ESC European Society of Cardiology

Europace (2021) **23**, 1847–1859
doi:10.1093/europace/euab125

BASIC SCIENCE

Effective termination of atrial fibrillation by SK channel inhibition is associated with a sudden organization of fibrillatory conduction

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Received 4 November 2020; editorial decision 18 April 2021; accepted after revision 22 April 2021; online publish-ahead-of-print 3 June 2021

Aims Pharmacological termination of atrial fibrillation (AF) remains a challenge due to limited efficacy and potential ventricular proarrhythmic effects of antiarrhythmic drugs. SK channels are proposed as atrial-specific targets in the treatment of AF. Here, we investigated the effects of the new SK channel inhibitor AP14145.

Methods and results Eight goats were implanted with pericardial electrodes for induction of AF (30 days). In an open-chest study, the atrial conduction velocity (CV) and effective refractory period (ERP) were measured during pacing. High-density mapping of both atrial free-walls was performed during AF and conduction properties were assessed. All measurements were performed at baseline and during AP14145 infusion [10 mg/kg/h ($n = 1$) or 20 mg/kg/h ($n = 6$)]. At an infusion rate of 20 mg/kg/h, AF terminated in five of six goats. AP14145 profoundly increased ERP and reduced CV during pacing. AP14145 increased spatiotemporal instability of conduction at short pacing cycle lengths. Atrial fibrillation cycle length and pathlength (AF cycle length × CV) underwent a strong dose-dependent prolongation. Conduction velocity during AF remained unchanged and conduction patterns remained complex until the last seconds before AF termination, during which a sudden and profound organization of fibrillatory conduction occurred.

Conclusion AP14145 provided a successful therapy for termination of persistent AF in goats. During AF, AP14145 caused an ERP and AF cycle length prolongation. AP14145 slowed CV during fast pacing but did not lead to a further decrease during AF. Termination of AF was preceded by an abrupt organization of AF with a decline in the number of fibrillation waves.

Keywords Atrial fibrillation • SK channel inhibition • AP14145 • Atrial mapping • Cardioversion

Introduction

Currently, available antiarrhythmic drugs (AADs) for the treatment atrial fibrillation (AF) lose their efficacy with AF progression and mainly convert recent-onset AF of <48 h.[1] The lack of atrial selectivity limits the use of more aggressive antiarrhythmic therapy, as many may AADs lead to ventricular proarrhythmia, particularly in patients with structural heart disease. A more effective and safe AF treatment might be achieved by targeting ion channels that are predominantly expressed in the atrium. The small conductance calcium-

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What's new?

- SK channel inhibition by AP14145 terminates 30 days of persistent atrial fibrillation (AF) in the goat.
- SK channel inhibition by AP14145 led to a dose-dependent AF cycle length prolongation.
- Just before cardioversion of AF, a sudden organization of fibrillatory conduction occurred.
- SK channel inhibition by AP14145 resulted in a slowing of the conduction velocity suggesting Class I antiarrhythmic properties.

activated potassium (SK or $K_{Ca}2$) channel has been proposed as such an atrial-selective target. Three subtypes of this ion channel have been identified, of which SK2 and SK3 are abundant in the human atria. SK channel opening is triggered by an increase in the intracellular Ca^{2+} concentration and therefore affects the action potential plateau and terminal repolarization in a Ca^{2+} dependent manner.[2] Inhibition of SK channels leads to prolongation of the action potential (AP) and atrial effective refractory period (ERP) in human muscle bundles[2] and shortens AF paroxysms.[3,4] In the canine left atrium, SK channel expression levels and SK current densities were enhanced after 7 days of AF-induced electrical remodelling.[3] Indeed, it has been shown that selective SK-channel inhibition could prolong AP duration and ERP in human atrial muscle bundles from chronic AF patients.[2]

In addition, it has been reported that SK channel inhibition may lead to a small depolarization of the resting membrane potential which was associated with a reduced AP upstroke velocity (dV/dt) and amplitude in human cardiomyocytes.[2,5] Moreover, the combination of a selective SK channel inhibitor (ICA) and sodium channel blockers synergistically prolonged ERP.[6] This reduction of excitability after SK channel inhibition, functionally a class I effect, could potentially act as an additional antiarrhythmic mechanism.

