Literature review: Drug-induced pro-arrhythmic risk via after-depolarisations.

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July 19, 2013

Contents

1 Introduction

Many therapies are withdrawn very late in the process of drug discovery due to side-effects that affect the normal cardiac rhythm, potentially causing fatal arrhythmias. This adds to the already considerable attrition rate of new drugs in the pharmaceutical industry [1]. Many drug compounds interact in some way with the cardiac ion channels, sometimes preventing the passage of ions. Since the beating of the heart is related in a complex and non-linear way to the actions of different individual ion channels in the many cell types and tissues found within the heart, predicting the effects of a drug block from experimental data is a non-trivial task. Computational modelling of the heart can be used to link the action of individual ion channels to behaviour at the cell level, the tissue level, and even over the whole heart, meaning that it is a potential method for predicting drug-induced cardiac side-effects.

In this review of the literature, I will first discuss the physiology and electrical systems of the heart, including the individual contribution of a selection of the many types of ion channel involved, followed by a brief history of the computational models of excitable cells, beginning with the first nerve cell model created by Hodgkin and Huxley over 60 years ago. Finally, I will discuss some of the mechanisms by which arrhythmias are thought to occur, and some recent modelling studies which have predicted arrhythmogenic risk from ion channel drug block experimental data.

2 Biological background

2.1 Physiology of the heart

Blood flow around the human body is maintained by the rhythmic and orderly contraction of each of the four chambers of the heart. The circulatory system is vital for the transport of oxygen and nutrients to all of the parts of the body. Defects in heart rhythm can cause fainting, low blood pressure and, in some cases, sudden death.

In a single cardiac cycle, first, blood flows into both atria of the heart from the pulmonary vein and the superior vena cava. Increasing pressure inside the atria forces the mitral and tricuspid valves to open, allowing blood into the ventricles. The walls of the atria then contract, forcing blood into the ventricles. The rising pressure in the ventricles closes the mitral and tricuspid valves and opens the aortic and pulmonary valves. The ventricles then contract from the bottom to the top, squeezing blood out of the heart. Blood from the right side of the heart is pumped into the lungs to be oxygenated, while the left side of the heart is used to supply the circulatory system of the rest of the body. The order and pace at which these events occur is vital for correct heart rhythm.

2.2 Electrophysiology of the heart

The contraction of the heart is triggered by a wave of electrical activity that travels from the group of pacemaker cells at the top of the right atrium. These cells are known as the sinoatrial node. The signal travels down through the muscle cells (myocytes) of both atria, causing them to contract. It then travels to the atrioventricular node at the centre of the heart and down, through the Bundle of His and the Purkinje fibres, to the bottom of the ventricles. Finally, the signal travels up the ventricles,

causing the myocytes in the walls of the ventricles to contract, squeezing blood out of the heart [23].

When an electrical current is carried in a wire, the charge is carried by electrons. In a biological system, charge is carried by ions in solution. The electrical signal that originates in the sinoatrial node is caused by the movement of charged ions across the cell membrane of the sinoatrial node cells. Ion channels, pumps, and exchangers are formed of membrane proteins which are specific to particular ions. These allow passage of ions from the inside to the outside of the cell, and vice versa, as well as transport between intracellular compartments. Mathematical cardiac electrophysiological models commonly only consider voltage-dependent gating of these ionic currents; however, the currents can also be dependent on stretch, temperature, ATP, and methylation. Currents formed of sodium, potassium, calcium, chloride, and some other ions are involved in the process of electrical excitation.

In the sinoatrial node, thanks to a complex interplay between different voltage-gated ion channels in the cell membrane, the membrane potential oscillates rhythmically over time, as in Figure 1.

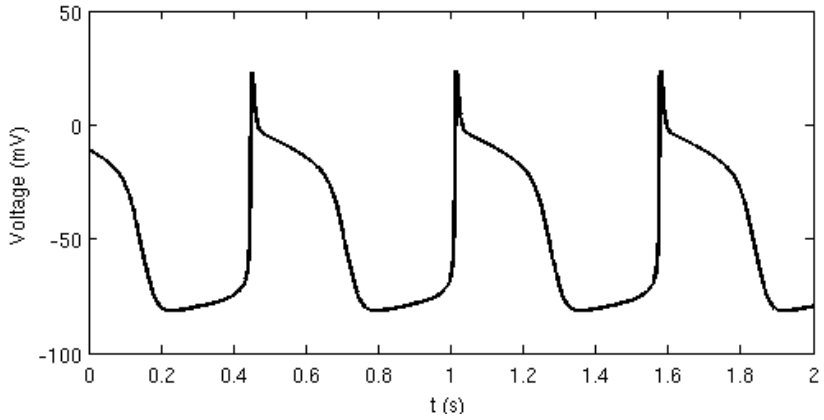

Figure 1: Graph of the action potential in a pacemaker cell [15]. Simulation performed in COR [8] using CVODE.

The atrioventricular node and Bundle of His all show oscillating currents in isolation, and can act as secondary pacemakers if the sinoatrial node is not functioning. The rest of the cells of the heart, however, have a stable resting voltage, as in Figure 2. This means that in the absence of stimulation, the cells will not depolarise.

