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Mechanisms and Drug Development in Atrial Fibrillation

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Abstract	505
I. Introduction	506
II. Definitions	506
III. Epidemiology of Atrial Fibrillation	507
IV. Genetics of Atrial Fibrillation	507
V. Pathophysiology of Atrial Fibrillation	509
A. Mechanisms of Atrial Fibrillation Initiation and Maintenance	509
B. Triggered Activity, Reentry, and the Initiation of Atrial Fibrillation	509
C. Ionic Bases of Triggered Activity	510
D. Ion Channels and Reentry in Atrial Fibrillation	511
VI. Status of Antiarrhythmic Drugs in Atrial Fibrillation	512
A. Atrial Selective Ion Channel Blockers	512
B. Acetylcholine-Activated Inward Rectifying K ⁺ Current and Atrial Fibrillation	513
C. Multichannel Blockade Drugs that Minimize Adverse Events	514
VII. New Drug Uses and New Molecular Mechanisms	514
A. Targeting Inward-Rectifying K ⁺ Channels, Insights from Chloroquine Use in Experimental Atrial Fibrillation	514
B. Intracellular Ca ²⁺ Regulation as a Target for Atrial Fibrillation Therapy	515
C. Gap-Junction Therapy Aiming at Homogenous Impulse Propagation	517
VIII. Novel Targets in Atrial Fibrillation	517
A. Ion Channels, Electrical Remodeling, and the Progression of Atrial Fibrillation	517
B. Remodeling and Upstream Therapies in Atrial Fibrillation	517
C. Nonpharmacologic Targets: Obesity, Adiposis, and Inflammation in Atrial Fibrillation	519
IX. Atrial Fibrillation and Sleep Apnea	520
X. Challenges and Opportunities in New Anti-atrial Fibrillation Drug Development	520
XI. Conclusion	521
References	521

Abstract—Atrial fibrillation is a highly prevalent cardiac arrhythmia and the most important cause of embolic stroke. Although genetic studies have identified an increasing assembly of AF-related genes, the impact of these genetic discoveries is yet to be realized. In addition, despite more than a century of research and speculation, the molecular and cellular mechanisms underlying AF

have not been established, and therapy for AF, particularly persistent AF, remains suboptimal. Current antiarrhythmic drugs are associated with a significant rate of adverse events, particularly proarrhythmia, which may explain why many highly symptomatic AF patients are not receiving any rhythm control therapy. This review focuses on recent advances in AF research, including its

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epidemiology, genetics, and pathophysiological mechanisms. We then discuss the status of antiarrhythmic drug therapy for AF today, reviewing molecular mechanisms, and the possible clinical use of some of the new atrial-selective antifibrillatory agents, as well as drugs that target atrial remodeling, inflammation and fibrosis, which are being

tested as upstream therapies to prevent AF perpetuation. Altogether, the objective is to highlight the magnitude and endemic dimension of AF, which requires a significant effort to develop new and effective antiarrhythmic drugs, but also improve AF prevention and treatment of risk factors that are associated with AF complications.

I. Introduction

Atrial fibrillation (AF) is the most common arrhythmia seen by the clinician. It affects more than 33 million people worldwide (Chugh et al., 2014) and is also the number one cause of hospitalization for arrhythmias (Hart, 2003; Heijman et al., 2014; Miyasaka et al., 2006). Prevalence increases with advancing age and so are its associated comorbidities, like heart failure (Sardar et al., 2016). AF is characterized by rapid and irregular activation of the atria without discrete P waves on the surface electrocardiogram (ECG). The pathophysiology of AF is complex, involving dynamic interactions among several factors, including substrate, triggers, and perpetuators, and the therapeutic approaches/strategies are informed by the disease progression from initiation of the abnormal electrical rhythm to its maintenance.

Many drugs have been tried in persistent AF with limited success (Camm et al., 2010). Several class IA, IC, and III drugs, as well as class II drugs (beta-blockers), are moderately effective in maintaining sinus rhythm after conversion of atrial fibrillation. However, they increase adverse events, including proarrhythmia, and some like disopyramide, quinidine, and sotalol, may increase mortality (Lafuente-Lafuente et al., 2015). In fact, antiarrhythmic drug therapy in general improves patients' symptom scores and exercise tolerance; however, large randomized trials have failed to show a mortality benefit with a rhythm-control compared with a rate-control strategy (Halsey and Chugh, 2014). Therefore, the availability of new oral anticoagulant drugs that overcome the intrinsic disadvantages of warfarin has shifted the focus of drug development toward enabling widespread application of effective thromboprophylaxis with oral anticoagulants, particularly in low-risk patients with AF (Lip et al., 2012; Kirchhof et al., 2016).

On the other hand, the development of new mapping and catheter-based ablation technologies, which have made the procedure safer, easier to perform, and more effective after a single attempt, has greatly improved the outcomes in patients with paroxysmal AF. However, success rates for persistent AF ablation remain far lower than paroxysmal AF and there is large variation

in the strategies used worldwide, (Cappato et al., 2005; Calkins et al., 2007; Matsuo et al., 2007) which highlights the need and offers new opportunities for the development of a new generation of drugs for the prevention and termination of AF.

This article is not intended as a comprehensive review of the pharmacology of antiarrhythmic drugs, as excellent reviews on the subject have appeared in the recent literature (Camm et al., 2012; Hanley et al., 2016; Kanagaratnam et al., 2017). Instead, we focus on recent advances in AF research, including its genetics, epidemiology, and pathophysiological mechanisms. We then discuss the status of antiarrhythmic drug therapy for AF today, reviewing molecular mechanisms and the possible clinical use of some of the new atrial-selective antifibrillatory agents (see Visual Abstract), as well as drugs that target remodeling and inflammation that are being tested as upstream therapies to prevent AF perpetuation.

II. Definitions

According to the 2016 and 2017 expert consensus documents (Kirchhof et al., 2016; Calkins et al., 2017), paroxysmal AF is defined as recurrent AF episodes that terminate spontaneously or with intervention within 7 days of onset; persistent AF is defined as continuous AF that is sustained beyond 7 days; long-standing persistent AF is defined as continuous AF of greater than 12-month duration. The first diagnosed AF refers to AF that has not been diagnosed before, irrespective of the duration of the arrhythmia or the presence and severity of AF-related symptoms. From a clinical point of view, the latter is important as more than 50% of patients with a first diagnosed AF episode will not experience recurrences over long-time follow up in the absence of antiarrhythmic drugs, cardiac structural abnormalities, and significant comorbidities (Pappone et al., 2008). Early persistent AF is a new term defined as continuous AF of more than 7-day duration but less than 3-month duration. The latter subdivision is reasonable for patients who are candidates for an AF ablation procedure, because better results are obtained with shorter duration of the persistent AF. The term permanent AF is defined as AF in which the presence of

ABBREVIATIONS: ACEI, angiotensin converting enzyme inhibitors; APD, action potential duration; ARB, angiotensin II receptor blockers; DAD, delayed afterdepolarization; DF, dominant excitation frequency; EAD, early afterdepolarization; EPL, eplerenone; FFA, free fatty acid; GWAS, genome-wide association studies; HF, heart failure; I_{K1} , inward rectifying K^+ current; I_{KACH} , acetylcholine-activated inward rectifying K^+ current; I_{Kr} , fast component of the delayed rectifier K^+ current; I_{Ks} , slow component of the delayed rectifier K^+ current; I_{Kur} , ultrarapid component of the delayed rectifier K^+ current; I_{Na} , fast inward Na^+ current; K_{2P} , two-pore K^+ channels; MRB, mineralocorticoid receptor blocker; PV, pulmonary vein; RAAS, renin-angiotensin-aldosterone system; SAC, stretch-activated channels; SK, small-conductance Ca^{2+} -activated K^+ channels; SNPs, single nucleotide polymorphisms; WK, Wenxin Keli.

the AF is accepted by the patient and physician, and no further attempts will be made either to restore or maintain sinus rhythm (Calkins et al., 2017).

III. Epidemiology of Atrial Fibrillation

AF represents a global health care problem that predominantly affects developed nations, North America being the region with the highest prevalence and incidence rates (Chugh et al., 2014). AF prevalence in North America almost triples the rates of the Asia-Pacific region. Overall, both incidence and prevalence rates have been progressively increasing in the world population (Colilla et al., 2013; Zoni-Berisso et al., 2014), which may result in more than 50 million AF patients worldwide by 2030. These estimations may even underestimate future AF burden worldwide. Data to make estimations have been obtained primarily from Western Europe and North America, with very limited representation from vast populations in developing countries (Colilla et al., 2013; Chugh et al., 2014; Zoni-Berisso et al., 2014). The latter underscores substantial differences in healthcare systems and resources among regions, which makes it difficult to detect and diagnose AF, especially silent AF episodes without symptoms (Fitzmaurice et al., 2007).

Current AF prevalence in the general adult population of Europe ranges from 1.9% to 2.9% depending on the country (Zoni-Berisso et al., 2014), although more importantly, the data are consistent with prevalence rates that double the reported data one decade earlier (Murphy et al., 2007). In the United States, the prevalence rate also increased for Medicare beneficiaries older than 65 years, with an absolute growth of 4.5% (from 4.1% to 8.6%) in the period 1993–2007 (Piccini et al., 2012). Moreover, AF prevalence varies with age and sex. In individuals younger than 50 years and older than 80 years, AF prevalence ranges from 0.1% to 10%–18%, respectively (Zoni-Berisso et al., 2014). Globally, AF prevalence is higher in men than in women, with a 1.5:1 ratio considering worldwide data (Chugh et al., 2014).

AF is frequently associated with cardiac disease and with cardiac/noncardiac comorbidity. The most frequent comorbidities are hypertension, heart failure (HF), diabetes, and obesity (Cea-Calvo et al., 2007; Di Pasquale et al., 2013; Zoni-Berisso et al., 2014). Moreover, one-third of AF patients have at least three associated comorbidities, with a low percentage of AF patients presenting with presumably no heart disease or comorbidities, although most studies do not provide information on the extent of diagnostic testing to rule out all risk factors and comorbidities currently associated with AF (Wyse et al., 2014).

