REVIEW

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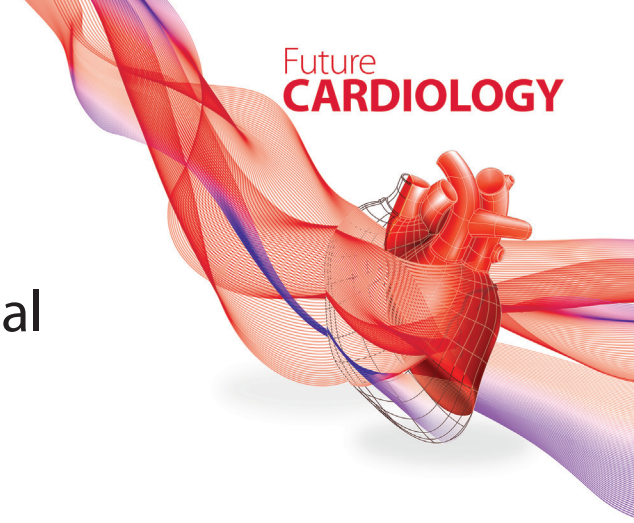

New antiarrhythmic targets in atrial fibrillation

Niels Voigt[1], Jordi Heijman[1] & Dobromir Dobrev*,[1]

Atrial fibrillation (AF) is the most common cardiac arrhythmia in developed countries. AF is associated with increased mortality and morbidity due to thromboembolism, stroke and worsening of pre-existing heart failure [1]. Currently available pharmacological therapies for AF suffer from unsatisfying efficacy and/or are associated with major side effects such as bleeding complications or proarrhythmia [2]. These limitations largely result from the fact that most of the currently available drugs were developed on an empirical basis, without precise knowledge of the molecular mechanisms underlying the arrhythmia. During the last decade substantial progress has been made in understanding the molecular mechanisms contributing to the initiation and maintenance of AF. This knowledge is expected to stimulate the development of safer and more effective drugs. Here, we review new antiarrhythmic drug targets, which have emerged based on this increasing knowledge about the molecular mechanisms of AF.

KEYWORDS

• atrial remodeling • delayed after depolarizations (DADs) • re-entry • triggered/ectopic activity

Background

Atrial fibrillation (AF), the most common cardiac arrhythmia in developed countries, is associated with increased mortality and morbidity due to thromboembolism, stroke and worsening of pre-existing heart failure [1]. Currently available pharmacological therapies have major limitations including unsatisfying efficacy and/or major side effects such as bleeding complications or proarrhythmia [2]. These limitations may be due to the fact that most of the currently available drugs were developed on an empirical basis, without precise knowledge of the molecular mechanisms underlying the arrhythmia. During the last decade substantial progress has been made in understanding the molecular mechanisms of AF. Here, we review new antiarrhythmic drug targets, which have emerged during the last years based on this increased knowledge.

Basic mechanisms of AF

It is generally assumed that ectopic activity and re-entry are the two major pathomechanisms playing a role in the initiation and maintenance of AF **(Figure 1)** [3–6]. Ectopic activity and re-entry often result from disease-related and/or AF-induced alterations in atrial tissue structure and function (atrial remodeling), which support the progression to more persistent forms of AF [7]. Ectopic activity describes abnormal impulse generation outside the sinus node. Experimental and clinical evidence suggests that high frequency ectopic activity alone can maintain several forms of AF as so called 'drivers' [8]. Under these conditions, 'drivers' are supposed to be localized particularly in the left atrium around the pulmonary veins. The surrounding tissue cannot keep up with the high frequency of the driver in a one-to-one manner and starts to fibrillate due to heterogeneous electrical impulse conduction ('fibrillatory conduction'). Radiofrequency ablation of the ectopic foci can usually

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successfully terminate these types of AF, thereby validating the importance of such 'drivers' for the maintenance of these AF forms [8].

Ectopic impulses can also trigger re-entry, the most widely accepted pathomechanism for AF maintenance [9,10]. It is defined as continuous electrical impulse propagation around an electrically inactive tissue area (Figure 2). Apart from the 'trigger,' which initiates re-entry, an appropriate arrhythmogenic substrate is necessary for the maintenance of re-entry. It ensures that the initially activated tissue area regains excitability while the electrical impulse travels around its circular path, thereby allowing the excitation wavefront to 're-enter' the circuit continuously [9]. The atria of a healthy heart are largely protected from re-entrant excitations by their long refractory period, during which the tissue cannot be re-excited. An arrhythmogenic substrate is characterized by a shortened refractory period, which is at the cellular level largely determined by the action potential (AP) duration (APD) and the postrepolarization refractoriness controlled by the kinetics of the cardiac Na^+-current [3]. Besides a shortened refractory period, slow electrical impulse propagation due to fibrosis or impaired electrical coupling between myocytes may facilitate re-entry by allowing more time for tissue to regain excitability, thereby contributing to the arrhythmogenic substrate [9].

In this review, we will first discuss novel pharmacological targets to inhibit the triggers, which initiate re-entry. Thereafter, we will focus on pharmacological modulation of the arrhythmogenic substrate. We will distinguish targets that may be directly responsible for triggered activity (direct targets) or the arrhythmogenic substrate and those that may influence the development of triggered activity and re-entry (indirect targets).