2.3 Sodium channels

The initial depolarisation at the beginning of an action potential is caused by the opening of sodium channels in the membrane, allowing positive sodium ions to flood into the cell. The sodium channels are voltage-gated, meaning that when the membrane voltage reaches a particular threshold, they open. This voltage change is triggered, in a non-pacemaker cell, by a current of ions from neighbouring cells via “gap junctions” - holes in the cell membrane. The ensuing flood of sodium ions makes the inside of the membrane less negative with respect to the outside, giving the membrane a positive voltage. Once the voltage has reached its maximum point, the voltage-gated

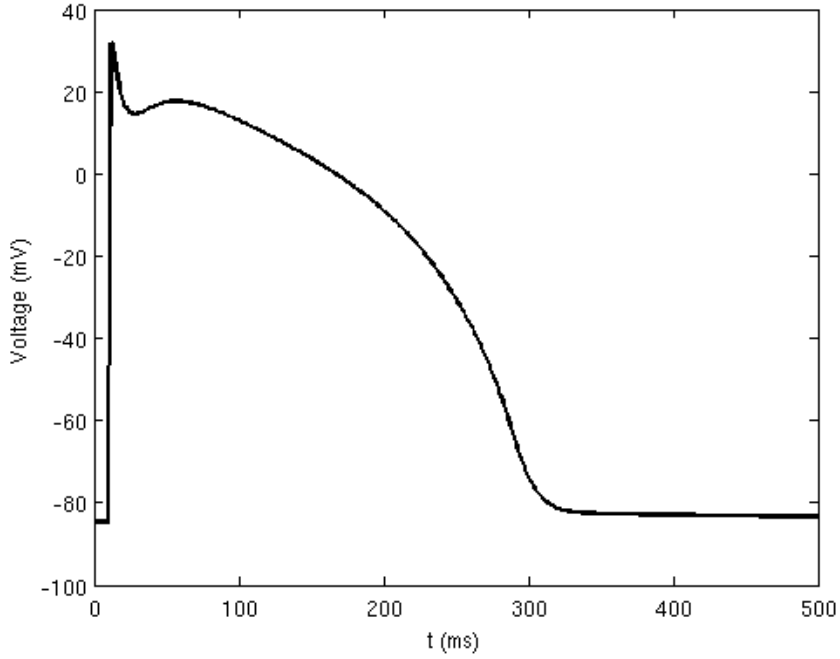

Figure 2: Graph of the action potential in a ventricular cell [3]. Simulation performed in COR using CVODE.

sodium channels are inactivated, preventing them from opening again until the action potential has finished.

2.4 Potassium channels

The repolarisation of the cell after the inital depolarisation is largely mediated by the action of the potassium channels. When potassium channels open, they allow the positive potassium ions out of the cell, returning the membrane voltage to its original resting value. The first channels to open are the transient outward potassium channels, which repolarise the cell during the early stage of repolarisation. These are followed by the opening of the slow rectifying delayed potassium channels, which are active during the plateau phase of the action potential. The rapid rectifying delayed potassium channels are responsible for the fast repolarisation of the cell after the plateau phase.

2.5 Calcium subsystem

The long-lasting (otherwise known as L-type) calcium channels are also triggered to open by the increase in membrane voltage during depolarisation. Through these calcium channels, positive calcium ions flow into the cell, slowing repolarisation and causing the plateau phase of the action potential.

All muscle cells contain a store of calcium ions inside a membrane-bound organelle called the sarcoplasmic reticulum. The membrane of the sarcoplasmic reticulum contains ion channels, called ryanodine receptors or RyR, which are specific for

calcium ions. These channels will allow calcium ion release from the sarcoplasmic reticulum only when there is a high local concentration of calcium ions on the cytoplasmic side of the membrane. This mechanism, termed "calcium-induced calcium release" (CICR) is what allows the electrical excitation of the cell to be linked to contraction.

2.6 Homeostasis

In a cardiomyocyte, once the membrane voltage has reached its resting value, the outward potassium channels all close, leaving only the inward potassium channel active. Additionally, in order to maintain the levels of sodium and potassium inside and outside the cell that are necessary for the correct electrochemical gradients to cause ion flow, the sodium-potassium pump in the cell membrane uses ATP to exchange two potassium ions for three sodium ions. This creates a high concentration of sodium outside the cell, and a high concentration of potassium inside the cell.

In pacemaker cells, once the membrane has fully repolarised, the membrane potential slowly rises until it reaches the threshold for the opening of the sodium channels and another action potential is triggered. This slow depolarisation is caused by a mixture of sodium and calcium ions flowing into the cell (the so-called "funny current"), and the resistance of the membrane to potassium ions increasing, stopping the repolarisation.

The sarco/endoplasmic reticulum calcium ATPase (SERCA) is a calcium-specific pump which transports calcium ions back into the sarcoplasmic reticulum after the cell has finished contracting, restoring calcium levels within the sarcoplasmic reticulum, ready for the next contraction [16].

2.7 Excitation-contraction coupling

The contraction of muscle cells is caused by the action of myosin on long filaments of a protein called actin. Myosin is a motor protein, meaning that it uses ATP to create movement in only one direction. Myosin binds to actin filaments and "walks" along the filament. Actin filaments and myosin heads are arranged in the cell such that the movement of myosin pulls actin filaments towards the centre of the cell, bringing the rest of the cytoskeleton with them, and causing the cell to contract. When a muscle cell is at rest, the myosin-binding sites of the actin filament are obscured by a protein complex called troponin, preventing contraction. The calcium ions released from the sarcoplasmic reticulum in CICR bind to troponin, allowing it to move away from the actin filament's active sites and allowing myosin heads to bind, causing contraction.