Recently, a new compound, AP14145, was developed that showed selective SK channel inhibiting properties in HEK293 cell lines.[4,7] AP14145 also exhibited potential for AF treatment, as it was able to terminate sustained AF and protect against its re-induction in a model where the clinically available drug vernakalant failed to terminate AF.[4] Here, we have studied the antiarrhythmic mechanisms of SK-channel inhibition by AP14145 during AF and regular rhythms in a goat model of persistent AF. We performed bi-atrial high-density mapping to obtain insight into the effects on conduction and termination of AF, including the potential Class I effect of AP14145 during AF.

Methods

Animal model and surgical procedures

All animal experiments were conducted according to both the Dutch (license number AVD107002016782) and the European regulations on animal experimentation. Eight goats were anaesthetized (induction: sodium thiopental, 10 mg/kg and maintenance of sufentanyl, 6 μg/kg/h and propofol, 10 mg/kg/h) and implanted with pericardial electrodes above the left atrium (LA) and a stimulator for AF induction (Medtronic Itrel II/III®, Medtronic plc., Minneapolis, MN, USA). After recovery from surgery, AF was maintained by 50 Hz pacing at 10 V, on: 1 s/off: 4 s (figure 1A).

A terminal experiment was performed after 30 days of AF maintenance under general anaesthesia (induction: sodium thiopental 10 mg/kg, maintenance: sufentanyl 6 μg/kg/h, propofol 10 mg/kg/h, and rocuronium 0.3 mg/kg/h). Standard Einthoven electrocardiogram (ECG) leads were recorded. Haemodynamic parameters were measured by a pressure-tip catheter (Sentron Europe BV, Roden, The Netherlands) in the left ventricle. A left-sided thoracotomy exposed the heart for electrophysiological measurements. Two mapping electrode arrays, each consisting of 249 unipolar electrodes (2.4-mm electrode distance), were placed on right atrial (RA) and left atrial free wall. Mapping-electrodes were kept in a fixed position during the whole experiment. Signals were recorded at a sampling frequency of 1039 Hz. Two defibrillation catheters were placed, one in the RA cavity and one in the coronary sinus, to allow internal direct current (DC) defibrillation. Goats were anticoagulated with 2500 IU/h heparin. One hour of stabilization was allowed before the start of the experimental protocol.

Experimental protocol

Stage 1, AF stability was assessed over a 30-min period. Unipolar atrial electrograms were recorded continuously in 60 s periods during the whole period. Next, AF was terminated by a DC shock (≤20 J, PhysioControl Lifepak 9B, Medtronic, Minneapolis, MN, USA) and ERP and conduction velocities (CVs) were assessed during pacing. The CV was measured by an S1–S1 protocol, applying 2X threshold, 40 cycles at a range of cycle lengths (450, 400, 350, 300, 275, 250, and 225 ms). Effective refractory period was measured using an S1–S2 protocol (8:1 ratio 4X threshold) at 250 and 400 ms cycle length. The S2 was incremented by 2 ms, until capture was observed. The last uncaptured beat was considered as the ERP. Atrial fibrillation was electrically terminated if AF was induced at one of the intermediate measurements to allow full accomplishment of the experimental protocol. After completion of the S1–S1 and S1–S2 measurements, AF was re-induced and sustained for 30 min.

Stage 2 (*Figure 1C*), the stability of AF was challenged by the SK channel blocker AP14145 (Acesion Pharma, Copenhagen, Denmark). One goat was excluded from AP14145 infusion due to lack of sustained AF and in one goat, AP14145 was infused at a rate of 10 mg/kg/h for 45 min and a cumulative dose of 7.5 mg/kg. In the remaining six goats with sustained AF, AP14145 was infused at a rate of 20 mg/kg/h for 45 min. Atrial fibrillation stability was monitored until a cumulative dose of 15 mg/kg was reached. Unipolar atrial electrograms were recorded during the whole period of drug infusion. Atrial fibrillation was re-induced by automated burst pacing (50 Hz, 1 s, 10 mA) whenever sinus rhythm occurred during the 45-min period of drug infusion.