Targeting abnormal atrial impulse formation

• Ca^{2+}-driven delayed afterdepolarizations & triggered activity in AF

In the healthy heart, extracellular Ca^{2+} enters the cytosol during each AP through L-type Ca^{2+}-channels. The thereby generated depolarizing $I_{Ca,L}$ current is the main determinant of the AP plateau, contributing importantly to the typical shape of the cardiac AP. In addition, the incoming Ca^{2+} binds to Ca^{2+}-release channels (ryanodine-receptor channels, type 2 = cardiac subtype; RyR2) located in the membrane of the sarcoplasmic reticulum (SR), the main Ca^{2+}-storage organelle, triggering a much greater

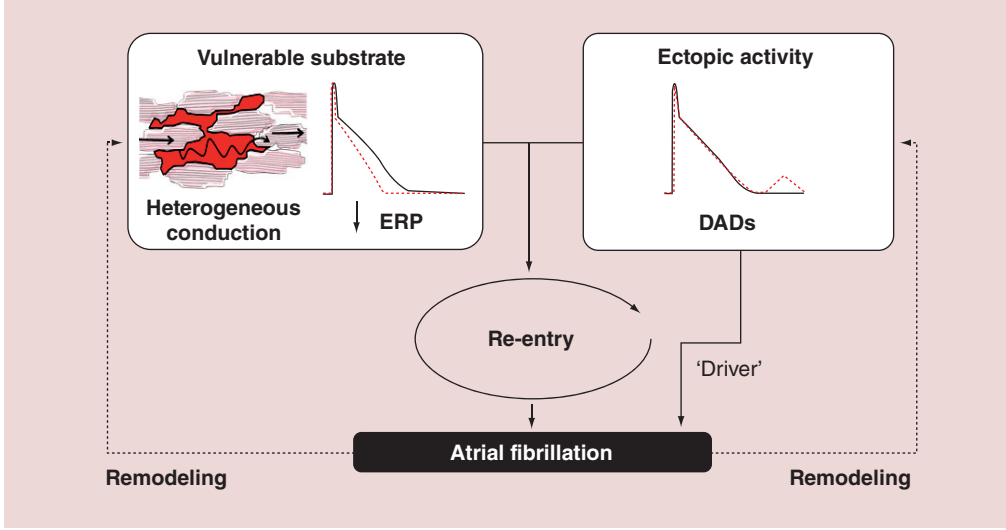

Figure 1. Basic concepts and general mechanisms of atrial fibrillation. Predisposing conditions can contribute to a vulnerable electrical and structural substrate and to the development of triggered activity. Triggered activity can initiate re-entry in a vulnerable substrate or can contribute to atrial fibrillation when occurring repetitively as a driver. When atrial fibrillation persists it causes atrial remodeling, further promoting and stabilizing its maintenance.
DAD: Delayed after depolarization; ERP: Effective refractory period.
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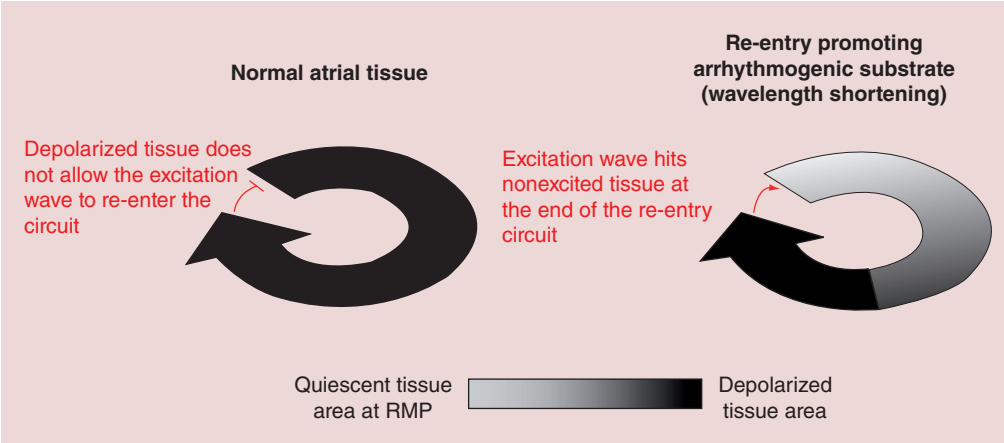

Figure 2. Basic concept of re-entry. In normal atrial tissue re-entry is unlikely to be maintained. Because of its long wavelength, which is defined as the product of conduction velocity and refractory period, the excitation wavefront encounters excited tissue (black area) and cannot re-enter (left panel). Re-entry maintenance can result from either shortened refractory period or slowed conduction, both resulting in a shortened wavelength, which allows the excitation wavefront to re-enter.
RMP: Diastolic resting membrane potential.

intracellular Ca^{2+}-release. The released Ca^{2+} binds to contractile proteins and initiates cardiac contraction. During diastole, Ca^{2+} is removed from the cytosol by the plasmalemmal Na^{+}–Ca^{2+}-exchanger (NCX1), which brings 3 Na^{+} ions into the cell per extruded Ca^{2+} ion, thereby resulting in a depolarizing inward current. In addition, the SR Ca^{2+}-ATPase (2a = cardiac subtype, SERCA2a) pumps Ca^{2+} back into the SR.

In patients with sinus rhythm, the open probability of atrial RyR2 channels is very low and there is little diastolic SR Ca^{2+} release (leak). In contrast, studies in patients with paroxysmal (pAF) [11] and chronic (>6 months persistent) AF (cAF) have shown an increased SR Ca^{2+}-leak and incidence of spontaneous Ca^{2+}-release events during diastole **(Figure 3)** [12–14]. The Ca^{2+} released during diastole activates NCX1, resulting in a transient inward current. The subsequent membrane depolarization, which is termed delayed after depolarization (DAD), may reach the threshold for AP initiation and thereby contribute to AF-promoting triggered activity.

Increased SR Ca^{2+}-leak can result from both increased SR Ca^{2+}-load and intrinsic RyR2 dysfunction. The high atrial rate in AF *per se* may increase the SR Ca^{2+}-load, promoting the occurrence of arrhythmogenic DADs. However, increased SR Ca^{2+}-leak and increased incidence of spontaneous SR Ca^{2+}-release events (SCaEs) persist in atrial myocytes from patients with cAF paced *in vitro* at 0.5 Hz, despite normal SR Ca^{2+}-load [14], suggesting an intrinsic RyR2 dysfunction in cAF. In accordance, single RyR2 channels from cAF patients reconstituted in lipid-bilayer membranes showed increased sensitivity to cytosolic (Ca^{2+}), resulting in higher channel open probability. These functional changes may result from RyR2 hyperphosphorylation, which may occur in cAF on both Ser2808, a PKA phosphorylation site and Ser2814, a Ca^{2+}/calmodulin-dependent protein kinase type II (CaMKII) phosphorylation site [14]. In addition, calmodulin and CaMKII protein levels, along with CaMKII autophosphorylation and oxidation are enhanced in cAF patients [13–14,16], pointing to an increased CaMKII activity in these patients. Although expression of PKA regulatory and catalytic subunits remain unchanged in cAF, increased cAMP levels may enhance PKA activity in cAF patients. Of note, inhibition of CaMKII, but not of PKA, reduces RyR2 open probability, RyR2 Ca^{2+}-leak and incidence of DADs in cAF patients suggesting that SR Ca^{2+}-leak and increased incidence of SCaEs in cAF predominantly result from CaMKII-mediated RyR2 hyperphosphorylation [14].