3 History of electrophysiological models

3.1 Hodgkin-Huxley squid giant axon model

The original electrophysiological model on which all cardiac models have been built was created in 1952 by Alan Hodgkin and Andrew Huxley [9]. They described the electrical signal that passes down the giant axon of a squid when it is excited. The giant axon, which is large enough to be manipulated easily, controls the water jet propulsion system of a squid.

The basis of this mathematical model is an understanding of the cellular features as components in an electrical circuit. The membrane of a neuron acts like a capacitor, which means that ions accumulate on one side of the membrane, allowing it to store charge. This makes one side of the membrane more positive than the other side, leading to a voltage across the membrane.

The flow of ions across the membrane acts as an electrical current. Three types of ionic current are considered in this model: the sodium (Na^+) ions that flow into the cell and cause depolarisation of the membrane, the potassium (K^+) ions that flow out to repolarise the membrane, and the "leak" current (a mixture of ions, including chloride ions), which flows in both directions.

The electrochemical gradients that power the flow of each type of ion are modelled as batteries, and the ion channels that permit ions to pass are represented by variable resistors.

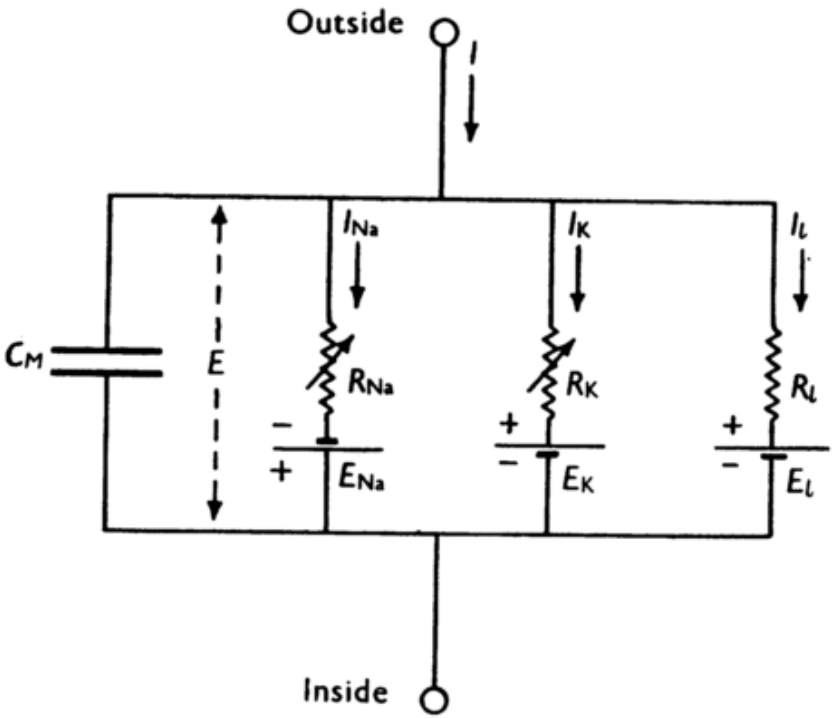

Figure 3: Diagram of circuit from Hodgkin & Huxley's paper

I is the stimulus current that the cell receives from outside.

I_{Na}, I_K, and I_l are the sodium, potassium and leak currents, respectively.

C_m is the capacitance of the membrane (i.e. its ability to store charge).

E is the membrane voltage.

R_{Na}, R_K, and R_l are the resistance of the membrane to letting each type of ion through. These values are usually quoted in terms of the conductance (g) of the membrane, where $g = \frac{1}{R}$.

E_{Na}, E_K, and E_l are the membrane potentials at which the flow of sodium, potassium or leak ions (respectively) through the membrane is zero.

Since the current across the capacitor depends on the change in voltage over time and on the capacitance ($I_{Capacitor} = C_m . \frac{dV}{dt}$), and the four components of

the current (that are wired in parallel) all add up to the stimulus current ($I_{stim} = I_{Capacitor} + I_{Na} + I_K + I_l$), the model's central differential equation can be created.

$$\frac{dV}{dt} = \frac{I_{stim} - I_{Na} - I_K - I_l}{C_m} \tag{1}$$

Where $V = E - E_{resting}$, i.e. the difference between the current membrane voltage and the usual, or "resting" voltage.

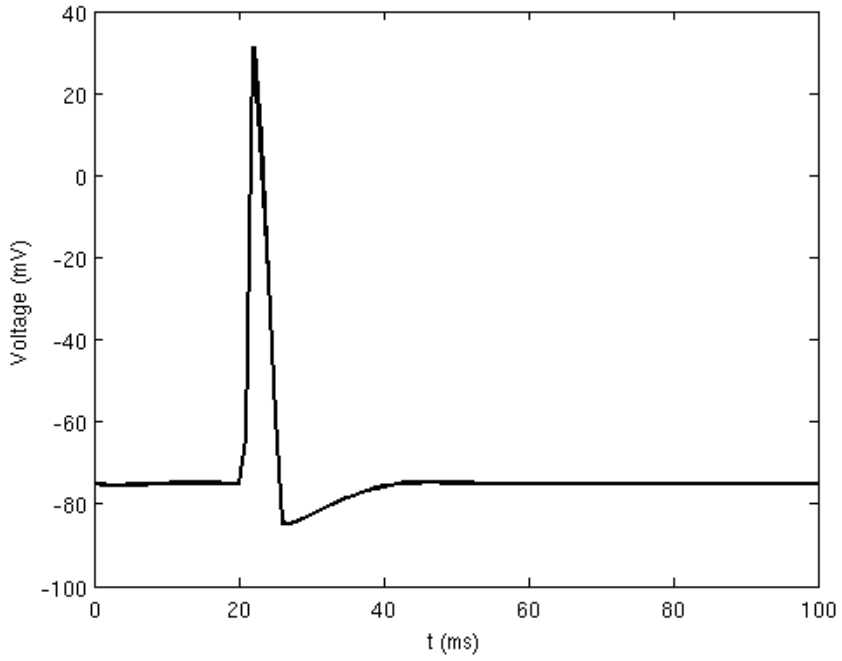

Figure 4: Graph of the action potential in a nerve cell [9]. Simulation performed in COR using CVODE.

