Antiarrhythmic Principle of SK channel Inhibition in Atrial Fibrillation

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THE THREE ORIGINAL PAPERS ARE


INTRODUCTION

The human heart beats more than two-billion times during an entire life (1) supplying the body with billions of litres of vital oxygenated blood, providing oxygen and energy to all cells and removing CO2. The heartbeat is therefore dependent on tightly regulated mechanisms to insure the maintenance of a regular heart rhythm, securing an appropriate blood circulation throughout life. These mechanisms include the opening and closing of various ion channels that cohesively generate the propagation of electrical signal conductance through the cardiac muscle. Ion channels are transmembrane spanning pore-forming proteins, present in both excitable and non-excitable cell-membranes, with the ability to selectively conduct the flux of specific ions either into- or out of the cell. In cardiac cells, called cardiomyocytes, it is primarily Na+, Ca2+ and K+ ion channels that are involved in cellular excitation and electrical signalling. Electrical signals known as action potentials are carefully controlled by the highly orchestrated gating of different types of ion channels (2). The shape and morphology of cardiac action potential is primarily governed by the summarized flux of Na+, Ca2+ and K+ ions entering and leaving the cell. Also Cl- channels play a role in cardiac electrophysiology, however, that aspect will not be covered in this thesis. The duration of the action potential determines the timeframe of cardiac contraction and subsequent relaxation. Within the action potential, the duration of excitation refractoriness serves as a protective mechanism from tetanic muscle activation, which would otherwise be potentially fatal. Ion channels come in many flavours, and while the diversity of Na+ and Ca2+ channels in the heart is limited, there exists a highly diverse distribution of K+ channels. This high diversity of K+ channels, together with a heterogeneous distribution, determines the differences in shape and morphology of action potentials observed from different regions of the heart (3). In figure 1, two distinct action potentials from the human heart are shown, one from the right atrium (left) and one from the inter-septal ventricle (right). The difference in shape between these two action potentials relies primarily on regional differences in the composition of the repolarizing K+ currents, with some being relatively atrial specific. Crucially, ion channels work through passive transport and can only conduct ions down their electro-chemical gradient; therefore, a prerequisite for cellular excitability is the maintenance of charged membrane potentials to drive the action potential. This is dependent on active transport via ATP driven carriers, such as the Na+/K+-ATPase and the Ca2+ pump, to transport ions against their electro-chemical gradient. The tightly regulated synchronicity of cardiac conduction can be disturbed causing dysrhythmic events. Such events are known as cardiac arrhythmias, a term which describes all cardiac rhythm disorders, whether they are supraventricular or ventricular arrhythmias. Several precursors may exist, that can be either inherited or acquired, such as structural abnormalities, electrical remodeling, genetic precursors, as well as pharmacological side effects, which all can serve as the underlining cause of arrhythmias. These aspects will be covered in the following theoretical sections. This thesis will focus on the supraventricular arrhythmia called atrial fibrillation (AF) and the possible anti-arrhythmic principle engaged through inhibition of a potassium channel known as the small conductance Ca2+ activated K+ (SK) channel.
Na\(^+\) and Ca\(^{2+}\) channels

Excitation of the cardiomyocyte, seen as the rapid depolarization in figure 1 constitutes phase 0 of the AP. It is mediated by the activation of voltage-gated Na\(^+\) channels conducting the \(i_{\text{Na}}\) current shown in figure 2, which is primarily conducted through Na\(_{v}1.5\) channels, encoded by the SCN5A gene. The rapid Na\(^+\) channel activation, observed as the initial downwards deflection, is followed by a fast voltage-dependent inactivation, setting the channel in a non-conducting, non-activatable state. The excitability of a cell is almost exclusively determined by Na\(^+\) channels being released from inactivation, which is both time and voltage dependent, however most release from inactivation happens at repolarized potentials. Thus, the longer the AP remains depolarized, the longer the cell will be in a refractory state. The time period where the cardiomyocyte is refractory is called the refractory period and represents a period where the cell cannot elicit a new action potential and is therefore unexcitable. In this thesis cellular refractoriness is measured as the effective refractory period (ERP), meaning the period during which the cell cannot elicit a new AP regardless of the force of excitation stimuli. Cellular excitability, which is dependent on the degree of Na\(^+\) channel inactivation in relation to the diastolic voltage potential, is an aspect of considerable importance in arrhythmogenesis which will be covered later in this thesis. As a response to membrane depolarization, influx of Ca\(^{2+}\) is initiated through activation of voltage-gated L-type Ca\(^{2+}\) (Ca\(_{v}1.2\) and Ca\(_{v}1.3\)) channels conducting the \(I_{\text{Ca}}\) current (5), which is largely responsible for the plateau phase 2 of the AP. The initial increase in intracellular Ca\(^{2+}\) concentration triggers calcium-induced calcium-release from intracellular stores in the sarcoplasmic reticulum (SARC), through activation of ryanodine receptors (6). Membrane depolarization by Na\(^+\) and Ca\(^{2+}\) influx is counteracted by activation of fast and delayed voltage-gated K\(^+\) channels to repolarise the cell.

**K\(^+\) channels are important in cardiac repolarization**

The ability of the myocardium to secure stable rhythmic electrical signalling is partly due to the delayed repolarization of cardiomyocytes. This relies mainly on the large diversity of cardiac K\(^+\) channels, but also on a particular redundancy in cardiac repolarization, in which one current is taking over if another one should fail. This redundancy has been named the “repolarization reserve” (7), and has in ventricular repolarization largely been attributed to the existence of three important cardiac K\(^+\) currents: \(I_{\text{Ks}}, I_{\text{Kr}}, I_{\text{KCa}}\) shown in figure 2. \(I_{\text{Ks}}\) and \(I_{\text{Kr}}\) are voltage-dependent currents which belong to the family of Kv channels and are named slow delayed rectifiers, due to their slow gating kinetics that secure long cardiac action potentials (100 – 1000 ms) (2). \(I_{\text{KCa}}\) belongs to the family of classical inward rectifiers, meaning that it prefers to conduct inward K\(^+\), while outward conductance is voltage-dependently blocked by intracellular polyvalent ions of the cytoplasm, such as Mg\(^{2+}\) and polyamines such as spermine and spermidine (8). Despite profound differences in the biophysical nature of these channels, they all share overlapping impact on the cardiac repolarization. In atrial repolarization more currents come into play, some of them being to some extent atrial specific or at least atrial predominant. This is the case for \(I_{\text{KACa}}, I_{\text{Ks}}, I_{\text{K1}}\), which will all be discussed thoroughly later in this thesis. The atrial diversity is possible the primary contributor to the characteristic spike and dome morphology observed in atrial APs, which is a definable feature separating them from ventricular APs, shown in figure 1.
Voltage gated potassium channels (Kv) consist of a superfamily of channels that conduct K⁺ ions as a response to voltage changes. There are several Kv channel subfamilies, among which are the transient outward voltage-gated K⁺ channels conducting (Iₒ) which governs the initial repolarization "notch" observed in phase 1 between the spike and plateau of the AP. The initial repolarization plays a functional role by increasing the driving force for Ca²⁺ into the cell. In atrial cardiomyocytes Iₒ is accompanied by the atrial specific ultra-rapid rectifier K⁺ current (Iₖur) and together these currents are responsible for the more prominent initial repolarization of the atrial-compared to the ventricular AP. Also among Kv channels we find the cardiac Kv11.1 (hERG1) channel, encoded by the KCNH2 gene, conducting the IKr current and the Kv7.1 channels encoded by the KCNQ1 gene, conducting the IKs current. Together IKr and IKs play a central role in the final repolarization phase 3 of the AP. Kv channels form tetrameric homo- and heteromeric complexes of α-subunits, each containing six transmembrane segments, in the assembly of functional channel structures. These protein subunits have intracellular N- and C-termini, the fourth transmembrane segment S4 functions as the voltage sensor, and the bringing together of four P-loops between SS and S6, line the membrane spanning pore (2). Studying these currents as presented in figure 2, it becomes clear that their individual gating kinetics have become highly specialized through evolution.

Kv11.1 (hERG1) channels can be in a closed, open or inactivated state, which are conformational transitions the channel will go through following membrane depolarization and repolarization. Upon depolarization the conformational transition from a closed to an open state is a relatively fast process, however the subsequent inactivation is much faster. As a consequence, the channel will almost immediately reach an inactivated and non-conducting state, not contributing substantially to the initial repolarization or plateau phase. However, upon repolarization, the channels are released from inactivation at a fast rate, while the conformational change from open to closed state named deactivation is a slow process (9, 10). This gives rise to the delayed “tail current” of IKr observed in figure 2, which is responsible for the relatively large contribution by IKr to the final part phase 3 of repolarization and to the early part of the diastolic phase 4.

