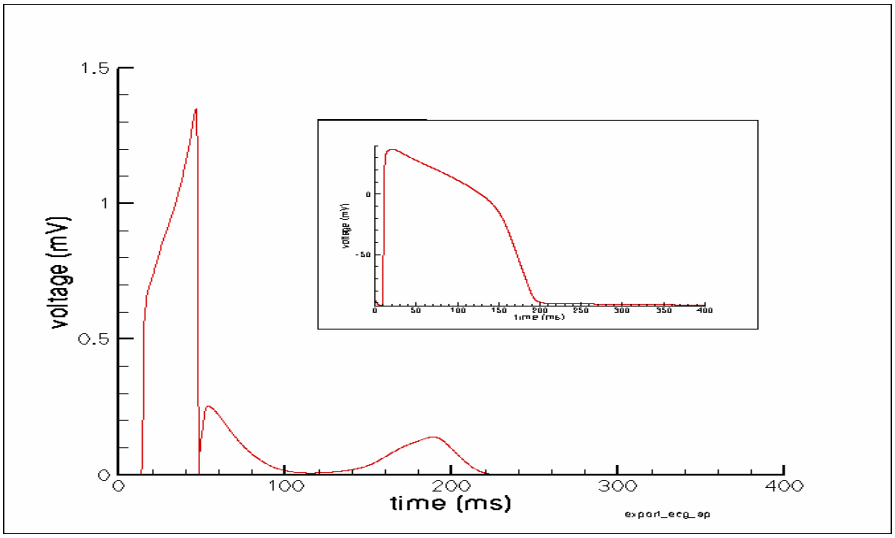

Figure 1. The baseline mathematically generated ECG of the cardiac tissue with action potential shown in the insert.

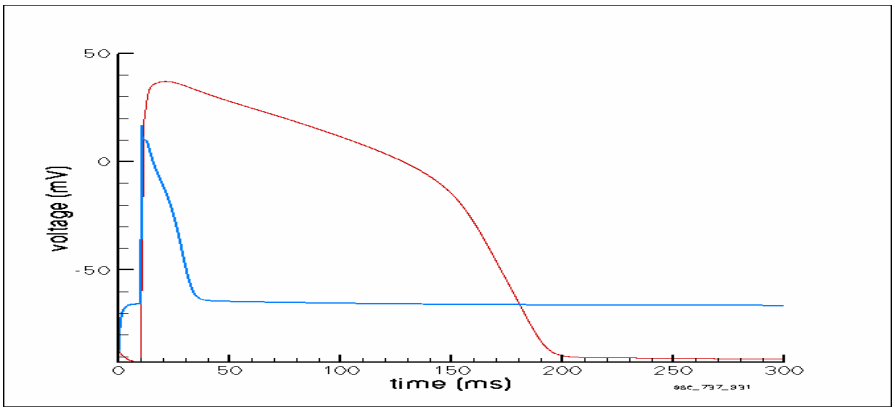

Figure 2. CN notably shortens the cycle length of affected tissue (blue trace) in comparison with the baseline (red trace).

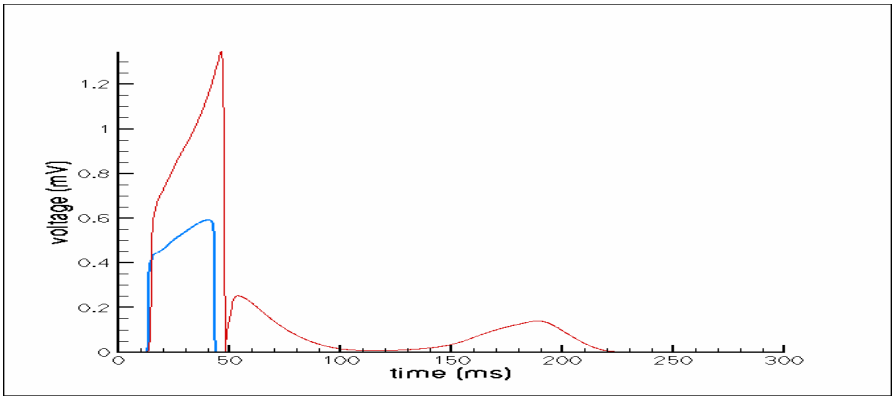

Figure 3. Simulated ECG (blue trace) of severely CN-affected cardiac tissue. $[Ca^{2+}]$ = 0.0009, [ATP] =1.0, K^{+}_{o} =12.0 mM/L. The baseline is shown in red.