3.1.1 Gating

One of the most interesting features of Hodgkin & Huxley's 1952 paper are its descriptions of a possible mechanistic basis for the permeability of the cell membrane to ions. This paper was written long before ion channels were discovered and characterised in mammalian cells, so the authors have modelled permeability as a function of charged particles that bind to ions and allow them to move across the membrane.

The opening, closing and inactivation of the ion channels is vital for the characteristic shape of the action potential in a nerve cell, as in Figure 4. The action of the ion channels in nerve cells are different to those in cardiac cells and proceed in the following way:

1) Sodium channels open and sodium floods into the cell (g_{Na} increases).

2) Potassium channels open, allowing potassium out of the cell (g_K increases).

3) Sodium channels are inactivated when the membrane voltage is at its highest ($g_{Na} = 0$).

4) Potassium channels close and sodium channels reactivate when the membrane voltage reaches its lowest ($g_K = 0$ and g_{Na} increase).

This means that the sodium and potassium conductances vary in a complicated way with changes in the membrane voltage. The "leak" current I_l is simply directly proportional to the membrane voltage.

$$I_l = \bar{g}_l(V - V_l) \tag{2}$$

The changes in conductivity for the sodium and potassium channels are modelled as "gates". The gates are represented by a dimensionless variable, which can be between 1 (fully active) and 0 (fully inactive). The rate of change of a gate x with time is defined as:

$$\frac{dx}{dt} = \alpha_x(1 - x) - \beta_x x \tag{3}$$

where α_x is the rate of the gate transitioning from inactive to active, and β_x is the reverse.

Unlike the rest of the model's features, the gating of the ion channels is not worked out from first principles. Instead, equations were fitted to match empirical data from experiments. Hodgkin and Huxley took measurements of each ionic current separately, while keeping the membrane voltage constant, over a variety of different voltages, and used the data to find α and β expressions for each gate as functions of voltage.

For the potassium channel, there is only one type of gate, called the "n" gate. The permeability of the membrane to potassium varies with the 4th power of n.

$$I_K = n^4 \bar{g}_K(V - V_K) \tag{4}$$

For the sodium channels, there are two types of gate. The m gate being active encourages flow of sodium ions through the membrane, but the h gate being active inactivates the sodium channels. I_{Na} is described by the following equations:

$$I_{Na} = m^3 h \bar{g}_{Na}(V - V_{Na}) \tag{5}$$

With these equations, the characteristic action potential of the nerve cell could be recreated, as in Figure 4.

3.2 Noble's Purkinje cell model

The seminal Purkinje fibre cardiac cell model was published in 1960 by Denis Noble [14] [15]. The sodium and leak currents in this model act in much the same way as in the original Hodgkin-Huxley formulation, with different rate constants. The potassium current is split into two components, which are modelled as rectifiers pointing in opposite directions. This means that the potassium ions flow easily into the membrane through the K1-type channels but not in the other direction, and potassium ions flow easily out of the cell through the K2-type channels, but not in the other direction. The two types of channel are active at different points during the cardiac cycle - the K1-type channels instantaneously become less active when the membrane is depolarised, and the K2-type channels become slowly more active when the membrane

is depolarised. The K2 equation is gated in a similar way to Hodgkin and Huxley's potassium channel n gate.

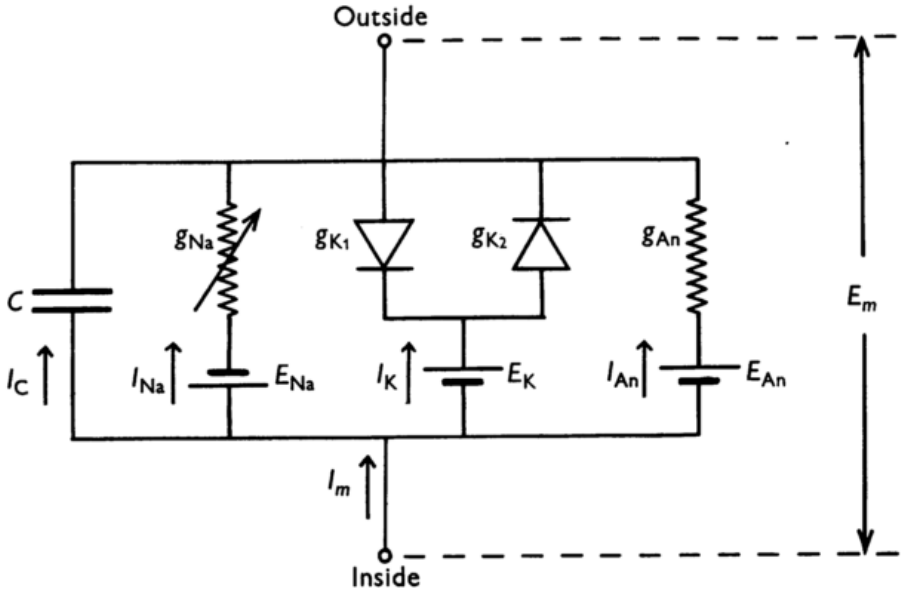

Figure 5: Diagram of the circuit from Noble's 1962 paper

The conductance of each chanel is calculated as follows:

$$g_{K_1} = 1.2e^{\frac{-E_m-90}{50}} + 0.0015e^{\frac{E_m+90}{60}} \tag{6}$$

$$g_{K_2} = 1.2n^4 \tag{7}$$

These equations recreated the characteristic action potential of a Purkinje cell, as in Figure 1.

