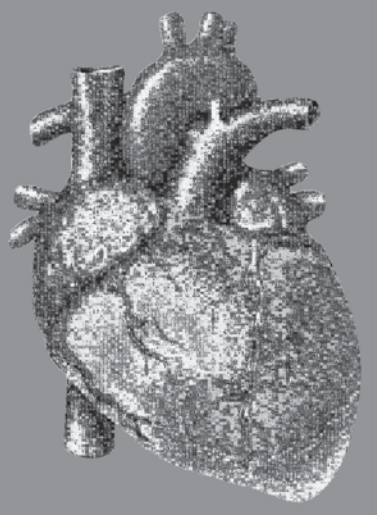
Chapter I

General introduction and outline of thesis



BACKGROUND

The human heart several billion cells of which the main functional or contractile unit is the cardiomyocyte.[1] For the heart to function normally (*i.e.* to deliver oxygenated blood, nutrients, immune cells and regulatory molecules to the organs and tissues) it relies on the coordinated rhythmic contractions of these cardiomyocytes upon electrical activation. In theory, given the large amount of cardiomyocytes constituting the heart, the number of sequences in which these cells could contract is enormous. However, only a small fraction of these theoretical sequences lead to the physiological forceful extrusion of blood from the heart.[2] Hence, tightly regulated electrical activation of these cardiomyocytes (cardiac electrophysiology) is essential for proper cardiac function. Concordantly, any significant perturbation of this regulation can, in theory, lead to dyssynchronous, irregular, overly fast or slow cardiac contractions, which are referred to as cardiac arrhythmias.

Cardiac arrhythmias are a major cause of morbidity and mortality throughout the world.[3, 4] Over the past decades, significant improvements have been made in anti-arrhythmic therapy. However, due to our incomplete understanding of the mechanisms underlying cardiac arrhythmias, treatment options are still far from optimal: Anti-arrhythmic drugs for instance, while sometimes effective in suppressing one arrhythmia, can display a tendency to provoke new/other arrhythmias.[5, 6] Furthermore, ablation techniques, whichrely on the intentional damaging of myocardium to prevent arrhythmias, are prone to complications due to the invasive nature of the techniques, while data on long-term outcome is currently lacking.[7, 8] Moreover, implantable devices, although effective, employ electric shocks to terminate arrhythmias, which are painful, traumatizing and cause tissue damage, while the use of this technology is limited by the high costs and complications such as infections and lead failures.[9-12] The limited preventive efficacy, the often invasive nature and the risk of adverse events/complications of current anti-arrhythmic treatment warrants a search for more specific, substrate-oriented treatment options. Hence, it is of critical importance to better understand the mechanisms underlying cardiac arrhythmias if we are to make progress in their treatment. In order to provide such understanding one should start by dissecting the basics of cardiac electrophysiology.

BASICS OF CARDIAC ELECTROPHYSIOLOGY

The cardiac cycle

During the normal cardiac cycle, deoxygenated blood from the body (systemic circulation) is collected in the right atrium, and pumped to the lungs by the right ventricles (the pulmonary circulation) where carbon dioxide is exchanged for oxygen. Subsequently, oxygenated blood is collected into the left atrium and pumped back into the systemic circulation by the left ventricle.

In order to complete this cycle to maximal efficiency, ventricular contraction must be delayed until the atria have completely emptied their contents into the ventricles to allow sufficient ventricular filling. Moreover, contraction of cardiomyocytes in the ventricles has to be coordinated so that ventricular contraction proceeds from apex to base to build up sufficient systolic pressure.[2]

The action potential

The initiation and coordination of cardiomyocyte contraction is governed by electrical signals called action potentials, which activate/excite the cardiomyocytes and stimulate contraction through a process called excitation-contraction coupling. Under normal conditions, action potentials develop spontaneously in the sinoatrial (or, in brief: sinus) node (the physiological cardiac pacemaker, located in the right atrium) and are propagated uniformly over both atria. Subsequently, the action potential travels through the atrioventricular node, where it slows down providing the critical delay between atrial and ventricular contraction, after which the ventricles are activated from apex to base through a specialized conduction network consisting of the His-bundle, left and right bundle branches and the Purkinje network.

The action potentials themselves are the result of the precisely timed opening and closing of mainly voltage-gated ion channels, located in the outer membrane (sarcolemma) of the cardiomyocyte. These ion channels allow selective passage of certain an- or cations across the sarcolemma down their electrochemical gradient. The difference in charge between the extracellular and intracellular environment of the cardiomyocyte (the transmembrane voltage or membrane potential) determines the conformation (*i.e.* open, closed or inactivated state) of the voltage-gated ion channels

and its consequent ionic conductance.[13-15] Under normal conditions, the cardiomyocyte membrane potential is negative at rest. However, action potentials propagated from neighboring cells can depolarize the cardiomyocyte until the threshold transmembrane voltage at which voltage-gated Na^+ (sodium) channels change from a closed state to an opened state, causing influx of Na^+.[16] The consequent, rapid further depolarization of the cardiomyocyte membrane (phase 0), marks the beginning of a new action potential. In turn, threshold voltages for several Ca^{2+} (calcium, inward current) and K^+ (potassium, outward current) channels are reached while the Na^+ channels are entering the inactivated state (phase 1)[16], leading to a transient plateau in membrane voltage (phase 2), characteristic for the cardiomyocyte action potential. As the main inward currents are inactivated at these depolarized membrane potentials, cardiomyocytes are resistant to new excitations (*i.e.* refractory), during this phase. Finally, when the outward current outweighs the inward current the cell repolarizes (phase 3) to its resting state (phase 4), enabling subsequent excitation by a new action potential (Figure 1).[14, 15]

