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REVIEW

Atrial Fibrillation

# From Atrial Small-conductance Calcium-activated Potassium Channels to New Antiarrhythmics

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## Abstract

Despite significant advances in its management, AF remains a major healthcare burden affecting millions of individuals. Rhythm control with antiarrhythmic drugs or catheter ablation has been shown to improve symptoms and outcomes in AF patients, but current treatment options have limited efficacy and/or significant side-effects. Novel mechanism-based approaches could potentially be more effective, enabling improved therapeutic strategies for managing AF. Small-conductance calcium-activated potassium (SK or KCa2.x) channels encoded by *KCNN1-3* have recently gathered interest as novel antiarrhythmic targets with potential atrial-predominant effects. Here, the molecular composition of small-conductance calcium-activated potassium channels and their complex regulation in AF as the basis for understanding the distinct mechanism of action of pore-blockers (apamin, UCL1684, ICAGEN) and modulators of calcium-dependent activation (NS8593, AP14145, AP30663) are summarised. Furthermore, the preclinical and early clinical evidence for the role of small-conductance calcium-activated potassium channel inhibitors in the treatment of AF are reviewed.

## Keywords

Antiarrhythmic drugs, atrial fibrillation, electrical remodelling, ion channel, small-conductance calcium-activated potassium channel

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AF is the most common cardiac arrhythmia, affecting >59 million people worldwide, contributing to increased morbidity and mortality.[1] Significant progress has been made in understanding the underlying molecular mechanisms of AF. However, traditional antiarrhythmic drugs were not specifically developed to target these mechanisms. As a result, novel mechanism-based approaches could potentially be more effective, enabling improved therapeutic strategies for managing AF.[2]

Essentially, an episode of AF can be initiated and maintained by focal ectopic (triggered) activity or re-entry mechanisms. Re-entry requires both a vulnerable substrate and an initiating trigger, typically caused by focal ectopic firing.[3–5] The most common causes of focal ectopic activity include delayed afterdepolarisations (DADs) and early afterdepolarisations. DADs are generated upon full repolarisation, and are small, spontaneous depolarisations that are able to promote ectopic firing (generation of a spontaneous action potential; AP) if they reach the firing threshold. DADs are caused by transient inward currents mainly mediated by the $Na^+/Ca^{2+}$ exchanger in response to a transient rise in diastolic cytoplasmic $Ca^{2+}$ concentration. This often occurs due to $Ca^{2+}$-handling abnormalities, such as spontaneous $Ca^{2+}$ release from the sarcoplasmic reticulum via ryanodine receptor type 2. Early afterdepolarisations occur before full repolarisation, during late phase 2 and early phase 3 of the AP, typically when the AP is excessively prolonged due to increased inward L-type $Ca^{2+}$ current or late $Na^+$ current, or reduced $K^+$ currents. The prolonged AP allows L-type $Ca^{2+}$ channels to recover from inactivation and reactivate, resulting in a depolarising current as $Ca^{2+}$ influx increases. Spontaneous ectopic activity can be transient, resulting in single atrial ectopic beats, or repetitive, leading to tachycardia and AF.[6]

Re-entry mechanisms are crucial for sustaining AF, and depend on the atrial refractory period and tissue conduction properties, with conduction slowing and/or block, as well as shortening of the refractory period promoting re-entry. Both focal ectopic activity and re-entry are promoted by various factors, including the rapid atrial activity caused by AF, genetic

and epigenetic factors, as well as comorbidities that alter the atrial tissue structure and function.[7–10]

With the increasing understanding of the fundamental mechanisms of AF, there is potential to develop new and improved antiarrhythmic approaches. Rhythm control therapy using antiarrhythmic drugs remains a cornerstone of AF management, despite the increasingly widespread use of ablation therapies, and has been shown to improve clinical outcomes when applied early in the disease process.[9,10] Emerging preclinical and early-phase clinical trials have suggested small-conductance $Ca^{2+}$-activated $K^+$ (SK)-channels as a promising atrial-preferential antiarrhythmic target for AF management.[9] Although several potential antiarrhythmic drugs are under clinical development, SK-channel inhibitors appear to be the most advanced antiarrhythmic agents.[9,11]

In this review, we focus on SK channels as a potentially important atrial-preferential drug target, highlighting their functional role and complex regulation in AF by reviewing data from both animal and human studies.

## Small-conductance $Ca^{2+}$-activated $K^+$ Channels

The family of $Ca^{2+}$-activated $K^+$channels is subdivided based on their single-channel conductance into three subtypes: big (~180 pS conductance), intermediate (~40 pS conductance) and small (~10 pS conductance).[12–14] The SK channels are further subdivided into SK1, SK2 and SK3 (KCa2.X) encoded by *KCNN1*, *KCNN2* and *KCNN3*, respectively.[14] SK channels are present in the plasma membrane of various cell types, where they regulate cellular excitability and influence the AP morphology, although SK channels could also locate in the mitochondrial inner membrane of cardiomyocytes.[15,16] The regulation of SK channel function is complex, and is governed by the tight control of their $Ca^{2+}$ sensitivity, and their channel trafficking and membrane targeting, as discussed in detail below.