Stage 3, at 45 min, the AF re-induction protocol was switched off. AP14145 infusion was continued, at a rate of 20 mg/kg/h, for the next 15 min. If AF did not spontaneously terminate at this point, it was electrically terminated by a DC shock. The S1–S1 and S1–S2 measurements of CV and ERP were performed as described above within the 15-min period. A graphical representation of the outline of the experimental design and measurements is depicted in *Figure 1B–D*.

Data analysis

The limb leads and left ventricular pressure were stored and analysed in IdeeQ software (Instrument Development Engineering and Evaluation, Maastricht University, Maastricht, The Netherlands). Recordings of unipolar atrial electrograms of 60 s acquired at the time points of 0, 5, 10, 15, 20, 25, and 30 min after the start of the drug infusion were used for analysis. Atrial electrograms were analysed offline using a custom-made algorithm (MATLAB 8.1; The Math-works, Inc., Natick, MA, USA) based on a probabilistic approach.[8] This algorithm identifies local deflections/

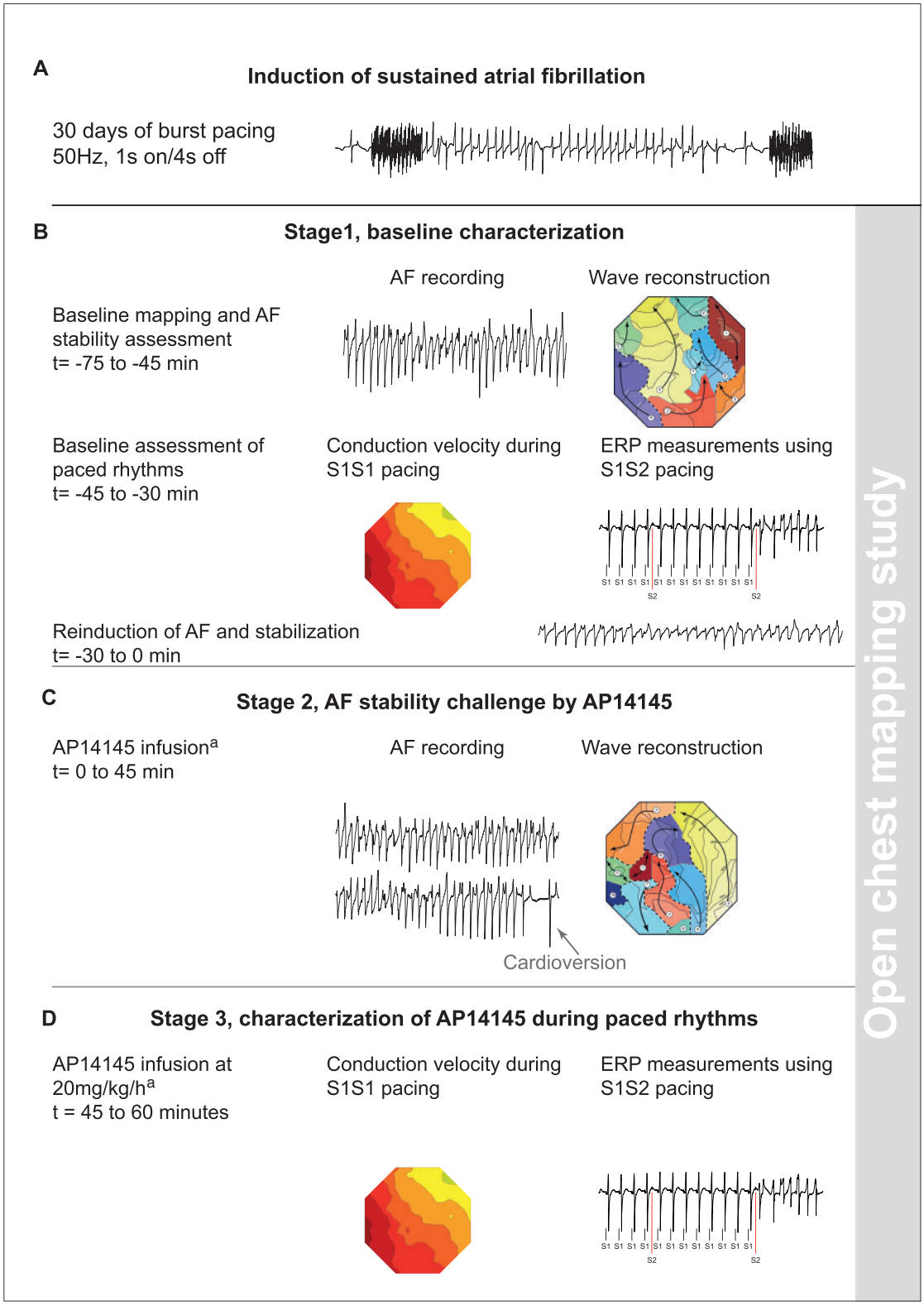

Figure 1 Graphical outline of the experimental design. (*A*) AF was induced and maintained by burst pacing for 30 days. At this point seven out of eight goats were in sustained AF. The goat with unstable AF was excluded from the rest of the study. (*B*) In an open-chest study, a baseline electrophysiological characterization was performed. Unipolar atrial electrograms were recorded during atrial pacing, to assess ERP and CV, and AF. (*C*) AF stability was challenged by the SK channel blocker AP14145. Unipolar atrial electrograms were recorded continuously during the intravenous administration of the drug. AF terminations were documented and if necessary, AF was re-induced. (*D*) Measurements of ERP and CV after 45 min of AP14145 administration. All ERP and CV measurements were performed during 20 mg/kg/h AP14145 infusion. [a]One goat was infused at a rate of 10 mg/kg/h and six goats at a rate of 20 mg/kg. AF, atrial fibrillation; CV, conduction velocity; ERP, effective refractory period.

activation times in the unipolar electrograms. Atrial fibrillation cycle length, CV during AF, and fibrillation waves were computed based on these activation times.