AF progression from paroxysmal or persistent episodes to permanent stages shows a slow but continuous trend that may reach 30% after 5 years of follow up despite appropriate clinical management (Jahangir

et al., 2007). Progression is often related to the development of underlying heart disease, which also increases the risk of adverse cardiovascular events and mortality (Jahangir et al., 2007; Potpara et al., 2012; Banerjee et al., 2013). Moreover, AF may be associated with symptoms, sometimes disabling, and often resistant to antiarrhythmic drug therapy as reported in real-life surveys, in which symptoms may persist in more than 50% of patients (Chiang et al., 2012; Kirchhof et al., 2014). The latter often requires hospitalization and invasive interventional procedures such as ablation, which leads to a significant impact on healthcare costs (Zoni-Berisso et al., 2014). Although acute decompensation of HF is the leading cause of hospitalization in the persistent and permanent forms of AF, proarrhythmic events from antiarrhythmic drugs are the most frequent causes of hospitalization among patients with paroxysmal AF (Chiang et al., 2012). Current antiarrhythmic drugs are associated with a significant rate of withdrawals due to adverse events (excluding proarrhythmia), which varies from 4% to 23% depending on the antiarrhythmic drug (Lafuente-Lafuente et al., 2015). The latter may explain why a large percentage (~50%) of highly symptomatic AF patients is not receiving any rhythm control therapy (Kirchhof et al., 2014). Altogether, the foregoing highlights the magnitude and endemic dimension of AF, which requires a significant effort to develop new and effective antiarrhythmic drugs, but also improve AF prevention and treatment of risk factors that are associated with AF complications.

IV. Genetics of Atrial Fibrillation

The risk of developing AF is intimately associated with clinical risk factors as diabetes, hypertension, HF, and smoking, among others (Benjamin et al., 1994; Schnabel et al., 2009). Integration of risk factors makes it possible to predict short-to-midterm AF risk development using a model based on clinical variables routinely used in a conventional clinical setting (Alonso et al., 2013). Emerging research in genetics during the first decade of the 21st century suggests that AF cases in the general population also have a significant genetic component, beyond traditional risk factors (Fox et al., 2004). Thus, the inclusion of familial AF (defined as occurrence of AF in a first-degree relative) as an additional variable further improves AF risk prediction based on clinical risk factors. The latter becomes especially relevant when familial AF is related to a first-degree relative younger than 65 years (Lubitz et al., 2010b). In fact, the risk of AF increases with decreasing age at AF onset in the youngest affected relative (Arnar et al., 2006; Oyen et al., 2012).

The genetic basis of AF varies from monogenic AF families and isolated AF cases, in which the reported mutations have large effect sizes and are directly responsible for causing the trait, to genetic variants with small effect sizes that confer an increased risk of

the disease. Although linkage analysis and genotyping can identify causative rare mutations related to AF, the use of recent next-generation sequencing technology in genetic association studies enables to investigate the co-occurrence of genetic variants among 100s of 1000s of single nucleotide polymorphisms (SNPs) in affected individuals. Both genetic approaches may have important clinical implications. Thus, mutations identified in monogenic AF pedigrees have shown either gain-of-function or loss-of-function in genes encoding potassium or sodium channel subunits (Chen et al., 2003; Xia et al., 2005; Das et al., 2009; Li et al., 2009), respectively, both leading to APD shortening and favoring reentrant activity. Rare mutations have been also associated with higher burden of triggered activity or enhanced action potential activity from intracardiac neurons (Olson et al., 2006; Scornik et al., 2006; Li et al., 2009). The later would theoretically increase acetylcholine release, leading to a heterogeneous functional substrate for AF initiation (Sarmast et al., 2003).

A large body of work suggests strongly that AF is heritable. Standard genetic techniques have led to the documentation of several chromosomal loci and genes in which rare mutations can cause dominantly inherited AF. Some such genes encode myocardial potassium (*KCNQ1*, *KCNA5*, *KCNE5*, *KCNJ2*, and *KCNE2*) and sodium (*SCN5A*, *SCN1B*, *SCN2B*, and *SCN3B*) channels, potassium-adenosine triphosphate channels (*ABCC9*), nucleoporin-155 (*NUP155*), and gap junction protein connexin 40 (*GJA5*) (Lubitz et al., 2010a; Judge, 2012). In addition, genome-wide association studies (GWAS) have suggested that, similar to other cardiovascular diseases, established AF loci only explain a moderate proportion of disease risk, suggesting that further genetic discovery, with an emphasis on common variations, in population-based studies could be of great value for AF risk assessment and potentially determine the response to therapies (Weng et al., 2017a). Recent data have shown that both genetic predisposition and validated clinical risk factors are associated with AF risk, which enables establishing that a low-risk clinical profile is associated with delay of AF onset, despite an underlying high polygenic risk (Andrade et al., 2014). In individuals older than 55 years of age from the Framingham cohort and predominant European ancestry, the average lifetime risk of AF was recently reported to be 37%, although the AF risk ranged from 20% to 50% among individuals in the lowest to the highest tertiles of both polygenic and clinical risk, respectively (Weng et al., 2018).

Some initial population-based studies have started to assess the influence of common SNPs related to AF on the response to antiarrhythmic drug therapy. A common SNP (rs10033464) on chromosome 4q25 (near the paired-like homeodomain transcription factor 2 gene; *PITX2*), has been independently associated with successful reduction in symptomatic AF burden (a composite score for frequency, duration, and severity of symptoms). Carriers

of the variant allele at rs10033464 responded favorably to class I antiarrhythmic drugs, whereas wild types responded better to class III antiarrhythmic drugs (Parvez et al., 2012). However, the mechanism for such a difference has not been elucidated; it may be mediated through effects of distant genes and particularly of *PITX2* (Gudbjartsson et al., 2007), which encodes a transcription factor (*PITX2c*) important for pulmonary vein development and determination of left and right atrial asymmetry during cardiac development. Heterozygous deletion of *PITX2c* (the cardiac isoform of *PITX2*) in mice has been associated with shortening of the left atrial APD and higher risk of AF (Kirchhof et al., 2011). Moreover, microarray analysis of *PITX2* null-mutant and control mouse hearts have revealed upregulation of *KCNQ1* (Abraham et al., 2010), a potassium channel gene that has been associated with gain-of-function mutations in familial AF. Data from atrial specific *PITX2*-deficient mice have also shown gain-of-function of *Kir2.1* (Chinchilla et al., 2011), which is intimately related to favoring reentrant activity, leading to AF (Van Wagoner, 2003; Girmatsion et al., 2009). Antiarrhythmic drug outcomes in patients with AF have been also related to the renin-angiotensin-aldosterone system and common angiotensin-converting enzyme insertion/deletion polymorphism. The deletion/insertion of a 287-bp intronic DNA segment resulting in double-deletion or insertion/deletion genotypes of the angiotensin-converting enzyme gene has been associated with failure of antiarrhythmic drug therapy in AF (Darbar et al., 2007). GWAS studies have identified more than 30 genetic loci in association with AF, suggesting previously unrecognized potential mechanisms of the disease (Bapat et al., 2018). For example, a recent GWAS study identified a novel AF locus comprising intronic and several highly correlated missense variants situated in the I-, A-, and M-bands of titin, which is the largest protein in humans and responsible for the passive elasticity of heart and skeletal muscle (Nielsen et al., 2018). This and other studies highlight the need to identify potential mechanisms whereby genetic risk loci act to increase the risk of AF during fetal heart development.

However, as more genes are screened, more AF patients are identified carrying multiple deleterious rare variants and/or combinations of rare and common variants in ion channel genes (Christophersen et al., 2017; Weng et al., 2017a; Nielsen et al., 2018). Such variants may differ in their functional expression as specific atrial substrates or conditions that can modify channel function and lead to AF (Mann et al., 2012). The latter complicates the use of specific antiarrhythmic drugs when attempting to associate genetic variants with potential therapeutic targets. Nevertheless, research efforts combining individual genetic profiles and clinical risk factors (Weng et al., 2018), along with atrial cell modeling (Mann et al., 2012), may help decipher the electrophysiological phenotype and the AF risk. Altogether, genetic information available highlights the fact that genotype may potentially enhance selection of

patients most likely to benefit from new mechanism-targeting therapies and aid in future clinical studies.

V. Pathophysiology of Atrial Fibrillation

A. Mechanisms of Atrial Fibrillation Initiation and Maintenance

The mechanisms that maintain AF in the human heart have been and continue to be a matter of intense debate. Despite more than 100 years of research and speculation, we are yet to fully understand its fundamental mechanisms and learn how to treat it effectively. In the late 1950s the seminal work of Moe and Abildskov (1959) led to the multiple wavelet hypothesis, which posited that AF is maintained by multiple coexisting electrical wavelets that propagate randomly and uninterruptedly throughout the atria. Moe and Abildskov postulated also that there were important requirements to allow fibrillation to be self-sustained. One such requirement was that a minimum number of propagating wavelets must be simultaneously exciting a tissue volume, a hypothesis that seemed to fit well with the excellent results obtained subsequently by Cox et al. (1991) using the surgical MAZE procedure. Briefly, the procedure consists of surgically compartmentalizing the atrium in small segments so that the volume that accommodates the minimal number of wavelets is larger than the new volume in each compartment. Therefore, AF becomes unsustainable due to the high probability of wavelet annihilation because of collision with the new boundaries. However, even if its assumptions were correct, the multiple wavelet hypothesis leaves many unresolved questions, including: What mechanisms explain the focal activity, wavebreak formation, and reentry that usually occur during AF? How does the multiple wavelet hypothesis explain the hierarchical spatiotemporal organization that has been consistently demonstrated in animal models and humans over the last 20 years of research (Vaquero et al., 2008)? More importantly, how does the multiple wavelet hypothesis explain the often successful termination of AF by pulmonary vein isolation and other highly localized ablation procedures in a significant number of patients? Finally, from a clinical point of view, the idea of randomness of wave propagation during AF introduces large uncertainties when trying to design effective therapy approaches, because whenever any target is addressed the outcome would be unpredictable.

Today, two main deterministic theories attempt to explain the mechanisms that control the complex patterns of wave propagation in AF. On the one hand, some authors postulate that high-frequency activation during AF depends on a widespread endoepicardial electrical dissociation that promotes complex bidirectional conduction between the two layers of the atrial walls that behave functionally independently (de Groot et al., 2010). The emergent waves propagating from deeper layers break

through each surface mimicking focal activity, whereas the dissociated propagation between endo- and epicardium promotes self-sustainment of the activity (de Groot et al., 2010, 2016). On the other hand, others support the theory of rotors, which gives predominance to a small number of localized functional reentrant sources (rotors) generating spiral waves (Jalife et al., 2002; Narayan et al., 2012). Such waves emerge at high frequency, propagating away from the rotor to interact with tissue heterogeneities and giving rise to complex patterns of nonuniform propagation termed “fibrillatory conduction” (Jalife et al., 2002). While rotors have been demonstrated in the atria of patients undergoing radiofrequency ablation (see Fig. 1), how both theories reconcile with the observed spatiotemporal organization during AF and whether they may even be combined to explain AF remains a matter of continuing debate (Allessie and de Groot, 2014; Narayan and Jalife, 2014). But the transcendent concept here is that randomness has lost its prominence as an explanation for AF maintenance, the research community now favoring singular mechanisms, which, although highly complex, are nevertheless consistent with the hierarchical organization of the atria during AF and potentially able to produce the necessary physical or pharmacologic tools to terminate AF more effectively (Calvo et al., 2017).