3.3 Subsequent heart cell models

In the 50 years since Noble's initial model was created, over a hundred cardiac cell models have been made, which use similar formulations for the behaviour of cardiac cells. Significant additions to the original model include the addition of the calcium subsystem, which was first introduced in the model created in 1975 by McAllister, Noble and Tsien [11]. This model added the calcium ion channels in the external membrane of the cell, as well as extra potassium ion channels.

The first model to allow change in the intracellular and extracellular concentrations of ions was created by DiFrancesco and Noble in 1985 [6]. This model also included the sodium-calcium and sodium-potassium exchangers on the outer membrane of the cell, and a mixed, two-way current of sodium and potassium ions (the "funny" current, which is responsible for the slow depolarisation of pacemaker cells).

Recent models have included the calcium subsystem in great detail (including T-tubules, the sarcoplasmic reticulum, RyR, the sarco/endoplasmic reticulum calcium ATPase (SERCA) and calcium-induced calcium release), as well as new formulations

for ion channel gating based on Markov models, and mechano-electric feedback in the form of stretch-activated current.

Additionally, cell models have been linked together to form 1-dimensional fibre models, and 2-dimensional tissue models, all of which have been linked together to form whole-heart models, and even to extrapolate upwards to the whole human, recreating the signature shape of the electrocardiogram [17].

4 Cellular substrates for arrhythmia

In heart cells in which the ion currents are perturbed from their normal state, through mutations, drug effects, or health conditions (for example, congestive heart failure), the rate of aberrant action potentials is higher. In some cases, the action potential can fire prematurely, or fail to fire on stimulus. Inappropriate depolarisation after the initial depolarisation can be classified as "early afterdepolarisation" (EAD) if the depolarisation occurs before the cell is fully repolarised, or "delayed afterdepolarisation" (DAD) if the depolarisation occurs after the cell is fully repolarised, but before the next external stimulus.

Abnormal electrical activity of individual cells in the heart can sometimes trigger a chamber-wide event called an arrhythmia, in which the heart beats too quickly, too slowly, or in the case of fibrillation, not at all. Arrhythmias are a common cause of sudden death. While arrhythmias can be caused by abnormal electrical activity at the tissue level, they can also be triggered by afterdepolarisations [20].

Torsades-de-Pointes is a particular type of arrhythmia that originates in the ventricles and is characterised by an unusual QRS complex, which appears as though it has been twisted around the "baseline" of the electrocardiogram.

EADs have long been linked to arrhythmias in patients with conditions which affect the action potential duration [24]. Such conditions, known as "Long QT syndromes" are characterised by an altered electrocardiogram, with an increased time between the Q and the T waves. Long QT syndromes, which can be congenital or triggered by drug interactions, are associated with a higher risk of arrhythmias, including Torsades-de-Pointes and ventricular tachycardia (abnormally fast beating), or ventricular fibrillation.

The action potential is also lengthened in patients with heart failure. This leads to a higher incidence of sudden death from ventricular fibrillation in these patients. There is some evidence to suggest that arrhythmias in heart failure are triggered by DADs in response to catecholamines (a family of signalling molecules that includes adrenaline) [20].

4.1 Delayed afterdepolarisations

A study by Fink, Noble, and Noble in 2011 [7] used cellular models to determine the factors that cause delayed afterdepolarisations (DADs) in a range of models that spanned multiple species and cell types. They examined particularly the role of intracellular calcium concentration, the sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA), the ryanodine receptor (RyR), and calcium concentration within the "dyadic subspace", a region of the cytosol that lies between the sarcoplasmic reticulum and the T-tubules.

DADs are a transient phenomenon, making it more difficult to find the parameter values that produce them. As well as provoking DADs in the whole cell model, sustained calcium oscillations in the isolated calcium subsystem of models were produced. Calcium oscillations were found for all models which showed DADs, and all models that showed calcium oscillations also showed DADs. The calcium subsystem of each model (comprising the sarcoplasmic reticulum, intracellular calcium, and calcium sequestering molecules such as calmodulin) was isolated from the whole cell model, by setting all outer membrane currents to zero and setting the intracellular concentrations of other ions to constant values. Since DADs, by definition, occur when the membrane potential is constant, and are triggered from inside the cell, the involvement of other membrane currents should not be necessary for the initial mechanism.

It was found that 23 models out of the 37 under investigation could produce DADs under a range of conditions. Preventing RyR from opening when the L-type calcium channel is closed was found to be a model feature which prevents DADs from occurring.