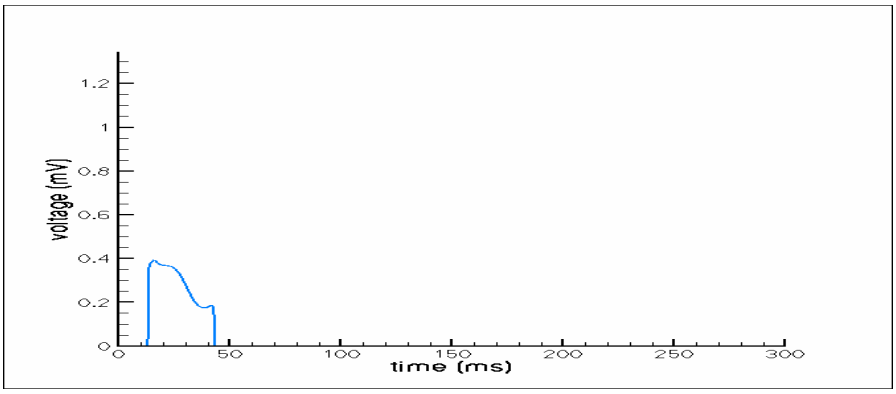

Figure 4. When the normoxic dormant currents are activated, further morphological changes in the ECG are evident.

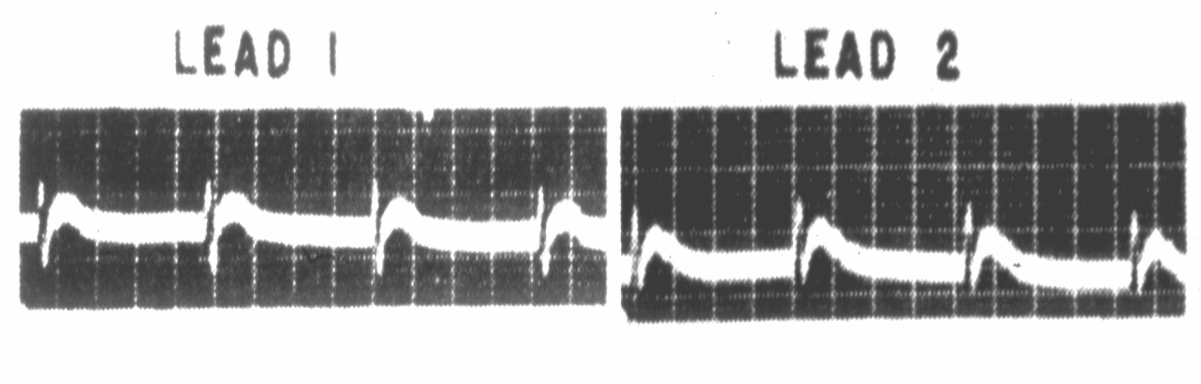

Figure 5. ECG from a CN intoxicated individual from Wexler et al. (1947).

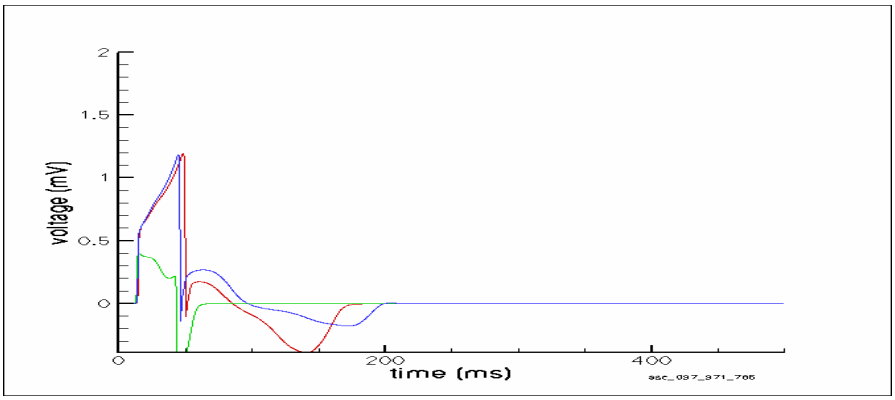

Figure 6. The effect of calcium overload, reduced availability of ATP and high external potassium concentration. The red trace shows the effect of a 50% overload of internal sodium. The blue curve represents potassium and calcium overload. The green ECG trace shows the effect of the activation of the chloride channel under ion concentration overload conditions. The reversal from normal direction of the membrane currents was noted.