IKr, which is conducted by the Kv7.1 channel is likewise activated upon depolarization, however this is a slow process with channel gating occurring at more depolarized potentials (right-shifted activation curve) showing only limited inactivation (11, 12). These properties allow IKr to slowly build up over time and to contribute primarily as a repolarizing current to phase 3 of the AP. These biophysical properties rely to a large extent on regulatory β-subunits of the KCNE family (KCNE1-5), with KCNE1 and KCNE4 as the most abundant in cardiac physiology contributing to the slow activation of native cardiac Ikr (13, 14). Ikr plays an important part in the AP shortening upon sympathetic stimulation, which is crucial during high heart rates (11). The particular importance of both Ikr and IKs in maintaining regular heart rhythm is reflected by functional mutations in either of these two ion channels or their regulatory subunits being the major cause of lethal phenotypes such as inherited Long-QT syndrome (15), thus KCNQ1 is sometimes referred to as K₄LQT1 (16). The primary cause of acquired Long-QT syndrome is due to unintended pharmacological blockage of hERG channels (17). In summary, cardiac K⁺ channel gating kinetics have become particularly specialized and differentiated through evolution, and their individual contribution is an important piece in the puzzle of cardiac repolarization.

Ion channels distribution

The multiple types of K⁺ channels and their highly differentiated and diverse distributions in cardiac myocytes contribute to the regional diversity in AP morphology observed between different cardiac cells (18). The atrial AP is generally shorter than the ventricular (3). Atrial myocytes have a mean diastolic resting membrane potential of approximately -78 mV, being ± 5 mV less negative than ventricular myocytes as depicted in figure 1, mainly due to a relatively lower Ikr (Kir2.1) expression (19, 20). Phase 1 is more prominent in the atria due to the presence of larger transient outward currents Iₒ and IKur (21). Compared to the ventricles the atrial AP exhibit slower phase 3 repolarization due to a smaller IKr and IKs and regional differences in Ikr and IKsk (20). The IKur current, which activates faster than Ikr, has been described exclusively in atrial tissue in a number of species, including humans (22, 23). IKur is expressed predominantly in the atrium relative to the ventricles in most species (20).

CARDIAC INNERRATION AND CONTRACTION

Autonomic regulation

In mammals, autonomic regulation of the heart is governed by changes in the tone of the sympathetic (adrenergic) and parasympathetic (cholinergic) nerves. Sympathetic and parasympathetic innervation dominates the inotropic (contractility) and chronotropic (rate) regulation of the heart. This innervation of the heart is known to be controlled initially from the CNS via the vagus nerve (the tenth cranial nerve) and sympathetic nerve innervation (arising from the thoraco-lumbar system). Sympathetic nervous stimulation activates β1-receptors through release of
epinephrine to the neuronal synapse, increasing heart rate and contractility. Parasympathetic nervous stimulation via the vagus nerve mediates release of acetylcholine from the synapse which activates the muscarinic (M2) G protein-coupled receptors in cholinergic nerve fibres, resulting in a decrease in heart rate and contractility (5). A moderate stimulating discharge takes place in the cardiac sympathetic nerves at rest, but there is a marked vagal discharge, called vagal tone, in humans and other large animals, which keeps the human heart rate at about 60 beats per minute at rest (24). The heart also contains an intrinsic nervous system including an atrial neural network. Disturbances in the balance of autonomic regulation is, to a large extent, involved in arrhythmogenesis; increased sympathetic activity increases intracellular Ca\(^{2+}\) and parasympathetic mediated heterogeneous abbreviation of the atrial ERP promotes the likelihood for arrhythmias (25).

**Calcium as a contractile messenger**

The existence of a plateau phase during the AP can basically be explained by the balance between inward Ca\(^{2+}\) and outward K\(^+\) flux. Initially, influx of Ca\(^{2+}\) happens through activation of voltage gated L-type Ca\(^{2+}\) channels (primarily Cav1.2, encoded by CACNA1C) (26), ubiquitously expressed in the heart across the tubular membrane, that trigger the release of Ca\(^{2+}\) from intracellular stores in the SARC into the cytosol via activation of ryanodine type 2 receptors (RYR2) located on the surface of the SARC (5). Ca\(^{2+}\) diffuses into myofibrils where it binds to troponin-C on the myofilaments which eventually causes contraction. This Ca\(^{2+}\)-dependent Ca\(^{2+}\)-release is a key element in the excitation-contraction coupling and a prerequisite for muscle function and contraction (27, 28). Relaxation of the myocyte is the reverse process, in which Ca\(^{2+}\) dissociates from the myofilaments and Ca\(^{2+}\) is extruded from the cytosol. Several mechanisms are involved in the clearance of cytosolic Ca\(^{2+}\), where the two major players are SERCA2a pumping Ca\(^{2+}\) back into the SARC and the NCX exchanging intracellular Ca\(^{2+}\) for extracellular Na\(^+\) “forward mode”. Cytosolic Ca\(^{2+}\) is also removed by Ca\(^{2+}\)-ATPase and the mitochondrial Ca\(^{2+}\) uniporter (28, 28, 29). The NCX also plays some part in the Ca\(^{2+}\) influx during the plateau phase of the AP, with a reversal potential around -30 mV resulting in Ca\(^{2+}\) influx and Na\(^+\) extrusion “reverse mode” (30). Dysregulation of intracellular calcium handling is a major contributor to arrhythmogenesis and has been suggested to play an important role in the initiation and maintenance of atrial arrhythmias (31).

**ATRIAL FIBRILLATION**

Atrial fibrillation (AF) is a supraventricular arrhythmia characterized by complex spatiotemporal organization and non-uniform electrical conduction (5). In AF, the atrial electrical activity is completely irregular and disorganized, as can be observed on the electrocardiogram figure 3. This is due to the presence of rapid electrical stimuli in areas other than the SA node within the atria. This will result in rapid and irregular atrial activity and, instead of contracting, the atria only quiver. Generally, the AV-node discharges at irregular intervals, thus the ventricles beat in an irregular rhythm (32), however at a lower rate compared with the atria due to the relatively long refractory period of the AV-node. AF is the most frequent cardiac arrhythmia seen in the clinic with a prevalence of approximately 2% - with more than 6 million Europeans and more than 6 million Americans affected (33-35). AF contributes significantly to cardiac morbidity and mortality (32, 36). The Framingham Heart study, one of the largest and most thorough clinical studies in cardiac medicine, predicted up to a twofold increase in the risk of death in patients with a history of AF, and embolic stroke is increased four- to fivefold (37, 38).

Clinically, AF is defined as an episode lasting longer than 30 seconds. AF can be categorized as: Paroxysmal AF; episodes of AF terminating spontaneously within 7 days or cardioverted within 48 hours. Persistent AF; episodes of AF lasting longer than 7 days or cardioverted after 48 hours. Longstanding persistent AF; defined as continuous AF of greater than 12 months' duration. Permanent AF; where restoration and maintenance of sinus rhythm (SR) has either failed or it has been decided not to attempt rhythm control (39). It is recognized that in many cases a particular patient may have AF episodes that fall into one or more of these categories (40). Patients suffering from AF, reported in Study I had been in AF for longer than 6 months and were in AF at the time of surgery; however fractionated in the sense of treatment regimens with some being on rate control and some being on rhythm control and with a larger fraction of patients undergoing ablation during the surgical procedure. The classification of this patient group therefore lies somewhere in between persistent and permanent AF, thus this patient group was defined as Chronic AF patients.

**Figure 3** Electrical propagation in the healthy heart in sinus rhythm left and during atrial fibrillation right. The impulse is initiated in the SA node and travels through the atria to the AV node, giving rise to the P wave prior to the QRS complex observed on the electrocardiogram (ECG) in the lower panels. After a short delay in the AV node the impulse travels through the bundle of His to the ventricular purekinje fibres. During atrial fibrillation the coordinated atrial conduction is disturbed by rapid electrical activity making the atria quiver and the ventricular conraction irregular. On the left ECG, from a patient in SR, the P wave represents the depolarization of the atria, next the QRS complex represents the depolarization of the ventricles and finally the T wave represents the repolarization of the ventricles. The right ECG from an AF patient, the P wave is replaced with fluctuations of the baseline, the ventricles beat irregularly, giving rise to irregular QRS complexes and the T wave becomes difficult to distinguish.

According to classical theory, for an arrhythmia to occur a substrate and a trigger is required. As a necessity for arrhythmia to establish, a triggering event must initiate self-sustained AP propagation. The substrate improves the possibility that a trigger will elicit an arrhythmia (41). In the following section, such theories will be reviewed.
Triggers of atrial fibrillation

The proximal cause of cardiac dysrhythmias can be abnormal impulse formation leading to focal ectopic arrhythmia generators figure 4. Such a triggering event is due to spontaneous discharge in any part of the myocardium external to the SA node and the AV node, called an ectopic focus, and may occur in abnormal situations, presenting the heart to increased automaticity. If an ectopic focus discharges, the result will be a beat that occurs before the next normal beat and transiently interrupts the cardiac rhythm (42). This is called an extrasystole or premature beat. If a focus discharges repetitively at a rate higher than that of the SA node, it produces rapid and regular tachycardia; called paroxysmal (meaning episodic) tachycardia or atrial flutter (24).