[8] Waves were defined by clusters of activation times that were connected in space and time by an apparent CV of ≥20 cm/s. A fibrillation wave was classified as breakthrough (BT) if the starting point occurred within the mapping array. In certain instances, multiple starting points are located in close proximity, potentially originating from a rugged wavefront or transmural conduction. To avoid overestimation of the number of wavefronts, territories of starting points with >90% overlap were merged to a single wave and wave type was assigned to the earliest activation. Within waves, the CV was calculated for each activation by fitting a plane through the spatially neighbouring activations. Waves of <3 electrodes were excluded from the analysis. The fifth percentile (p5) of the AF cycle length distribution was used as a surrogate measure for ERP during AF. The wavelength during pacing was defined as the product of CV and ERP. The pathlength during AF was defined as the product of CV and AF cycle length.

Statistics

Statistical analysis was performed using IBM SPSS statistics V.25.0. Single repeated parameters were tested with a paired sample *t*-test. The dose- or time-dependent effects on AF properties and pacing cycle length dependent effects on CV were tested with a linear mixed-effects model using a diagonal covariance structure with atrium, cumulative dose, and cycle length considered as fixed variables and animal taken as a random variable. Results are reported as mean ± standard deviation (SD). A *P*-value <0.05 was considered statistically significant.

Results

The effects of AP14145 on atrial fibrillation stability and global electrocardiogram parameters

One goat did not develop stable AF after 30 days of burst pacing and was excluded for the rest of the study. All sustained AF goats remained in stable AF in the 30-min window after all surgical manipulations and prior to drug infusion. Atrial fibrillation terminated in five of seven goats at a range of 3.7–9.7 mg/kg of AP14145, as shown in *Figure 2A*. Of the two goats that did not terminate AF, one had an infusion rate of 10/mg/kg/h and the other a rate of 20 mg/kg/h. In four out of five goats that cardioverted, AF became very unstable as demonstrated by the multiple AF inductions that were required to maintain AF (*Figure 2A*). RR intervals did not change during sinus rhythm (*Figure 2A*) or AF (data not shown). Electrocardiogram parameters were assessed during regular pacing to avoid necessity of corrections for rate. No changes in the QRS duration or in the QT time were observed in the applied range of pacing cycle lengths (450–250 ms) (*Figure 2C* or *D*). Small trends towards increased left ventricular systolic pressure or in dP/dt_{max} were observed after the administration of AP14145. No drug-induced ventricular arrhythmias were observed up to the maximal dose of 20 mg/kg AP14145.