### Structure and $Ca^{2+}$ Sensitivity of Small-conductance $Ca^{2+}$-activated $K^+$ Channels

The structure of the SK channels is similar to most other $K^+$ channels, with four α-subunits containing six transmembrane domains (S1–S6) with both the N- and C-terminal ends orientated intracellularly (*Figure 1*). SK channels assemble as homo- or hetero-tetramers to form a functional channel with a $K^+$-permeable pore that is also the target of several SK channel inhibitors (discussed below). Although their topology resembles that of voltage-gated $K^+$ (channel with six transmembrane domains, the S4 segment that acts as voltage sensor in voltage-gated $K^+$ channels contains fewer positively charged arginine residues, making the SK channels largely voltage-independent.[14,15]

The $Ca^{2+}$ sensitivity of SK channels is a critical determinant of their function, enabling them to respond to intracellular $Ca^{2+}$ levels, and contribute to the regulation of membrane potential dynamics and ionic homeostasis.[14,15] It also represents a second option for pharmacological modulation of SK channels, as discussed below. All three SK channel subtypes are activated within milliseconds (5–15 ms) by $Ca^{2+}$ in the submicromolar range ($K_{0.5}$: 300–700 nM).[14,17–19] Unlike other $Ca^{2+}$-activated $K^+$ channels, SK channels do not have obvious $Ca^{2+}$-binding sites in their primary structure. Instead, SK channels rely on an interaction between their C-terminal domain and the $Ca^{2+}$-sensor and signalling molecule calmodulin (CaM; *Figure 1*).

### Gating Behaviour of Small-conductance $Ca^{2+}$-activated $K^+$ Channels

The interaction between SK channels and CaM is facilitated by the CaM-binding domain (CaMBD), a highly conserved 90 amino acid region located in the C-terminal region of SK channels.[18,20] CaM associates with the CaMBD of the SK channel in both $Ca^{2+}$-dependent and $Ca^{2+}$-independent manners.[21] Gating is initiated when intracellular $Ca^{2+}$ concentrations increase, leading to $Ca^{2+}$ binding by the N-lobe of CaM. This binding induces an interaction between two adjacent CaMBDs, resulting in the formation of a CaMBD dimer complex. As each functional SK channel incorporates four CaM molecules, two CaMBD dimer complexes are formed, which together drive the opening of the channel. Channel deactivation occurs upon $Ca^{2+}$ dissociation from CaM, highlighting the critical role of CaM in both activating and deactivating SK channels.[21,22] Data on SK single-channel kinetics are sparse, but have shown complex gating kinetics, including two open times and three closed times, as well as two distinct behaviours characterised by high and low open probabilities. These behaviours can switch spontaneously and are influenced by the actual $Ca^{2+}$ concentration, with higher $Ca^{2+}$ levels promoting high open probability states.[19]

High-resolution stimulated emission depleted imaging and quantitative analyses have shown that SK2 channels are located in close vicinity to L-type $Ca^{2+}$ channels and cardiac ryanodine receptor type 2 channels, allowing a tight $Ca^{2+}$ regulation of SK channels. This proximity suggests a functional relationship, where local $Ca^{2+}$ movements from L-type $Ca^{2+}$ current and ryanodine receptor type 2 channels precisely regulate SK channel activity, although a subfraction of SK channels may also operate outside of such functional units.[23]

Besides CaM, the SK channel macromolecular complex contains additional key regulatory proteins (*Figure 1*), including the catalytic and regulatory subunits of casein kinase type 2 (CK2α and CK2β) and protein phosphatase type 2A (PP2A). These regulate SK channel gating by tuning the degree of CaM phosphorylation at threonine 80. In particular, PP2A-mediated dephosphorylation of CaM at threonine 80 increases the $Ca^{2+}$ affinity and the functional gating of SK channels (*Figure 1*).[24]

### Dynamic Trafficking of Small-conductance $Ca^{2+}$-activated $K^+$ Channels

SK channel trafficking and membrane targeting are highly dynamic processes that depend on interactions with several key cytoskeletal proteins (*Figure 1*).[25,26] CaM is not only crucial for gating, but also for the assembly, trafficking and targeting of SK channels.[18] Several studies indicated that the $Ca^{2+}$-independent interaction between the C-lobe of CaM and the CaMBD on the SK channel is necessary for proper membrane expression.[20,22,27,28] The N-terminal region of SK2 channels interacts with the C-terminal region of filamin A, which also supports membrane targeting of SK channels in cardiomyocytes. SK2 channel trafficking is also highly dependent on intracellular $Ca^{2+}$ levels, with increased $Ca^{2+}$ levels enhancing SK2 channel surface expression, especially when co-expressed with α-actinin-2.[25]

### Small-conductance $Ca^{2+}$-activated $K^+$ Channel Distribution and Localisation in the Heart

Numerous studies have confirmed the expression of SK channels not only in atrial and ventricular cardiomyocytes, but also in pulmonary vein (PV) cardiomyocytes, and in atrioventricular and sinoatrial nodal cells of different species.[17,29–38] SK2 protein expression appears significantly higher in human atrial compared with ventricular tissue, and both SK1 and SK2 messenger RNA (mRNA) were more abundantly expressed in mouse atrial compared with ventricular tissue, while the SK3 mRNA was similar in both regions.[17,39] However, there have been some discrepancies between

studies in terms of expression levels in different regions of the heart, which is most likely caused by different detection techniques, as well as by the degree of cardiac remodelling in different animal and patient cohorts. However, mRNA and protein data do not provide information about the functionality of these channels. Although SK channel transcripts have been found in several regions of the heart, functional SK channels appear to be preferentially located in the atria, which makes them a potential target for treatment of atrial arrhythmias with minimal ventricular side-effects.