B. Triggered Activity, Reentry, and the Initiation of Atrial Fibrillation

Better agreement exists currently among clinicians and scientists regarding the mechanisms of AF initiation. Although the numerous *in vivo* and *in vitro* studies have not conclusively defined a mechanism, both triggered activity and reentry have been suggested. In 1998 Haissaguerre et al. (1998) first demonstrated in human patients that the atrial sleeves in the pulmonary veins harbor the vast majority of the ectopic electric triggers that initiate AF. Since then, multiple researchers have confirmed Haissaguerre’s results and also identified additional ectopic trigger locations (e.g., the superior vena cava and the vein of Marshall) (Enriquez et al., 2017). With time, the procedure involving electrical isolation of the pulmonary veins (PVs) revolutionized the field and became universally accepted as the gold standard to prevent AF initiation and maintenance. The outcomes of ablation procedures are consistent with both mechanisms. Substantial evidence emerged, also connecting PV ectopic triggers to the initiation of reentry in sustained AF. In a cholinergic model of AF, Klos et al. (2008) showed that the initiation of reentry and AF by impulses emanating from a PV is rate dependent. At critically high rates, propagation into the posterior wall of the left atrium becomes discontinuous, leading to conduction block and wavebreak formation with curling of the wave front around a pivoting “singularity point” that becomes the rotor that organizes the fibrillatory activity. Sustained functional reentry stabilizes as a single or a pair of

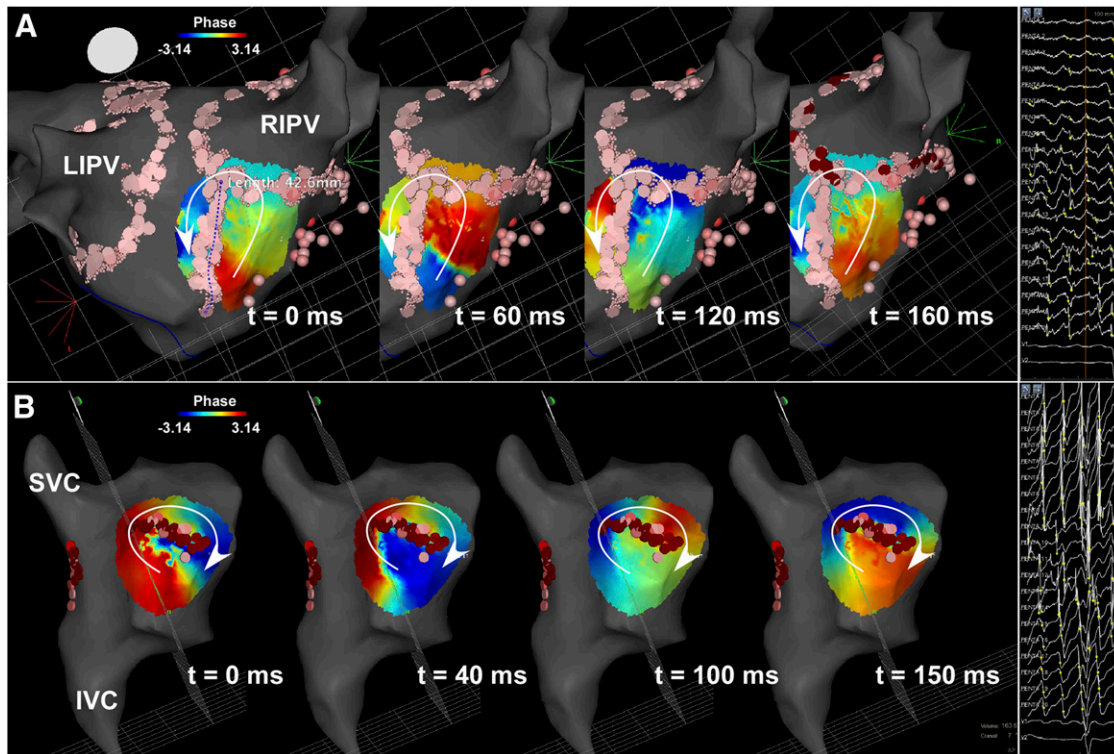


Fig. 1. Examples of rotor domains localized in human atria and their ablation. (A) A rotor domain, defined as an anatomic location displaying repetitive rotational activity organized by a meandering singularity point, is displayed at the floor of the left atrium. The ablation line (pink dots) crosses the distribution of the singularity points up to the right circumferential pulmonary vein isolation. (B) A rotor domain in the lateral wall of the right atrium. The ablation line (pink and red dots) crosses the distribution of the singularity points on the right atrium lateral wall. Corresponding unipolar recordings are displayed on the right hand side. IVC, inferior vena cava; LIPV, left inferior pulmonary vein; RIPV, right inferior pulmonary vein; SVC, superior vena cava. Reproduced with permission from Calvo et al. (2017).

counter-rotating “rotors” at or near the PV antrum, with centrifugal spiral wave activation to the rest of the atrium. The latter suggests that heterogeneities in the atrial substrate lead to dynamic interaction with high-frequency electrical inputs. Under critical conditions it would be possible to initiate functional reentry that will become the source of the first emanating wavelets. The preferential site for reentry formation would depend on the distribution of heterogeneities, moving away from the PV antrum as the substrate becomes more complex in the structurally affected atria (i.e., myocardial fibrosis) (Tanaka et al., 2007). Organization in the frequency domain of patients with AF seems to follow the same distribution. Patients with paroxysmal AF and no structural heart disease tend to display the highest frequency sites concentrated at or near the PV antrum, whereas patients with persistent AF, whose arrhythmogenic substrate is often more complex, display a more widespread distribution of highest frequencies that may be localized in either the left or the right atrium (Sanders et al., 2005; Atriencia et al., 2014).

Topics of high relevance to AF initiation and maintenance are the histologic composition of the PV sleeves and the role of specific cell types in generating ectopic activity that triggers AF. As will be discussed below, atrial-like cardiomyocytes comprise most of the evidence. But some researchers call attention to additional

cell types including nodal-like P cells, transitional cells, and large Purkinje-like myocytes, all of which have been suggested to participate in the architecture of the PVs of patients with a history of AF (Perez-Lugones et al., 2003). In addition, the role of the cardiac neural network has been gaining much attention during the last several years. Neuronal plexi surrounding the PVs may contribute to modulate electrophysiological properties of PV cells in a variety of ways, including through vagal influxes that shorten the APD and create conditions for high-frequency reentry (Arora, 2012; Wickramasinghe and Patel, 2013). An intriguing recent observation is the demonstration of interstitial cells of Cajal as a constitutive part of the architecture of the PVs in patients with a history of AF (Gherghiceanu et al., 2008; Morel et al., 2008). In the gastrointestinal system, the interstitial cells of Cajal are well known drivers of pacemaker activity promoting peristalsis. To what extent such cells may be the source of ectopic activity in humans remains under discussion. To the best of our knowledge, no studies have yet addressed the electrophysiology or the function of these interesting cells.

C. Ionic Bases of Triggered Activity

The pulmonary veins are a major source of ectopic electrical triggers (Haissaguerre et al., 1998). When the triggered waves propagate into the left atrium they may

break as they interact with the atrial tissue and engender the rotors that sustain AF (Kalifa et al., 2006; Klos et al., 2008). After the seminal work by Haïssaguerre et al. (1998) demonstrating the effectiveness of isolation of the PV in the treatment of paroxysmal AF in patients, many researchers have endeavored to understand why the atrial muscular sleeves penetrating the PV walls are arrhythmogenic. There is substantial agreement that the mechanism leading to PV discharges in animal models and patients with AF is mainly triggered activity, which is defined as cardiac impulse initiation that is dependent on afterdepolarizations (Cranefield and Aronson, 1988). Afterdepolarizations are oscillations in membrane potential that follow the upstroke of an action potential. The two kinds of afterdepolarizations known to cause triggered activity are early afterdepolarizations (EADs) and delayed afterdepolarizations (DADs). EADs occur early, i.e., during phase 2 or 3 of repolarization of the action potential, whereas DADs are delayed until repolarization is complete or nearly complete (Wit and Boyden, 2007). When an EAD or DAD is large enough to reach the threshold potential for activation of a regenerative inward current, a triggered action potential ensues. EAD- and DAD-induced triggered activity is always preceded by at least one action potential.

Although the evidence supporting triggered activity as an initiator of AF is not conclusive, special conditions of PV electrophysiology might facilitate EADs and DADs. Pulmonary vein muscle fibers have a smaller I_{K1} and thus less negative membrane potential and a slower phase-0 upstroke velocity than atrial muscle. This difference is more prominent in the distal than proximal veins (Hocini et al., 2002; Ehrlich et al., 2003; Wang et al., 2003). PVs also have shorter APD associated with larger delayed rectifier K^+ currents (I_{Kr} and I_{Ks}). To date only one study in the normal dog heart has described what has been interpreted as triggered activity, arising just proximal to the venous ostium in the presence of isoproterenol, with an increased rate after rapid pacing (Arora et al., 2003). However, a role for triggered activity is uncertain because no pacemaker potentials or afterdepolarizations were demonstrated. EAD-induced triggered activity is usually dependent on APD prolongation, with reactivation of inward Ca^{2+} or Na^+ current during the plateau phase (Fozzard, 1992). Studies using microelectrode recordings in tissue preparations have suggested, however, that simultaneous activation of cholinergic and adrenergic receptors, which reduces action potential duration, can result in triggered activity emerging from EADs during phase 3 of repolarization (Schauerte et al., 2001; Patterson et al., 2006). But these were *in vitro* conditions in which nonsustained reentry could not be ruled out. Therefore, although the autonomic nervous system may sometimes be involved in AF (Schauerte et al., 2001; Pappone et al., 2004), late phase 3 EAD-triggered activity caused by the

above mechanisms remains to be demonstrated under physiologic conditions.