SR Ca^{2+}-uptake is controlled by a multiprotein complex consisting, among other things, of SERCA2a, and the two inhibitory proteins phospholamban (PLB) and sarcolipin. PLB is

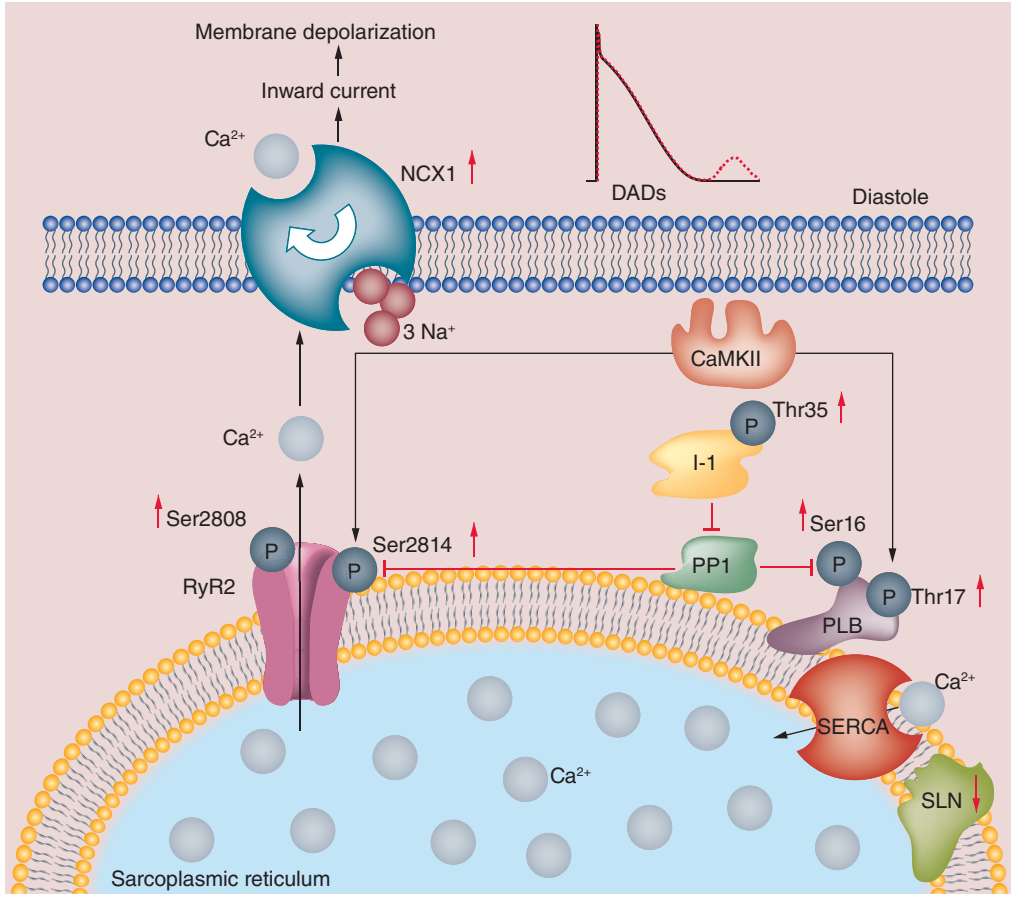

Figure 3. Molecular basis of increased diastolic sarcoplasmic reticulum Ca $^{2+}$-leak in atrial fibrillation. Abnormal spontaneous sarcoplasmic reticulum Ca^{2+}-release events through ryanodine-receptor subtype-2 channels (RyR2) during diastole can activate the Na^+–Ca^{2+}-exchanger (NCX1), which brings three Na^+ ions per extruded Ca^{2+} ion into the cytosol, creating a depolarizing inward current (I_{ti}). The resulting membrane potential depolarization (delayed after depolarization, DAD) may trigger ectopic action potentials thereby facilitating the induction or maintenance of AF. Altered expression and/or phosphorylation of sarcoplasmatic reticulum proteins (SERCA2a, PLB, SLN) may contribute to the increased SR Ca^{2+}-leak in AF. See text for details. Red arrows indicate changes in protein expression or phosphorylation associated with cAF.
Ser2808/Ser2814: Phosphorylation sites of RyR2; Ser16/Thr17 phosphorylation sites of PLB; Thr35, phosphorylation site of I-1.
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hyperphosphorylated in cAF, which may reduce its inhibitory effects on SERCA2a [14]. In cAF, the expression of sarcolipin is also reduced and the increased CaMKII activity would be expected to cause hyperphosphorylation of sarcolipin at Thr5. Together, these alterations in PLB and sarcolipin are expected to contribute to the maintained SR Ca^{2+}-load, which plays a permissive role for the development of SCaEs, DADs and triggered activity [17].

We recently identified a similar cellular phenotype, showing Ca^{2+}-handling abnormalities and increased susceptibility to DADs and triggered activity, in atrial myocytes from patients with paroxysmal AF (pAF) [11]. The underlying mechanisms, however, were distinct from those in cAF, with greater SR Ca^{2+}-load, increased RyR2 expression and single-channel open probability, but unaltered RyR2 phosphorylation [11].