## Functional Role and Remodelling of Cardiac Small-conductance $Ca^{2+}$-activated $K^+$ Channels in AF

The highly-selective SK channel inhibitor, apamin, was instrumental for the detection of SK currents ($I_{SK}$) in the heart, showing a significantly larger $I_{SK}$ in atrial compared with ventricular cardiomyocytes in mice.[17] Subsequently, Özgen et al. demonstrated that early electrical remodelling induced by short-term burst pacing protocols mimicking ectopic activity in rabbit PVs, known to trigger paroxysms of AF, is linked to an enhanced expression and trafficking of SK channels to membrane sites.[37] This led to an increased $I_{SK}$ that correlated with a shortening of AP duration (APD) in PVs, suggesting that SK2 channels play a prominent role in the development of a pro-arrhythmic substrate in AF.[37] The important role of SK channels in the PVs was further validated in another canine model, where SK2 channels were more abundant in PVs compared with the left atrium at baseline, along with a larger $I_{SK}$ in the PVs compared with the left atrium. Upon 7 days of atrial tachypacing, $I_{SK}$ was increased in both the left atrium and PVs, which was attributed in part to a higher single-channel open probability.[40]

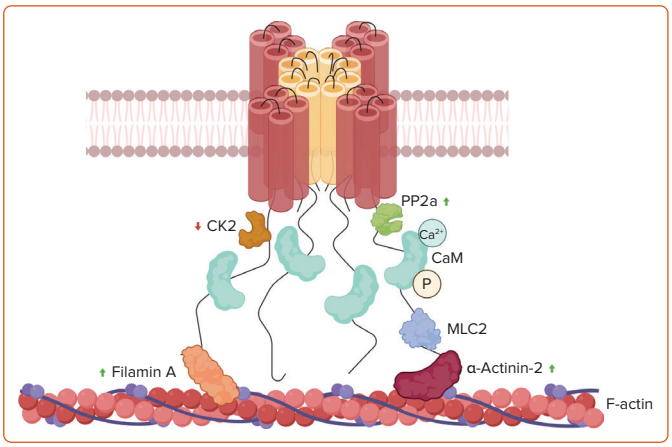
In humans, genome-wide association studies have pointed to a potential causal role of SK channels in AF.[39,41] We could show that SK2 channels were more abundant at the sarcolemma of atrial cardiomyocytes from patients with persistent (chronic) AF (cAF), and carried a greater $I_{SK}$ in atrial cardiomyocytes from cAF patients. This enhanced membrane localisation of SK channels was sensitive to inhibitors of anterograde (latrunculin A) and retrograde (primaquine) protein trafficking, which eliminated the differences in SK2 membrane levels and $I_{SK}$ between cAF and control cardiomyocytes, suggesting enhanced trafficking/membrane targeting as a potential mechanism of enhanced $I_{SK}$ in cAF patients. Our study also validated the highly dynamic nature of SK2 channel trafficking, showing that rapid electrical activation (5 Hz for 10 min) in control cardiomyocytes increased SK2 membrane localisation and $I_{SK}$, reaching the levels observed in cAF cardiomyocytes.[29]

## Small-conductance $Ca^{2+}$-activated $K^+$ Channels as a Novel Antiarrhythmic Drug Target

In isolated dog and monkey hearts, apamin produced an increase in cardiac force and resistance, and was able to restore normal rhythm, pointing to potential antiarrhythmic effects. When injected in the animals, apamin did not lead to any significant changes in blood pressure or ECG characteristics. The antiarrhythmic properties of apamin were observed for 90 min, and the authors even concluded that the long-term antiarrhythmic properties of apamin should be further explored.[42] However, the purity of the apamin used in this early study was later questioned, and the existence of cardiac $Ca^{2+}$-activated $K^+$ channels was doubted for years.[43,44]

Since then, apamin has been used in numerous experiments to selectively block SK channels, and various other compounds modulating SK channel activity have been developed. The compounds belong to two groups: SK channel pore blockers and negative modulators. Pore channel blockers achieve this by directly inhibiting ion permeation through SK channels, while negative modulators reduce the activity of SK channels by decreasing their sensitivity towards $Ca^{2+}$. Besides apamin, which is the most potent and selective SK channel blocker allosterically binding to the external pore region of the channel, UCL1684 and ICA/ICAGEN are SK channel blockers acting by binding to the apamin binding site (*Figure 2*).[45–47] The best known negative channel modulators of SK channels are NS8593, AP14145 and AP30663 (*Figure 2*).[48–50]