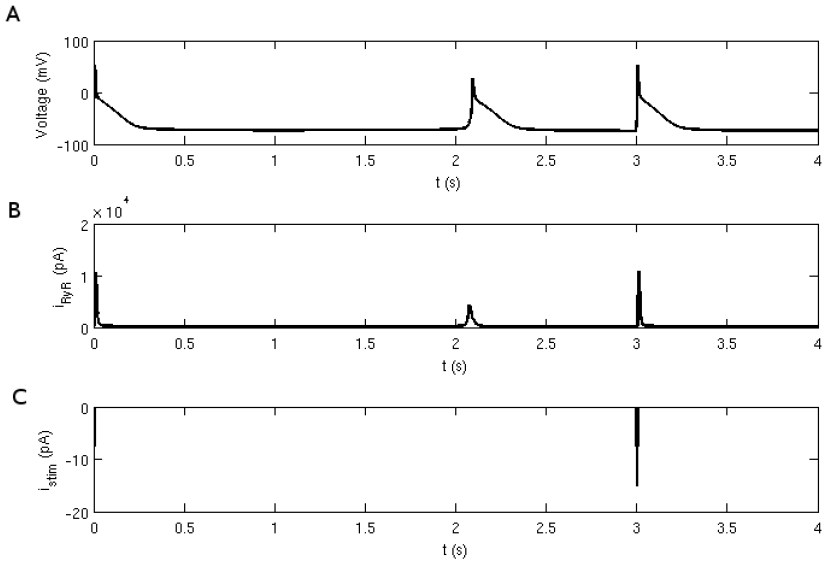

Figure 6: A DAD occuring in the Maleckar09 model, induced by increasing RyR's open probability by 50% [10]. A) shows the membrane voltage, B) the calcium current through RyR, and C) the stimulus current. The second depolarisation is a DAD, and occurs in the absence of stimulus. Simulation performed in COR using CVODE.

4.1.1 Contribution of sarcoplasmic reticulum calcium concentration

While excessively high calcium concentration (or "calcium overload") in the sarcoplasmic reticulum had previously been thought to be the main trigger of RyR opening, causing DADs, decoupling of the RyR channel opening from the sarcoplasmic reticulum calcium concentration allows DADs to occur, indicating that the sarcoplas-

mic reticulum's calcium concentration may not be the main causal factor. The concentration of calcium in the dyadic subspace was found to be vital for RyR opening in these experiments.

Similarly, by modulating the calcium levels in the models, the opening of the RyR channels was found to be more sensitive to changes in dyadic calcium than in sarcoplasmic reticulum calcium.

In agreement with experimental data [5], an increase in activity of SERCA was shown to protect against DADs. This also points towards the importance of the dyadic subspace calcium concentration in DAD formation, since SERCA pumps calcium ions into the sarcoplamic reticulum from the cytosol, thus removing calcium from the dyadic subspace.

However - given that, for a DAD to occur, large amounts of calcium must be released from the sarcoplasmic reticulum - the paper concluded that sarcoplasmic calcium was still a vital ingredient for the formation of DADs. Instead of triggering calcium-induced calcium release, the sarcoplasmic reticulum calcium concentration determines the amplitude of the DAD.

4.1.2 DAD mechanism

While calcium release from the sarcoplasmic reticulum is necessary for DADs, it is not always sufficient. The actions of the sodium-calcium exchanger and the calcium-activated chloride current could have depolarising effects by providing a transient inward current.

Additionally, blocking of the sodium-calcium exchanger was shown to prevent intracellular calcium oscillations under some cellular conditions, but modifying some parameters could bring back the oscillations, indicating that the state of the cell is significant in this mechanism.

The mechanism for DADs that was suggested by the authors is as follows: the cellular concentration of calcium is high, including in the dyadic subspace. This leads to RyR channels opening, triggering calcium-induced calcium release, the amplitude of which depends on the calcium concentration in the sarcoplasmic reticulum. This causes the cell to depolarise prematurely.

4.2 Early afterdepolarisations

Early afterdepolarisations, which happen before the cell has repolarised, have been thought to be caused by a reduction in the cell's capacity to repolarise - a phenomenon called "reduced repolarisation reserve". This can be caused by a net increase in currents into the cell, a net decrease in currents out of the cell, or a mixture of both. In this section, I will discuss two papers that have used simulation to probe the particular cellular currents that have an effect on EAD formation.

Qu *et al* [21] used non-linear dynamical theory to explore why not all conditions and drugs that promote action potential duration prolongation cause EADs, by examining the relationship between a theoretical current I and the membrane voltage V. The authors discussed the need for a quasi-equilibrium state (termed the *p-state*) for the current at the plateau voltage - i.e. $I = 0$ at a voltage other than at the resting membrane potential of the cell.

This means that if the membrane potential is close to the p-state - within an area called the "window region" - the membrane potential will oscillate around the p-state voltage rather than returning to the resting potential, causing EADs. In addition to a quasi-equilibrium state, the current in question must be active when the voltage is in the window region. This means that any "gates" on the current must not be closed or inactivated during the time whent the voltage reaches the window region. The speed at which gates open and close is also vital to the production of EADs. A current with a steep steady-state activation curve will make oscillations around the p-state, and thus EADs, more probable. Below, I will discuss in more detail the ramifications of these findings for different types of current.

Noble, Noble & Garny [19] used a more holistic approach to study the effects of perturbing cellular currents in the context of biologically-relevant conditions, particularly concentrating on extracellular potassium concentration and on ion channels which are known to be affected by mutations or drug blocks.