Direct targets

Targeting RyR2 Ca^{2+}-leak may be a promising novel therapeutic approach in both pAF and

cAF patients, although the divergent molecular mechanisms suggest a need for antiarrhythmic drugs targeting specific molecular abnormalities in pAF versus cAF. Tetracaine, a local anesthetic drug which blocks Na^+-channels with imperfect selectivity, has also been shown to block RyR2-channels [18]. Tetracaine stabilizes the RyR2 channels in their closed state, lowering its open probability and reducing the occurrence of small nonpropagating Ca^{2+}-release events (Ca^{2+}-sparks). This increases SR Ca^{2+}-overload, promoting the occurrence of SCaEs and eventually DADs, which may limit the antiarrhythmic effect of tetracaine. In contrast, the class-IC antiarrhythmic drug flecainide blocks RyR2 channels in the open state, thereby reducing the mean open time of the channel [18]. Although flecainide increases the occurrence of nonarrhythmogenic Ca^{2+}-sparks, it does not increase SR Ca^{2+}-load by providing a kind of 'Ca^{2+} overflow valve.' This new mechanism may contribute to the anti-AF effect of flecainide in AF patients, in addition to the reduction in atrial excitability due to its well-known Na^+-channel blocking effects [19]. Similar to flecainide, newer drugs based on the structure of the β-blocker carvedilol have been shown to inhibit RyR2-mediated SR Ca^{2+}-leak and prevent potentially arrhythmogenic SCaEs and related DADs [20]. Together, these substances may provide possible lead structures for the development of novel antiarrhythmic agents targeting AF-associated RyR2 dysfunction [4,5].

FKBP12.6, a protein found in the macromolecular RyR2 complex, stabilizes the RyR2-channel closed state, reducing SR Ca^{2+}-leak. FKBP12.6 knockout mice present increased SR Ca^{2+}-leak and enhanced susceptibility to pacing-induced AF [4,21]. The benzothiazepine derivate JTV-519 (K201) stabilizes the FKBP12.6-RyR2 interaction and reduces SR Ca^{2+}-leak and AF inducibility in canines with sterile pericarditis [22]. S107, a JTV-519 derivate with less off-target effects, reduces SR Ca^{2+}-leak and AF susceptibility in mice with mutated RyR2 channels found in patients with catecholaminergic polymorphic ventricular tachycardia [23]. These findings strongly point to the FKBP12.6-RyR2 interaction as a promising therapeutic target to prevent proarrhythmogenic SR Ca^{2+}-leak in AF patients [21].

Since diastolic SCaEs activate NCX1, creating a depolarizing transient membrane current (I_{ti}), NCX1 inhibition seems a straightforward strategy to inhibit triggered activity in AF patients. However, inhibition of Ca^{2+}-extrusion from the cytosol via NCX1 (forward mode = Ca^{2+}-efflux) will allow SERCA2a to pump more Ca^{2+} back into the SR, thereby creating a higher SR Ca^{2+}-load, which should also increase the occurrence of SCaEs [24]. Since NCX1 activity is voltage-dependent, it can also act in a so-called 'reverse' (Ca^{2+}-influx) mode at more depolarized membrane potentials. In this mode, NCX1 contributes to Ca^{2+}-influx during the AP and shortens APD because of its electrogenic nature. Several benzyloxyphenyl analogs such as KB-R7943, SEA0400, SN-6 and YM-244769, are potent NCX1 inhibitors and are assumed to block NCX1 more effectively in the reversed mode. This approach limits Ca^{2+}-overload and reduces the occurrence of DADs and triggered activity [25]. In addition, blocking reverse-mode NCX1 prolongs the AP, thereby targeting the re-entry-maintaining substrate as well (see below). However, currently available NCX1 blockers present imperfect selectivity and their value for treatment of AF cannot yet be fully evaluated.

Indirect targets

Indirect targets attempt to prevent disease-related remodeling processes and the related cellular signaling pathways in contrast to directly influence the activity of ion-channels or transporters. CaMKII hyperactivity appears to contribute critically to the SR Ca^{2+}-leak in cAF. Accordingly, CaMKII suppression reduces the inducibility of AF in mice with mutated RyR2, by reducing SR Ca^{2+}-leak [26]. However, systemic inhibition of CaMKII (e.g., with KN-93) or its cofactor calmodulin (for instance with W-7) could have deleterious effects on memory, fertility and cardiac contractility [5]. Therefore, targeting specific molecular mechanisms that are involved in the augmented CaMKII activity associated with cAF, may result in a more cardiac- and pathology-specific CaMKII inhibition. It has been shown that oxidative stress accompanies cAF and that CaMKII oxidation at Met281/282 residues contributes to its increased activity in cAF [16]. Prevention of oxidation-mediated CaMKII activation is assumed to contribute to the reported beneficial effects of statins, angiotensin-converting enzyme inhibitors and angiotensin-II type 1 receptor blockers in cAF [1].

Although activity of protein phosphatases is higher in atrial tissue from cAF patients [27,28],

RyR2 hyperphosphorylation may also result from locally reduced phosphatase activity in the SR fraction. In patients with cAF, phosphorylation of inhibitor-1 (I-1), a specific SR-located inhibitor of PP1, is increased at the Thr35 site (and therefore more active), suggesting that PP1-dysfunction due to increased I-1 activity contributes to RyR2-hyperphosphorylation and SR Ca^{2+}-leak [27]. Although not directly proven, I-1 inhibitors may therefore be another interesting novel approach to target SR Ca^{2+}-leak, SCaEs and DADs in cAF patients [29].