### Figure 1: Major Components of the Small-conductance $Ca^{2+}$-activated $K^+$ Channel Macromolecular Complex That Regulates Small-conductance $Ca^{2+}$-activated $K^+$ Channel Targeting and Function




*The channel's $Ca^{2+}$ sensitivity is controlled by phosphorylation of threonine 80 on calmodulin by casein kinase type II and its dephosphorylation by protein phosphatase type 2A. Channel trafficking and membrane targeting are regulated by interactions with several cytoskeletal-associated proteins, including α-actinin-2, filamin A and myosin light chain type 2. CaM = calmodulin; CK2 = casein kinase type II; MLC2 = myosin light chain type-2; PP2A = protein phosphatase type 2A.*

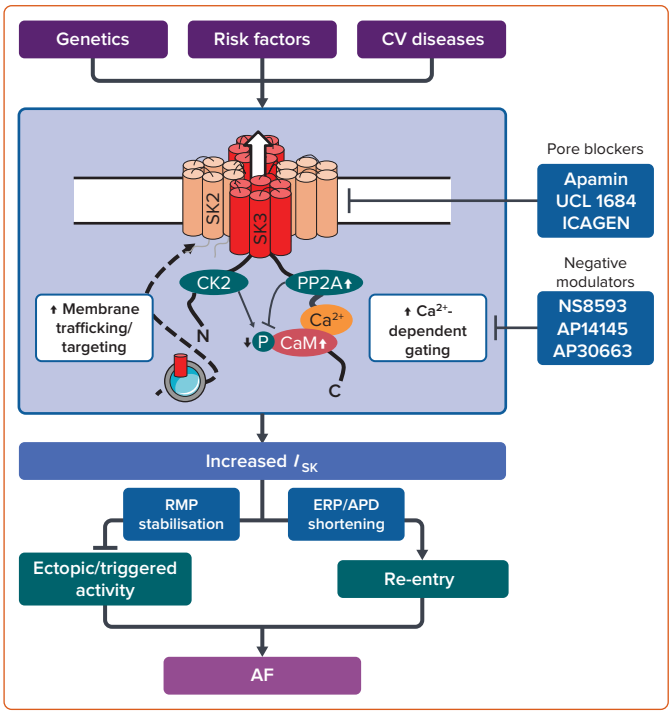
Although both types of inhibitors reduce $I_{SK}$, which typically prolongs APD, each inhibitor uniquely regulates the gating kinetics and unitary current of SK channels. These distinct biophysical characteristics are likely key to understanding the distinct functional effects of these inhibitors during the dynamic $Ca^{2+}$-dependent channel activation *in vivo*.[51] These compounds have been tested for their antiarrhythmic properties in numerous animal models (*Table 1*), as discussed below.

## Animal Studies of Small-conductance $Ca^{2+}$-activated $K^+$ Channel Inhibition

Overexpression of SK3 channels in mice significantly shortened atrial cardiomyocyte $APD_{90}$, which increased susceptibility to inducible atrial arrhythmias, providing direct evidence on the link between SK channels and AF (*Figure 2*).[52] Studies in rats revealed antiarrhythmic effects of pharmacological SK channel inhibition.[53,54] Injection of NS8593 (5 mg/kg), which reduces $Ca^{2+}$ sensitivity of SK channels, significantly shortened the duration of inducible AF *in vivo*, comparable to the effect of amiodarone. Importantly, the SK channel inhibition did not affect the QT interval, indicating an atrial preferential action without ventricular proarrhythmic effects. In isolated rat hearts, NS8593 (10 µmol/l) also prolonged the atrial effective refractory period (aERP), and was able to terminate AF and prevent its reinduction by burst pacing.[53]

Subsequently, experiments in large-animal models (dogs, pigs, goats and horses) validated the antiarrhythmic effects of SK channel inhibition. In

## Figure 2: Role of Small-conductance $Ca^{2+}$-activated $K^+$ Channel Upregulation in AF and Potential Consequences of Small-conductance $Ca^{2+}$-activated $K^+$ Channel Inhibition




*Small-conductance $Ca^{2+}$-activated $K^+$-channel current can be upregulated due to changes in $Ca^{2+}$-dependent gating and increased membrane trafficking/targeting, driven among other things by genetics, risk factors (including female sex) and cardiovascular diseases. Increased small-conductance $Ca^{2+}$-activated $K^+$ current can promote re-entry by shortening atrial action potential duration and effective refractory period, but can also inhibit ectopic/triggered activity by stabilising the resting membrane potential. Small-conductance $Ca^{2+}$-activated $K^+$ channel inhibitors reverse these effects, thereby potentially showing both pro- and antiarrhythmic effects, although currently available preclinical and clinical data suggest a predominant antiarrhythmic effect. Furthermore, small-conductance $Ca^{2+}$-activated $K^+$ channel inhibitors can have distinct mechanisms; for example, directly blocking the channel pore or reducing $Ca^{2+}$-dependent activation, with potentially distinct electrophysiological effects. APD = action potential duration; CaM = calmodulin; CK2 = casein kinase type 2; CV = cardiovascular; ERP = effective refractory period; $I_{SK}$ = small-conductance $Ca^{2+}$-activated $K^+$ current; PP2A = protein phosphatase type 2A; RMP = resting membrane potential.*