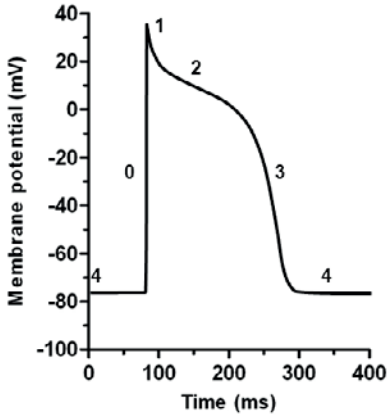


Figure 1. *Schematic representation of the action potential phases. 0: rapid depolarization of membrane potential caused by Na-⁺influx. 1: early repolarization caused by Na+ channel inactivation. 2: plateau phase resulting from calcium channel opening. 3: repolarization as a consequence of potassium channel activation. 4: resting phase*

Excitation contraction-coupling

The Ca^{2+} entering the cell during the action potential is a key regulating element for excitation-contraction coupling, serving as a signal for Ca^{2+}-induced Ca^{2+} release from an intracellular Ca^{2+} store called the sarcoplasmatic reticulum (SR) through specialized Ca^{2+} channels called ryanodine receptors located in the SR membrane.[17] Inside the sarcomere (the contractile element of the cardiomyocyte consisting of a multitude of proteins including troponins, tropomyosin, myosin and actin) the resulting increase in cytoplasmic Ca^{2+}, through its binding to tropinin-c, causes a conformational change in the tropomyosin complex enabling binding of the myosin head to the actin filament. This allows a conformational change in the myosin to occur, which pulls the actin filaments towards the centre of the sarcomere causing contraction of the cardiomyocyte.[18, 19] Upon hydrolysis of ATP bound to myosin, myosin releases its binding to actin, after which it reverts to its initial configuration. Simultaneously, intracellular Ca^{2+} is transported back to the SR by the sarco/endoplasmatic reticulum ATPase, moving the tropomyosin complex back in its original position, while preparing the SR for the next cycle.

Intercellular communication

To coordinate contraction throughout the entire heart, action potentials are propagated between cardiomyocytes by specialized intracellular channels called gap junctions.[20, 21] Gap junctions consist of hexamers of proteins called connexins that form transmembrane hemichannels (connexons) which connect to connexons of juxtaposed cells.[22] Different subtypes of connexins make up gap junctions in a tissue- and site-specific fashion. In mammalian hearts, ventricles mainly express connexin43 and connexin45, whereas connexin40, connexin43 and connexin45 are found in the atria and conduction system.[23] The resistance provided by the gap junctions, which are clustered together at the intercalated discs (the microscopic cross bands that connect the opposing short

ends of cardiomyocytes), roughly approximates the resistance provided by the cytosol. Gap junctions thus allow the passing of depolarizing current from activated cardiomyocytes to resting cardiomyocytes in order to trigger new action potentials. This causes the action potential to propagate as a wave of excitation.[14]

In summary, during normal cardiac rhythm, action potentials are initiated at the sinus node and propagated from cardiomyocyte to cardiomyocyte throughout the heart via gap junctions, stimulating the near simultaneous contraction of cardiomyocytes in the atrium followed by those in the ventricles. Any disruption in this sequence at the molecular, cellular or tissue level can disrupt normal cardiac electrophysiology and potentially lead to cardiac arrhythmias.

CARDIAC ARRHYTHMIAS

Cardiac arrhythmias comprise a wide range of conditions which can be subdivided into two categories by heart rate being either too low (*i.e.* bradyarrhythmias), or too high (*i.e.* tachyarrhythmias). The altered rhythm, especially when irregular, can lead to an abnormal awareness of the heartbeat (palpitations). More importantly, as the cardiac output is determined by the stroke volume × heart rate, both bradyarrhythmias (though limiting heart rate) and tachyarrhythmias (through limiting diastolic filling and thereby stroke volume) can severely impair cardiac function. In addition, stasis of blood secondary to the arrhythmia can lead to potentially lethal thromboembolic events. As such, cardiac arrhythmias provide a significant contribution to morbidity and mortality throughout the world.[3, 4]

Bradyarrhythmias arise when impulse generation at the sinus node is abnormally slow or atrioventricular conduction is impaired. Tachyarrhythmias, which are the main focus of this thesis, can also occur through altered (fast) impulse generation at the sinus node (sinus tachycardia). Sinus tachycardias, however, are usually the result of an increase in the body's oxygen demand, and are therefore benign. The most dangerous tachyarrhythmias originate from outside the sinus node and can occur as a consequence of alterations in impulse initiation and conduction, resulting in either high-frequency focal or reentrant activation.[14, 24]

FOCAL TACHYARRHYTHMIAS

Focal tachyarrhythmias are rhythm disturbances in which a single focus or multiple ectopic (*i.e.* outside the normal dominant pacemaker site, the sinus node) foci residing in the atria or the ventricles override the activity from the sinoatrial node by firing action

potentials at a higher frequency, resulting in tachycardia. Several theories regarding the mechanisms underlying the spontaneous firing from ectopic foci have been postulated during the past decades, which include enhanced automaticity, abnormal automaticity and triggered activity.