Effective refractory period and conduction velocity during regular pacing

Atrial ERP was measured at a cycle length close to the fibrillation cycle length (250 ms) and at a cycle length closer to physiological sinus cycle length (400 ms). *Figure 3* illustrates a profound ERP increase at both cycle lengths, 72 ± 27 ms at 250 ms, $P = 0.02$ and 83 ± 15 ms at 400 ms, $P < 0.001$. No rate-dependent effects on ERP were observed. At baseline, the CV varied from 77 to 73 cm/s at a cycle length range from 225 to 450 ms. AP14145 significantly reduced CV at most investigated cycle lengths ($P < 0.001$). Moreover, a significant positive rate-dependent effect of AP14145 was present ($P < 0.001$), leading to about 20% slowing of the CV at the shortest cycle lengths. The wavelength at the cycle length of 400 ms increased by +5.7 ± 1 cm, ($P < 0.001$). However, at a cycle length of 250 ms, no change in wavelength was observed.

The effect of AP14145 on atrial fibrillation properties

Figure 4 presents the dose-dependent changes by AP14145 on AF. Atrial fibrillation cycle length and the 5th percentile of the AF cycle length presented a robust dose-dependent increase which persisted throughout all cumulative concentrations. At baseline (0 mg AP14145), a right-to-left gradient of AF cycle length was present ($n = 6$, <0.001). The gradient decreased somewhat at higher dosages of the drug but remained significantly different. Goats started to cardiovert (grey area in the figure) at moderate increases of AF cycle length. The goat that failed to cardiovert to sinus rhythm by AP14145 had an equivalent magnitude and steepness of AF cycle length increase in the LA compared with the goats that cardioverted but the steepness of AF cycle length increase in RA levelled off at a cumulative dose of 5 mg/kg. The left atrial CV during baseline AF was lower compared to the CV measured during fast pacing, 58 ± 4.6 vs. 73 ± 5.6 cm/s ($P < 0.01$), respectively. Conduction velocity did not change despite the robust increase in AF cycle length and cumulative dose of AP14145. In line with the stable CV and increasing AF cycle length, a consistent and significant increase in pathlength occurred in both atria with a trend towards stronger increases in the RA.

Next, we evaluated the type of conduction patterns (peripheral vs. BT) and complexity of conduction in both atria (measured as the total number of waves). Surprisingly, the level of complexity showed only a trend towards fewer waves in the LA and no change was present in the RA. Increased number of BT events or focal activity may be a source for the persistent high level of complexity. Breakthrough waves were therefore investigated for incidence and spatial distribution. Similar to the total number of waves, only a trend towards fewer BTs was found in the LA, while no change was observed in the RA. Breakthrough waves occurred at all electrodes within the mapping area with certain sites of a higher BT activity. During AP14145 infusion, no sites of increased BT incidence were observed and the general spatial distribution of BTs remained stable. In the Supplementary material online, a more detailed report on the distribution of BTs is presented.

A sudden transition in fibrillation pattern just prior to cardioversion

Except for an AF cycle length and pathlength increase, no considerable changes in conduction properties were observed during the infusion of AP14145. We hypothesized that shortly before cardioversion, marked changes in fibrillation pattern occurred. We therefore analysed the final 10 s prior to the (first) cardioversion. *Figure 5* depicts four snapshots of conduction patterns during AF in a