dogs, NS8593 significantly prolonged aERP in both control animals and dogs subjected to atrial tachypacing for 7 days. The aERP prolongation was stronger in the tachypaced (~90%) compared with the control (~15%) group. This difference likely reflects the high atrial rate-dependent upregulation of channel trafficking to the membrane and the enhanced $Ca^{2+}$ sensing of SK channels under these conditions.[29,37] Overall, both inducibility of AF and the duration of inducible AF episodes were significantly reduced by the SK channel blocker, NS8593.

The majority of research on SK channels in large animal models has been performed in pigs (*Table 1*). Diness et al. showed that the SK channel inhibitor, AP14145, which also reduces the $Ca^{2+}$ sensitivity of SK channels, successfully converted AF to sinus rhythm and prevented reinduction of AF in all tested pigs. Both AP14145 and vernakalant significantly prolonged aERP, and reduced AF duration without affecting ventricular ERP or blood pressure.[55] The same group subsequently demonstrated that the first $Ca^{2+}$ sensitivity-reducing SK channel blocker enrolled in a clinical trial (AP30663) significantly increased aERP in a dose-dependent manner, with a ≥30 ms prolongation achieved at 5 mg/kg, indicating the minimal efficacious dose. AP30663 successfully converted AF to sinus rhythm in six out of 10 pigs with vernakalant-resistant AF, and prevented reinduction in four of these six pigs. AP30663 caused a significant decrease in heart rate without a clear dose-dependent pattern. The QTc increased by ~11 ms at the highest dose applied, suggesting a dose-dependent effect on QTc, although this increase was relatively small, and its clinical significance remains uncertain.

In 2015, Haugaard et al. demonstrated that the SK channel blocker, NS8593, significantly prolonged the aERP in healthy, anaesthetised horses, with the most pronounced effects at lower atrial stimulation rates. NS8593 achieved a 100% cardioversion rate for acutely induced AF, and significantly reduced both vulnerability to AF and the duration of inducible AF episodes. Cardiac biopsies showed equivalent mRNA levels of SK channel isoforms in both atria and ventricles, but there were no drug-induced effects on QRS duration, QTc or heart rate, indicating a minor functional role of SK channels in ventricular tissue under normal conditions (*Table 1*).[56]

## Ex Vivo and In Silico Human Studies Employing Small-conductance $Ca^{2+}$-activated $K^+$ Channel Inhibitors

Several studies have addressed the potential role of SK channels in patients with AF (*Table 2*). At the mRNA and protein level, most studies point towards either no change or downregulation of SK channel isoforms. However, the majority of the functional studies indicate an upregulation of $I_{SK}$ in patients with AF. The discrepancies between studies may be related to heterogeneities in patient cohorts and stratification of patients, genetic variability and concomitant medication. Furthermore, technical differences in the experimental setups, including the use of different SK channel blockers ranging from the channel pore blocker, apamin, to $Ca^{2+}$-dependent gating modulators, such as NS8593, may have influenced the individual studies. However, the primary distinction appears to be the differences between whole-cell mRNA/protein data and functional data, underscoring the importance of post-translational regulation of SK channel trafficking/membrane targeting and $Ca^{2+}$ sensitivity as key mechanisms of altered SK channel function (*Table 2*).

Inhibition of $I_{SK}$ produced a slight APD prolongation in atrial monolayers obtained from human induced pluripotent stem cell-derived cardiomyocytes, or adult human atrial cardiomyocytes from patients without persistent AF.[57,58] However, in a recent study, we demonstrated that the upregulation of $I_{SK}$ is associated with decreased CaM phosphorylation at threonine 80, likely due to the accompanying upregulation of dephosphorylating PP2Ac in cAF cardiomyocytes, in line with previous evidence of increased PP2Ac expression and activity in patients with cAF.[24,29,59,60] Consistent with the increased $I_{SK}$, apamin prevented the AF-promoting APD shortening in cAF cardiomyocytes, suggesting that the inhibition of upregulated $I_{SK}$ in AF patients could constitute a potential treatment option.[29]

Cardiomyocyte $Ca^{2+}$ handling is remodelled in AF patients, which should impact the $Ca^{2+}$-dependent gating of SK channels.[61,62] Computer models incorporating the changes in atrial cardiomyocyte $Ca^{2+}$ handling were employed to validate the effects of $I_{SK}$ inhibition with fixed intracellular $Ca^{2+}$ or during a control or AF-specific $Ca^{2+}$ transient, confirming the APD-prolonging effect under all conditions.[29] Subsequent *in silico* analyses confirmed that $I_{SK}$ activation shortens atrial APD and ERP, and slightly increases the propensity for alternans in both control and cAF conditions.[63] Moreover, $I_{SK}$ inhibition in 2D virtual tissue was able to stop sustained arrhythmia-maintaining rotors in the presence of simulated acetylcholine (ACh) concentrations up to 0.01 µM.[64] Conversely, increased $I_{SK}$ counteracts