Targeting re-entry maintaining substrate

• ERP & APD

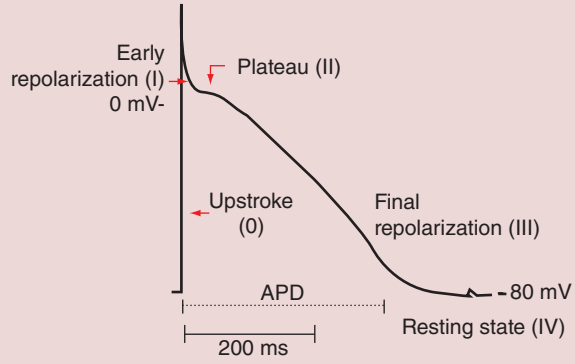
Shortening of APD is a major hallmark of atrial remodeling during cAF. The typical shape of a cardiac AP is determined by the balanced interaction of depolarizing inward currents and repolarizing outward currents **(Figure 4)**. Therefore, the shortened APD in cAF patients can be explained by a reduction of depolarizing inward currents such as $I_{Ca,L}$, or an increase in repolarizing outward inward-rectifier K^+-currents I_{K1} and constitutive $I_{K,ACh}$ [28,30]. A shortened APD promotes re-entry-maintaining circuits. As such, classical class III antiarrhythmic drugs such as amiodarone, dofetilide, sotalol and ibutilide, which are commonly used for pharmacological cardioversion, aim to prolong the atrial AP by blocking repolarizing K^+-currents. However, because of limited chamber selectivity, these drugs are associated with ventricular side effects such as QT-prolongation and ventricular arrhythmogenesis. Therefore, there is hope that atrial- and pathology-specific targets, which will be described hereafter, will facilitate the development of safer and more effective drugs to treat AF.

Direct targets

I_{Kur} activation upon membrane depolarization is very rapid ('ur' = 'ultra rapid') and thereby contributes to the early repolarization (phase I) of the atrial AP. However, since I_{Kur} inactivates very slowly, it may also contribute to the late repolarization (phase III). Blocking these channels therefore results in APD prolongation and may be antiarrhythmic. Since the underlying channel subunit Kv1.5 is selectively expressed in the atria but not in the ventricles, I_{Kur} may present an interesting drug target against AF. Initial promising results with relatively selective inhibitors have shown that inhibition of I_{Kur} can reduce AF inducibility in dog models [31]. However, since I_{Kur} is downregulated in cAF patients and rapid rates during AF result in accumulation of inactivated I_{Kur}-channels, it is unclear whether selective I_{Kur} inhibition alone will be effective to terminate the arrhythmia [31].

Inward-rectifier K^+-channels contribute to the late AP repolarization and to the resting membrane potential. Increased inward-rectifier K^+-currents have been shown to contribute to APD shortening in cAF patients and to stabilization of re-entrant excitations [3]. In addition to increased I_{K1}, $I_{K,ACh}$, which is physiologically activated by the vagal neurotransmitter acetylcholine, develops agonist-independent (constitutive) activity in cAF and thereby contributes to the increased total inward-rectifier K^+-current [30,32–33]. Since $I_{K,ACh}$ is expressed in the atria but not in the ventricles, $I_{K,ACh}$ represents an interesting potential atrial-specific drug target of AF. NTC-801 is a selective $I_{K,ACh}$-blocker that effectively prevents atrial tachypacing or vagally-induced AF, and which is currently investigated in a Phase II clinical trial [31]. However, because of the expression of $I_{K,ACh}$ channels in the sino–atrial and atrio–ventricular nodes, in Purkinje-fibers and the CNS, general inhibition of $I_{K,ACh}$ may cause unwanted side effects in these systems [31]. Therefore, understanding the molecular basis of the development of agonist-independent constitutive $I_{K,ACh}$ activity may provide atrial-selective and pathology-specific drug targets. First results from patients with cAF and a dog model of atrial tachycardia-induced remodeling suggest that a dysbalance between PKC isoforms contributes to constitutive $I_{K,ACh}$ [34]. Whereas classical PKC isoforms with inhibitory effects on $I_{K,ACh}$ may be reduced in cAF, stimulatory novel PKC isoforms seem to be enriched in the plasma membrane of atrial myocytes from cAF patients. Whether targeting PKC dysbalance in patients with cAF is feasible and may provide an antiarrhythmic strategy is currently unclear.

Ca^{2+}-dependent small-conductance K^+ channels (SK-channels) are predominantly expressed in the atria in a number of species including human, and therefore represent potential atrial-selective drug targets **(Figure 4)**. Experimental studies in guinea pigs, aged hypertensive rats and dogs with atrial tachycardia remodeling showed that inhibition of SK-channel mediated currents prevents burst pacing-induced AF [35]. The role of SK-channels in cAF patients is less clear. Both upregulation and downregulation of SK-current



	Upstroke (phase 0)	Early repolarization (phase I)	Plateau (phase II)	Final repolarization (phase III)	Resting state (phase IV)
Major contributing currents	I_{Na}		$I_{Ca,L}$		
		I_{tor} I_{Kur}		I_{Ks}, I_{Kr}, (I_{Kur}, I_{NCX}) I_{K1}, $I_{K,ACh}$	I_{K1}, $I_{K,ACh}$
Potential atrial selective novel drug targets		I_{Kur}		$I_{Kur,}$ SK-channels, K_{2P}3.1, $I_{K,ACh}$	$I_{K,ACh}$

Figure 4. Ion currents determining the shape of an atrial action potential. The action potential is controlled by ions flowing through ion channels. The action potential upstroke (phase 0) results from a large sodium current (I_{Na}) with subsequent Ca^{2+}-entry through L-type Ca^{2+}-channels ($I_{Ca,L}$). During the plateau (phase II) there is a balance between inward and outward currents. Repolarization is governed by several K^+-currents including transient outward (I_{to}), ultra-rapid (I_{Kur}), rapid (I_{Kr}) and slow (I_{Ks}) delayed-rectifier K^+-currents. I_{to} and I_{Kur} underlie early depolarization (phase I) and together with I_{Kr} and I_{Ks} determines late depolarization (phase III), which brings the myocyte to the resting state (phase IV). The resting potential is determined by inward-rectifier K^+-current (I_{K1}) and is modulated by acetylcholine-regulated K^+-current ($I_{K,ACh}$).
APD: Action potential duration.

was observed in cAF patients, indicating that more studies are required to evaluate the potential role of SK-channels as new antiarrhythmic drug targets [5].