Ischemia induced depolarization or increased sympathetic activity can cause pacemaker activity to be initiated at an ectopic focus either in the atria or ventricles leading to afterdepolarizations. Early afterdepolarizations (EADs) or delayed afterdepolarizations (DADs) shown in the lower part of figure 4 can act as triggers for the generation of abnormal impulses or extrasystoles. L-type Ca\(^{2+}\) channel inactivation, and particularly the release from inactivation, are important features in the formation of afterdepolarizations. Reactivation of L-type Ca\(^{2+}\) channels in the late phase 2 and phase 3 of the AP gives rise to EADs that can serve as triggers for arrhythmic events (43, 44). Unintended activation of the Na\(^+\)/Ca\(^{2+}\) exchanger (NCX) during the diastolic interval, resulting in a net inward gradient, could potentially give rise to DADs (45). EADs can also be a result of treatment with conventional class III anti-arrhythmic drugs that prolong the QT interval, which will be explained thoroughly later.

A substrate for arrhythmia can be an abnormality in the conduction system that permits wave excitation to propagate continuously within a closed circuit. This can be described as a situation of electrical reentry and is a general cause of paroxysmal arrhythmias (32). Abnormalities allowing reentry, for instance cellular or electrical remodeling, can mediate unfavorable electrical or anatomical changes of the conduction system. This could be the case in tissue regions of fibrosis, ischemia or infarct. Such anatomic conditions can ultimately disturb the normal pathways of conduction, causing acceleration, delay, fractionation or rotation of the propagating impulse. These are all essential co-players in arrhythmogenesis (46, 47). Furthermore, several other factors can contribute to the initiation and maintenance of AF, including hypertension, heart failure and cardiac valve diseases (48).

Whether or not an impulse propagates is dependent on the ERP of the cells it encounters. Normally an impulse spreads in every direction and the tissue immediately behind each branch of the impulse is refractory (24). If the ERP is shortened or if the propagating impulse is blocked in a tissue region, the heart will become prone to reentry arrhythmias. To this end, heterogeneity in refractoriness is seen as a strong enhancer of arrhythmia vulnerability (49). Reentry may occur in the absence of an anatomical substrate, if a functional blockage is present. This is dependent on both a unidirectional block, and a sufficiently long circuit path length. The unidirectional block allows the impulse that initiates the circuit movement to only propagate in one direction. The path length of the circuit enables the impulse to reach tissue that has recovered from inactivation and is no longer in a refractory state. This process can continue indefinitely and will allow self-sustainable, continuous propagation of the electrical signal which will cause re-entry arrhythmias. The hypothesis that AF is a result of multiple re-entry wavelets was proposed more than 50 years ago, and has been the predominant theory since (50).

MECHANISMS OF ATRIAL FIBRILLATION

Traditional cardiac theory involves two main mechanisms of AF. 1) One or more fast depolarizing foci, generating abnormal impulses, which function as triggers of arrhythmia, and 2) a fibillation prone heart that allows reentry to occur in one or more tissue circuits (51, 52). More recently the spiral wave theory, involving rotors and circulating spiral waves of excitation in the arrhythmogenesis of AF, has gained acceptance (53). In fact, these theories are probably connected and somehow linked to the arrhythmogenesis and progression of AF. The more we learn about the pathophysiological nature of arrhythmias the closer we get to unraveling the complexity of its genesis and maintenance.

Reentry arrhythmias

A state of multiple reentry wavelets circulating the atria has traditionally been the theory and understanding of how supraventricular arrhythmias could perpetuate and be sustainable (32, 50). According to the leading circle theory (54), the wavelength (WL) equals the path length an impulse travels during one refractory period. The WL is therefore defined as the product of the velocity of the propagating impulse (CV) and the duration of the ERP (WL = CV x ERP). The possibility for reentry to occur demands that the impulse survive until the refractory period is over. The WL determines the shortest possible circuit path length, the “leading circle”, in which reentry can exist. If the circuit is shorter than the wavelength the propagating impulse will encounter refractory

Substrate for atrial fibrillation
tissue and extinguish once it returns to its point of origin. In figure 5 the concept of the leading circle is depicted, where the wavelength shown by the thick arrow will propagate continuously through excitable tissue. Once stimulating efficacy is just enough to reach excitation threshold, the circulating wavefront will excite tissue ahead, which is still relatively refractory. Thus the leading circle leaves no fully excitable gap. This tight fit ensures that the length of the circuit pathway equals the wavelength of the circulating impulse (54). Impulses traveling through shorter circuits, such as the centripetal impulses shown by thinner arrows will encounter refractory tissue and extinguish. These centripetal wavelets constantly keep the central core in a refractory state. Impulses moving in larger circuits will be dominated by activation originating from the shortest possible reentry circuit; the leading circle. Thus, a short wavelength would allow reentry to occur even in circuits of short path lengths. Short refractory periods or slow CV would shorten the length of the excitation wave, thus increasing self-sustainability of a circulating wave ultimately leading to reentry arrhythmias.

There has been a general consensus in the field for ectopic foci, leading circle reentry circuits, and multiple reentry circuits all being part of the arrhythmogenesis of AF (32). The multiple-wavelet hypothesis, as the mechanism of reentrant AF, proposes that fractionation of the wave fronts as they propagate through the atria results in self-perpetuating "daughter wavelets". The number of wavelets present at any time depends on the ERP, mass, and conduction velocity in different parts of the atria (55).

Another important arrhythmogenic factor in reentry is electrophysiological heterogeneity of the cardiac tissue. Spatial atrial AP/APD heterogeneity occurs within and between atrial regions and play a role in atrial reentrant arrhythmias (49). In this context, vagal stimulation shortens atrial APD in a spatially heterogeneous fashion (58), producing important proarrhythmic effects (59).

In experimental models of AF, high vagal activity has been utilized by stimulating vagal nerve activity, either directly using electrodes (50), by hypoxia, or pharmacologically by injecting acetylcholine, thus producing less refractory heart tissue in order to initiate AF.

Animal models (60-62) and clinical studies (63) suggest an important role of the left atrium in AF. This may partly be due to accelerated left atrial repolarization (64), which shortens ERPs, favoring reentry (32). However, at the end of last century it was discovered that abnormal activity in left atrial pulmonary vein and superior vena cava also triggers AF (65-67). The hypothesis suggests that maintenance of AF may depend on the periodic activity of a small number of rotors in the posterior left atrial wall/pulmonary vein region. These rotors activate the atria at exceedingly high frequencies resulting in fibrillatory conduction (53). Today AF is often managed clinically by catheter ablation of ectopic foci located in the pulmonary veins or in the left atrium, however success rates are still far from ideal (68).

Spiral waves

The spiral wave theory has provided insights to the fundamental properties of cardiac reentry, bridging the gap between theoretical and experimental arrhythmia models. According to this theory, reentry is maintained through the ability of circulating spiral waves to perpetuate in an environment that is sufficiently excitable to support the angle of the spiral curvature. A spiral wave can be described as an excitation wavefront rotating continuously around an excitable core centre. Different to the leading circle, the spiral wave operates with a curved wavefront, as depicted in figure 5, which is important for its existence. Depolarization is initiated in the core centre, shown by the dashed circle. The curvature of the depolarizing wavefront is depicted by the solid blue line and the direction of impulse propagation by the arrows. The convex nature of the wavefront causes a source-to-sink mismatch, as one cell has to excite more than one cell in front of it, which will slow the velocity of propagation. The curvature of the wavefront will determine the velocity of propagation and cause CV heterogeneity. Behind the wavefront the repolarizing front will follow, shown by the red line. The gray area in between the two fronts is in state of refractoriness. Because of the curvature of the spiral wave, the depolarization and repolarization fronts will meet at the tip of the wave. This area is named the phase singularity and is depicted at the point where the blue and red fronts meet. This represents an area that is highly excitable, however, unexcited due to the biophysical nature and rotation of the spiral wave (56). This stands in contrast to the leading circle theory of reentry, where the core is constantly excited and maintained in a refractory state. While the leading circle theory fails to explain the effects produced by Class I antiarrhythmic drugs, the spiral wave theory predicts that block of Na⁺ can be antiarrhythmic by reducing the excitability of the spiral core centre. Inhibition of I_{Na} will cause an increase of the core size and decrease the curvature, along with a reduction in the source-to-sink mismatch, which together will diminish the driving source of the spiral wave and terminate the arrhythmia, despite a decrease in wavelength (56, 57).