### Table 1: Small-conductance $Ca^{2+}$-activated $K^+$ Channel Inhibitors Tested in Animal Models of AF

| Compound | Species | AF Cardioversion Rate (%) | | | Electrophysiological Parameters | | | | Side-effects | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Acute | Paroxysmal | Persistent | aERP | AFR | AFCL | CV | QT/QTc | QRS | Others |
| **SK channel blockers** | | | | | | | | | | | |
| Apamin | Rat[54] | | | | ↔ | | | | | | HR ↔ |
| UCL1684 | Guinea pig[53] | 100% | | | | | | | | | |
| | Rat[54] | | | | ↑ | | | | | | HR ↔ |
| ICAGEN | Guinea pig[53] | 100% | | | | | | | | | |
| **SK channel modulators** | | | | | | | | | | | |
| NS8593 | Rat[53] | 100% | | | ↑ | | | | | | |
| | Guinea pig[53] | 33–100% | | | | | | | | | |
| | Dog[40] | | | | ↑ | | | | | | |
| | Horse[56,89] | 100% | | 0% | ↑ | ↑ | | ↓ | ↔ | ↔ | Tremors |
| AP14145 | Guinea pig[90] | | | | ↑ | | | | ↔ | | |
| | Pig[55] | | 100% | 50–100% | ↑ | | | | | ↔ | Vomiting<br>LV ERP ↔ |
| | Goat[91] | | | 71% | ↑ | | ↑ | ↓ | ↔ | ↔ | |
| AP30663 | Pig[92] | | | | ↑ | | | | ↔ | | HR UCL |

*aERP = atrial effective refractory period; AFR = AF rate; AFCL = AF cycle length; CV = conduction velocity; HR = heart rate; LV ERP = left ventricular effective refractory period.*

DAD development by enhancing the repolarisation force that opposes the $Ca^{2+}$-dependent depolarisation.[63] Thus, inhibition of $I_{SK}$ in human atrial cardiomyocytes might have both anti- and proarrhythmic effects; reducing re-entry, while promoting triggered activity (*Figure 2*). Consistent with the latter, SK2 knockout in mice prolonged $APD_{90}$ in association with increased occurrence of early afterdepolarisations and a higher susceptibility to inducible AF *in vivo*, while no ventricular arrhythmias occur.[65] Thus, depending on the prevailing arrhythmia mechanism in individual patients, SK channel inhibition may exert anti- or proarrhythmic effects, which may offset each other in some patients. This hypothesis warrants further extensive proof and validation in both preclinical and clinical studies in different subpopulations of patients.

## Small-conductance $Ca^{2+}$-activated $K^+$ Channel Inhibitors Employed in Clinical Studies

The first clinically tested SK channel blocker AP30663 inhibits SK channels by reducing $Ca^{2+}$ sensitivity. Thus, in the presence of AP30663, larger increases in intracellular $Ca^{2+}$ concentration are required to activate SK channels, which reduces their function, thereby prolonging the aERP. *In vitro* patch-clamp studies have shown that AP30663 inhibits all three SK channel isoforms, but exhibits a slightly lower potency for KCa2.1, which is less abundantly expressed in human atrial tissues compared with KCa2.2 and KCa2.3. In heterologous expression systems, AP30663 had minimal effects on Kir3.1/Kir3.4 (underlying the ACh-activated inward-rectifier $K^+$ current; $I_{K,ACh}$), $K_V1.5$ (underlying the ultrarapid delayed-rectifier $K^+$ current), $K_V7.1$/KCNE1 (underlying the slow delayed-rectifier $K^+$ current), $K_V4.3$/KChiP2 (underlying the transient outward $K^+$ current), Kir2.1 (underlying the basal inward-rectifier $K^+$ current) channels and L-type $Ca^{2+}$ current. Although AP30663 inhibited the rapid delayed-rectifier $K^+$ current mediated by $K_V11.1$ channels, the inhibition was significantly weaker compared with its effect on SK channels. The compound also exhibited minor effects on peak $I_{Na}$, but significantly inhibited the late $I_{Na}$, which may help suppress ventricular arrhythmias.[66]

The first clinical trial investigated the safety, tolerability, pharmacokinetics and pharmacodynamics of AP30663. This randomised, single ascending dose, double-blind, placebo-controlled Phase I study enrolled 47 healthy male subjects aged 18–45 years, who received AP30663 IV in ascending doses across six cohorts. AP30663 was generally well tolerated. Of the 34 adverse events related to AP30663, most were mild, temporary and associated with infusion site reactions, such as vein hardening, redness and pain. These reactions were mitigated in later cohorts by modifying the administration procedure and formulation. There were no serious adverse events or discontinuations due to adverse events. Electrocardiographic monitoring revealed no significant effects on P-wave duration, PR interval, RR interval or QRS duration. However, a dose-dependent, transient prolongation of the QTcF interval was observed.[67]