D. Ion Channels and Reentry in Atrial Fibrillation

The spatial distribution of ion channel functional expression is heterogeneous throughout the atria (Vaquero et al., 2008; Swartz et al., 2009; Voigt et al., 2010). In addition, the amount of remodeling ion channels undergo with time in AF is also heterogeneous (Nattel et al., 2007). Such heterogeneities are responsible for the different electrophysiological properties of different regions of the atria in terms of conduction velocity, APD, and refractory period, all of which condition the ability of each region to maintain sustained reentry at different frequencies in response to the remodeling process. A study using specimens of human atrium demonstrated that chronic AF reduces the transient K^+ outward current and the ultrarapid component of the delayed rectifier current (I_{Kur}) (Caballero et al., 2010). In addition, the slow component of the delayed rectifier K^+ current (I_{Ks}) is increased, which results in significant APD abbreviation. Importantly, such changes were quantitatively different between right and left atria, which may explain the propensity of one atrium to sustain more stable reentry at a higher frequency than the other. Functional reentry is extremely sensitive to changes in the ionic currents with three main factors being the most affected: 1) the width of the wave front, 2) the size of the rotor core (i.e., excitable tissue that remains unexcited forming the pivoting center) and 3) the excitability and refractoriness of the tissue ahead of the wave front (Vaquero et al., 2008). Increasing potassium inward currents leads to shortening of the APD, which allows faster spin of the rotor because of increased net hyperpolarizing currents during phases 2 and 3 of the AP. The latter agrees well with two observations: first 1) the significantly higher dominant frequency in persistent than paroxysmal AF of animals (Martins et al., 2014) and human patients (Sanders et al., 2005), and 2) the increased atrial frequency of activation after adenosine infusion (Atienza et al., 2006). In the heart, adenosine interacts with the A1 receptor and the ACh-activated inward rectifying K^+ current (I_{KACh}) via intracellular signaling through G-proteins, producing an increased net hyperpolarizing current during phases 2 and 3 of the action potential to shorten the APD (Sarmast et al., 2003).

Sustained AF leads to downregulation of the depolarizing I_{Na} and calcium currents via reduced expression of the alpha subunit of the sodium channel ($Na_v1.5$) and the alpha subunit of the calcium current ($Ca_v1.2$), respectively (Martins et al., 2014). The latter changes work to slow the AF frequency by increasing the sink-source imbalance at the rotor core (Vaquero et al., 2008). However, unlike the inward currents, sustained AF results in an increased functional expression of the Kir2.1 channel (Martins et al., 2014), the molecular equivalent of the inward rectifier potassium current I_{K1} ,

which would have the opposite effect. Together with $I_{K_{ACH}}$, I_{K1} governs the dynamics of functional reentry (Pandit and Jalife, 2013; Calvo et al., 2014). For example, I_{K1} elevation yields faster and more stable rotors because of both a greater outward conductance at the core and a shortened action potential duration in the core vicinity, as well as increased excitability, in part due to faster recovery of I_{Na} (Samie et al., 2001; Noujaim et al., 2007). The latter should result in a larger rate of increase in the local conduction velocity as a function of the distance from the core in persistent compared with paroxysmal AF. In combination, these results strongly suggest that I_{K1} upregulation establishes a substrate for stable and very fast rotors (Noujaim et al., 2007). Of interest, the remodeling process also promotes an increase in I_{Ks} and postrepolarization refractoriness, which facilitates wave-break formation and spatially distributed complex patterns of fibrillatory conduction of the wave fronts emanating at high rate from the core (Muñoz et al., 2007).

VI. Status of Antiarrhythmic Drugs in Atrial Fibrillation

A. Atrial Selective Ion Channel Blockers

During the last decade, drug interaction with ion channels that are selectively expressed in the atria emerged as a promising therapy able to control AF without significant consequences to the electrophysiology of the ventricles (Ehrlich et al., 2008). The spectrum of targets spans from the ultrarapid delayed rectifier K^+ current (I_{Kur}) (Walsh, 2015) and the constitutively active acetylcholine (ACh)-activated K^+ current ($I_{K_{ACH}}$) (Dobrev et al., 2005) to newly proposed mechanisms like atrial-selective Na^+ channel inhibition (Burashnikov et al., 2012) and interaction with the small-conductance Ca^{2+} -activated K^+ (SK) channels (Diness et al., 2010), the two-pore K^+ (K_{2P}) channels (Schmidt et al., 2017a), and the stretch-activated channels (SAC) (Franz and Bode, 2003) (Visual Abstract and Table 1).

I_{Kur} is a transient outward current that rapidly inactivates, leaving a sustained outward current after continued depolarization (Wang et al., 1993). I_{Kur} is present only in the atria, but some I_{Kur} blockers have minor unspecific effects in the ventricles. Several drugs have demonstrated that efficient I_{Kur} inhibition promotes AF termination and precludes reinduction with some differences between them (Linz et al., 2007) (Blaauw et al., 2004; Stump et al., 2005). But the relevance of I_{Kur} as an antifibrillatory target has been brought into question by the fact that a highly selective I_{Kur} blocking agent was ineffective in prolonging atrial refractory periods during sinus rhythm (Pavri et al., 2012) and also because I_{Kur} is downregulated in the human atria during persistent AF (Caballero et al., 2010). However, it has been proposed that because of its atrial selectivity, I_{Kur} blockade might contribute to optimize the anti-AF effects of Na^+ -channel blockers

(Aguilar et al., 2015). Thus, in the future, the combined therapy might more efficiently control AF, provided drug development works to overcome the substantial adverse effects of I_{Na} blockers that have been demonstrated in diverse clinical scenarios (Echt et al., 1991).

Although the voltage dependence of inactivation or blockade of I_{Na} seems quite similar in human atrial and ventricular cardiomyocytes (Furukawa et al., 1995), differences in the AP configuration between both cell types may explain atrial selectivity of some I_{Na} blockers (Hancox et al., 2016). Ranolazine is an open-state blocker that becomes trapped in the inactivated state (Zygmunt et al., 2011), which together with the fact that in atrial cells the Na^+ channel spends more time in the inactivated state than the ventricles explains atrial selectivity. Some experimental studies demonstrated significant effects on AF physiology, leading to reduced frequency of fibrillatory waves and AF termination (Black-Maier et al., 2017). But there is controversy regarding the role of ranolazine in the treatment of human AF, with recent studies demonstrating just borderline effects in terms of clinical efficacy (De Ferrari et al., 2015).

Wenxin Keli (WK) is a Chinese herb extract that includes five main components: *Nardostachys chinensis* batal extract, *codonopsis*, *notoginseng*, amber, and *Rhizoma polygonati* (Xue et al., 2013). WK appears to exert atrial selective inhibition of I_{Na} because of differences in AP configuration in atria versus ventricles, i.e., the atria have a more negative steady-state inactivation, a less negative resting membrane potential, and shorter diastolic intervals than the ventricles (Burashnikov et al., 2007). Consequently, the drug effectively suppresses AF in experimental models (Hu et al., 2016). WK has been tested in limited clinical scenarios, like AF associated with hyperthyroidism, in which WK seems to be as efficacious as sotalol in preventing AF perpetuation (Meng et al., 2015). However, the clinical information is very limited (Chen et al., 2013), and further evidence is needed before its use may be generalized.

The small-conductance Ca^{2+} -activated K^+ (SK) channels contribute to repolarization in atrial cardiomyocytes during the latter phase of the action potential, an effect that seems larger than in the ventricles (Xu et al., 2003). In experimental models, upregulation of SK channels leads to APD shortening after rapid atrial pacing and promotes atrial arrhythmias (Tsai et al., 2016). Recently, the SK channel inhibitor AP14145 was shown to be effective in controlling AF resistant to vernakalant in pigs (Diness et al., 2017). However, there is evidence of downregulation of SK channels during human AF (Yu et al., 2012) and no indication of an effect on the atrial APD of patients with AF (Skibsbjerg et al., 2014), which raises substantial doubt about the role of SK blockers in the treatment of human AF. In addition, there is concern regarding possible proarrhythmia, which might limit the translation to clinical testing of

TABLE 1
Some characteristics of antiarrhythmic drugs that show atrial specificity

Blocker or Modulator (Drug)	Target	Comments
BMS914392	I_{KACH}	Resulted ineffective in clinical studies Potential adverse/vagolytic effects
MK-0448	I_{Kur}	Appears ineffective
I_{Na} Blockers	I_{Na}	Ranolazine resulted ineffective in clinical studies Wenxin-Keli appears effective but needs additional studies
Multichannel blockers	I_{Na}	Celivarone resulted ineffective in clinical studies
	I_{Kur}	Budiodarone appears effective but need for additional studies
	I_{KACH}	Vernakalant seems effective for acute conversion to sinus rhythm
AP14145:	I_{Kr}	SK channels are downregulated in human AF No effect on APD of patients with AF
	I_{Ks}	
	SK	
SAC Blockers	SAC	Gadolinium is effective in experiments Selective agents have not been tested in the clinic
K_{2p} Blockers	K_{2p}	Amiodarone and Dronedarone Selective analogs are under development but have not been tested in the clinic
IP3 modulators	$IP3$ Receptor	Not clinically tested as yet Potential systemic effects due to nonspecificity
Dantrolene	$RyR2$	Not clinically tested in AF as yet Potential hepatotoxicity on chronic oral administration
Rotigaptide	$Connexin$	Not clinically tested as yet Improvement of conduction velocity does not correlate with AF vulnerability

drugs blocking SK channels (Hsueh et al., 2013; Hancock et al., 2015). The two-pore K^+ (K_{2p}) channels are responsible for background currents in human atrial cardiomyocytes, and in patients with chronic AF some isoforms (e.g., $K_{2p3.1}$) are upregulated and thought to contribute to APD shortening (Schmidt et al., 2015). The pharmacology of K_{2p} channels has been gaining attention over the last 10 years; the interested reader may find extensive reviews in the relevant literature (Ravens and Wettwer, 2011; Hancox et al., 2016). Notably, commonly used antiarrhythmic drugs such as amiodarone (Gierten et al., 2010) and dronedarone (Schmidt et al., 2012) block $K_{2p3.1}$ channels. Yet selective agents are still under development and have not been tested in the clinic (Flaherty et al., 2014).

The stretch-activated nonselective cation channels (SACs; permeable to Na^+ , Ca^{2+} , and K^+) are another group of channels whose molecular structure is still not entirely known but whose functional behavior has been well-characterized (Riemer et al., 1998). SACs inhibition by the GsMTx4 peptide (a tarantula spider venom toxin) suppresses AF in experimental models (Bode et al., 2001). In addition, atrial stretch correlates with shortened APD and refractoriness promoting AF (Ravelli and Allessie, 1997). By blocking SACs (Chang et al., 2007), gadolinium reduces AF vulnerability in a dose-dependent manner (Bode et al., 2000) and decreases spontaneous firing from the pulmonary veins in animal models. However, selectivity has not been demonstrated, because SACs are also functionally active in the ventricles and modulate susceptibility to ventricular fibrillation (Barrabés et al., 2013; Calvo and Jalife, 2013).