Enhanced automaticity

Enhanced automaticity occurs when cells with pacemaking ability, such as those in the sinus node, increases their rate of spontaneous discharge. In these pacemaker cells, the so-called "funny current" (I_f, governed by the HCN family of ion channels), causes periodic diastolic depolarisations in resting membrane potential, which lead to action potential generation if the threshold voltage is reached.[25] Hence, enhanced automaticity can occur through an increase in the slope of diastolic depolarization (by increased HCN channel activity), lowering of the threshold voltage, or depolarization of the maximal diastolic membrane potential (MDP). The presence of I_f and enhanced automaticity in cardiomyocytes in the pulmonary vein sleeves (probably because of shared embryonic origin between pulmonary vein and nodal myocytes) has been suggested as a mechanism in the initiation of atrial fibrillation.[26] However, further evidence for enhanced automaticity as a mechanism of tachyarrhythmias is lacking.

Abnormal automaticity

Abnormal automaticity can arise in cells lacking pacemaking ability, when the MDP is depolarized to the threshold voltage for inward currents. MDP depolarization however precludes inward currents through the fast Na^+ channels as these are inactivated at depolarized MDPs.[16] Hence, the upstroke of action potentials generated through abnormal automaticity relies on Ca^{2+} currents. Abnormal automaticity is suggested to play a role in for example post-myocardial infarction ventricular tachycardias, as a consequence of depolarized surviving subendocardial Purkinje fiber cardiomyocytes.[27]

Triggered activity

During triggered activity, single action potentials generated in a normal fashion "trigger" a second (ectopic) action potential in the absence of an extra-stimulus. Afterdepolarizations, which can be categorized as early or late, are regarded as the prime mechanisms underlying the double activations characterizing triggered activity.

During an early afterdepolarization (EAD), depolarizing force is reactivated during phase 2 (the plateau phase) or phase 3 (the repolarizing phase) of the action potential, reversing repolarization. EADs occur if the net outward current, required to repolarize the cardiomyocyte, during phase 2 or 3 of the action potential is diminished. Given this reduced repolarization reserve, currents that can increase progressively as the membrane potential depolarizes allow the generation of the early afterdepolarization

upstroke. For phase-2 EADs, currents that meet this criterion in the voltage range of action potential phase-2 (approximately -30 to 0 mV) are the L-type Ca^{2+} current (I_{CaL}), the Na^{+}-Ca^{2+} exchanger current (I_{NCX}) and the late Na^{+} current (I_{Na}).

I_{CaL} can exhibit this property because at membrane potentials between -30 and 0 mV the steady state activation and inactivation curves (plotting the state of the activation gate and the inactivation gate as values between open and closed as a function of the membrane potential) of I_{CaL} overlap (I_{CaL} window current).[28] In other words, within this window the probability of the Ca^{2+} to be open and not inactivated are both greater than 0 at steady state. As such, if the membrane potential lingers between these values, I_{CaL} is allowed to recover from inactivation.[28, 29] Reduced repolarization reserve is in this case essential as deinactivation of the I_{CaL} is in part time-dependent.

In forward mode, NCX transports one Ca^{2+} ion out of the cell in exchange for the import of three Na^{+} ions. During repolarization when intracellular Ca^{2+} is elevated through sarcoplasmatic reticulum Ca^{2+} release, inward I_{NCX} increases, impairing repolarization. However, as inward I_{NCX} becomes smaller when the membrane potential increases, I_{NCX} alone is unsufficient for EAD induction. Yet, if through reduced repolarization reserve I_{CaL} increases, further Ca^{2+} release from the sarcoplasmatic reticulum is stimulated, in turn stimulating I_{NCX} forward mode. Hence, synergistic interaction between I_{CaL} and I_{NCX} during phase 2 of the action potential provides an additional mechanism opposing repolarization during phase 2 of the action potential.[29] The resulting net depolarizing current can generate a triggered beat in adjacent tissue, given sufficient local excitability.

Finally, the Na^{+}-current has been suggested to play a role in EADs including those occurring during phase 3 of the action potential. Although, under normal conditions I_{Na} is inactivated during the repolarization and plateau phases of the action potential,[16] in several diseased states sustained channel activity been observed. This sustained activity is ascribed to three mechanisms being failure to inactivate (called channel bursting),[30] increase of the (normally very narrow) I_{Na} window current,[31, 32] and an increase in the rate of channel recovery from inactivation relative to deactivation (referred to as non-equilibrium).[32, 33] Through these channel gating abnormalities the so-called late I_{Na} can also lead to reactivation of depolarizing current and subsequent triggered action potentials.

In contrast to EADs, delayed afterdepolarizations (DADs) occur after full repolarization of the action potential (*i.e.* in phase 4). DADs are thought to depend on spontaneous sarcoplasmatic reticulum Ca^{2+} release events (SCREs). In the case of SCREs, Ca^{2+}-dependent currents such as the I_{NCX} and the Ca^{2+}-activated Cl^{-} (chloride, outward) current are activated upon the increase of cytosolic Ca^{2+}.[29] Again, if the resulting current depolarizes the cell and adjacent tissue to threshold voltage an action potential is triggered.

REENTRANT TACHYARRHYTHMIAS

As mentioned earlier, during normal heart rhythm, action potentials (starting at the sinus node) are uniformly propagated from right to left atrium, and from apex to base in the ventricles. Next, the cardiomyocytes repolarize after which any subsequent activation is again dependent on the pacemaker activity of the sinus node. However, under specific conditions, the action potential can follow an alternative route, which loops back upon itself. As such, subsequent activations are no longer dependent on the sinus node, but on the action potential returning at the beginning of the loop. The resulting self-perpetuating high-frequency activation is referred to as a reentrant tachyarrhythmia.