Two-pore domain K^+-channel (K_{2p}) isoform K_{2p}3.1 is selectively expressed in atria and inhibition of K_{2p}-channels prolongs atrial APD [36]. Both amiodarone and dronedarone block also K_{2p}3.1-channels, which may contribute to their AF-specific efficacy [37]. However, the exact role of K_{2p}-channel-mediated current in AF pathophysiology is unclear and needs further evaluation.

Indirect targets

In addition to the traditional approach to directly target ion channels to normalize APD in cAF patients, a novel therapeutic approach may be the modulation of regulatory pathways, which control the expression and function of ion channels and contribute to the development of AF-promoting atrial remodeling.

During AF the higher cytosolic Ca^{2+}-concentration resulting from the rapid atrial rate and increased SR Ca^{2+}-leak stimulates several cellular remodeling processes. For instance, the activation of the Ca^{2+}-dependent phosphatase calcineurin dephosphorylates NFAT. Upon dephosphorylation, NFAT is translocated to the nucleus and, among others, inhibits transcription of L-type Ca^{2+}-channels. Activated NFAT contributes also to the cAF-associated I_{K1}-increase by downregulating atrial microRNAs (miR-26, miR-101), which have inhibitory effects on the expression of the I_{K1}-channel subunit Kir2.1 [4]. Therefore, inhibition of the Ca^{2+}-dependent phosphatase calcineurin or its cofactor calmodulin (e.g., with W-7) would be expected to limit the cAF-associated downregulation of $I_{Ca,L}$ and upregulation of I_{K1}, thereby targeting two major re-entry-favoring mechanism in AF [29].

Besides dysregulation of L-type Ca^{2+}-channel gene expression by NFAT, post-translational modifications of L-type Ca^{2+}-channel proteins may

also contribute to reduced $I_{Ca,L}$ in cAF. Increased dephosphorylation of L-type Ca^{2+}-channels protein phosphatases is also involved in the $I_{Ca,L}$ reduction in cAF. Therefore, targeting local phosphatase and kinase complexes to restore L-type Ca^{2+}-channel phosphorylation may at least partially correct the re-entry-promoting $I_{Ca,L}$ dysfunction in cAF [28,29].

• Conduction velocity & structural remodeling

Impulse propagation in the heart is principally controlled by the availability of depolarizing force through cardiac Na^+-channels, electrical conductivity between cardiac myocytes through gap junction channels comprised connexins, and cardiac tissue architecture. Reduced Na^+-currents, decreased gap junction conductance or cardiac muscle-bundle discontinuities due to increased fibrosis reduce conduction velocity (CV) and promote re-entry [4,5].

Recent evidence has suggested that Ca^{2+}-dependent remodeling also plays a critical role in CV slowing and structural remodeling. Acute elevations of intracellular Ca^{2+} can inhibit cardiac Na^+-channels, reducing cardiac CV, whereas chronic elevation of intracellular Ca^{2+} resulting from increased SR Ca^{2+}-leak has been shown to reduce expression of the Nav1.5 α-subunit of the Na^+-channel [38,39]. In addition, very high intracellular Ca^{2+} levels may also reduce gap junction conductance [38]. In AF, connexin expression relocalizes to the lateral cell membranes, thereby reducing longitudinal CV and increasing conduction heterogeneities [3]. Finally, atrial fibrosis, resulting from excessive deposition of extracellular matrix proteins, predominantly by cardiac myofibroblasts, is a major component of atrial structural remodeling. Patients with cAF have significantly increased levels of fibrosis [40] and several animal models have provided important insights into the molecular mechanisms of fibrosis, highlighting the involvement of a large number of signaling pathways that provide potential targets for therapeutic interventions [7].

Direct targets

A number of peptides aiming to improve cardiac conduction by targeting gap junction channels have recently been developed [4–5,29]. Although currently not further pursued in clinical studies, compounds such as rotagaptide, which have shown promise in large-animal studies, may provide a basis for the development of new compounds targeting gap junctions. It has also been shown that reduced connexin-43 expression was associated with increased fibrosis, suggesting a potential to beneficially affect both CV and structural remodeling by direct targeting gap junction channels [41].

Indirect targets

Several potential therapeutic targets for atrial structural remodeling have recently been identified in cardiac myofibroblasts. Cardiac fibroblasts contain several ion-channels that allow Ca^{2+}-entry in response to a range of stimuli, thereby promoting fibrogenesis. Recent research has suggested major roles for transient-receptor potential (TRP) channel type M7 and C3. TRPM7 and TRPC3 channel expression are increased in fibroblasts during AF and may play a major role in fibrogenesis [42,43]. Inhibition of TRPC3 reduced the AF duration in dogs with atrial tachycardia remodeling [43]. These channels may therefore provide indirect therapeutic targets to prevent AF-promoting structural remodeling.

TGF-β1 is a major profibrotic signaling molecule [7]. Pirfenidone is a drug that inhibits TGF-β1 signaling, among many other targets, and has been shown to prevent AF in canines with heart failure [44]. Although it is not further developed for AF, pirfenidone suggests that TGF-β1 is another potential therapeutic target for structural remodeling [5].

Conclusion & future perspective

During the last decade substantial progress has been made to understand the molecular pathophysiology of AF. Given the wide range of molecular changes from ion channels to intracellular signal-transduction molecules, and the numerous cell types involved (myocytes, myofibroblasts), it is very unlikely that one single molecular target can cover all pathological abnormalities. Furthermore, it has been shown that different types of AF present distinct forms of atrial remodeling. Thus, atrial myocytes from pAF patients do not present classical hallmarks of electrical remodeling such as APD shortening, $I_{Ca,L}$-downregulation and I_{K1}-upregulation, although they show an increased incidence of SCaEs and DADs [11]. The latter were not due to RyR2-hyperphosphorylation like in cAF, but due to increases in SERCA2a-activity and RyR2-expression, pointing to atrial remodeling of a different sort. These findings suggest that therapeutic approaches will have different efficacy in various types of AF. Therefore, future studies will

need to substratify AF patients based on different arrhythmia signatures in order to identify appropriate therapeutic drug targets.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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EXECUTIVE SUMMARY