Figure 5 (left) the leading circle concept: Activity establishes itself in the smallest pathway that can support reentry, shown as the tight fit between the wavelength and the circuit length. Inside the leading circle, centripetal wavelets (small arrows) emanating from it constantly maintain the central core in a refractory state. (Right) Spiral wave model: Schematic drawing of a spiral wave with the activation front shown in blue and the repolarization front in red, with the gray area being refractory tissue. The outer arrows depict the direction of the depolarization front. The point at which the red and blue curves meet has an undefined voltage state and is usually referred to as the phase singularity point. Inspired by Comtois, et al 2005 (56).
Atrial fibrillation begets atrial fibrillation

AF induced atrial remodeling plays an important part in the maintenance, advancement and perpetuation of AF (69). AF causes both structural and electrically remodeling of the atria. This remodeling increases the likelihood that AF becomes self-sustainable and permanent. This was demonstrated in a goat model of atrial tachypacing leading to remodeling of the atrium, induction of AF and increased duration of AF, once it was introduced (70). When AF is present and sustainable it gets increasing difficulty to manage. A phenomenon frequently observed in the clinic known as “AF begets AF”. Atrial tachycardia remodeling is important in the arrhythmogenesis of AF. It causes non-uniform remodeling of atrial refractoriness, which plays a significant role in increasing atrial vulnerability to AF induction and duration (71, 72). Atrial tachycardia abbreviates atrial refractoriness and decreases the WL, primarily by \( i_{\text{ca}} \) downregulation and increased inward rectifier \( K^+ \) currents (47). The reduced WL caused by APD abbreviation decreases the size of functional reentry circuits and promotes multiple circuit reentry. Atrial tachycardia remodeling also causes contractile dysfunction and the formation of afterdepolarizations, mainly via \( Ca^{2+} \) handling abnormalities, which may promote AF maintenance (73). Progression in electrical remodeling causing AP alterations as a consequence of AF duration is evident in atrial tissue taken at different stages of disease progression. An example of this is shown in figure 6. Here APs are recorded in atrial tissue from SR, persistent AF and chronic AF patients. As the disease progresses electrical remodeling abbreviates the AP, the characteristic spike-and-dome morphology disappears which gives rise to a more triangulated shape, the AP peak is augmented and lastly the RMP gets hyperpolarized. Electrical remodeling in AF also causes loss of the rate adaptation shortening of the atrial APD as a response to increasing heart rates. Structural remodeling as a consequence of AF comes in forms of increased levels of non-conductive atrial fibrotic tissue, dilatation and hypertrophy (74, 75), which all could serve as substrates promoting the perpetuation of AF.

MANAGEMENT OF ATRIAL FIBRILLATION

Two principal strategies exist for the management of AF: Rhythm control and rate control. Rhythm control has traditionally been the primary treatment paradigm in AF, and is based on the principle of restoration and maintenance of SR. For patients, where rhythm control has failed or not been an option, rate control has been the second line treatment. Rate control is based on controlling the ventricular rate by using drugs that prolong the AV-node conduction, such as beta blockers, calcium channel blockers or digoxin (40). Several large scale clinical studies including (AFFIRM, HOT CAFE, STAF, RACE and PIAF trials) comparing the effects of rate control versus rhythm control found that rhythm control was not superior to rate control in reducing the risk of hard endpoints such as stroke and death (76–80). These results came as a rather discouraging surprise, primarily since it was always believed that maintenance of SR would reduce the burden of embolic stroke, a direct consequence to the fibrillating atria, thereby reducing mortality. One explanation for this rather contra-intuitive result is that currently available drugs applied in rhythm control are ineffective in keeping the patients in SR and furthermore exhibit poor safety profiles including both cardiac and extra-cardiac toxicities, such as ventricular proarrrhythmia (81, 82) and hyperthyroidism (83). A study reanalyzing the independent risk factors of the AFFIRM trial, demonstrated that SR as an independent predictor, was associated with a reduction in the risk of death, and concluded that the beneficial effects of drugs used in rhythm control are offset by their adverse effect (84). Today’s antiarrhythmic drug development strives to find an effective drug for maintaining SR with limited side-effects.

Traditionally, management of AF utilizes drugs that alter the electrical properties of ion channels in the heart. Pharmacologically, these drugs have been classified on the basis of which ion channel or receptor they block. Accordingly, class I block \( Na^+ \) channels, class II block \( \beta \)-adrenergic receptors, class III block \( K^+ \) channels and class IV block \( Ca^{2+} \) channels (85). Often anti-arrhythmics fall into two or more of these classifications and are thus referred to as multiple ion channel blockers (86). The principles of antiarrhythmic drug effects include suppression of excitability and prolongation of the ERP. Excitability can be reduced by blocking \( Na^+ \) channels or by reducing \( \beta \)-adrenergic innervation and the ERP can be prolonged either by slowing of repolarization (class III), or by enhancing post-excitatory refractoriness (class I). The widespread use of conventional class III antiarrhythmic
agents has been limited by their potentially fatal ventricular proarrhythmic effects (85). Also, the clinical use of some class I drugs has been restricted in light of results from the CAST study, which revealed higher mortality risk in the treated population compared to control population (87). Existing antiarrhythmic drugs approved for the treatment of AF exhibit moderate efficacy for AF termination and suppression and have significant associated adverse effects, resulting in poor patient tolerance, which limits their use (88, 89). This apparent need for safe pharmacological therapies has generated the development of several exciting drugs for the medical management of AF. However, the challenge of proving efficacy and safety in large randomized controlled trials will remain for any promising new agent (89). Alternative treatment such as pulmonary vein isolation or implantable cardioverting defibrillators have been shown in some studies to be more efficacious and safer than drug treatment in AF (90). Nevertheless, there is hope that fast developing knowledge on the pathophysiology of AF will lead to safer and better drugs targeting underlying mechanisms (32). Ongoing drug development has focused on increased safety by targeting ion channels specifically expressed in the atria. However, the number of such atrial-specific ion channels is limited. So far the ion channels responsible for the acetylcholine-activated current (I_{ACh}) and the ultra-rapid delayed rectifier potassium current (I_{Kr}) have been the main targets in the search for an atrial-specific antiarrhythmic drug, however available compounds that exhibit exclusive selectivity for these currents are limited (91). Furthermore, translatability of successful experimental effects to clinical efficacy remains questionable (92, 93).

Proarrhythmic effects of prolonging repolarization

The antiarrhythmic principle of prolonging cardiac repolarization is a two-edge sword. The inhibition of potassium channels can predispose to tachy-arrhythmias due to the fact that the late phase of cardiac AP is highly susceptible to abnormal excitation (94). This intimidating paradigm was first recognized in the SWORD (Survival With Oral D-sotalol) trial, assessing the efficacy of the class III antiarrhythmic drug D-sotalol, which was terminated due to an increased mortality in the drug treated patients relative to the placebo treated control group (82). It has later been recognized, that most conventional class III agents induce the risk of initiating polymorphic ventricular tachycardia named “Torsade de pointes” (Tdp). During repolarization the heart muscle becomes vulnerable. The vulnerable period occurs at a time when some of the myocardium is depolarized, some is incompletely repolarized and some is completely repolarized. These are excellent conditions for arrhythmias to establish. A possible mechanism of the proarrhythmic effect of QT prolongation is the resulting occurrence of EADs. These are depolarizations of the AP initiated prior to completion of repolarization of the previous AP figure 4 (95). It is documented that EADs contribute to the induction of polymorphic ventricular Tdp arrhythmias, which can generate ventricular fibrillation (VF) and eventually cardiac death (10). EADs are also implicated in the reinduction of AF immediately after its termination (96). EADs are dependent on both prolongation of the APD, and recovery of the Ca^{2+} current through L-type channels, that carry the depolarizing charge (95). It has been shown that not only the QT prolongation is responsible for the induction of arrhythmia, but also the simultaneous occurrence of other risk factors abbreviated TRIAD (Triangulation, Reverse use-dependency, Instability and Dispersion) of the action potential (97). Studies show, that lengthening of APD without instability or triangulation is not proarrhythmic in itself but rather antiarrhythmic (98).

As described by others, the atrial versus ventricular activities of Class III agents are different according to the K’ channel blocking profile (99). These findings support the potential of selectively modulating atrial versus ventricular refractoriness by targeting appropriate K’ channel subtypes. Ultimately, the identification and targeting of an appropriate K’ channel subtype or mix of subtypes may result in the achievement of atrial-selective effects for the treatment of supraventricular arrhythmias.

Atrial specific targets

Differences in the balance of inwardly and outwardly ion currents between atrial and ventricular cells, which is mainly the result of regional differences in ion channel distribution, opens an opportunity for pharmacological atrial-selective targeting. As explained above, antiarrhythmic drug therapies are often associated with simultaneous deleterious effects on the ventricles, which has brought about the identification of drug targets specifically or predominantly associated to the atria. The I_{Kr} (100), the I_{ACl} (93, 101) and both peak and late atrial Na’ currents (102) have become potential targets in antiarrhythmic drug development, and agents targeting these currents have been under clinical evaluation, while others have been drawn back for undisclosed reasons (103). However, clinical evidence in converting AF to SR or reducing AF burden remains to be demonstrated for selective I_{Kr} blockers (93).

Atrial selectivity can also be achieved by targeting Na’ channels in an atrial-selective manner, through state-dependent blocking properties enabling a higher degree of block in the atria compared to the ventricles. This is reported for molecules selectively inhibiting Na’ channels that are rapidly activated in situations such as AF or atrial flutter (104), such an effect is referred to as use-dependency. It has also been suggested that atrial selective properties of Na’ channel blockers are brought about by atrial-ventricular differences in the biophysical properties of the Na’ channel and differences in the morphology of atrial and ventricular APs. Steady-state inactivation of I_{Na} is more negative in atrial than in ventricular myocytes (105). As a consequence of the more depolarized resting membrane potential in atrial cells and the more negative steady-state inactivation, a larger fraction of Na’ channels are inactivated during diastole of atrial cells compared to ventricular cells. The fraction of resting channels is therefore smaller in atrial versus ventricular cells at RMPs. As much of the recovery from Na’ channel block occurs during the resting state of the channel, atrial cells show a greater accumulation of use-dependent Na’ channel block (21).