In the subsequent human Phase II clinical trial, AP30663 demonstrated significant efficacy in converting recent-onset AF to sinus rhythm. The study involved 66 patients who received IV infusions of AP30663 at doses of 3 or 5 mg/kg, or a placebo. Cardioversion rates were 42% for the 3-mg/kg dose and 55% for the 5 mg/kg dose, compared with 0% for the placebo group. These conversion rates are comparable to those obtained with the approved antiarrhythmic drug, vernakalant (51.7% for patients with short-duration paroxysmal AF[68]), and the new inhaled formulation of flecainide (46.9% in patients without previous exposure to flecainide[69]). The mean time to cardioversion was 47 min for the 3-mg/kg dose and 41 min for the 5 mg/kg dose. A dose-dependent, transient increase in the QTcF interval was observed, with the highest dose causing an increase of 18.8 ± 4.3 ms. This effect was mediated through increases in the J-point to T-peak interval and T-peak to T-end interval subintervals, without affecting the QRS duration.[50] These results require confirmation in larger studies. Moreover, the clinical relevance of effectiveness for acute cardioversion of a single AF episode may be limited in view of the high rate of spontaneous cardioversion.[70] Nevertheless, these results represent an important milestone, providing support for the further development of these SK channel inhibitors towards formulations that can ultimately also be used for long-term rhythm control. Currently, a Phase I trial (NCT06066099) is being conducted with a second-generation oral lead compound, AP31969, indicated for sinus rhythm maintenance. The estimated date of completion is September 2024.

## Table 2: *Ex Vivo* Human Studies of Small-conductance $Ca^{2+}$-activated $K^+$ Channel Expression and Function in AF Patients

| Reference | Sample | mRNA | | | Protein | | | Function | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | KCNN1 | KCNN2 | KCNN3 | SK1 | SK2 | SK3 | EP | $[Ca^{2+}]i$ | Blocker/conc | Outcome |
| **RA** | | | | | | | | | | | |
| Heijman et al. 2023.[29] | Tissue | −17% | −17% | +41%* | −13% | −1% | +41% | $I_{SK}$ | 500 nmol/l<br>1,000 nmol/l | Apamin<br>(100 nmol/l) | 500 nmol/l: +397%* [−110 mV];<br>+544%* [+30 mV]<br>1,000 nmol/l: +705%* [−110 mV] |
| | CM | −38% | −37%* | +49% | +32% | −14% | +20% | APD | 500 nmol/l | Apamin<br>(100 nmol/l) | Ctl: +11%*<br>cAF: +39%* |
| Rahm et al. 2021.[30] | Tissue | −78%* | −55%* | −48%* | | | | | | | |
| Darkow et al. 2021.[33] | Tissue | −29% | −30% | −3% | | | | | | | |
| Yu et al. 2020.[93] | | | | | | | | $I_{SK}$ | 500 nmol/l | Apamin<br>(100 nmol/l) | 110 mV: +281%*; +60 mV: +119%* |
| Shamsaldeen et al. 2019.[94] | | | | | | | | $I_{SK}$ | Unknown | Apamin<br>(100 nmol/l) | +10 mV −14.3% membrane current by apamin |
| Fan et al. 2018.[31] | Tissue | −76%* | −63%* | −74%* | −29%* | −39%* | −45%* | $I_{SK}$ | 500 nmol/l | Apamin<br>(100 nmol/l) | 110 mV: +134%*<br>+60 mV: +120%* |
| Skibsbye et al. 2014.[32] | Tissue | −19%* | −53%* | −40%* | | | | $I_{SK}$ | 300 nmol/l | ICAGEN<br>(100 µmol/l) | Ctl: −28% versus TMC<br>cAF: −23% versus TMC |
| | | | | | | | | APD | 35 nmol/l | ICAGEN<br>(1 µmol/l) | Ctl: +13% versus TMC<br>cAF: −1% versus TMC |
| Wang et al. 2014.[95] | | | | | | | | $I_{SK}$ | 500 nmol/l<br>1,000 nmol/l | Apamin<br>(100 nmol/l) | −130 mV: +134%* [500 nmol/l]<br>−130 mV: +43%* [1,000 nmol/l] |
| Yu et al. 2012.[93] | CM | −84%* | −84%* | −3% | −71%* | −45%* | −24% | $I_{SK}$ | 900 nmol/l | Apamin<br>(100 nmol/l) | −120 mV: −53%*<br>+80 mV: −55%* |
| Li et al. 2011.[96] | | | | | | | | $I_{SK}$ | 500 nmol/l | Apamin<br>(100 nmol/l) | −130 mV: +167%* |
| Ling et al. 2013.[97] | Tissue | | | | | | −46%* | | | | |
| Gaborit et al. 2005.[98] | Tissue | | −9% | | | | | | | | |
| **LA** | | | | | | | | | | | |
| Heijman et al. 2023.[29] | Tissue | | | | +9% | +30% | +38% | | | | |
| Rahm et al. 2021.[30] | Tissue | +91% | −31% | −4% | | | | | | | |
| Yu et al. 2012.[93] | CM | −67%* | −55%* | −5% | −49%* | −63%* | −8% | $I_{SK}$ | 900 nmol/l | Apamin<br>(100 nmol/l) | −120 mV: −53%*; +80 mV: −59%* |
| | | | | | | | | APD | Perforated patch | Apamin<br>(100 nmol/l) | Ctl: +61%<br>cAF: +17% |