4.2.1 Sodium channels

Sodium channels are inactivated when the membrane potential reaches its highest point. This allows the membrane to repolarise. The channels must then recover from inactivation before the membrane can depolarise again. Certain sodium channel gene mutations cause faster recovery, delaying repolarisation and making EADs more likely. This mutation can be represented in cardiac models by modifying the h-gate for the sodium channels, shifting the inactivation curve by a certain voltage.

In the Noble paper [19], by shifting the inactivation curve of the sodium channels, 84% of simulations produced an EAD. To mimick the drop in blood potassium levels after strenuous exercise (hypokalemia), the extracellular potassium levels were lowered [12]. This increased the EAD incidence to 91%. A third protocol involving sodium ions was the addition of or increase in the persistent sodium current. This modification lead to an EAD rate of 86%.

4.2.2 L-type calcium channels

The L-type calcium channel is responsible for the slow inward calcium current during repolarisation. L-type calcium channel blocker drugs are commonly used for their anti-arrhythmic properties. In the Qu paper [21], increasing the window current of the L-type calcium channel is found to promote quasi-equilibrium states, and thus EADs, at the plateau voltage. Similarly, reducing the window current can eliminate EADs.

In the Noble paper [19], the L-type calcium channel's conductance was increased to attempt to provoke EADs. This modification yielded EADs in 73% of simulations. Since an increase in the conductance of a channel causes a proportional increase in the window current, this finding matches the results from the Qu paper. In addition, to mimic conditions found in the body during (rather than after) vigorous exercise, simulations were also performed with an increased L-type calcium channel conductance as well as an *increased* extracellular potassium concentration (a condition known as hyperkalemia). These conditions reduced EADs, with only 67% of simulations showing EADs.

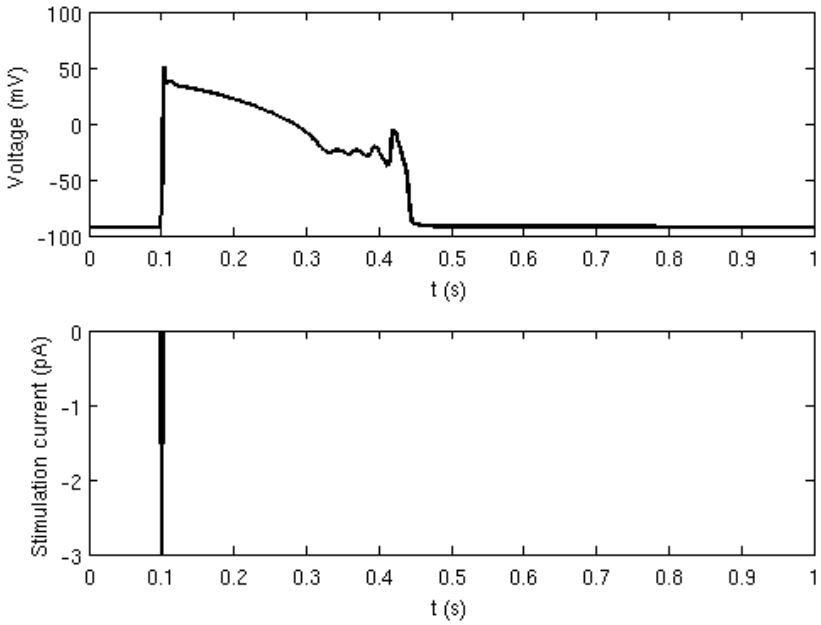

Figure 7: EAD occurring after fast sodium channel inactivation curve shift of 18mV in the Noble98 model [18]. The EAD begins at around 0.3 seconds. Simulation performed in COR using CVODE.

4.2.3 Potassium channels

The rapid rectifying potassium current (I_{Kr}), which in humans is coded for by the hERG gene, is of particular interest when discussing drug-induced arrhythmias, due to its role in acquired long QT syndrome [4]. Drugs which interact with hERG channels are unlikely to make it to market; however, many hERG blockers do not cause arrhythmias, and many arrhythmias are caused by drugs which do not affect hERG at all [13].

In the Noble paper [17], the I_{Kr} current was reduced, completely blocked, or in some cases, adjusted to a negative value. This lead to EADs in only 42% of cases. When combined with a reduction in extracellular potassium levels, this figure rises to 66%.

The Qu paper states that I_{Kr} prevents EADs by opposing steady-state inward currents and preventing a quasi-equilibrium state at the plateau voltage. However, since I_{Kr} has a steeply sloped steady state activation curve, it may make the p-state less stable, increasing the probability that EADs will occur in the case of a prolonged action potential.

4.2.4 Specialised models

In patients with congestive heart failure, the pumping action of the heart is insufficient to provide appropriate blood flow [23]. In the Noble paper [19], in addition to the protocols applied to a range of models, specialised congestive heart failure variants of some models were created. The variants used ion current values taken from humans

with congestive heart failure, as well as congestive heart failure models in rabbit, rat, guinea pig and dog, to mimic the effects of heart failure in a non-species-specific way. Many ionic current values were modified, including the action of the sodium-calcium exchanger.

In addition, RyR mutations were investigated. Mutations that cause increased sensitivity to calcium concentration have been shown to cause DADs, and could potentially lead to an increased incidence of EADs. Similarly, exposure of ventricular myocytes containing wild-type RyR to catecholamines (such as adrenaline) can cause increased calcium-induced calcium release by RyR.

The RyR and congestive heart failure model variants successfully produced EADs, but could only be applied to a relatively small number of existing models, as many lack the necessary components - namely, a sufficiently detailed RyR channel model, and representation of the currents that are involved in congestive heart failure.

