# Organophosphonate-Induced Toxicity: Computer Study of Reentry in Atrial Tissue

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## Abstract

*The electrophysiologic changes of organophosphonate (OP)-produced acetylcholine (ACh) overload in atrial tissue was studied by computer simulation. Changes in the action potential dynamics due to the heterogeneity of the substrate resulting from OP deposition in the tissue were produced, and the conditions for the generation and persistence of reentry waves were determined. It was shown that modulation of cardiac cell membrane currents can be used to modulate conditions leading to reentry suggesting avenues to prophylaxis.*

## 1. Introduction

Organophosphorous compounds comprise both nerve agents and insecticides which are highly toxic substances with severe cardiovascular ramification upon exposure. An estimated 98% of the contacting agent is distributed within and is transported by the vasculature and is primarily deposited in tissues of the cardium and thoracic areas. For a review see Baskin and Whitmer [1], Roth et al. [2], and Hassler et al. [3].

Toxicity is manifested when organophosphonate (OP) binds to AChE preventing ACh hydrolysis and resulting in an accumulation of ACh in the tissue. This overload causes negative inotropy and arrhythmia not only in the atria (bradyarrhythmia), but also in the Purkinje fibers, and produces electrical conduction anomalies. In addition, the absence of cholinergic agonists increases the calcium influx and stimulates its uptake in the sarcoplasmic reticulum (SR), thereby increasing the intracellular calcium. Accompanying this process is an increase in the vagal tone in the presence of high norepinephrine produced by reflex mechanisms that may contribute to the generation of atrial fibrillation. Cholinergic stimulation of atrial tissue reproducibly induces atrial fibrillation (AF) [4 - 7].

The physiological events that culminate in AF upon cholinergic stimulation are only incompletely understood. Some of the known factors ancillary to AF are

(1) non-uniform shortening of the myocardial refractory period [7],
(2) production of intra-atrial conduction delays,
(3) bradycardia that increases susceptibility to reentry,
(4) shortening of the atrial action potential duration,
(5) cholinergic stimulation may be a sine non quo for the preparation of the subtrate where atrial reentry may occur,
(6) inhomogeneous repolarization may be necessary for atrial arrhythmia,
(7) heterogeneity of the substrate.

OP toxicity is accompanied by elevation of the concentration of $[K^+]_o$, the extracellular potassium concentration that influences the transmembrane resistance in Purkinje fibers and $[Na^+]_o$ whose presence mimics ischaemia. In the atria, a biphasic behavior of conduction velocity as a function of potassium concentration has been noted. Moderate elevation of extracellular potassium (up to 8 mM) increases the conduction velocity; but in the concentration range of 8-16 mM, it progressively decreases, and the eventual termination of conduction is observed. Changes in the action potential due to toxicology-produced shifts in potassium concentration also form the basis of an updated model of soman-produced cardiac toxicity [8].

The following sections describe a computer study of the effect of ACh overload on the electrophysiology of atrial tissue, especially the processes, through reentry, leading to AF.

## 2. Methods and materials

### 2.1. Prior work

Two models of AF have been advocated the rapidly discharging focus and reentrant excitation. In this study we discuss the latter, where atrial fibrillation is manifested by rapid, irregular action potential waves of variable wavelength and timing [9-11] along reentrant paths extinguishing and reestablishing themselves. These irregular waves prevent the proper functioning of the atria due to the shortening of the refractory period and the loss of the normal lengthening of refractoriness when the heart slows down. The wave of excitation returns to the tissue it

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previously exited and reexcites it, hence the name reentry. Until quite recently, it was believed that below a certain mass of atrial tissue reentry, one of the forms of AF could not be sustained. However, the work of Wakimoto et al. [12] showed that even in murine atria, AF is inducible. The breakup of the spiral waves signals the transition from tachycardia to fibrillation.

Moe [13] and his collaborators were the first to provide a mathematical description of atrial fibrillation. Several electrophysiological models of the atria, such as those of Ramirez et al. [14], Lindblad et al. [15], and Nygren et al. [16], based on the Hodgkin and Huxley formalism and patch clamp data, are now available. These models allow *in silico* studies of the effect of changes in the membrane currents caused by pathological conditions and their effect on the subsequent changes in the action potential to be studied without resort to *in vivo* experiments.