- Triggered activity and re-entry are the most accepted pathomechanisms of atrial fibrillation.
- Spontaneous SR Ca^{2+}-releases due to RyR2 dysfunction activate NCX1, thereby contributing to generation of delay after depolarizations, triggered activity and arrhythmia susceptibility.
- Modulation of CaMKII activity and targeting protein-phosphatase activity within the SR microdomain may modulate SR Ca^{2+}-leak and represent possible indirect targets to reduce SR Ca^{2+}-leak.
- The re-entry-maintaining arrhythmogenic substrate is characterized by shortened action potential duration, slowed conduction and increased tissue heterogeneity.
- Ion channels located in fibroblasts such as TRPM7 and TRPC3 could represent promising novel therapeutic approaches to target atrial structural remodeling.
- Pharmacological inhibition of I_{Kur}, constitutively-active $I_{K,ACh}$, SK- and $K_{2P}3.1$-channels may represent an atrial-selective approach to prolong action potential duration and target the arrhythmogenic electrical substrate.
- In cAF patients, dephosphorylation of nuclear factor of activated T lymphocytes inhibits transcription of $I_{Ca,L}$-channel subunits and increases expression of I_{K1}-channel subunits via downregulation of I_{K1}-inhibitory miRNAs (miR-26 and miR-101). Modulation of nuclear factor of activated T-lymphocytes activity may therefore represent a future approach to target multiple processes involved in atrial fibrillation-associated atrial electrical remodeling.

References

1 Camm AJ, Lip GY, De Caterina R *et al.* 2012 focused update of the ESC Guidelines for the management of atrial fibrillation: an update of the 2010 ESC Guidelines for the management of atrial fibrillation. Developed with the special contribution of the European Heart Rhythm Association. *Eur. Heart J.* 33(21), 2719–2747 (2012).

2 Camm J. Antiarrhythmic drugs for the maintenance of sinus rhythm: risks and benefits. *Int. J. Cardiol.* 155(3), 362–371 (2012).

3 Wakili R, Voigt N, Kaab S, Dobrev D, Nattel S. Recent advances in the molecular pathophysiology of atrial fibrillation. *J. Clin. Invest.* 121(8), 2955–2968 (2011).

4 Dobrev D, Carlsson L, Nattel S. Novel molecular targets for atrial fibrillation therapy. *Nat. Rev. Drug Discov.* 11(4), 275–291 (2012).

5 Heijman J, Voigt N, Dobrev D. New directions in antiarrhythmic drug therapy for atrial fibrillation. *Future Cardiol.* 9(1), 71–88 (2013).

6 Heijman J, Voigt N, Nattel S, Dobrev D. Cellular and molecular electrophysiology of atrial fibrillation initiation, maintenance, and progression. *Circ. Res.* 114(9), 1483–1499 (2014).

7 Nattel S, Burstein B, Dobrev D. Atrial remodeling and atrial fibrillation: mechanisms and implications. *Circ. Arrhythm. Electrophysiol.* 1(1), 62–73 (2008).

8 Haissaguerre M, Jais P, Shah DC *et al.* Spontaneous initiation of atrial fibrillation by ectopic beats originating in the pulmonary veins. *N. Engl. J. Med.* 339(10), 659–666 (1998).

9 Comtois P, Kneller J, Nattel S. Of circles and spirals: bridging the gap between the leading circle and spiral wave concepts of cardiac re-entry. *Europace* 7(Suppl. 2), 10–20 (2005).

10 Atienza F, Martins RP, Jalife J. Translational research in atrial fibrillation: a quest for mechanistically based diagnosis and therapy. *Circ. Arrhythm. Electrophysiol.* 5(6), 1207–1215 (2012).

11 Voigt N, Heijman J, Wang Q *et al.* Cellular and molecular mechanisms of atrial arrhythmogenesis in patients with paroxysmal atrial fibrillation. *Circulation* 129(2), 145–156 (2014).

12 Hove-Madsen L, Llach A, Bayes-Genis A *et al.* Atrial fibrillation is associated with increased spontaneous calcium release from the sarcoplasmic reticulum in human atrial myocytes. *Circulation* 110(11), 1358–1363 (2004).

13 Neef S, Dybkova N, Sossalla S *et al.* CaMKII-dependent diastolic SR Ca^{2+} leak and elevated diastolic Ca^{2+} levels in right atrial myocardium of patients with atrial fibrillation. *Circ. Res.* 106(6), 1134–1144 (2010).

14 Voigt N, Li N, Wang Q *et al.* Enhanced sarcoplasmic reticulum Ca^{2+} leak and increased Na^{+}-Ca^{2+} exchanger function underlie delayed after depolarizations in patients with chronic atrial fibrillation. *Circulation* 125(17), 2059–2070 (2012).

15 Voigt N, Dobrev D. Cellular and molecular correlates of ectopic activity in patients with atrial fibrillation. *Europace* 14(Suppl. 5), v97–v105 (2012).

16 Purohit A, Rokita AG, Guan X *et al.* Oxidized Ca^{2+}/calmodulin-dependent protein kinase II triggers atrial fibrillation. *Circulation* 128(16), 1748–1757 (2013).

17 Nattel S, Dobrev D. The multidimensional role of calcium in atrial fibrillation

pathophysiology: mechanistic insights and therapeutic opportunities. *Eur. Heart J.* 33(15), 1870–1877 (2012).

18 Hilliard FA, Steele DS, Laver D *et al.* Flecainide inhibits arrhythmogenic Ca^{2+} waves by open state block of ryanodine receptor Ca^{2+} release channels and reduction of Ca^{2+} spark mass. *J. Mol. Cell. Cardiol.* 48(2), 293–301 (2010).

19 Aliot E, Capucci A, Crijns HJ, Goette A, Tamargo J. Twenty-five years in the making: flecainide is safe and effective for the management of atrial fibrillation. *Europace* 13(2), 161–173

20 Zhou Q, Xiao J, Jiang D *et al.* Carvedilol and its new analogs suppress arrhythmogenic store overload-induced Ca^{2+} release. *Nat. Med.* 17(8), 1003–1009 (2011).