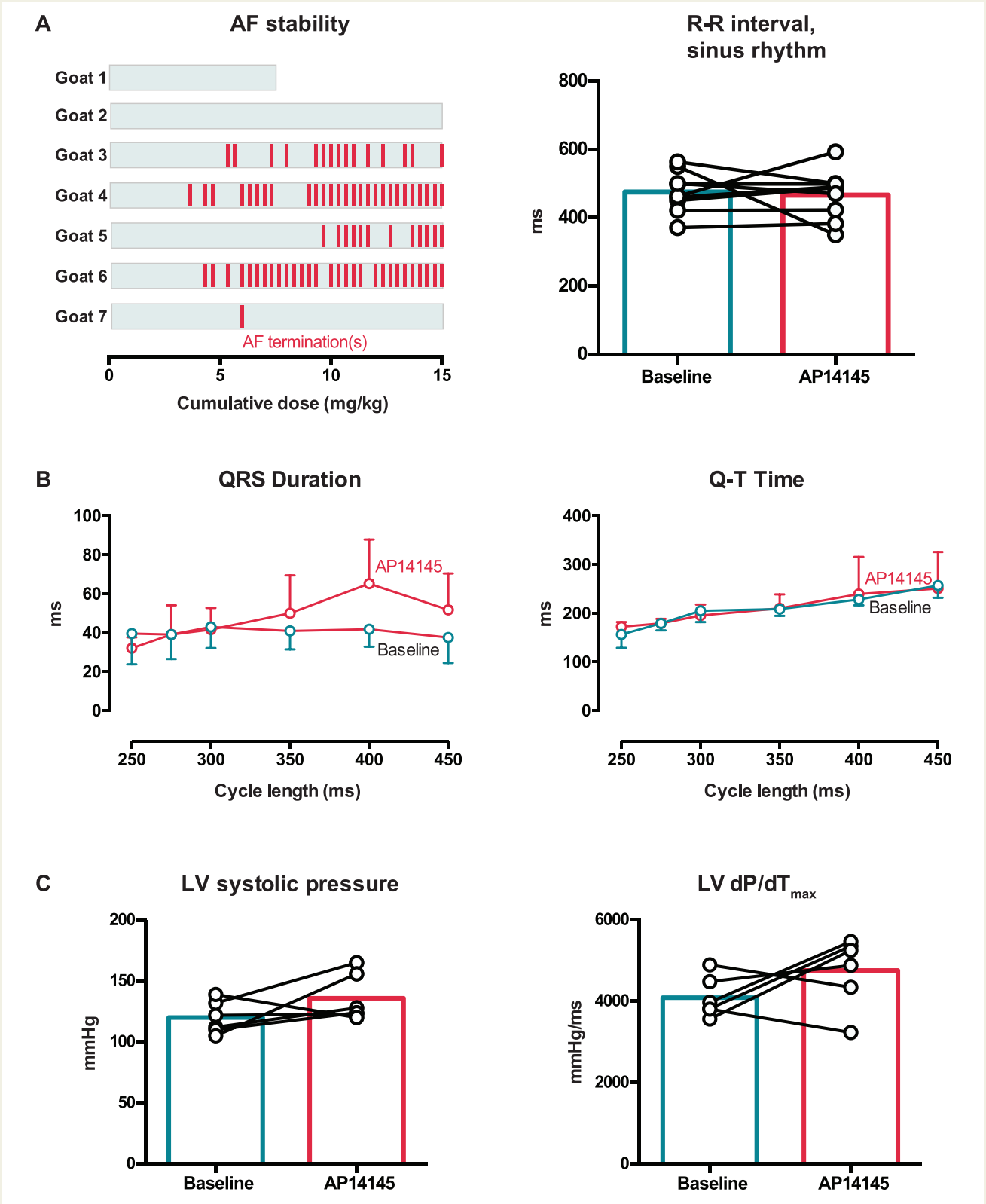

Figure 2 The effects of AP14145 infusion on AF stability and global ECG parameters. (*A*) The left panel depicts, per goat, the number of AF inductions (red lines) that were required to maintain AF during continuous AP14145 infusion. AF became highly unstable in four out of five the goats that cardioverted. The right panel illustrates the lack of effect of AP14145 (red bar) on the sinus cycle length. (*B*) The QRS durations and QT times were determined during pacing. AP14145 (red lines) did not significantly affect the QRS duration and QT time. Mean ± SD, $n = 6$. (*C*) At a cycle length of 400 ms, AP14145 led to a trend towards higher systolic pressure and rate of pressure rise. Mean ± SD, $n = 6$. AF, atrial fibrillation; ECG, electrocardiogram.