Unidirectional block

Paramount to the initiation of reentrant tachyarrhythmias is the occurrence of unidirectional conduction block, where antegrade propagation of the action potential is (partially) blocked but retrograde propagation through the same area is not. If after reexcitation of the previously blocked area, the area in which antegrade propagation occurred is repolarized (hence no longer refractory) the wavefront of excitation propagated from the retrogradely activated tissue can enter the self-perpetuating reentrant loop. Unidirectional block can occur as a consequence of source-sink mismatches, anatomical obstacles or critically timed extra-stimuli.[14]

Source-sink mismatching

If an action potential is to propagate between cardiomyocytes, the charge provided by the first cell in the sequence should exceed the charge required to excite subsequent cells. If this condition is met, the so-called safety factor of conduction (SF) exceeds 1. Permutations of the required relation between tsource and sink (*i.e.* the charge required exceeds the charge provided, referred to as source-sink mismatch), cause the SF to fall below 1, and conduction block to occur.[14]

As myocardial tissue structure is inherently heterogeneous (owing to varying wall thickness, trabeculation, [micro]vascularization), which is emphasized in the diseased heart (as a result of fibrosis), the heart contains multiple area in which thin pathways (isthmuses) lead into large expansions. These areas are prone to antegrade conduction block as cells at the leading tip of the wavefront have to activate more cells in front of it, resulting in local small source/sink ratios.[34] Here, conduction block will be unidirectional as current provided by cells at the expansion will be relatively large compared to the charge necessary to excite cells at isthmus during retrograde conduction (Figure 2). Moreover, as the area connecting the isthmus to the expansion is prone to EAD formation (as a consequence of electrotonic current [*i.e.* passive spread of charge] flowing from expansion to isthmus during the activation of the expansion and early repolarization of

the isthmus leading to local prolongation of the action potential plateau),[35] conduction through isthmuses provide an important substrate for arrhythmia initiation.

Logically, source-sink mismatches can also occur through areas of decreased excitability (*i.e.* a reduction in the provided charge) at the source. The consequent conduction block will be unidirectional if in this area excitability is asymmetrically decreased, such that a gradient in excitability exists in one direction, while excitability abruptly decreases in the opposite direction. Action potentials entering from the gradient side (anterograde) are blocked because when the wavefront arrives at the area of least excitability (most sink), the excitability of the source is lowest as well. In retrograde direction, excitability of the source will be maximal when arriving at the point of least excitability allowing retrograde action potential propagation. As such, impulses are conducted more easily from a rapidly conducting tissue to a slowly conducting tissue than in the opposite direction (Figure 2).[14, 36]

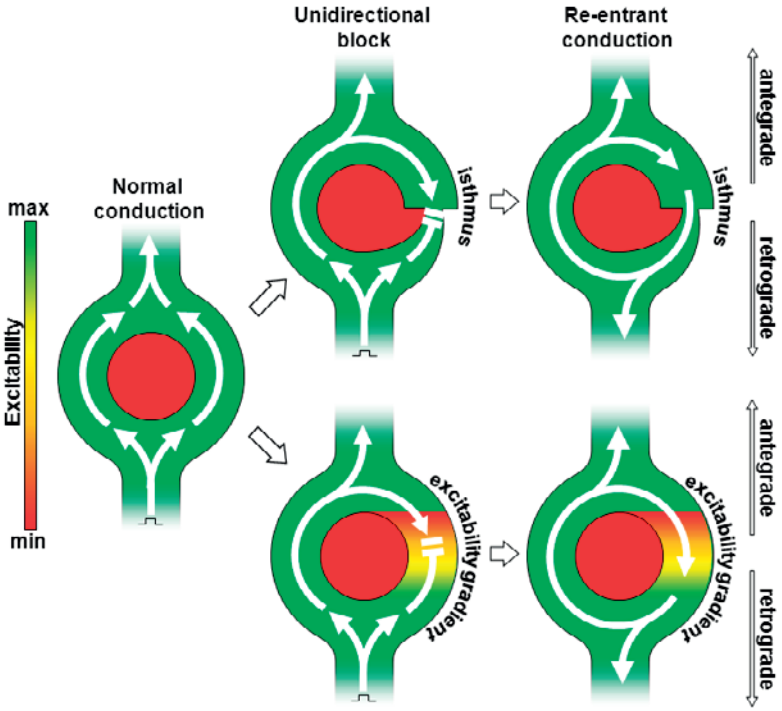


Figure 2. *Schematic representation of a myocardial tissue containing an anatomical obstacle during normal AP propagation (left panel), unidirectional block caused by a critical isthmus (upper middle panel) or an excitability gradient (lower middle panel) followed by reentrant conduction (right panels). Arrows indicate direction of AP propagation, double white lines indicate conduction block.*