I_{Kur}

The ultra-rapid rectifier K’ channel (K_{r1.5}) encoded by KCNA5, which conducts the I_{Kur} current, has for some time been considered an interesting drug target candidate for atrial selective treatment of AF, since the current seems to be absent in the human ventricle (106). Despite a reduction in I_{Kur} current levels as a consequence of AF remodeling (107), new evidence describes that selective I_{Kur} inhibition promotes larger effect on APD and ERP in human AF tissue compared to SR (108), probably due to an overall reduction in atrial repolarization reserve in AF. In 2010 Vernakalant was marketed in the EU, approved as an infusion drug for the conversion of recent onset AF. As a possible atrial-
selective drug, Vernakalant was reported to potently block among other currents, I\textsubscript{Kur} (109) in the atria, however recent data from human tissue studies report only minor effects on I\textsubscript{Kur} while the main antiarrhythmic mechanism of vernakalant is suggested to be mediated primarily through rate dependent block of Na\textsuperscript{+} channels (110). The increasing interest in this target has, however, led to the development of compounds more or less selectively inhibiting I\textsubscript{Kur} (111), showing effective antiarrhythmic properties in various animal models including dogs (112) and pigs (113). Newly developed compounds targeting I\textsubscript{Kur} appear with more attractive selectivity profiles (114). However, the potential clinical benefit of this atrial-selective target in antiarrhythmic therapy still needs to be confirmed.

**I\textsubscript{K,ACH}**

The cardiac acetylcholine activated inwardly rectifying current I\textsubscript{K,ACH} conducted through the G protein coupled K\textsuperscript{+} channels complex is composed by heteromeric assembly of Kir3.1/GIRK1 and Kir3.4/GIRK4 encoded by KCNJ3 and KCNJ5 (115) (116). I\textsubscript{K,ACH} constitutes another interesting atrial selective antiarrhythmic target in AF. Even though it has been proposed predominantly in the atria (117, 118), also human ventricular I\textsubscript{K,ACH} has been reported (119). I\textsubscript{K,ACH} activation hyperpolarizes RMP and shortens atrial action potentials and ERP, thereby contributing to maintenance of AF by promoting reentry. Selective block of I\textsubscript{K,ACH} has revealed clear antiarrhythmic effects in different in vivo models of experimental AF (120). While the activatable part of I\textsubscript{K,ACH} like most other K\textsuperscript{+} currents (with the exception of I\textsubscript{K1}) is downregulated in permanent AF (121), I\textsubscript{K,ACH} draws particular interest, since its constitutively active current is upregulated in AF (122), and together with I\textsubscript{K1} might account for the more hyperpolarized RMP in AF tissue (93). A recent study described the difficulties in translating antiarrhythmic results obtained in animal studies to those in man. Despite high antiarrhythmic efficacy of specific I\textsubscript{K,ACH} blockers documented in dog models of experimental AF, it is questionable whether such drugs have any effect in man. The authors conclude that translation of effects observed in animal models should be interpreted with precaution (92).

**SMALL CONDUCTANCE CA\textsuperscript{2+} ACTIVATED K\textsuperscript{+} (SK) CHANNELS**

Sequence analysis of the cloned channels has revealed three subfamilies of Ca\textsuperscript{2+}-activated K\textsuperscript{+} channel subunits that had originally been classified according to their single channel conductance: big conductance BK (KCNMA1/KCa1.1), intermediate conductance IK (KCNN4/KCa3.1) and small conductance SK1, SK2, SK3 (KCNN1, KCNN2, KCNN3/KCa2.1, KCa2.2, KCa2.3, respectively) channels (123), where the focus here is on the latter. The IUPAC nomenclature is (KCa2.x), however, for simplicity the trivial names (SK1-3) have been used. SK channels are formed by tetrameric assembly of four α-subunits each having six transmembrane segments, with intracellular N- and C-termini and the S5 and S6 aligning the pore (124). SK channels are expressed in mammals in various tissues, including nervous system, vasculature, skeletal muscle, smooth muscle and cardiac tissue (125-129). Cardiac SK subunits have been shown to form both homo- and heteromultimeric channel complexes (130, 131). Even though it has been proposed by some (132), SK channels have not been demonstrated to be regulated by β-subunits (133).

**Figure 7** Topological model of a single SK α-subunit with six transmembrane spans ending the plasma membrane with both N and C terminus on the intracellular side. The calmodulin (CaM) molecule is constitutively bound to the intracellular C-terminus and functions as the channel’s Ca\textsuperscript{2+} sensor. Three subtypes of SK α-subunits exists (SK1-3) which go together in four to form the tetrameric channel complex. Both homomeric and heteromeric SK channel complexes have been reported to exist.

SK channels are activated by a rise in intracellular Ca\textsuperscript{2+} ([Ca\textsuperscript{2+}]\textsubscript{i}), with the three subtypes exhibiting similar sensitivities for Ca\textsuperscript{2+} activation yielding half maximal activation at approximately 300 nM [Ca\textsuperscript{2+}]\textsubscript{i}, with a Hill coefficient between 4 and 5 (134). This gives rise to a fast activating, moderate inwardly rectifying K\textsuperscript{+} current (135-137). SK represent a unique class among Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels since they are exclusively gated by Ca\textsuperscript{2+} in a time and voltage independent manner, thereby integrating changes in intracellular Ca\textsuperscript{2+} with changes in K\textsuperscript{+} conductances (124). The activation is not mediated through direct binding of Ca\textsuperscript{2+} to the channel complex but rather through binding of Ca\textsuperscript{2+} to calmodulin (CaM) which is constitutively bound to the calmodulin-binding-domain at the C-terminal of each α-subunits, serving as the channels’ Ca\textsuperscript{2+} sensor (138). SK channel activity follows the free Ca\textsuperscript{2+} concentration in proximity to the channels making the I\textsubscript{KCa} current activity strongly depends on the distance to its regional Ca\textsuperscript{2+} source (139).

SK channels were first reported in non-innervated skeletal muscle where the afterhyperpolarization of the membrane potential was found to be mediated by I\textsubscript{KCa} (140). It was later shown in various neuronal cell types that SK channel activation constitutes the intermediate phase of afterhyperpolarization and in turn regulates changes in cellular excitability (128, 141). SK channels play an important role in setting the firing frequency in neuronal tissue (142), and as a consequence of specific pharmacological blockade, neuronal excitability can be increased (143).

Functional coupling of L-type Ca\textsuperscript{2+} channels and SK channels has been described in cardiac tissue via the cytoskeletal protein α-actinin2 (144). Also, in neuronal transmission, close interaction between SK channels and voltage-gated Ca\textsuperscript{2+} channels has been shown to play a critical role in regulating excitatory synaptic transmission (128, 145). As the level of neuronal activity rises, the more SK channels are likely to become activated, due to the rise in [Ca\textsuperscript{2+}]\textsubscript{i}, providing a negative feedback mechanism for neuronal excitability (128).
**Pharmacology of SK channels**

Various toxins have been used in the identification and characterization of Ca\(^{2+}\) activated K\(^+\) channels (146), one of them being the highly selective 18-amino-acid bee-venom toxin called apamin (140), which was one of the first toxins described to show selective block of K\(_{Ca}\) channels (147). SK channels are selectively blocked by apamin in concentrations ranging from 100 pM–10 nM (134), which distinguish them from all other K\(_{Ca}\) channels. The three SK channels show subtype-specific affinity for apamin induced inhibition; a feature which has been used for determining the expression pattern of SK channel subtypes in native tissue (148). SK channels can be blocked by a number of pharmacological agents besides apamin, including several scorpion toxins, such as scyllatoxin and tamapin (128). Lei-Dab7, a synthetic derivative of Leiurotoxin selectively blocks SK2 channels (149). Compounds such as tubocurarine and UCL1684 mimic the structural elements of these selective SK neurotoxins whereas N-(pyridin-2-yl)-4-(pyridin-2-yl)thiazol-2-amine (ICA) has been suggested to act by blocking the channels through its chelation to a cation (150). All the above mentioned compounds displace [125I]-apamin binding and are considered as pore blockers acting at the apamin binding site (151).

A novel class of selective SK channel inhibitors that do not block the channel pore has also been described (151, 152). The SK [125I]-apamin binding site is not displaced by these compounds in binding studies, and they still inhibit SK channels when point mutations of essential amino acids have disrupted the apamin binding site (151). Representing a compound from this structural class, the pharmacology of (R)-N-(benzimidazol-2-yl)-1,2,3,4-tetrahydro-1-naphthylamine (NS8593) has been studied in detail, showing that it indiscriminately modulates all SK1-3 subtypes negatively by decreasing the sensitivity towards Ca\(^{2+}\), rightward shifting the activation curve for Ca\(^{2+}\), only slightly affecting the maximal Ca\(^{2+}\) activated I\(_{Ca}\) current (152). As mentioned, blockade of SK channels by apamin formed the basis for their characterization (140), and provided knowledge into the subunit composition due to the differential apamin sensitivity of SK channel subunits. SK2 channels are the most sensitive to apamin (IC\(_{50}\) 0.03–0.14 nM), followed by SK3 channels (IC\(_{50}\) 0.6–4 nM), with SK1 channels being the least sensitive (IC\(_{50}\) in the 0.1– 12 nM range (136, 148). Sensitivity of these channels to apamin has been suggested partly to be dependent on the expression system used (136, 153). The binding site for apamin is located in both the pore region, between S5 and S6, and at a serine residue located in the extracellular region between S3 and S4 (154). SK channel block by apamin has been suggested as a possible therapeutic in cognitive disorders, improving memory and learning by increasing synaptic plasticity (127). This optimism was later dampened, though, by the simultaneous toxic effects produced by SK channel inhibition, leading to neuronal over-excitability causing epileptic activity and tremors as a consequence (155).