21 Dobrev D, Voigt N, Wehrens XH. The ryanodine receptor channel as a molecular motif in atrial fibrillation: pathophysiological and therapeutic implications. *Cardiovasc. Res.* 89(4), 734–743 (2011).

22 Kumagai K, Nakashima H, Gondo N, Saku K. Antiarrhythmic effects of JTV-519, a novel cardioprotective drug, on atrial fibrillation/flutter in a canine sterile pericarditis model. *J. Cardiovasc. Electrophysiol.* 14(8), 880–884 (2003).

23 Shan J, Xie W, Betzenhauser M *et al.* Calcium leak through ryanodine receptors leads to atrial fibrillation in 3 mouse models of catecholaminergic polymorphic ventricular tachycardia. *Circ. Res.* 111(6), 708–717 (2012).

24 Ozdemir S, Bito V, Holemans P *et al.* Pharmacological inhibition of Na/Ca exchange results in increased cellular Ca^{2+} load attributable to the predominance of forward mode block. *Circ. Res.* 102(11), 1398–1405 (2008).

25 Dobrev D. Atrial Ca^{2+} signaling in atrial fibrillation as an antiarrhythmic drug target. *Naunyn Schmiedebergs Arch. Pharmacol.* 381(3), 195–206 (2010).

26 Chelu MG, Sarma S, Sood S *et al.* Calmodulin kinase II-mediated sarcoplasmic reticulum Ca^{2+} leak promotes atrial fibrillation in mice. *J. Clin. Invest.* 119(7), 1940–1951 (2009).

27 El-Armouche A, Boknik P, Eschenhagen T *et al.* Molecular determinants of altered Ca^{2+} handling in human chronic atrial fibrillation. *Circulation* 114(7), 670–680 (2006).

28 Christ T, Boknik P, Wohrl S *et al.* L-type Ca^{2+} current downregulation in chronic human atrial fibrillation is associated with increased activity of protein phosphatases. *Circulation* 110(17), 2651–2657 (2004).

29 Heijman J, Dewenter M, El-Armouche A, Dobrev D. Function and regulation of serine/threonine phosphatases in the healthy and diseased heart. *J. Mol. Cell. Cardiol.* 64, 90–98 (2013).

30 Dobrev D, Friedrich A, Voigt N *et al.* The G protein-gated potassium current $I_{K,ACh}$ is constitutively active in patients with chronic atrial fibrillation. *Circulation* 112(24), 3697–3706 (2005).

31 Milnes JT, Madge DJ, Ford JW. New pharmacological approaches to atrial fibrillation. *Drug Discov. Today* 17(13–14), 654–659 (2012).

32 Voigt N, Heijman J, Trausch A *et al.* Impaired Na^+-dependent regulation of acetylcholine-activated inward-rectifier K? current modulates action potential rate dependence in patients with chronic atrial fibrillation. *J. Mol. Cell Cardiol.* 61, 142–152 (2013).

33 Voigt N, Maguy A, Yeh YH *et al.* Changes in I K, ACh single-channel activity with atrial tachycardia remodelling in canine atrial cardiomyocytes. *Cardiovasc. Res.* 77(1), 35–43 (2008).

34 Makary S, Voigt N, Maguy A *et al.* Differential protein kinase C isoform regulation and increased constitutive activity of acetylcholine-regulated potassium channels in atrial remodeling. *Circ. Res.* 109(9), 1031–1043 (2011).

35 Qi X, Diness J, Brundel B *et al.* Role of small conductance calcium-activated potassium channels in atrial electrophysiology and fibrillation in the dog. *Circulation* 129(4), 430–440 (2014).

36 Schmidt C, Wiedmann F, Voigt N *et al.* Upregulation of K(2P)3.1 K+ current causes action potential shortening in patients with chronic atrial fibrillation. *Circulation* 132(2), 82–92 (2015).

37 Schmidt C, Wiedmann F, Schweizer PA, Becker R, Katus HA, Thomas D. Novel electrophysiological properties of dronedarone: inhibition of human cardiac two-pore-domain potassium (K2P) channels. *Naunyn Schmiedebergs Arch. Pharmacol.* 385(10), 1003–1016 (2012).

38 Heijman J, Wehrens XH, Dobrev D. Atrial arrhythmogenesis in catecholaminergic polymorphic ventricular tachycardia-is there a mechanistic link between sarcoplasmic reticulum Ca^{2+} leak and re-entry? *Acta Physiol. (Oxf)* 207(2), 208–211 (2013).

39 King JH, Wickramarachchi C, Kua K *et al.* Loss of Nav1.5 expression and function in murine atria containing the RyR2-P2328S gain-of-function mutation. *Cardiovasc. Res.* 99(4), 751–759 (2013).

40 Van Brakel TJ, Van Der Krieken T, Westra SW, Van Der Laak JA, Smeets JL, Van Swieten HA. Fibrosis and electrophysiological characteristics of the atrial appendage in patients with atrial fibrillation and structural heart disease. *J. Interv. Card. Electrophysiol.* 38(2), 85–93 (2013).

41 Jansen JA, Van Veen TA, De Jong S *et al.* Reduced Cx43 expression triggers increased fibrosis due to enhanced fibroblast activity. *Circ. Arrhythm. Electrophysiol.* 5(2), 380–390 (2012).

42 Du J, Xie J, Zhang Z *et al.* TRPM7-mediated Ca^{2+} signals confer fibrogenesis in human atrial fibrillation. *Circ. Res.* 106(5), 992–1003 (2010).

43 Harada M, Luo X, Qi XY *et al.* Transient receptor potential canonical-3 channel-dependent fibroblast regulation in atrial fibrillation. *Circulation* 126(17), 2051–2064 (2012).

44 Lee KW, Everett THT, Rahmutula D *et al.* Pirfenidone prevents the development of a vulnerable substrate for atrial fibrillation in a canine model of heart failure. *Circulation* 114(16), 1703–1712 (2006).