Interestingly, some K_{2p} channel isoforms exhibit mechanosensitive responses (Schmidt et al., 2017a), which adds to the complexity in the role of stretch in the pathophysiology of AF (Ninio and Saint, 2008; Yamazaki et al., 2009). The mechanosensitive isoform $K_{2p2.1}$ is downregulated, whereas the $K_{2p10.1}$ isoform is upregulated in AF, which might contribute to APD changes in patients with concomitant heart failure (Schmidt et al., 2017a). In addition, $K_{2p3.1}$ is upregulated in patients with severe systolic dysfunction and chronic AF but not in patients in sinus rhythm (Schmidt et al., 2017b). However, further investigation is needed to confirm a significant role of mechanosensitive K_{2p} channels in human atrial physiology and AF pathophysiology. In addition, translation to clinical testing is also limited by the fact that specific inhibitors of mechanosensitive K_{2p} channels are currently not available.

B. Acetylcholine-Activated Inward Rectifying K^+ Current and Atrial Fibrillation

Vagal control of the heart is mediated in part via the inwardly rectifier potassium acetylcholine activated channel responsible for I_{KACH} , which is constitutively upregulated during AF (Cha et al., 2006) and promotes sustained AF in the absence of cholinergic mediators (Kovoor et al., 2001; Klos et al., 2008). Acetylcholine activates I_{KACH} via its interaction with M2 receptors and intracellular G-protein transducers (see Visual Abstract), promoting APD shortening and AF maintenance. In humans and animal models, I_{KACH} activation via adenosine infusion significantly shortens APD (Sarmast et al., 2003) and increases the dominant frequency at anatomic

regions believed to contain the AF drivers (Atienza et al., 2006). On the contrary, I_{KACH} blockade significantly prolongs APD and atrial refractoriness, leading to AF termination in animal models (Cha et al., 2006). Commonly used antiarrhythmics like dronedarone and ibutilide nonspecifically block I_{KACH} , which explains in part their antifibrillatory properties (Ravens et al., 2013). However, development of selective I_{KACH} blockers for AF treatment in humans will require avoiding molecules with significant vagolytic adverse effects at therapeutic doses. Recently, BMS914392, a potent and selective oral inhibitor of I_{KACH} has been tested in the clinic (Podd et al., 2016). BMS914392 failed to maintain sinus rhythm in patients with paroxysmal AF when administered at the doses required to avoid neurologic adverse effects. Other molecules have been evaluated experimentally with favorable results (Kockskämper et al., 2008; Machida et al., 2011), but to the best of our knowledge they have not been clinically tested.

C. Multichannel Blockade Drugs that Minimize Adverse Events

Multichannel-blockade drug therapy derives from the well-known antiarrhythmic efficacy of amiodarone, which may be considered the most effective drug preventing AF recurrences, although it is also very effective in terminating AF (Lip et al., 2012). However, the significant extra cardiac adverse actions of amiodarone are also very well-known and substantially limit its clinical use (Singh, 2008).

Dronedarone was derived from the molecular structure of amiodarone, attempting to minimize its toxicity (Doggrell and Hancox, 2004). Dronedarone does not contain the iodine moieties of amiodarone, which has substantially less toxic effects on the thyroid and other organs. Dronedarone's electrophysiological profile is similar to amiodarone, although it has been shown to be a 10 times more potent blocker of the sodium current in human atrial myocytes and up to 100 times more potent as a blocker of I_{KACH} in guinea pig atrial myocytes (Guillemare et al., 2000; Lalevée et al., 2003). Yet, despite its promising profile, dronedarone shows a very limited ability to terminate AF in both experiments and the clinic (Burashnikov et al., 2010; Le Heuzey et al., 2010). The different clinical outcomes of amiodarone and dronedarone in the treatment of AF remain unexplained. However, it might be related to the fact that the active metabolite of amiodarone (*N*-desethylamiodarone) accumulates in cardiac tissue (Holt et al., 1983), whereas dronedarone's active metabolite (*N*-debutyldronedarone) does not accumulate. Indeed, the antiarrhythmic effects of *N*-desethylamiodarone are presumably superior to *N*-debutyldronedarone (Talajic et al., 1987). Amiodarone's interaction with thyroid hormone, which depends on the iodide moieties that are absent in dronedarone, may also contribute to amiodarone's antiarrhythmic properties. Finally, it is important that dronedarone is

contraindicated in patients with HF and New York Heart Association functional class III-IV after the report of the prematurely terminated ANDROMEDA trial, which showed an increase in mortality in the dronedarone group compared with placebo (Køber et al., 2008).

Celivarone is a noniodinated benzofuran derivative that is pharmacologically related to dronedarone and amiodarone. Celivarone blocks I_{Kr} , I_{Ks} , I_{KACH} , I_{Kur} , and I_{CaL} (Gautier et al., 2004). It also inhibits β 1-adrenoceptor-mediated increases in heart rate and α -adrenoceptor and angiotensin-II-mediated increases in blood pressure. Interestingly, celivarone has been reported to be as potent as dronedarone and approximately threefold more effective than amiodarone in restoring sinus rhythm in vagally induced AF in dogs (Gautier et al., 2005). However, the drug failed to show efficacy preventing AF recurrences and terminating AF in the clinic (Khitri et al., 2012).

Budiodarone is another chemical analog of amiodarone with similar multichannel blocking properties (Arya et al., 2009). Unlike amiodarone, budiodarone undergoes rapid metabolism by plasma and tissue esterases, which potentially makes it less susceptible to drug-drug interactions with drugs that inhibit CYP450-mediated metabolism (Juhász and Bodor, 2000). A preliminary randomized trial in patients with paroxysmal AF and dual-chamber pacemaker devices (capable of storing and quantifying AF burden) reported that budiodarone significantly reduced AF and atrial flutter burden after 12 weeks of treatment compared with placebo (Ezekowitz et al., 2012).

Despite enormous difficulties in the development of new antiarrhythmic drugs that have a multichannel blockade profile but lack extracardiac adverse effects, the broad cardiac safety profile of amiodarone and its strong antiarrhythmic effects are encouraging investigators and industry to continue developing similar molecules. Over the last several years, vernakalant, a multichannel blocker with relatively selective effects on I_{Kur} , some blocking actions on I_{KACH} and I_{Na} , and minor effects on other ionic channels at therapeutic concentrations (Dorian et al., 2007), has demonstrated successful in acutely converting AF to sinus rhythm when administered intravenously (Camm et al., 2011). It has also been tested orally for the prevention of AF recurrences postcardioversion, with modest efficacy when compared with placebo (Torp-Pedersen et al., 2011). Nevertheless, the good safety profile displayed by vernakalant has motivated additional studies toward developing new molecules with a similar profile.

VII. New Drug Uses and New Molecular Mechanisms

A. Targeting Inward-Rectifying K^+ Channels, Insights from Chloroquine Use in Experimental Atrial Fibrillation

Inwardly rectifying K^+ currents (I_{K1} , I_{KACH} , and I_{K-ATP}) play important roles in controlling rotor dynamics

and stabilizing fast reentrant activity (Pandit and Jalife, 2013). Although I_{K-ATP} is prominent in ischemia (Noma, 1983) and I_{KACH} underlies vagally mediated reentry in paroxysmal AF (Noujaim et al., 2010), I_{K1} is the most relevant inwardly rectifying K^+ current controlling cardiac fibrillation. In fact, both specific and preferential I_{K1} blockade have consistently been shown to terminate cardiac fibrillation in different experimental models, including both AF and ventricular fibrillation (Warren et al., 2003; Noujaim et al., 2007, 2010; Filgueiras-Rama et al., 2012). Although attractive, I_{K1} -blockade is currently not used to treat patients with AF, because to our knowledge, none of the antiarrhythmic drugs available to date block I_{K1} specifically at therapeutic concentrations. In addition, the fear that reducing I_{K1} would reduce the resting membrane potential during diastole and consequently increase propensity for triggered arrhythmias likely limits chloroquine's appeal. Interestingly, based on the expression profile of the Kir2.x subfamily of inwardly rectifying K^+ channels, Kir2.3 transcripts are mostly concentrated in the human atria rather than the ventricles (Schram et al., 2002). Protein crystallization, molecular modeling, and interactions with modulators (e.g., Phosphatidylinositol 4,5-bisphosphate or PtdIns(4,5)P₂, PIP₂) (Du et al., 2004) might lead to new pharmacological strategies focusing on selective blockade of Kir2.3 subunits. The latter becomes especially relevant in the setting of congestive HF, in which I_{K1} downregulation might be associated with increased susceptibility to ventricular arrhythmias (Pogwizd et al., 2001).

The potential impact of developing clinically relevant atrial-specific I_{K1} blockers has been highlighted by the use of chloroquine, an antimalarial quinolone, which as a relatively selective I_{K1} blocker has been shown to be highly effective in terminating AF in various experimental models (Noujaim et al., 2010; Filgueiras-Rama et al., 2012; Takemoto et al., 2018). At the structural level, the molecule interacts with electronegative residues (amino acids F254, D259, and E224) in the inner vestibule of the intracellular domains of Kir2.1 (Pegan et al., 2005; Noujaim et al., 2010). Such interactions cause a disruption of the channel upon chloroquine binding, which leads to a break in the ion permeation pathway (Noujaim et al., 2011). Chloroquine also interacts with negatively charged residues within the ion permeation pathway of the intracellular domain of Kir3.1 and Kir6.2, the channels responsible for the I_{KACH} and I_{K-ATP} , respectively, which likely provides additional synergistic antiarrhythmic properties. Although chloroquine blockade of Kir3.1 is similar to Kir2.3 through interaction with amino acids D260 and F255 in the ion-permeation pathway (Fig. 2) (Takemoto et al., 2018), the lack of D259 and E224 in Kir6.2 and its substitution for neutral polar residues leads to a decrease in chloroquine's ability to block the channel (Noujaim et al., 2010). Beyond the specific molecular interactions, chloroquine blockade of

the inward rectifying K^+ channels may partially explain the drug's antiarrhythmic power in such pathophysiological scenarios as vagally mediated AF or persistent AF in the presence of constitutively active I_{KACH} and I_{K1} increase (Noujaim et al., 2010; Makary et al., 2011; Martins et al., 2014; Takemoto et al., 2018). Chloroquine's ability to terminate persistent AF in Langendorff-perfused hearts explanted from a clinically relevant sheep model is remarkable (Filgueiras-Rama et al., 2012; Takemoto et al., 2018). It supports the concept that combined I_{K1} and I_{KACH} blockade may be synergistic and play a crucial role in terminating persistent AF; although chloroquine terminated 100% of the AF episodes, selective blockade of constitutively active I_{KACH} by tertiapinQ (Ramu et al., 2004) failed to terminate AF in one-third of episodes. Moreover, the effects of chloroquine on the dynamics of AF occurred more rapidly than those of tertiapinQ (Takemoto et al., 2018).