The Noble paper provided a very thorough overview of the suitability of particular models for the investigation of EADs by a variety of biologically-relevant modifications: namely, the recovery speed of fast sodium channels, the addition of the persitent sodium current, the increase in L-type calcium channel conductance, hERG block, congestive heart failure, RyR mutation and hypokalemia. For all protocols, hypokalemia increased the likelihood of EADs, and hyperkalemia reduced it. In addition, hypokalemia often increased the number of EADs produced in each simulation, and reduced the threshold values for other parameters at which EADs could be provoked.

5 Predicting Torsades-de-Pointes

Torsades-de-Pointes is a particular form of arrhythmia that occurs in the ventricles of the heart, and can cause sudden death. It is correlated with an increase in the length of the QT interval in the electrocardiogram [22], which corresponds to the wave of depolarisation that travels up both ventricles, and the wave of repolarisation that follows. Drug interactions with the cardiac ion channels can cause Torsades-de-Pointes.

The prediction of arrhythmias by *in silico* modelling of ion channel blocks has obvious implications for the pre-clinical testing of new drugs. Currently, blocking of hERG (the channel that mediates the rectifying potassium current I_{Kr}) is used as an early indicator of pro-arrhythmic potential. However, a study by Mirams *et al* in 2011 [13] created an improved measure of a compound's propensity for causing Torsades-de-Pointes arrhythmias, using simulated action potential duration as a metric. This approach takes into account the contributions of multiple ion channels to the shape and length of the action potential, and classifies drugs into discrete risk categories, based on their effect on action potential duration.

Drug block of ion channels can be modelled as a reduction in the channel's conductance, affected by the concentration of the drug and the IC_{50} value. IC_{50} is a measure of the inhibition the channel's action, defined as the concentration of drug at which the channel's activity is halved. The change in conductance for a channel j is described by this equation:

$$g_j = g_{control,j} \left[1 + \left(\frac{[D]}{[IC_{50}]} \right) \right]^{-1} \tag{8}$$

Where g_j is the conductance of the drug-blocked channel, $g_{control,j}$ is the conductance of the channel when there is no drug present, and $[D]$ is the concentration of the drug.

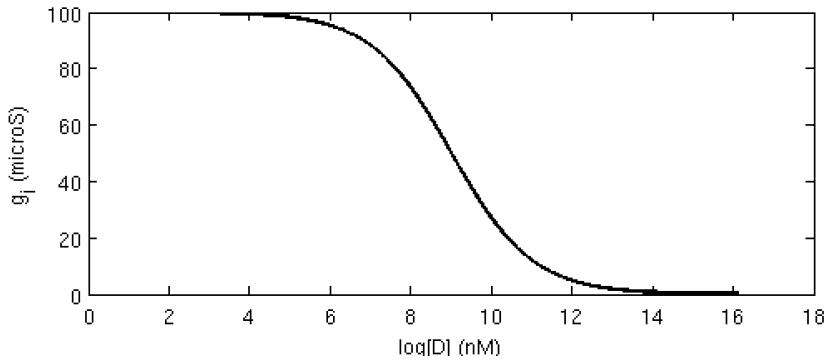

Figure 8: Dependence of channel conductance on drug concentration

6 Conclusion

The link between drug block of ion channels and pro-arrhythmic risk via prolongation of action has been investigated to create an improved measure of pharmaceutical safety [13] [2]. However, simulations incorporating mechanistic basis for this effect have yet to be used for drug safety studies. Since early and delayed afterdepolarisations have been linked to the onset of arrhythmias [20], and there are a large number of existing cardiac models from various species that can show EADs [19] and DADs [7] in response to physiologically-relevant perturbations, an investigation that links drug block of ion channels to the instigation of EADs and DADs could provide an improved measure of drug-induced arrhythmic risk.

7 Glossary of terms and abbreviations

- **Action potential** – A change in the membrane voltage of an excitable cell that triggers an action
- **Axon** – The conductive part of a nerve cell
- **Bradycardia** – Slow heartbeat
- **CICR** – Calcium-induced calcium release – the release of calcium from the sarcoplasmic reticulum when there is a high concentration of calcium in the cytoplasm, which links excitation to contraction
- **Conductance** – The inverse of resistance

- **DAD** – Delayed afterdepolarisation – a rise in the membrane potential after the membrane has repolarised
- **Depolarisation** – A rise in the membrane potential
- **EAD** – Early afterdepolarisation – a rise in the membrane potential before the membrane has fully repolarised
- **Fibrillation** – Desynchronised contraction of heart cells
- **Ion channel** – A protein within the cell membrane that allows charged ions to pass through
- **Long QT syndrome** – A family of heart conditions that all show an increased time between the Q and T waves of the electrocardiogram
- **Myocyte** – Muscle cell
- **Purkinje fibres** – Fibres within the ventricles that conduct the electrical impulse down to the bottom
- **RyR** – Ryanodine receptor – the ion channel that releases calcium from the sarcoplasmic reticulum when triggered by cytoplasmic calcium
- **Sarcoplasmic reticulum** – A membrane bound organelle which stores calcium
- **SERCA** – The sarco/endoplasmic reticulum calcium ATPase – A calcium pump that transports calcium into the sarcoplasmic reticulum
- **Sinoatrial Node** – The group of pacemaker cells situated in the right atrium
- **Tachycardia** – Fast heartbeat
- **Torsades-de-Pointes** – An arrhythmia that affects the ventricles, with an unusual QRS complex on the electrocardiogram

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