Anatomical obstacles

Reentry can be induced when the wavefront of action potential propagation interacts with an anatomical (inexcitable) obstacle (*e.g.* fibrotic strands of myocardium or coronary vasculature) through a process called vortex shedding. Vortex shedding depends on the fact that conduction velocity relies on the curvature of the wavefront, such that increased wavefront curvature decreases conduction velocity (strongly curved wavefronts require an increased number of cells to be activated from a single source, leading to relative mismatching in the source-sink relationship, reducing conduction velocity).[34] At the critical wavefront curvature, absolute source-sink mismatching occurs and conduction velocity becomes zero. Logically, the value of the critical wavefront curvature depends on local excitability (*i.e.* the source). For an action potential wavefront to propagate towards tissue behind an anatomical obstacle, the wavefront has to curve around the obstacle. If at a certain local excitability, the wavefront curvature necessary to excite all tissue behind the obstacle is below the critical curvature, the wavefront will circumnavigate the obstacle to proceed in the initial antegrade direction. However, if the wavefront curvature necessary to excite all tissue behind the obstacle exceeds the critical curvature (*i.e.* if the obstacle has sharp edges, or excitability is locally decreased), propagation will only proceed distal from the obstacle at a wavefront curvature that allows the SF to be >1. Hence, the wavefront detaches from the obstacle (*i.e.* the obstacle sheds the wavefront), after which tissue behind the obstacle can be activated in a retrograde fashion, allowing formation of a reentrant loop (*i.e.* vortex).[34, 37]

Extra-stimuli in the vulnerable window

Structural heterogeneities are no prerequisite for the induction of reentrant conduction. The classical method to induce reentrant conduction in homogenously behaving myocardial tissue involves provoking the collision of a wavefront (from an extra-stimulus) with the wavetail of another perpendicularly conducted wave (*i.e.* crossfield stimulation). During crossfield stimulation, anterograde action potential propagation of the extra-stimulus is blocked where it meets refractory tissue from the wavetail of the preceding action potential. However, behind the wavetail of the first action potential, the wavefront of the extra-stimulus will encounter excitable tissue. As a result, the second wave can turn retrogradely into the recovering cells at the area previously blocking antegrade conduction, forming an reentrant loop. As such, extra-stimuli (arising from sites with enhanced automaticity, triggered activity or external electrical stimulation), when applied in the vulnerable window (*i.e.* the time, space or voltage window in the action potential in which unidirectionality can be induced; stimuli applied before or after the vunerable window will culminate in bidirectional block and conduction, respectively) can underlie reentry in the absence of structural heterogeneities (*i.e.* functional reentry) (Figure 3A-E).[38] However, it should be noted that the presence of structural heterogene-

ities in repolarization will significantly prolong the vulnerable window, increasing the chance of reentry initiation in the presence of abnormal extra-stimuli.

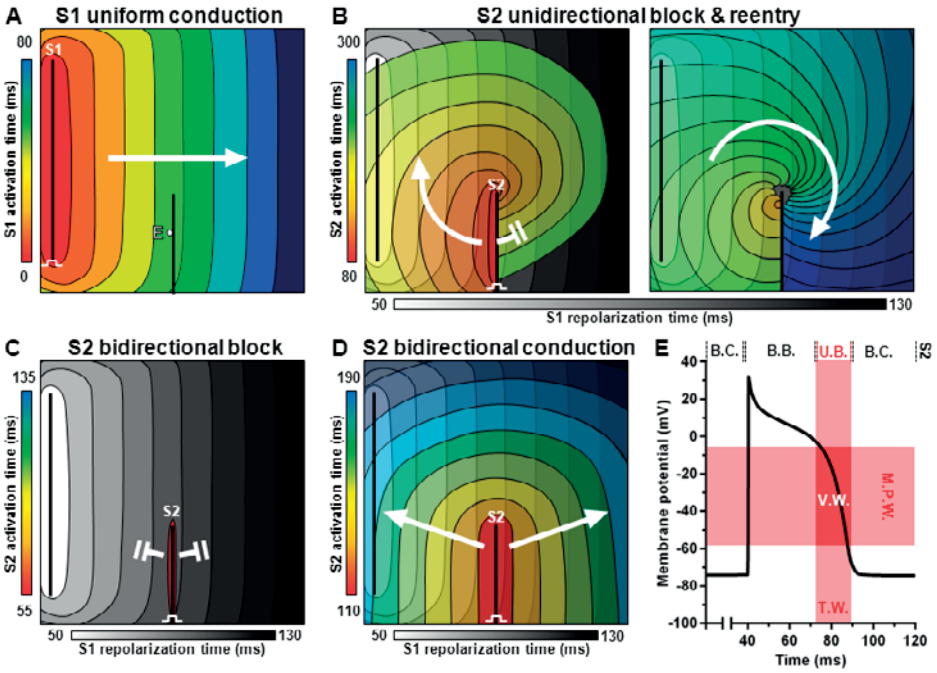


Figure 3. *Schematic representation of a myocardial tissue square during (A) an S1 stimulus from a line shaped electrode leading (left black line) to uniform conduction and subsequent S2 stimulus from a line shaped electrode (right black line) timed during (B) the vulnerable window, leading to reentry, (C) before the vulnerable window, leading to bi-directional block or (D) after the vulnerable window, leading to bidirectional conduction. (E) Schematic representation of the action potential in point E in subfigure A. V.W.: Vulnerable window. M.P.W.: membrane potential window. T.W.: Time window. B.C.: Bidirectional conduction. B.B.: Bidirectional block. U.B.: Unidirectional block. All indicated times represent the time from the start of the S1 pacing stimulus. Isochrones are spaced 10ms apart. White arrows indicate the direction of AP conduction, double white lines indicate conduction block.*