A number of SK channel enhancers also exist, which enhance both the calcium sensitivity and open probability of SK channels, including; dichloro-EBIO (DCEBIO) and NS309 (156-158). Recent structural studies have revealed the possible binding pocket of these positive modulators to be located at the interface between the channel α-subunit and calmodulin (159).

**CARDIAC SK CHANNELS**

Even though SK channels have not traditionally been considered important in cardiac tissue, it has in the last decade been recognized that these channels play a role in cardiac electrophysiology. Initial ideas of putative cardiac SK channels, reported more than 40 years ago, were later put aside due to contradictory views in 1983 (160). More than a decade later the first evidence of SK3 subunits and I\(_{Ca}\) currents were reported by Wang, et al. to exist in myocytes derived from a rat ventricular cell-line (161). These results rekindled the attention in the cardiac SK channel, and in 2003 Xu, et al. confirmed the presence of functional SK2 channels in human and mouse hearts (162). Moreover, this study demonstrated a marked differential expression of SK2 channels exhibiting predominant distribution in the atria, which was in accordance with the apamin-sensitive current being significantly larger in atrial compared to the ventricular myocytes (162). The cardiac presence of all three SK channel subtypes was documented in 2005 by Tuteja et al., who also confirmed the atrial selective distribution of both SK1 and SK2 channel subtypes in mouse hearts. Today, SK channels have been demonstrated to have functional importance in atrial cardiomyocytes of various species, including mice, rats, guinea pigs, rabbits, dogs and human (94, 162-167). While the existence of cardiac SK channels is undisputable, the role they play is still a matter of debate and consensus has not at present been established. In contrast to the hyperpolarizing effects of SK channels in neurons and vascular tissue, in cardiomyocytes the SK current has been speculated to contribute towards the late phase of the cardiac repolarization (162, 164). This idea has however been contradicted by others, stating that SK channels have little or no effect on cardiac repolarization (168). In theory cardiac SK channels coupling [Ca\(^{2+}\)] to K\(^+\) conductance through I\(_{Ca}\) would make a lot of sense and explain a feedback mechanism reacting upon excessive Ca\(^{2+}\) release. Functional SK channels have also been reported to exist in nodal tissue, such as the AV-node. An increase in [Ca\(^{2+}\)] may under certain pathologic conditions produce profound changes in AV-node conduction. For example, during AF, the increased frequency increases [Ca\(^{2+}\)], which will potentiate SK current conduction. SK channel may therefore represent an attractive target of modulation during AF (129).

**SK channels in atrial fibrillation**

The first evidence of SK channels playing a role in electrical remodeling in response to rhythm disturbances was demonstrated by Ozgen, et al. in 2009. This study in rabbits showed increased trafficking of SK2 channels to the cardiomyocyte membrane leading to increased apamin-sensitive current and APD abbreviation as a response to intermittent burst pacing at the pulmonary vein-atrial interface, possibly providing the basis for an arrhythmogenic substrate (163). Further evidence was given by Li, et al. in 2009 exploiting a SK2 knock-out mouse model, in which they reported prolongation of the atrial APD and increasing vulnerability towards extra-stimuli induced AF. It should be noted though, that the likelihood of APD and ERP prolongation promoting the susceptibility towards re-entry arrhythmias has been questioned by others (169). In contrast, overexpression studies of SK2 channels in mice resulted in shortening of APs in the AV-node and increase in the spontaneous firing frequency, while ablation of SK2 channels led to the opposite result (129). This implies SK channels as an interesting target in modifying AV nodal conduction in atrial arrhythmias such as AF. These studies, although
being controversial, clearly demonstrate the association of SK channels in AF arrhythmogenesis. The first evidence directly linking SK channels to clinical AF was demonstrated by Ellinor, et al. in 2010 in a genome-wide association study, reporting that common genetic variants in the KCNQ3 gene encoding the SK3 channel are associated with lone AF (170). Later studies have confirmed and expanded this association (171, 172). In 2010 and 2011 our group published a series of papers demonstrating the antiarrhythmic effects of SK channel inhibition in various ex vivo and in vivo small animal models of experimental AF (165, 173, 174), one paper included in this thesis. The antiarrhythmic principle of SK channel inhibition in AF was confirmed in a canine in vivo model in 2014 (166). However, proarrhythmic effects of SK channel block has also been proposed, supposedly as a result of increased APD heterogeneity in the canine left atrium (175). Clinically, the role of SK channels is conflicting, since both $I_{\text{KCa}}$ upregulation (176) and downregulation (177) have been reported in persistent and permanent AF patients. This particular issue will be discussed later in the thesis.

**SK channels in heart failure**

SK channels are expressed predominantly in the atria of various species, and are attributed with a predominant impact in atrial electrophysiology, while exerting no or only limited functional effect in the ventricles. Recent reports, however, describe functional ventricular importance of SK channels under pathophysiological conditions such as heart failure in humans (178, 179) as well as in animal models (180-183). Although most studies show a consistent upregulation of SK channels and $I_{\text{KCa}}$ during heart failure, which supposedly serve as a protective mechanism securing sufficient ventricular repolarization reserve (180), this upregulation seems to be heterogeneously distributed between myocardial layers (179). $\beta$-adrenergic inhibition can completely reverse the upregulation in $I_{\text{KCa}}$, indicating that the degree sympathetic innervation is causal to heart failure remodeling. Several studies utilizing optical mapping in the intact isolated rabbit heart, show apamin to prolong the ventricular AP rendering the ventricles prone to arrhythmia. However, in one study, apamin eliminated recurrent ventricular fibrillation (181). It has also been proposed that the ventricular upregulation of $I_{\text{KCa}}$ acts as a safety feature eliminating DADs by reducing triggered activity (180). In the same way as the physiological role of $I_{\text{KCa}}$ in the atria is controversial and debated, the pathophysiological role of $I_{\text{KCa}}$ in the ventricles in heart failure is also disputed.

The latter results and discussion section will evaluate the three studies; in respect to their contribution to the scientific field and to the overall understanding of the role of atrial SK channels in SR and AF and as an antiarrhythmic target.

**AIM**

The role of SK channels and possible functional $I_{\text{KCa}}$ current in cardiac electrophysiology has in recent years been debated and has been a subject of particular controversy. At the time when the experimental work for this thesis project started, literature on the subject was not overwhelming. The initial part of the project objectives was built upon the, at that time, newly published evidence by Diness, et al. demonstration antiarrhythmic effects by SK channel inhibition in small animal models of experimental AF. Based on the hypothesis that SK channels might be increasingly activated during AF where the intracellular Ca$^{2+}$ concentration is elevated, the purpose with this thesis was formed. The aim with this thesis was therefore to expand our knowledge on antiarrhythmic mechanisms mediated through SK channel inhibition and to investigate whether SK channels and $I_{\text{KCa}}$ are functionally important in the human heart, thus constituting a therapeutic potential target in the treatment of AF.

**Research questions:**

What is the role of cardiac SK channels, and do they comprise a pathophysiological functionality rather than a physiological role, being silent during normal heart rhythms, however, increasingly activated during AF?

What mechanisms could explain the antiarrhythmic effects produced by SK channel inhibition?

Are functional SK channels and $I_{\text{KCa}}$ present in human atrial tissue under physiological conditions contributing to atrial repolarization and do they play a role in AF related electrical remodeling?

Can inhibition of cardiac SK channels and $I_{\text{KCa}}$ constitute a novel atrial-selective antiarrhythmic principle with a therapeutic potential?

Could $I_{\text{KCa}}$ be functionally important in human ventricular repolarization, thus comprising a potential hazardous target in the treatment of supraventricular arrhythmias?

**METHODS**

Throughout the three studies discussed, various experimental techniques and methods have been exploited to conduct the in vivo, in vitro, ex vivo and molecular studies which form the basis for this thesis. One particular method of measuring APs in isolated cardiac tissue has been instrumental in the genesis of a series of ex vivo experiments, both in human and animal tissue, which has been of fundamental importance for the novel findings reported in this thesis. The methods setup is schematically presented in figure 8. A part from the published results a series of unpublished findings obtained with human atrial tissue utilizing this methodological approach have briefly been presented in the introduction section.

**Fig 8** Schematic presentation of the Steiert organ bath. The cardiac tissue is placed in an organ bath superfused with oxygenated Tyrodes buffer, with a stimulating electrode fixed at one end. A sharp, high resistance recording glass electrode, connected to an amplifier is delicately lowered into the contracting tissue impaling the first cellular layers. At one point an intracellular voltage recording can be captured once a sufficiently resistant seal between the pipette tip and the impaled cell is established.