## 2.2. The simulation setup

The dynamics and integrity of the action potential propagation in atrial tissue, 3 cm x 3 cm in size, whose electrophysiological behavior is described by the Nygren et al. model [16], was studied. For the computations, the tissue was represented as a slab of 300 x 300 x 1 nodes. The stimulus was applied to the left hand edge and consisted of a pulse of 40 $\mu A/cm^2$ of 2 ms in duration. The presence of OPs was modeled by square patches of tissue having a $[K^+]_0$ 10.8 mM in the first case and then 25.0 mM in a single patch centrally located in the second case instead of ambient 5.4 mM. This is based on the observation that hyperkalemia is a manifestation of OP toxicity. This is coarse graining the problem since localized sodium and calcium concentration deviations due to OP presence are also indicators of the change of the state of the cell. Pacing was used to ensure stable initial conditions.

## 3. Computational approach

Cardiac tissue is usually modeled as a bidomain, that is as two interpenetrating domains representing the inside and the outside of the cell, separated by a membrane through which the current transits from one domain to the other. In each of these domains, the potential is described by

$$\nabla \cdot D_i \nabla \phi_i = \beta I_m - I_{si} \qquad (1)$$

$$\nabla \cdot D_e \nabla \phi_e = -\beta I_m - I_{se}. \qquad (2)$$

Here, the $\phi_i$ and $\phi_e$ are intracellular and interstitial potentials, $D$'s are the diffusion tensors, and the $\beta$ is the cellular surface to volume ratio. $I_m$ is the membrane current, and $I_{si}$ and $I_{se}$ are the current source densities. When the diffusion tensors of the two domains are proportional to each other, as was assumed here, the bidomain equations simplify to the monodomain model. Monodomain calculations are considerably less expensive to perform. For the calculations reported here, fiber orientation (one of the diffusion matrix entries) was assumed to be uniform.

Two other sets of equations need to be solved: equations describing the gating of the ion channels and equations for the concentration changes of the ions. Generically, gating equations may be written as follows:

$$dy/dt = (y_\infty - y)/\tau_y, \qquad (3)$$

where y represents a gating variable and $\tau_y$ the time constant. The model also relies on the solution of ion concentration equations. For calcium, for example,

$$d[Ca]_i/dt = -10^{-4} I_{si} + 0.07(10^{-4} - [Ca]_i). \qquad (4)$$

The quantities in the square brackets are the ion concentrations and $I_{si}$ represents the L-type $Ca^{++}$ current. No-flux boundary conditions were enforced at the edge of the tissue.

The solution consists of three steps: (1) calculation of the ionic currents, $I_m$ , (2) the determination of the transmembrane potential $V_m$, the difference between the intra and the interstitial potentials, and (3) the upgrading of the field potentials. In step (3), conjugate-gradient was the solution method used.

The calculations were carried out with the code CardioWave on the computer assets of the Major Shared Resource Center (MSRC) at Aberdeen Proving Ground, MD.

In parallel mode, a typical calculation on 16 nodes of the Origin 3000, with 300 MHz IP27 processors and data cache size of 32 Kbytes, required 7 hr and 15 min non-dedicated time. This time was improved with a larger number of processors, but the speedup was not linear.

## 4. Results

Figure 1 shows the process leading to reentry, a precursor to AF in atrial tissue. Panels (a)-(c) show the action potential, entering from the left and traversing the tissue with potassium overload in a 1 $cm^2$ region starting at the left boundary along the axis of the tissue. An identical, high potassium concentration region was located along the lower boundary starting 1 cm from the left boundary. The action potential is seen to accelerate

in the regions of high potassium concentration and, as seen in panel (c), undulation of the front curvature appears, sign of incipient breakup, and precursor of atrial fibrillation.

Figure 2 shows the action potential encountering a cardioplegic, 1 $cm^2$. region of high potassium concentration in the tissue. Note the considerable, a 270% increase, in the wave velocity. In Figure 3 the action potential encounter with a high potassium concentration is shown when a partial block of $i_{Kr}$, and $i_{Ks}$ in the tissue is present. This gives an indication of the effect of drugs like azimilide that block $i_{Kr}$ and $i_{Ks}$, thereby prolonging APD and counteracting reentry and the transition to AF.

## 5. Discussion

In this study we examined the conditions, in terms of membrane currents, that promote atrial reentry and which in turn can be modulated to terminate reentry of OP-affected cardiac tissue.

One of the underlying assumptions was that OP-induced ACh overload results in patches of tissue with $[K^+]_0$ in excess of normal values. When an action potential encounters such a region, reentry can occur. Conditions through the modulation of membrane currents were sought that could block reentry. This is important, since it is believed that the morphology of AF is a progression from tachycardia, through the breakup of spiral waves in the atrial tissue, to fibrillation.