Anatomical and functional reentry

For many years it has been known that reentrant conduction can occur around an anatomical (inexcitable) obstacle, such as a post-myocardial infarction scar.[39] To allow its maintenance, in such an anatomical reentrant circuit (*i.e.* circus movement reentry), reentry cycle length has to exceed a critical value to prevent wavefront-wavetail interaction, and consequent spontaneous reentry termination. Therefore, reentrant conduction depends on conduction velocity and refractory period, in such a way that the path length of the reentrant circuit must exceed the wavelength of excitation (*i.e.* conduction velocity×refractory period, which determines the size of the refractory zone behind the wavefront). Under such conditions, an area of excitable tissue (*i.e.* an excitable gap) is

present between the wavetail and the wavefront.[40] The anatomical fixation of the reentrant circuit gives rise to a monomorphic appearance of the resultant tachyarrhythmia on electrograms.[41] These characteristics can hold true for, for example, atrioventricular reentrant tachycardias, and macroreentrant atrial and monomorphic ventricular tachycardias.

As indicated above reentry can also occur without any anatomical obstacle, giving rise to the term functional reentry. Due to the source-sink relationship, the maximal velocity of a convex wave can never exceed that of a flat front. Hence, in case of homogeneous basal excitability and stable reentry cycle lengths throughout the tissue, wavefront curvature increases (*i.e.* making sharper curves), while conduction velocity has to decrease from the periphery towards the center of a rotating wave. As such, functional reentry acquires the form of an Archimedean spiral.[34]

The functional core (center of rotation) is formed where the wavefront curvature hits the critical value and conduction velocity becomes zero.[34] Since functional reentry does not rely on an anatomical obstacle, during such reentrant activation the functional core can meander throughout the tissue, giving rise to polymorphic electrograms as can be seen in polymorphic ventricular tachycardias, atrial fibrillation and ventricular fibrillation.[41] Meandering occurs as a consequence of the relationship between wavefront curvature and conduction velocity. When excitability and critical curvature are high, the wavefront will make sharp turns around the functional core, causing it to meet its refractory tail, decreasing excitability as well as the consequent critical curvature. As the critical curvature is decreased, the wavefront detaches from the wavetail, increasing excitability, after which the curvature of the path followed by the tip of the spiral wave (at the core) will again increase. As such, the recurrent changes in critical wavefront curvature will underlie (cycloidal) meandering of the spiral wave. However, if the length of the pivoting trajectory at the core exceeds wavelength, propagation of the wavefront near the core is not affected by the wavetail, precluding meandering. As such, the area circumvented by the spiral wave tip is -in theory- never excited, and the spiral wave contains a fully excitable gap.[34] Thus, at conditions of low excitability, functional reentry circuits can be fixed, giving rise to monomorphic electrograms only.[34, 41] Hence, the stability of spiral waves seems to depend on the currents determining excitability near the core, such as I_{Na} and the inward rectifier K^+ current (I_{K1}).[42] As collision of the spiral wave tip at the core of the spiral wave to anatomical boundaries or a spiral wave tip of an opposite chirality can lead to extinguishing of the spiral wave (as well as the arrhythmic high-frequency activation originating from the spiral wave), altering the stability of the spiral wave core can be very relevant. However, still a lot of controversy exists around the actual state of the spiral wave core (being either unexcitable, inexcitable, continuously excited or never excited),[40] as well as the possible ways to facilitate its destabilization or termination. Moreover, as functional reentrant arrhythmias can become anatomical by pinning to

anatomical obstructions, even the border between anatomical and functional reentry is blurred in practice[41], illustrating the difficulty in dissecting the electrophysiology underlying cardiac arrhythmias.

CURRENT TREATMENT OF CARDIAC ARRHYTHMIAS

Symptomatic treatment

Many of the treatments available for cardiac arrhythmias depend on the alleviation of symptoms or prevention of complications secondary to the arrhythmia. A prime example is oral anticoagulation, to prevent the occurrence of thromboembolic events as a consequence of stasis of blood (and hypercoagulability) through impaired atrial wall movement at excessively high atrial activation frequencies in the treatment of atrial fibrillation.[43] Measures to prevent high ventricular activation rate (rate control) by slowing atrioventricular conduction in patients suffering from atrial fibrillation are another widely used form of symptomatic treatment. Importantly, several studies show superiority with regard to survival of anti-coagulation and rate control strategies over strategies aiming to regain sinus rhythm (rhythm control).[44, 45] However, regaining or preventing any deviations from sinus rhythm can be a reasonable and sometimes imperative goal in the treatment of tachyarrhythmias, especially when hemodynamic instability is involved or expected. Moreover, prevention of the arrhythmia itself can prevent further myocardial remodelling,[46] leading to increased susceptibility to arrhythmias, as well as the associated complications such as thromboembolic events.[43] These rhythm control strategies include anti-arrhythmic drug intake, ablative treatment and device therapy.