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RESULTS AND DISCUSSION

The principle of functional SK channels in the heart is not new. Already in the 1970’s researchers reported the possible existence of $I_{\text{SK}}$ in the heart (160), however this became disputed due to conflicting results and the difficulties in providing convincing evidence identifying cardiac $I_{\text{SK}}$ and the notion was put a side for more than two decades. It was put forward by Eisner et al. more than 30 years ago that a possible existence of a cardiac $\text{Ca}^{2+}$ activated $K^+$ current would be difficult to dissect: “However, the evidence that such channels exist is equivocal. This is partly because of technical problems, for example the difficulty of identifying an individual ionic current amongst the many currents that exist in the heart.”(160). Today, even though we have more selective pharmacological tools available enabling us to study specific ion current, this statement still holds true and the strive for providing substantial evidence in the identification of cardiac $I_{\text{SK}}$ continues.

Study I

1. Skibsbye L, Diness JG, Sorensen US, Hansen RS, Grunnet M. The duration of pacing-induced atrial fibrillation is reduced in vivo by inhibition of small conductance $\text{Ca}(2+)$-activated $K(+)$. The experimental work presented in this study was brought about by an extended and refined method reported in (165). Presenting a simple in vivo arrhythmia rat model, Study I demonstrates antiarrhythmic effects produced by two small molecule SK channel inhibitors along with various antiarrhythmic drugs, whereas the $\text{Ca}^{2+}$ channel inhibitor verapamil induced proarrhythmic effects. This study demonstrates that treatment with the SK channel inhibitors (NS8593 and UCL1684) is equally effective in shortening the duration of induced AF, compared with high concentrations of the multi-channel blocking drug ranolazine, which is approved for the management of chronic angina but also used clinically for the cardioversion of newly onset AF. Dose-response relationship in reducing AF duration resulted in a calculated half maximal effective concentration (IC50) of (1.58 mg/kg) for NS8593. Apamin was not convincingly effective in reducing AF burden in this model. The antiarrhythmic effects for all compounds, but lidocaine, were associated with prolongation of the ERP. Analysing data on experimental baseline AF duration as a function of ERP, we found a negative correlation between AF duration and ERP. This correlation was found to fit a semi-logarithmic relationship, with initial short ERP giving rise to long AF durations, while initial long ERP resulted in short AF durations. Interestingly, this study documented the antiarrhythmic effects mediated by the rapidly unbinding class I antiarrhythmic drug lidocaine, independent of ERP changes. This is interesting in regards to the mechanistic interpretation, favouring the theory of the spiral wave reentry, as explained in detail earlier, where a reduction in excitability of the spiral core centre, leading to the destabilization of primary rotors, could explain reentry failure (56, 184).

Study II:

2. Skibsbye L, Poulet C, Diness JG, Bentzen BH, Yuan L, Kappert U, et al. Small-conductance calcium-activated potassium (SK) channels contribute to action potential repolarization in human atria. Cardiovasc Res 2014;103:156-67. (167) In light of the findings presented in Study I we sought to identify if SK channels, reported to be present in human cardiac tissue, would play any functional importance in human cardiac electrophysiology. With the hypothesis that SK channels might be of importance in human atra, we wanted to investigate to which level SK channels are expressed in the human atra and whether $I_{\text{SK}}$ inhibition would inflict changes on the human atrial AP from patients in SR and chronic AF. From the electrophysiological work performed in this study we included 65 patients in SR, 22 patients in AF and received ventricular biopsies from 15 patients. The patient characteristics are show in table 1. The functional experimental work in human cardiac tissue and isolated cells presented in this study was primarily performed in Professor Ursula Ravens’ laboratory, Technical University Dresden, Germany. The biochemical and molecular work was performed at University of Copenhagen.

<table>
<thead>
<tr>
<th>Tabel 1 Patient characteristics</th>
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Table 1: Patient characteristics.
downregulation (177). However, whether functional SK channels are up- or downregulated in clinical AF is still controversial. Conflicting data has been reported, demonstrated both scenarios (176, 177, 185, 186). Recent evidence report micro-RNA’s being involved in electrical remodeling in AF (187), and report a link between upregulation of miRNA-499 leading to downregulation of SK3 protein in AF patients (177). It might be plausible that the duration of AF represent a determining factor to the expressional level of atrial SK channels. Short periods of AF could result in an initial upregulation of SK current by increased membrane trafficking, as it has been shown experimentally (163, 166, 188). However, the mechanism behind long-term AF remodeling resulting in downregulation of SK channel and I_{KCa} to a degree below basal levels still remains unclear. Such a bell-shaped phenomenon has, to my knowledge not been reported in cardiac remodeling, and if so, it would indeed complicate potential therapeutic interventions, utilizing SK inhibition as a concept, due to the inadequate AF duration assessment in clinical AF. While in experimental models of AF, telemetric tools have made it possible to monitor the exact duration of AF, however, the clinical situation is rather different and it is often unknown for how long patients have been in AF, due to the nature of this disease. Paroxysmal AF may come and go, while progression into more persistent and eventually permanent AF is often evident (69). Also, asymptomatic AF (silent AF) can be present for an indefinite duration prior to clinical diagnosis. Furthermore, high inter-subject variability in atrial electrophysiology in both SR and AF patients (189) along with high heterogeneity in AF progression and remodeling impedes the medical elucidation (190).

In the present study we demonstrated using immunohistochemistry and confocal microscopy that SK2 channels are present in the membrane and along the Z-lines of atrial SR myocytes. Although still present in AF myocytes the subcellular expression was irregular and clear membrane localization was absent, while the overall immuno-signal was much weaker compared to SR myocytes. It should be noticed that the cellular and subcellular structure was markedly changed in chronic AF tissue, showing elongated myocytes with long narrow nuclei and a disrupted Z-line structure. This atrial structural remodeling is reported to be induced by AF increased cellular stretch, hypertrophy along with increased interstitial fibrosis (191). This provides a morphological substrate for supporting arrhythmia and hence the persistence of AF.

After initial results documenting the presence of SK channels in human atria, we performed a series of experiments in single isolated cardiomyocytes in the attempt to dissect the I_{KCa} current. This proved to be difficult, since the recordable atrial inwardly rectifying current is a mixture of I_{KCa}, I_{K1}, I_{KACH}, I_{KATP} and possibly other currents. Utilizing specific pharmacological tools, we dissected a current sensitive to low concentrations (1 μM) of NS8593 and ICA. We believe that this current is specific I_{KCa} current, however, we could only identify this sensitive fraction of current in isolated cardiomyocytes from patients in SR and not in chronic AF. These findings were in accordance with low concentrations of NS8593 and ICA producing prolongation of SR but not AF APD in isolated cardiomyocytes. To further establish evidence of functional I_{KCa} current in the human atria we performed a series of experiments, measuring APs in intact atrial trabeculae muscle dissected from human right atrial appendages. The effects on the atrial APs mediated by NS8593 and ICA are clearly demonstrated in Study II. One limitation in studying pharmacological effect in multiple cellular preparations is the fact that the tissue itself encompasses a physical barrier difficult for lipophilic compounds to permeate by simple diffusion. This led to the necessity for long incubation protocols and relatively high drug concentration, high above the IC50 values reported for their primary target, when expressed in a single-cell context. This, of course limits an interpretation of highly selective targeting in this study. The investigations, however, showed little to no effect on the APs from chronic AF patients, and also in human ventricular muscle preparations we did not observe any functional importance of I_{KCa} inhibition. Interestingly, we observed a positive shift in the atrial RMP, which could explain part of the post-repolarization-refractoriness observed in SR tissue after I_{KCa} inhibition, serving as an indirect Na⁺ block thereby decreasing cellular excitability. This hypothesis led to the study design in Study III, with the aim of combining intracellular AP measurements with mechanistically analysis in a setting of high atrial frequency and arrhythmia.

Study III:


This study presents a model, where inducible runs of AF can occur in the isolated right atrium. By measuring intracellular APs in a situation of arrhythmia, enables the opportunity of studying electrophysiological parameters, otherwise not possible using monophasic-AP electrodes, optical mapping, ECG or other electrophysiological techniques. In this model, application of the most specific small molecule SK channel blocker ICA (167) inhibited the induction of AF, an antiarrhythmic effect which was accompanied with changes in the atrial AP. Apart from APD and ERP prolongation also a concentration-dependent depolarization of the RMP was observed. A number of parameters were carefully studied in this model, enabling us to predict upon Na⁺ availability both prior and subsequent to SK channel inhibition. Both upstroke velocity and upstroke amplitude were significantly decreased along with a slowing in conduction velocity, indicating Na⁺ channel block. However, automatized patch-clamping experiments on heterologously expressed Na_{p}1.5 channels revealed that ICA did not inhibit the sodium current at low concentrations, however at 30 μM - ICA gave rise to a use-dependent inhibition of I_{Na}. The observed depolarizing effect is indicating that the I_{KCa} current might also be active during the diastolic phase and that part of the antiarrhythmic effect observed by I_{KCa} inhibition is generated as an indirect effect of reduced sodium channel availability and reduction in excitability that expectedly will follow a depolarization of the membrane potential.