### 5.1. OP-toxicity modeled as extracellular potassium concentration

Changes in $K^+$ ion movement are central to the expression of OP-induced cardiac toxicity. An ancillary aspect is that the accumulation of $K^+$ ions in the interstitial space may play an important role in the overall behavior. The reasoning is as follows: excessive parasympathetic stimulation results in excessive ACh release. ACh binds to $M_2$ muscarinic receptors, activates the G-protein, and contributes to the hyperpolarization of the SA node. When ACh activates $i_{K,ACh}$, $K^+$ exits the cell. The OP-induced ACh overload results in more receptors than usual being bound, also leading to an increase in the external concentration of potassium ions.

At high $[K^+]_0$, similar to the condition of ischemia or during strenuous exercise, opening of less than 1% of $i_{K,ATP}$ channels causes significant shortening of AP.

Excessive concentration of potassium ions changes the concentration gradients and the response of the Na-K pump. In the initial stages of the accumulation of the potassium ions, the cells are more excitable, but as the membrane potential drops below the excitation threshold, the generation of an action potential is no longer possible. Hyperkalemia suppresses automaticity of the heart slows conduction, and leads to bradycardia.

### 5.2. Modulation of reentry in the atria

Qu et al. [17] noted in their study of reentry in ventricular tissue that the condition for spiral wave breakup, and thus the condition for VF, was reached when the slope of the action potential restitution curve exceeded 1. Flattening the restitution curve can exert an anti-fibrillatory effect.

In the atrial tissue studies reported here, modulation of the potassium currents can modify the processes leading to reentry. This points to Class III drugs that prolong APD as possible candidates for treatment of OP-caused atrial arrhythmias.

## 6. Summary

Computer experiments to simulate reentry in atrial tissue with OP-induced ACh overload were performed. The presence of the OP was modeled as local potassium overload. The experiments showed the transition of the wave to the precursor to atrial fibrillation. It was demonstrated that modulation of membrane current, by blocking some of the potassium channels, affects changes leading to reentry. This suggests possible new approaches to prevention and treatment of OP toxicity. Planned experiments in the near future should help to validate this approach.

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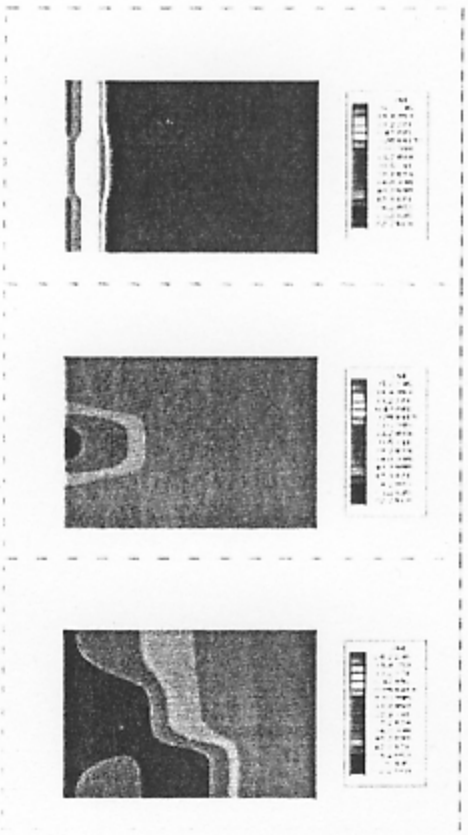

Figure 1. Action potential in atrial tissue with two subregions of $[K^+]_0$ equal to 10.8 mM.

Figure 2. Action potential encountering a cardioplegic region of high potassium concentration.

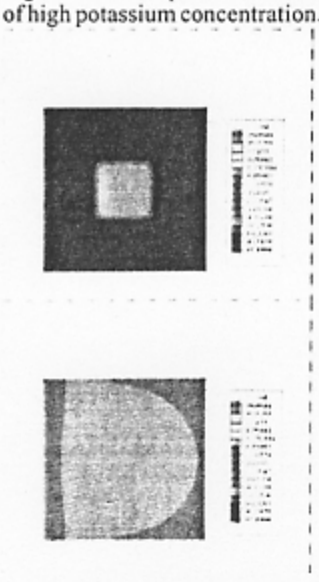

Figure 3. Effect of partial block of $i_{Kr}$ and $i_{Ks}$ shown in the lower panel at t = 300 ms.