Anti-arrhythmic drugs

The least invasive treatment method to attain rhythm control involves the use of pharmacological agents (anti-arrhythmic drugs) to modulate ion channel function. Anti-arrhythmic drugs can be categorized according to the Vaughan Williams classification as being either Na^+ channel blocking (class I), blocking sympathetic activation (class II), K^+ channel blocking (class III) or Ca^{2+} channel blocking (class IV). Anti-arrhythmic drugs acting through other or unknown mechanisms are categorized as class V drugs.[47]

Class I drugs exert their effect through the blockade of Na^+ channels, which decreases excitability and results in conduction slowing. As the stability of tachyarrhythmias based on functional reentry depends on the interplay between I_{K1} and I_{Na}, class I drugs can destabilize spiral waves leading to their termination.[42] In addition, blockade of I_{Na} is implicated in the prevention of triggered activity (EADs) based on late I_{Na}.[48] Moreover, conduction slowing can be anti-arrhythmic in reentrant tachyarrhythmias maintained or initiated by conduction through critical isthmuses. A reduction of Na^+ channel avail-

ability can lead to conduction block at the critical isthmus through enlargement of the source-sink mismatch present at the distal expansion (see also subheading *Source-sink mismatching*),[34] breaking up the reentrant circuit. However, it is the same effect causing the slowing of conduction, that can in theory promote the induction of reentry, by allowing time for repolarization of antegradely conducting tissue before return of the retrograde wavefront (as discussed in subheading *Unidirectional block*).[14]

Class II drugs are mainly used to control ventricular rate (see the subheading *Symptomatic treatment*).

The class III drugs' mode of action involves slowing repolarization by blocking K^+ channels in order to prolong the refractory period. As circus movement reentry exists at the grace of its path length exceeding the wavelength of excitation (preventing blockade of the wavefront on the refractory wavetail), prolonging the area occupied by the wavetail increases the chance of arrhythmia termination (see also subheading *Anatomical and functional reentry*). Moreover, I_{K1} has been implicated in the maintenance of functional reentry. Hence, blockade of this current can induce drift of the spiral wave resulting in its termination.[42] Inversely, also class III drugs come with a pro-arrhythmic downside, as slowing of repolarization can increase the chance of arrhythmia initiation through triggered activity (via EADs, as discussed in subheading *Triggered activity*).[14]

As EADs and DADs largely depends on the deinactivation of Ca^{2+} channels (see subheading *Triggered activity*), class IV drugs are thought to prevent such triggers of tachyarrhythmias. However, the negative effects of Ca^{2+} channel blockers on vascular tone and cardiac inotropy deem its use undesirable in a large number of patients that often suffer from mechanical dysfunction of the heart, coinciding with their pro-arrhythmic substrate, and are hence prone to hemodynamic instability.[49] Moreover, as blockade of the Ca^{2+} channel shortens the action potential plateau, the refractory period is abbreviated. Hence, the use of Ca^{2+} channel blockade also comes with the risk of increased vulnerability to circus movement reentry.[14]

Taken together, theoretically all anti-arrhythmic drugs can have both pro- and anti-arrhythmic effects. The limited efficacy and safety of current anti-arrhythmic drug therapies support this notion. This result, which may be explained by our incomplete understanding, of pro-arrhythmic mechanisms, as well as their interplay, provided the incentive for the development of different anti-arrhythmic interventions such as (cathether) ablation and device therapy.

Ablation-based therapy

Ablation-based therapy involves techniques that aim to alter the substrate or prevent triggers for cardiac arrhythmias, by damaging pro-arrhythmic tissue using radiofrequency energy or cryogenic cooling. Ablation can be performed either surgically (when performed in combination with other procedures which necessitate primary surgical/

open chest access) or catheter-based (*i.e.* transluminal). Targets for ablation include anatomically defined substrates for circus movement reentry, microreentrant circuits, areas of slow conduction (critical isthmuses) or sources of focal activation.[50, 51] Moreover, ablation can prevent atrial arrhythmias through compartmentalization of the functional atrial myocardium below the critical mass (*i.e.* the minimal amount of tissue necessary to allow perpetuation of the arrhythmic wavefronts) rendering the resulting electrically isolated atrial areas too small to maintain the arrhythmia.[50]

As such, ablation-based therapies can be very effective in battling cardiac arrhythmias. Single procedure success rates can be as high as 90% for both atrial fibrillation and ventricular tachycardia.[50-52]

As ablation-based therapy aims to break up the pro-arrhythmic substrate locally, overall electrophysiology is maintained. Hence, ablative treatment can overcome the pro-arrhythmia associated with anti-arrhythmic drugs. However, as the pro-arrhythmic features in the non-ablated tissue remain, new arrhythmias may arise after the procedure requiring repetitive ablation. In addition, while macroreentrant circuits and focal sources are feasible targets for ablation of ventricular arrhythmias, compartmentalization to target smaller or functional circuits is difficult to achieve (due to ventricular wall thickness) and undesirable (due to the consequent impairment of ventricular function). Moreover, little evidence exists for the ablation of functional reentrant circuits (other than achieved by compartmentalization. Of note: triggers of functional reentry cán be prevented). Hence, the efficacy of ablation in ventricular arrhythmias decreases with their complexity. Moreover, ablation techniques comprise highly invasive procedures, which can result in serious complications arising from damage to cardiac structures, thermal or cryogenic injury to adjacent extracardiac structures or thromboembolism.[53]

Device therapy

The most effective direct method for regaining sinus rhythm (*i.e.* to achieve electrical cardioversion or defibrillation) is by the application of a high-energy electric shock. Electric shocks can be delivered by implanted and external devices. Exposure of the cardiomyocytes in the heart to the electric field between the shocking electrodes causes a gradient in extracellular membrane voltage, while the intracellular voltage is thought not to change appreciably, due to the relatively high impedance of the sarcolemma. As a consequence, the transmembrane potential drops in a linear fashion along the extracellular voltage gradient (with hyperpolarization and depolarization at the cells' ends facing the anode and cathode, respectively). If the depolarization at their cathodic ends causes cells to reach threshold membrane voltage, an action potential is initiated. Being applied over the entire heart, the high energy shocks hence serves to depolarize a critical mass of the myocardium simultaneously. As a result, the wavefronts maintaining arrhythmic activity are extinguished through their collision with shock-excited wave-

backs. Subsequently, after synchronized repolarization from the shock, the sinus node is allowed to reestablish normal rhythm.[54]