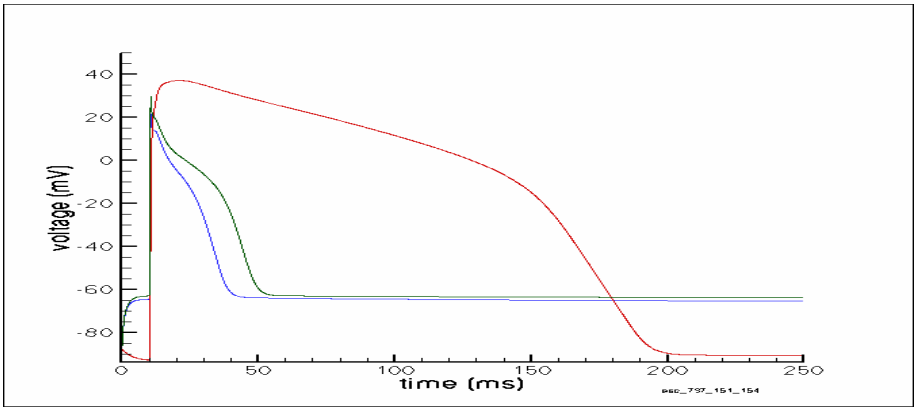

Figure 7. The sodium concentrations returned to normal and with internal calcium at 0.0004 mM/L, the CL is improved but tachycardia still is in evidence.

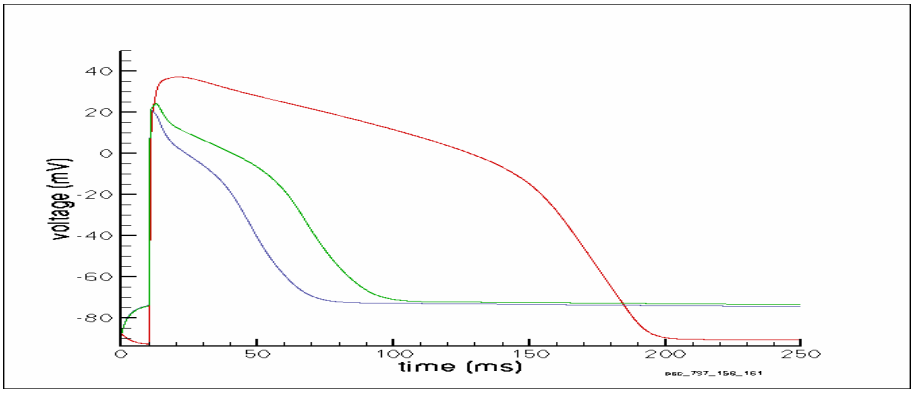

Figure 8. Elimination of the exterior potassium overload helps (green curve), but baseline behavior is not achieved.

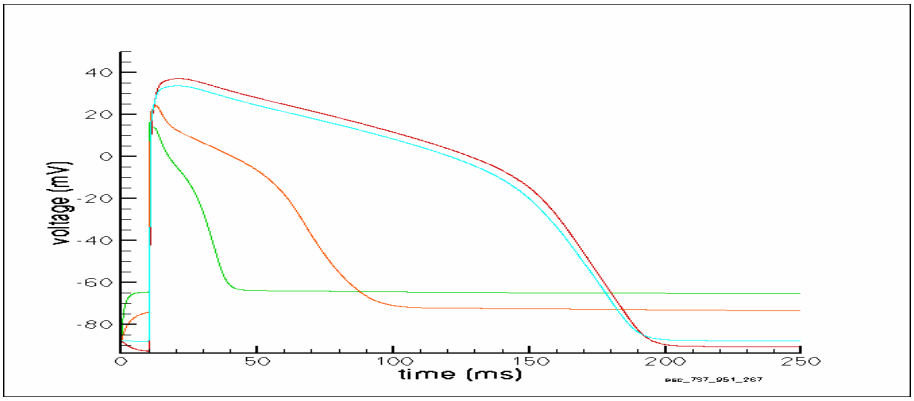

Figure 9. Blocking the swelling-activated chloride current stops the tachycardia and restores baseline-like conditions, light blue curve from that of CN-affected state, shown in green. The orange curve shows that ion concentration changes help, but do not restore baseline conditions.