There is limited but significant evidence for chloroquine's ability to terminate AF in the clinical setting. In a study of 64 patients with arrhythmias published 60 years ago in the *New England Journal of Medicine* (Burrell and Martinez, 1958), 31 patients had AF and were treated with chloroquine, 18 of these patients with AF responded favorably with restoration of sinus rhythm. The dosages of chloroquine used in that study were well-tolerated, despite being considerably greater than those recommended at the time for other indications (Burrell and Martinez, 1958). However, chronic chloroquine administration is not free of systemic effects (AlKadi, 2007). Nevertheless, the foregoing suggests that revisiting chloroquine and its analogs as a starting point toward the development of a new generation of drugs capable of selectively blocking inward rectifying K^+ channels might be an interesting opportunity for pharma and for AF therapy.

B. Intracellular Ca^{2+} Regulation as a Target for Atrial Fibrillation Therapy

Ca^{2+} regulation is a major determinant of cellular electrophysiology in the heart, with some differences between atria and ventricles, which might result in a certain degree of drug selectivity. In the atria, inositol 1,4,5-triphosphate (IP₃) receptor (IP₃R) signaling is more developed (Kockskämper et al., 2008), and atrial cells display a much less developed T-tubule system than the ventricles. Consequently, in contrast with the ventricles, the most prominent calcium waves are observed at the subsarcolemma of atrial cardiomyocytes (Sheehan and Blatter, 2003). Interestingly, IP₃R colocalizes with the cardiac ryanodine receptor (RyR2) at the subsarcolemma of atrial cardiomyocytes (Visual Abstract). The latter suggests the possibility that IP₃R modulates RyR2 and Ca^{2+} release in some animal models (Mackenzie et al., 2002) and also in the human atria (Liang et al., 2009) and may explain the Ca^{2+} sparks leading to triggered activity under various pathologic conditions (Opel et al., 2015). The intracellular concentration of IP₃ increases after activation of

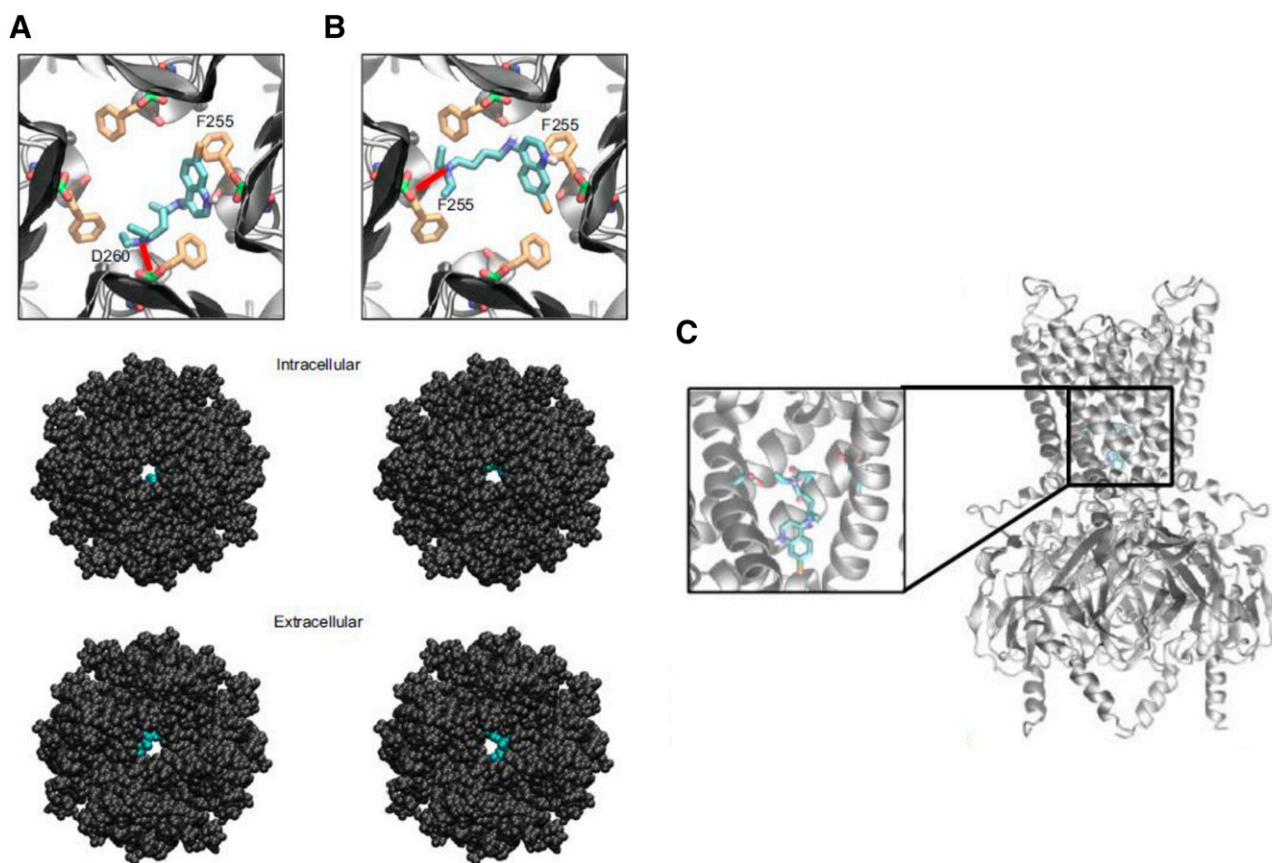


Fig. 2. Docking of chloroquine in the ion permeation pathway of the Kir3.1 channel. (A and B) Two lowest energy poses. Top: Magnified view of the binding poses of chloroquine (cyan sticks) in Kir3.1. The D260 and F255 residues from each of the 4 Kir3.1 subunits are shown in green and orange sticks, respectively. (A) The amine nitrogen of chloroquine forms a hydrogen bond (red line) with the side-chain of D260 in 1 subunit, whereas the aminoquinoline ring of chloroquine is involved in an aromatic-aromatic interaction with the phenylalanine ring of F255 in the adjacent subunit. (B) The amine nitrogen of chloroquine hydrogen bonds (red line) the carbonyl oxygen of F255 in 1 subunit, whereas the aminoquinoline ring of chloroquine is involved in an aromatic-aromatic interaction with the phenylalanine ring of F255 in the opposing subunit. Middle, bottom: van der Waals representations of the channel bound to chloroquine (cyan), viewed from the intracellular and extracellular sides, respectively. (C) Computational model of chloroquine docking in the aqueous region of the transmembrane domain in the homology model for Kir3.1 using the PDB of Kir3.2 (PDB ID: 3SYO). Chloroquine (in cyan) binds the transmembrane domain of the channel at residue. Modified with permission from figures 4 and 9 of Takemoto et al. (2018).

G-proteins via different membrane receptors; i.e., the muscarinic (M3) receptor, the endothelin-1 receptor ET-A, the angiotensin II receptor AT-I, and the α 1-adrenergic receptor (Tinker et al., 2016). IP3R activation in turn increases RyR2 permeability, promoting Ca^{2+} sparks and triggered activity. Some regulators of G-protein signaling accelerate the intrinsic GTPase activity and deactivate G-protein-mediated signaling, which in turn decreases the intracellular concentration of IP3. Recently, global deletion of the regulator of G-protein signaling 4 in the mouse resulted in a higher rate of AF inducibility in addition to abnormal Ca^{2+} release in atrial cardiomyocytes even under basal conditions (Opel et al., 2015). It was therefore suggested that modulating the G-protein-IP3- Ca^{2+} signaling axis may be a potential target for treating AF. However, there are doubts about selectivity, because the axis is functionally active in many cellular processes and cell types, leading to potential adverse events in the heart and other organs. To our knowledge, no drug that modulates the G-protein-IP3- Ca^{2+} signaling axis has been tested for AF treatment in humans.

Altered RyR2 kinetics and downregulation of the L-type calcium current, both already occurring during atrial remodeling in AF, contribute to abnormal Ca^{2+} handling and APD alternans and may facilitate triggered activity and wavebreak formation (Chang et al., 2014). Dantrolene increases the threshold for spontaneous Ca^{2+} release, decreasing Ca^{2+} leak from RyR2 (see Visual Abstract) (Jung et al., 2012). At clinically applicable and well-tolerated doses in humans, dantrolene effectively decreases the frequency of Ca^{2+} waves and the spontaneous Ca^{2+} transients and suppresses triggered activity in human atrial cells (Hartmann et al., 2017). Therefore, dantrolene is emerging as a potential drug able to control AF initiation. Recently, simulation studies suggested that RyR2 altered kinetics may also be crucial in underlying the complex behavior of functional reentry during AF and in governing of the transition from stable reentrant sources to an unstable and short-lasting multispiral state promoting wavelet formation (Chang and Trayanova, 2016). However, to the best of our knowledge there are yet no clinical data confirming a potential role for dantrolene in preventing

AF or modulating physiologic conditions that promote AF termination in patients.

C. Gap-Junction Therapy Aiming at Homogenous Impulse Propagation

Consistently, atrial heterogeneity and slow conduction are common factors underlying AF initiation and maintenance (Jalife, 2011). Gap-junctions, comprised of proteins called connexins, connect myocardial cells to each other through low-resistance pathways. Therefore, connexin dysfunction may potentially lead to AF. More specifically, Cx40 may be the most relevant target for therapy among the connexin family, because it is abundantly expressed in the atria but not the ventricles (Gros et al., 1994). A relatively new group of antiarrhythmic agents classified as antiarrhythmic peptides improve gap-junctional conductance. Of these, rotigaptide has been shown to improve conduction velocity in several animal models (Haugan et al., 2006; Shiroshita-Takeshita et al., 2007), which did not always correlate with a decrease in AF duration or vulnerability (Shiroshita-Takeshita et al., 2007). Similar results have been obtained with other antiarrhythmic peptides (e.g., GAP-134) (Rossman et al., 2009). Yet to date it is unknown whether this strategy might be clinically relevant.

Even though the rationale behind the antiarrhythmic peptides seems potentially beneficial in AF, the effects may not be homogeneous throughout the atria. This would not only enhance intrinsic atrial electrophysiological heterogeneities, but together with fibrosis and ion channel remodeling may counterbalance any potential benefits. An alternative to drug therapy may be the use of gene transfer to deliver Cx43 to the atrial tissue. Two different strategies using injection/electroporation or atrial painting of adenoviruses encoding Cx43 or Cx40 have been reported in a porcine model of AF based on rapid atrial pacing (Bikou et al., 2011; Igarashi et al., 2012). Both reports showed that AF could be prevented for a short-term period using such strategies.