*Experiments were performed in ruptured patch-clamp unless otherwise indicated. *Significant differences versus Ctl. APD = action potential duration; cAF = long-standing persistent ('chronic') AF; conc = concentration; Ctl = sinus rhythm control; CM = cardiomyocyte; EP = Electrophysiology; $I_{SK}$ = (drug-sensitive) small-conductance calcium-activated potassium current; LA = left atrium; mRNA = messenger RNA; RA = right atrium; TMC = time-matched control.*

## Conclusion

The functional atrial predominance of SK channels, along with an extensive set of preclinical data on their antiarrhythmic potential, makes them a promising target for novel AF therapy. Nevertheless, potential proarrhythmic effects in some patient subpopulations and under specific disease conditions by 'dormant' ventricular SK channels, as well as extra-cardiac side-effects, for example, related to the expression of SK channels in the brain, should be considered.[71] Fortunately, the small molecules that are currently available do not seem to pass the blood–brain barrier, and do not exert serious neurological adverse effects in animal models or in the currently conducted clinical trials. Clearly, further large-scale clinical trials are necessary to ultimately demonstrate the efficacy and validate the safety of SK channel blockers.

In the present review, we focused primarily on the role of small-conductance $Ca^{2+}$-activated $K^+$ channels (SK1-3). It is noteworthy that intermediate-conductance $Ca^{2+}$-activated $K^+$ channels (also known as SK4) have also been implicated in AF-promoting atrial remodelling.[72,73] Inhibition of SK4 has shown antiarrhythmic effects in small and large animal models.[72–74] The structural similarity between SK1-3 and SK4 is low, and inhibitors of SK1-3, such as NS8593, do not inhibit SK4, while the SK4 inhibitor, BA6b9, does not affect SK1-3, positioning these channels as distinct targets.[74,75] However, although SK4 channels were identified in human left-atrial tissue with immunostaining, SK4 currents could not be detected with patch-clamp recordings in human right-atrial cardiomyocytes of AF patients.[29,74] Since no clinical studies have been performed with SK4 inhibitors in patients with AF, the putative anti-AF efficacy of selective SK4 inhibitors in humans remains uncertain.

Besides SK channels, the selective inhibition of other apparently atrial-predominant targets, including TASK-1 and Kir3.1/Kir3.4, constitutes an additional antiarrhythmic approach that needs further investigation and

validation.[76–79] Like SK channels, both TASK-1 and Kir3.1/Kir3.4 are preferentially expressed in the atria, and the corresponding currents ($I_{K2P}$ and $I_{K,ACh}$) are modulated in patients with cAF, promoting re-entry and making these channels interesting targets for rhythm control of persistent AF.[11,76,78] Upregulation of TASK-1 is due to increased TASK-1 protein levels in AF compared with sinus rhythm.[76] By contrast, protein levels of Kir3.1/Kir3.4 are reduced in AF, resulting in a smaller agonist-induced peak $I_{K,ACh}$. However, Kir3.1/Kir3.4 channels develop agonist-independent activity in AF, contributing to a constitutive component ($I_{K,AChc}$) that promotes ERP shortening.[78]

Both TASK-1 and Kir3.1/Kir3.4 are interesting targets from an approved antiarrhythmic drug development perspective. TASK-1 provides interesting options for drug repurposing, with previous work identifying the Food and Drug Administration-approved respiratory stimulus, doxapram, as a potent TASK-1 inhibitor with antiarrhythmic properties in human atrial cardiomyocytes, large animal models and computer simulations.[80] In contrast, drug development efforts have resulted in a highly potent bioengineered peptibody inhibiting Kir3.1/Kir3.4.[81] In aged mice, this peptibody reduced the AF inducibility by blocking $I_{K,AChc}$.[81]

Ultimately, it is unlikely that any single target will provide highly effective therapeutic effects in a condition as heterogeneous as AF. Different comorbidities modulate atrial remodelling in specific manners, producing distinct forms of AF-promoting atrial cardiomyopathy.[8,82–88] Given these different mechanisms driving AF, the identification of the predominant arrhythmia mechanisms operative in an individual patient and tailoring the antiarrhythmic therapies based on this information will likely be essential to provide safe and effective rhythm control therapy. In agreement, preclinical data suggest that the remodelling of SK channels may be distinct in patients with different comorbidities (e.g. the absence or presence of heart failure[29]), which could potentially affect the antiarrhythmic effectiveness of SK channel inhibitors. However, at present, there are no clinical data on the efficacy of SK channel inhibition in defined patient subpopulations. When single approved antiarrhythmic drugs are ineffective, combinations of drugs may need to be applied to inhibit multiple targets, keeping the dose of the individual compounds low enough to prevent cardiac and extra-cardiac side-effects. However, this can be done only if intrinsically safe and effective compounds are clinically available. The compounds currently being developed against SK channels may represent a first step in this direction. ❑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