While implantable and external defibrillators provide very effective rescue therapy, preventing arrhythmic complications (*e.g.* sudden cardiac death in the case of ventricular fibrillation), *electrical shocks* constitute very painful and stressful events. As device therapy does not prevent arrhythmias from occurring, recurring arrhythmias and accompanying shocks remain a problem. Moreover, the electrogram characteristics of some benign rhythms are not readily distinguishable from malignant tachyarrhythmias through programmable algorithms in implantable devices and sensing itself can occur inaccurately (for instance when the repolarization wave or T-wave is recorded as being an extra activation, doubling the sensed activation frequency). As a consequence, inappropriate shocks are known to occur in up to 10-20% of patients.[55-57] Hence, device therapy is associated with a reduced quality of life and even increased mortality rate.[57, 58] In addition, to date, no appropriate device therapy is possible for supraventricular tachycardias, mainly because many of the patients would require multiple painful shocks a day.

Taken together, anti-arrhythmic drug, ablative therapy and device therapy can be effective in the treatment of cardiac arrhythmias. However, all are associated with non-trivial adverse effects. By furthering our understanding of the pro-arrhythmic mechanisms more specific, substrate-oriented therapies may be developed in the future, allowing increased anti-arrhythmic efficacy while decreasing its harmfulness.

AIM AND OUTLINE OF THESIS

To be able to improve anti-arrhythmic treatment efficacy, it is essential to comprehend the electrophysiological mechanisms underlying the initiation (triggers), maintenance (substrate) and termination of cardiac arrhythmias as discussed in **Chapter I.**

Therefore, the aim of this thesis was to develop and utilize *in vitro* and whole heart *ex vivo* models of ventricular tachycardia/fibrillation and atrial fibrillation to investigate their pro-arrhythmic mechanisms and to provide novel rationales for (1) more substrate- or trigger- oriented, (2) more specific and (3) less hazardous treatment strategies.

Cardiac pathological remodelling is a complex agglomerate of multiple processes that aim to compensate for altered biomechanical strain, as occurs after, for example, myocardial infarction and aortic stenosis. There is a clear association between cardiac remodelling and arrhythmias. However, as pathological remodelling constitutes multiple simultaneous processes, including cardiac fibrosis and cardiomyocyte hypertrophy, no distinction can be made between the contribution of these separate processes to the pro-arrhythmic phenotype. Hence, in **Chapter II** the differences between the pro-

arrhythmic triggers and substrates provided by mechanisms specific for pathological cardiomyocyte hypertrophy and cardiac fibrosis are investigated. Moreover, the influence of these specific mechanisms on anti-arrhythmic strategies is evaluated.

Ventricular fibrillation is not compatible with life in part because of the presence of multiple reentrant circuits (constituting a highly complex arrhythmia) within the ventricle(s) which causes dyssynchronous myocardial contractions resulting in insufficient cardiac output. Moreover, highly complex cardiac arrhythmias are associated with decreased defibrillation success rate. Hence, decreasing the number of rotors (*i.e.* the complexity) maintaining ventricular fibrillation can be an important step in improving its treatment. Hence, **Chapter III** describes a study into the electrophysiological characteristics that determine complexity of fibrillation and how they can be utilized to destabilize and ultimately terminate reentrant arrhythmias using a wide range of pharmacological agents.

Pharmocological treatment of atrial fibrillation is hampered by the ventricular pro-arrhythmia associated with the use of anti-arrhythmic drugs. As the atrial ion channel targets of these drugs overlap with the ion channels expressed in the ventricles, these drugs alter ventricular electrophysiology, possibly leading to increased arrhythmia susceptibility. Hence, to improve treatment of atrial fibrillation atrium-specific targets are needed. Moreover, contribution of these targets to atrial pro-arrhythmia needs to be elucidated. Therefore, in **Chapter IV** he effects of blockade or downregulation of the Kir3.x superfamily of K^+ channels, which were found to be expressed in atrial but not ventricular cardiomyocytes, is investigated.

Electroshocks, applied by external and internal defibrillators, are an effective way to terminate reentrant activity underlying both atrial and ventricular fibrillation. However, these shocks are very painful, associated with tissue damage and not always effective. To make better use of electrical shock therapy it seems essential to decrease the energy requirements for successful defibrillation. For atrial fibrillation decreased efficacy of electrical shock therapy is related to the atrial remodelling associated with persistent atrial fibrillation. A constituent of this remodelling is constitutive activation of the acetylcholine-dependent K^+ current (governed by the Kir3.x channels). Therefore, in **Chapter V** the contribution of this current in setting the energy threshold for atrial defibrillation was studied, as well as the mechanisms associated with the interaction between this current and defibrillation threshold.

In **Chapter VI**, a new method of cardioversion/defibrillation that does not rely on the application of electrical shocks is explored. Here, the hypothesis that the depolarizing current, necessary for resynchronizing the atrium during reentrant tachyarrhythmias (in order to regain normal rhythm), could be provided by inserting and (shocklessly) activating light-sensitive ion channels in cardiomyocytes was tested.

In conclusion, **Chapter VII** summarizes the findings of this thesis. Moreover, results are discussed with special emphasis on their translational perspectives.

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