VIII. Novel Targets in Atrial Fibrillation

A. Ion Channels, Electrical Remodeling, and the Progression of Atrial Fibrillation

AF frequently starts as paroxysmal AF, with spontaneous termination often occurring within 48 hours. Some patients suffer paroxysmal AF episodes indefinitely, but a significant number ($\approx 40\%$) progress to persistent AF within 10 years follow up (Padfield et al., 2017). AF-mediated remodeling involves changes at the structural, ionic, and mechanical levels that favor the initiation, maintenance, and perpetuation of AF (Nattel and Harada, 2014; Jalife and Kaur, 2015). Electrical remodeling, manifested by shortening of the atrial APD and refractoriness and loss of APD adaptation to changes in frequency, is known to develop within the first few days of AF (Allessie et al., 2002; de Vos et al., 2010) and to contribute to progressive prolongation of the duration of

AF episodes (“AF begets AF”) (Wijffels et al., 1995). Several ion channel modifications underlying such electrical changes have been described in animal models and humans (Van Wagoner et al., 1999; Nattel et al., 2008; Caballero et al., 2010; Voigt et al., 2010; Heijman et al., 2014). However, until recently, the sequence and the way such changes integrate to perpetuate AF had not been elucidated, particularly in humans. Structural remodeling at the cell and tissue levels also contributes to intra-atrial conduction disturbances and increase susceptibility for AF, yet its role in progression from paroxysmal to persistent AF remains unknown. Finally, atrial mechanical function is significantly altered, with reduced contractility and electromechanical dissociation. Recent studies using a clinically relevant ovine model showed that during intermittent right atrial tachypacing, not only the duration of AF episodes increased as expected, but also the dominant excitation frequency (DF) increased gradually during a 2-week period in both left and right atria, until it stabilized at a time that coincides with the onset of persistent AF (Martins et al., 2014). Thereafter, the DF remained stable during a 12-month follow up. Both the increases in the AF episode duration and the concomitant DF acceleration were associated with I_{CaL} and I_{Na} downregulation, and I_{K1} upregulation, along with corresponding changes in gene expression and ion-channel protein subunits. Structural remodeling in the form of cellular hypertrophy, atrial dilatation, and interstitial fibrosis also developed in the course toward AF stabilization (Martins et al., 2014). Consistent with the above findings, other studies have demonstrated numerous transcriptional changes in ion channel expression (Deshmukh et al., 2015), including upregulation of *KCNJ2* and *KCNJ4* (encoding Kir2.1 and Kir2.3 subunits, respectively, which contribute to I_{K1}) and downregulation of *CACNA1C* (encoding the I_{CaL} α -subunit), *CACNAB2* (a I_{CaL} β -subunit) (Deshmukh et al., 2015; Nattel, 2015), and *CACNAC1D*, which is atrial specific, and its absence in mice leads to impaired calcium homeostasis and increased AF susceptibility (Mancarella et al., 2008). Therefore, the progressive AF-related remodeling leading to DF increase in the animal model (Martins et al., 2014) is also consistent with the observation that the AF frequency is usually higher in patients with persistent than paroxysmal AF (Sanders et al., 2005). Sustained AF shortens APD and effective refractory period, decreasing the wavelength and facilitating the acceleration and stabilization of sustained reentry. The main determinants of APD shortening are the decrease in I_{CaL} and increase I_{K1} (Dobrev et al., 2001; Martins et al., 2014).

B. Remodeling and Upstream Therapies in Atrial Fibrillation

Diverse pathologies such as hypertension, heart failure, or coronary heart disease, among others, can promote atrial remodeling, both electrically and structurally. In this context, “upstream” therapies aim to act

on atrial remodeling and/or factors that promote it to control the burden of AF. More specifically, one of the most relevant objectives within upstream therapies is the control of atrial fibrosis, which is associated with the perpetuation of the arrhythmia.

Most of the scientific evidence focuses on inhibitors of the renin-angiotensin-aldosterone system (RAAS), such as angiotensin converting enzyme inhibitors (ACEIs) or angiotensin II receptor blockers (ARBs). The stimulation of angiotensin II receptors promotes nicotinamide adenine dinucleotide phosphate oxidase 2-dependent reactive oxygen species formation (Matsushima et al., 2014), producing oxidative stress and inflammation. In addition, angiotensin II activates an intracellular signaling cascade, leading to the activation of mitogen protein kinases to generate cardiomyocyte hypertrophy, apoptosis, and fibroblast proliferation, all favoring the perpetuation of AF, potentiated by higher concentrations of angiotensin II in patients with AF (Bedecs et al., 1997; Novo et al., 2008).

In addition to the traditional ACEIs and ARBs, mineralocorticoid receptor blockers (MRBs) have also been shown to effectively reduce the incidence of new-onset AF in systolic heart failure (HF) and to have beneficial effects on mortality in patients with cardiac disease (Lavall et al., 2014). From the mechanistic point of view, recent experimental results show clearly that the MRB eplerenone (EPL) significantly mitigates structural remodeling during the transition to persistent AF (Takemoto et al., 2017). Animals treated with EPL had significantly smaller atrial size and less cellular hypertrophy and fibrosis than animals given sugar pills. Atrial size is an important determinant of clinical AF (Takemoto et al., 2017). Experience in patients and numerical data suggest that dilated atria have a higher probability for initiation and maintenance of rotor-driven fibrillatory activity (Pandit and Jalife, 2013). Myocyte hypertrophy was also diminished by EPL. The data are consistent with studies in mouse models of chronic pressure overload or myocardial infarction, in which deletion or inactivation of the MR gene attenuated left ventricular dilatation, cardiac hypertrophy, and development of HF, whereas MR overexpression in cardiomyocytes induced ventricular remodeling, development of HF, and pro-arrhythmogenic effects (Ouvrard-Pascaud et al., 2005; Fraccarollo et al., 2011). Some RAAS pathways affected by ACEIs, ARBs, and MRBs are illustrated schematically in Fig. 3.

Several retrospective studies from clinical trials suggest a beneficial effect of the inhibition of RAAS in the prevention of AF, especially in patients with ventricular hypertrophy or ventricular dysfunction. Among others, the EMPHASIS-HF study randomized 2743 patients with heart failure (ventricular ejection fraction $\leq 35\%$) and functional class New York Heart Association \geq II to receive EPL or placebo, the main objective of which was to analyze the incidence of new-onset AF during follow

up. The investigators observed a significant reduction of new onset AF in the group randomized to EPL (2.7% vs. 4.5%) (Zannad et al., 2011). These data are supported by a recent meta-analysis that included 14 studies (five randomized clinical trials and nine observational studies) and 5332 patients, of whom 2397 received MRBs (EPL or spironolactone), which showed that the incidence of AF in this group was significantly lower compared with patients who did not receive this treatment (8.5% and 18.6%, respectively) (Neefs et al., 2017). Although the specific mechanism of action was not an objective of the work, the inhibition of fibrosis and atrial structural remodeling might be highly involved, as has been demonstrated in the animal model of persistent AF in sheep, with high similarity to clinical AF (Takemoto et al., 2016).

In line with the above findings, the RACE-3 study (Rienstra et al., 2018), whose results were presented at the congress of the European Society of Cardiology in August 2017, included patients with mild-to-moderate heart failure for less than 1 year and persistent AF with less than 5 years of history and episodes lasting less than 6 months, who were selected for electrical cardioversion. Patients were randomized to receive intensive treatment against cardiovascular risk factors and upstream therapy or perform conventional management. Upstream therapy included statins, ACEIs or ARBs, MRBs, and a cardiac rehabilitation program for 11 weeks. The main result of the study was that 75% of the patients within the intensive treatment group were in sinus rhythm during a year of follow up (including Holter registry of 7 days) compared with 63% in the group that received conventional management. Notably, there was no difference in antiarrhythmic drug use or the number of electrical cardioversions between the two groups. The results further increase the value of substrate control and comorbidities in the prevention of AF recurrences. It should be noted that the greatest differences between both groups were observed in therapy with MRBs (85% vs. 4%, in intensive vs. conventional therapy groups, respectively), which highlights their relevant role in the prevention of AF in patients with an obvious structural substrate.

Other studies such as GISSI-AF seemed to show conflicting data, since valsartan did not reduce the incidence of AF in secondary prevention. However, it is important to consider that half of the patients in both groups were under treatment with ACEIs (a fact that was not considered a possible confounding factor), and the patients had good ventricular function and, therefore, a low structural substrate and less influence of fibrosis-causing factors (Disertori et al., 2009).

A recent study demonstrated that intracardiac serum levels of the profibrotic protein galectin-3 (Gal-3) are greater in patients with persistent than paroxysmal AF and that Gal-3 is an independent predictor of atrial tachyarrhythmia recurrences after a single ablation procedure in some patients (Takemoto et al., 2016). In addition, upstream therapy targeting Gal-3 using a