Measuring both ERP and CV in this study enabled us to establish a relationship of the path length traveled by the conducting impulse during the duration of one refractory period. Interestingly, we observed effects on both these two parameters that are responsible for determining the WL of excitation propagation. While a prolonged ERP augments the WL which in principal serves antiarrhythmic, the observed slowing in conduction velocity would abbreviate the WL favoring reentry arrhythmias. In this study we recognized despite a decrease in CV that the calculated wavelength (CV × ERP) was increased from 18.3 mm at baseline values to 31.8 mm, after I_{KCa} inhibition, a prolongation of 74%.
Curiously, it is hard to accept that the relatively small sized atrial tissue could encompass the existence of such long circuit paths. If traveling at normal conduction velocities reentry would seem quite unlikely since the circulating path would have to be very long. However, the ability of impulses being able to travel at slower velocities and also that the refractory period is not fixed in time are described phenomena (193) and plausible explanations to these observations. The refractory period can be heterogeneously distributed over the myocardium and the traveling impulse might linger in a small path in the myocardium for a longer time traveling at slow velocities (193), thus the necessity for long circuits to sustain reentry can be neglected.

The relationship between the wavelength and the path length of a tissue circuit is schematically presented figure 5. In relation to ICA effects, at baseline the wavelength is shorter than the circuit path length leaving a relative excitable gap for the circulating impulse to sustain reentry. ICA prolongs the wavelength to a degree where the propagating impulse will encounter refractory tissue and extinguish, leaving the circuit not functional for reentry to perpetuate. This understanding has for many years been the theory for mechanism of action of most antiarrhythmic drugs. From a different theoretical perspective, drawn from the spiral wave model of reentry, ICA mediated effects on indirect Na+ channel inhibition would also result in antiarrhythmic effects. As explained in both Study II and III, ICA application resulted in a reduced cellular excitability. A reduced excitability in the core centre would lead to destabilization of primary spiral wave rotors, and eventually result in reentry failure.

Besides elucidating and interpreting the mechanistic theory underlying the antiarrhythmic effects produced by I_{KCa} inhibition, this study also demonstrated intact contractile function of atrial tissue strips following drug application inhibiting I_{KCa}, which is in line with results obtained in trabeculae muscle from human atrial tissue. Relieving an antiarrhythmic compound from negative inotropic effects, which otherwise could reveal contraindications, in respect to its medical utility, serves interesting in respect to the possible therapeutic potential.

**Therapeutic evaluation**

The findings reported in Study II concerning SK channel downregulation and possible loss of functional importance in chronic AF patients, is from a therapeutic and drug-development perspective somewhat discouraging. However, it is a well-known fact, that when AF is persistent, it gets increasingly difficult to manage using presently available pharmacological options, wherefore a reduction in drug efficacy is an expected outcome in the treatment of chronic AF. When it comes to antiarrhythmic drug development the room for improvement is relatively large. The newest marketed antiarrhythmic drug in Europe, vernakalant, approved for pharmacological conversion of recent-onset AF (AF < 7 days) (194) shows a success rate in converting AF less than 40% of the overall patient population (195, 196) but is, however, considered superior in efficacy for cardioversion compared with the first-line treatment drug amiodarone (197). Furthermore, vernakalant is contraindicated in a number of patient populations including patients suffering from moderate to severe heart failure (198), which represent a substantial fraction of AF patients, thus limiting its therapeutic use. Other antiarrhythmic drugs such as propafenone, flecainide, or ibutilide can rarely be used in patients with AF, since they are contraindicated in structural heart disease due to the risk of proarrhythmic ventricular effects (199). The potential for a new antiarrhythmic drug, such as an SK blocker, can therefore be raised by improvement of either clinical efficacy or safety compared to presently marketed drugs. The fact that classical as well as newly marketed drugs exhibit low efficacy for cardioversion of AF and maintenance of SR combined with moderate safety-profiles, leaves the market open for better and safer antiarrhythmic drugs. Future experimental and clinical studies shall demonstrate whether a drug utilizing the concept of inhibiting I_{KCa} in an atrial-selective manner encompasses improved efficacy and safety profiles in the strive to advance treatment of patients suffering from this frequent arrhythmia.

**Perspectives**

Calcium dysregulation in AF is an important aspect which has only been briefly touched upon in the present thesis. It is evident, that in permanent AF, Ca2+ leak from the SAR and over-activity of NCX can lead to Ca2+ sparks and DADs promoting ectopic activity. It has been suggested that Ca2+/calmodulin-dependent protein kinase type-II (CaMKII), which is upregulated in AF, plays an important role in Ca2+ dysregulation and arrhythmogenesis. This includes phosphorylation mediated over-activity of L-type Ca2+ channels, NCX, RYR2, SK and GIRK channels (200). It has furthermore been reported that heart failure-induced atrial Ca2+ handling abnormalities lead to a Ca2+-wave arrhythmogenic substrate, important in the mechanism of AF associated to heart failure (201). A recent study demonstrates that in addition to interactions with cytoskeletal proteins, also intracellular Ca2+ enhances the membrane expression of SK channels (202). Studying Ca2+ dynamics in situations of arrhythmia along with Ca2+ handling over short and long-term AF and the implications this might have on I_{KCa} is needed for better understanding the pathophysiological function of I_{KCa} and its role in AF related electrical remodeling.

In a future study, it would be obvious to conduct an evaluation of SK2 and SK3 protein levels in paroxysmal AF patients both with and without heart failure and to access whether in such patient cohorts I_{KCa} might be upregulated and potentially involved in the genesis, maintenance and progression into persistent and permanent AF.

The challenge of providing therapeutic evidence from animal models that is translatable to clinical efficacy is a considerable problem in the development of antiarrhythmic drugs. In the attempt to develop better and safer therapeutics for treating the growing population of AF patients, the pharmaceutical industry is dependent on reliable and translatable pre-clinical evidence before the advancement into clinical trials. Therefore, there exist a need for developing large animal models of AF, resembling the clinical situation in terms of AF progression, atrial remodeling and heterogeneity amongst patient populations. This is of course a very challenging task, which demands collaboration between research groups in this scientific field, providing synergetic outcomes of already ongoing research.
CONCLUSION

This thesis provides substantial evidence for the presence of SK channels in human atrial tissue, and the functional importance of \( i_{\text{KCa}} \) in human atrial electrophysiology, prior to dramatic electrical remodeling that lead to the downregulation of SK channels in human chronic AF. Furthermore, results demonstrate that the antiarrhythmic mechanism produced by SK channels inhibition is related both to the direct slowing of repolarizing current but also by indirect effects on \( i_{\text{Ca}} \) associated RMP changes, all leading to a marked prolongation of the ERP and decreased excitability. To which degree SK channel regulation contributes to the overall remodeling in AF and the regulating mechanisms, is at present unknown. This thesis discusses possible antiarrhythmic implications of SK channel inhibition and \( i_{\text{KCa}} \) as a possible therapeutic target in AF.

SUMMARY

Atrial fibrillation (AF) is a cardiac arrhythmia which affects millions of people in Europe alone. This arrhythmia is associated with increased morbidity and mortality and it gets increasingly difficult to treat when allowed to persist for longer periods. Pharmacologically, AF therapy is limited by suboptimal efficacy and the risk of both cardiac and extracardiac side effects. Current antiarrhythmic drugs work by inhibiting several ionic currents in the heart, while new drug development pursues atrial-selectivity by targeting ion currents predominantly present in the atria, with the purpose of reducing the risk of ventricular proarrhythmic effects and other toxicities. Among such targets are the ion channels conducting the acetylcholine activated potassium current (\( i_{\text{KACN}} \)) and the ultra-rapid delayed rectifier potassium current (\( i_{\text{kur}} \)). During the last decade, another possible atrial specific target has gained attention, which is the small conductance Ca\(^{2+}\) activated K\(^{+}\) (SK) channel that conducts the \( i_{\text{KCa}} \) current. Recently, our group and others have documented \( i_{\text{KCa}} \) inhibition to be antiarrhythmic in a number of in vivo and ex vivo animal models both in restoring sinus rhythm and in preventing AF induction, however the exact antiarrhythmic mechanisms have not been elucidated. SK channels have furthermore been reported to be involved in electrical remodeling in patients suffering from AF and heart failure, while their exact function in these diseases remains unknown.

In this thesis the aim was to explore the antiarrhythmic mechanisms generated by \( i_{\text{KCA}} \) inhibition and to study whether SK channels and \( i_{\text{KCa}} \) is functionally present in human atrial and ventricular tissue. The results presented in this thesis, confirm the antiarrhythmic effects of several SK channel inhibitors and show the presence of selective \( i_{\text{KCa}} \) current in atrial muscle preparations and isolated cardiomyocytes from cardiac patients, while ventricular \( i_{\text{KCa}} \) current was not detected. In atrial tissue from patients in chronic AF SK channels along with the detectable \( i_{\text{KCa}} \) current was downregulated.

In conclusion, the present thesis and the three published papers report evidence for the existence of functional SK channels and \( i_{\text{KCa}} \) predominantly present in human atria and provide elucidation of the possible antiarrhythmic mechanisms of \( i_{\text{KCa}} \) inhibition.

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