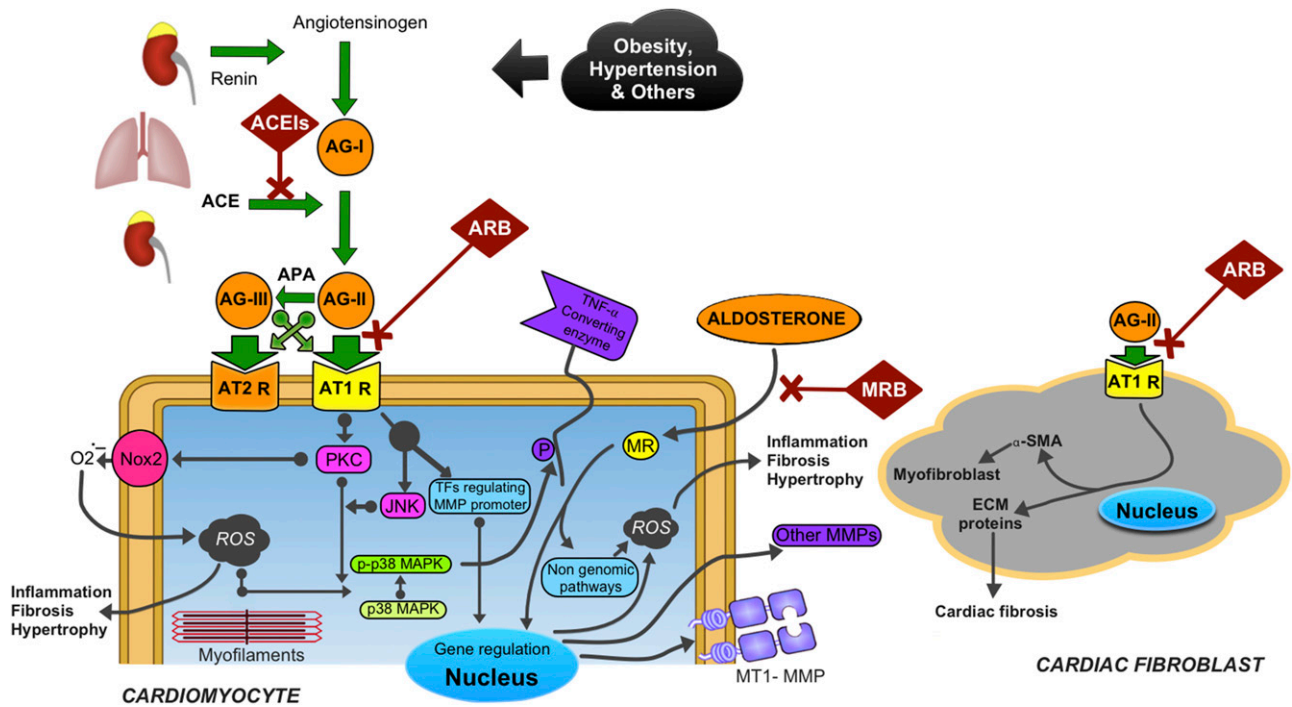


Fig. 3. Diagrammatic representation of the main effects exerted by renin-angiotensin-aldosterone system blockers. ACEIs, ARBs, and MRBs block inflammatory and profibrotic pathways that promote atrial remodeling and favor AF initiation and maintenance. ACEIs, angiotensin-converting enzyme inhibitors; AG-I, angiotensin I; AG-II, angiotensin II; AG-III, angiotensin III; APA, aminopeptidase A; ARB, angiotensin II type 1 receptor blocker; AT1R, angiotensin II type 1 receptor; AT2R, angiotensin II type 2 receptor; ECM, extracellular matrix; JNK, c-Jun N-terminal kinases; MMP, matrix metalloproteinase; MR, mineralocorticoid receptor; MRB, mineralocorticoid receptor blocker; MT1-MMP, membrane type 1 metalloproteinase; Nox2, nicotinamide adenine dinucleotide phosphate oxidase 2; P, phosphorylation; p38 MAPK, p38 mitogen-activated protein kinase; PKC, protein kinase C; ROS, reactive oxygen species; SMA, α -smooth muscle actin; TFs, transcription factors; TNF- α , tumor necrosis factor- α .

relatively low intravenous dose of the galactomannan GM-CT-01 reduced both structural and electrical remodeling as well as AF burden in a sheep model of persistent AF in the absence of comorbidities (Martins et al., 2014; Takemoto et al., 2016). However, Gal-3 inhibition did not restore sinus rhythm in the long term. Nevertheless, the study provided a solid proof of concept in support of upstream AF prevention therapy. Through its effects promoting gene transcription via cytokine-mediated signaling pathways (de Boer et al., 2009; Mackinnon et al., 2012), Gal-3 might represent but one of multiple potential targets for the prevention of either structural and/or electrical remodeling and AF perpetuation.

Less evidence exists in favor of therapies such as polyunsaturated omega-3 fatty acids or the inhibitors of 3-hydroxy-3-methylglutaryl-CoA reductase (statins) in isolation. To summarize, the dietary supplement with omega-3 polyunsaturated fatty acids could play a role in the prevention of AF in patients with evident structural substrate and presence of atrial remodeling, with high levels of circulating inflammatory biomarkers, and in the context of low circulating levels of the own polyunsaturated fatty acids omega-3 (Salvador-Montañés et al., 2012). In relation to statins, data from a meta-analysis suggest that statins may contribute to the prevention of AF in the postoperative period of cardiac surgery (Kuhn et al., 2014).

None of the agents described as “upstream” therapies to date have specific approval as an antiarrhythmic treatment against AF, but they are widely used drugs in cardiovascular disease and many of their current indications (e.g., hypertension) include most pathologies associated with AF.

C. Nonpharmacologic Targets: Obesity, Adiposis, and Inflammation in Atrial Fibrillation

Upstream therapies not only involve pharmacological agents, but also other modifiable factors and measures such as the control of body weight, the treatment of obstructive sleep apnea, or the treatment and control of local or systemic inflammatory processes, which act on the structural or functional substrate that favors AF. Thus, obesity is a known risk factor for the development of AF; an increased body mass index, the presence of metabolic syndrome, and the diameter of the abdominal circumference have been identified as predictive factors for the development of AF (Zhang et al., 2009; Tanner et al., 2011). Current evidence from randomized clinical trials indicates that loss of weight gain is associated with a decrease in AF recurrences, as well as in the severity of the symptoms and the number of episodes (Abed et al., 2013). Therefore, the practice of moderate physical activity and avoiding excessive weight gain constitutes a preventive strategy for AF, as well as

recurrences after a first episode, as also evidenced by the CARDIO-FIT study (Pathak et al., 2015).

Cardiac adipose tissue consists of epicardial fat, overlying the epicardium, and the pericardial fat, situated outside the visceral pericardium and on the external surface of the parietal pericardium (Iacobellis, 2015). Under normal conditions, epicardial adipose tissue provides biochemical, mechanical, and thermogenic protective input to the myocardium (Iacobellis and Bianco, 2011). However, under pathologic conditions, epicardial fat can locally affect the myocardium, and recent studies suggest that, in addition to systemic adiposity, the volume of the pericardial fat of the entire heart, and particularly of that overlying the atria, may represent an even more important risk factor for AF. Many studies, including the Framingham Heart Study, have reported a relationship between the amount of adipose tissue that accumulates around the atria and the risk and maintenance of AF (Al Chekatie et al., 2010; Thanassoulis et al., 2010; Haemers et al., 2017). Also, epicardial fat is associated with AF severity and ablation outcomes (Wong et al., 2011). However, the underlying mechanisms linking AF to adipose tissue have not been elucidated.

In addition to being a rich source of free fatty acids (FFA), fat tissue can secrete many proinflammatory cytokines (Iacobellis and Bianco, 2011; Iacobellis, 2015) that can freely diffuse into the adjacent myocardium (Mazurek et al., 2003; Iacobellis et al., 2005; Greulich et al., 2012). For instance, the secretome of human epicardial adipose tissue can induce atrial fibrosis, an effect that is mediated by Activin A (Venteclef et al., 2015). Epicardial adiposity and plasma levels of FFAs are elevated in AF (Rennison and Van Wagoner, 2009). FFAs have been shown to disrupt t-tubular architecture and to remodel properties of membrane ionic currents in sheep atrial myocytes, which might help explain their proarrhythmic effects (O'Connell et al., 2015). Crosstalk between adipose and myocardial tissue has been demonstrated by the observation that rapid atrial pacing or AF induces the expression of several genes able to regulate adipose tissue accumulation (Chilukoti et al., 2015). Adipose tissue can also infiltrate the myocardium and contribute to its functional disorganization as described for the right ventricle (Burke et al., 1998; Pouliopoulos et al., 2013). The inflammation of atrial tissue contributes to arrhythmogenic remodeling and could be a target of antiarrhythmic therapies. Corticosteroids have a potent anti-inflammatory effect and have been shown to be effective against AF in animal and clinical studies, but their potential adverse effects are a limitation for their use, especially in the long term.

IX. Atrial Fibrillation and Sleep Apnea

Atrial arrhythmias, including AF, are highly prevalent in patients with moderate to severe obstructive sleep apnea, which is an independent risk factor for the

development of AF. Some of the mechanisms involved include hypoxia, hypercapnia, increased atrial intracavitary pressure, inflammation itself, and negative intrathoracic pressure during the inspiratory effort of apnea. It is especially relevant that therapy with continuous positive airway pressure during sleep hours reduces or even eliminates the recurrence of atrial arrhythmias, not only AF (Filgueiras-Rama et al., 2013).

X. Challenges and Opportunities in New Anti-atrial Fibrillation Drug Development

The global AF market size was valued at USD 6.1 billion in 2012 and is expected to reach 8319 million by 2020 (<https://www.alliedmarketresearch.com/atrial-fibrillation-market>). Fibrosis and electrical remodeling are now recognized to be major causes of AF perpetuation with consequent increase in morbidity and mortality (Dzeshka et al., 2015). The future growth of this market and the rising prevalence of AF in emerging Asian economies will ensure the success of future efforts for the discovery of novel biomarkers that could help advance preventative therapies that target specific mechanisms and signaling pathways involved in atrial remodeling and AF perpetuation and/or recurrence of the arrhythmia. Therefore, future research in AF mapping and ablation should be complemented by fundamental basic and translational science innovations in drug discovery, with the objective of preventing remodeling upstream, thus reducing AF burden, increasing the success of AF termination, and preventing recurrences. At the very least, such studies should dramatically improve our current understanding of the mechanisms underlying AF progression and perpetuation and help improve ablation outcomes.

There is a need to develop new and more effective therapies to cure or at least prevent the progression of AF to a chronic stage. The highly complex molecular underpinnings of atrial remodeling leading to persistent AF makes target identification challenging. Yet only a profound and complete understanding of the mechanisms involved in the maintenance and perpetuation of AF will allow us to generate more specific prevention and/or treatment of this dangerous and debilitating disease. Animal models play an important role in the study of the pathophysiology of AF, including molecular basis, ion-current determinants, anatomic features, and macroscopic mechanisms, as well as in the development of new therapeutic approaches, whether drug-based, molecular therapeutics, or device related (Nishida et al., 2010). Unfortunately, the scientific challenges to translational AF research taken together with the financial risks have led to significant reductions in the investment in treatments for new cardiovascular drugs both in the United States and globally (Fordyce et al., 2015). Many pharmaceutical companies have stopped almost entirely their antiarrhythmic drug

research programs, focusing on therapeutic areas like oncology and Alzheimer's disease (DiMasi et al., 2010; Pammolli et al., 2011; Fordyce et al., 2015). However, reproducibility and collaboration among basic scientists, clinicians, and industry will be critical for translation into effective therapies.

XI. Conclusion

The prevalence and burden of AF worldwide call for an urgent increase in investment in basic and translational research within industry, academia, and government. Significant advances in science and new approaches to translation are needed, so that vital scientific challenges are overcome, including increasing the understanding of AF mechanisms, identifying and validating targets, developing predictive biologic and computational models, recognizing reliable biomarkers for patient stratification and as endpoints for clinical trials, preventing proarrhythmia toward clearing regulatory pathways, and ensuring the reliability and reproducibility of published data, as well as data sharing and collaboration.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Calvo, Filgueiras Rama, Jalife.

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