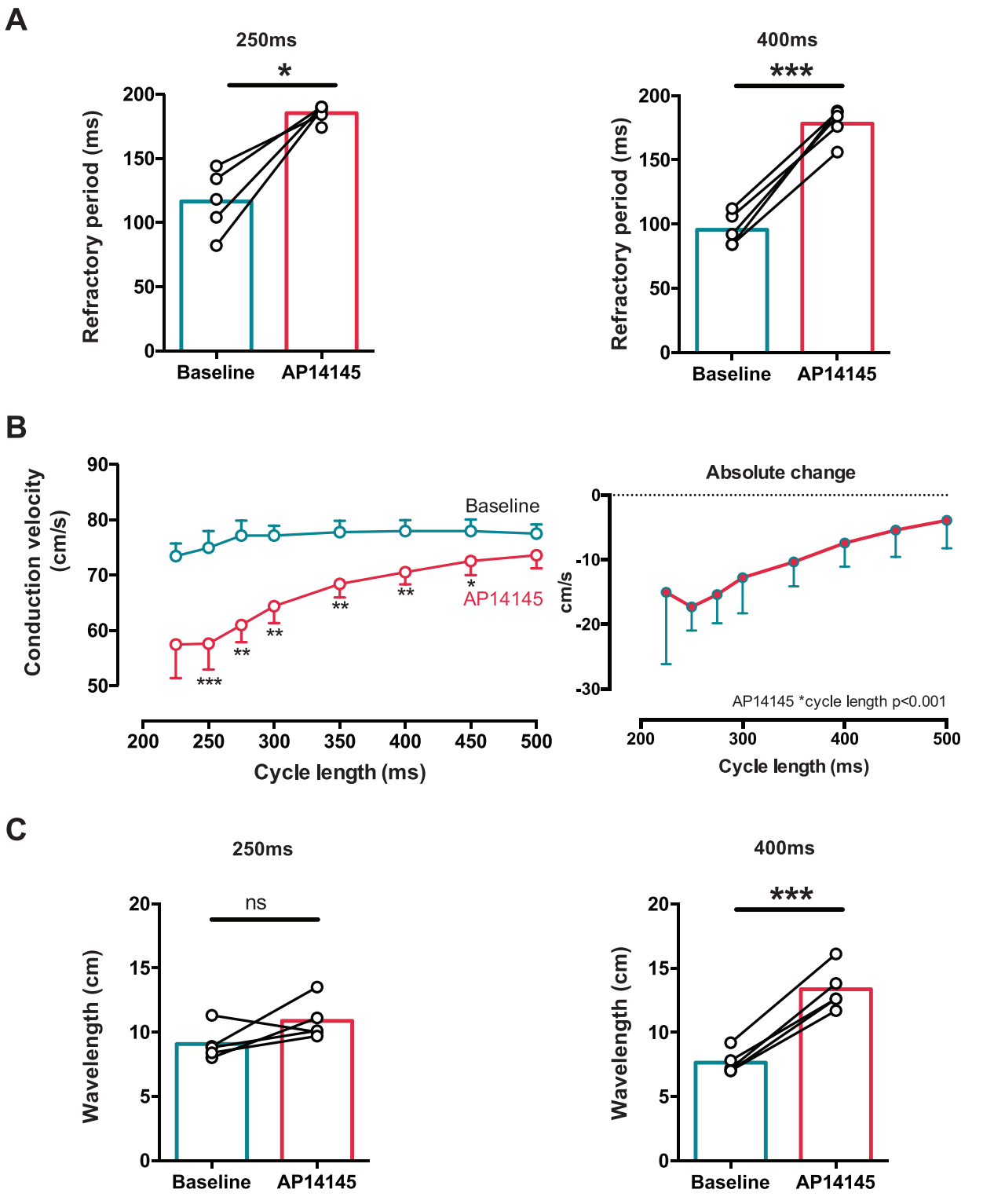

Figure 3 Electrophysiological effects of AP14145 during pacing. (*A*) Refractory period at a cycle length close to the AF cycle length (250 ms) and at a cycle length close to the sinus cycle length (400 ms). AP14145 significantly increased effective refractory periods at both cycle lengths. (*B*) Left atrial CV at a range of cycle lengths. AP14145 significantly reduced CV at most cycle lengths. From the absolute individual CV differences, it can be appreciated that AP14145 had a pronounced rate-dependent effect on CV. *B*. The wavelength, calculated as a product of ERP and CV. At a cycle length of 400 ms, a significant increase was observed. However, no significant changes were observed at 250 ms. AP14145 vs. baseline $*P < 0.05$, $***P < 0.001$, $n = 6$. AF, atrial fibrillation; CV, conduction velocity; ERP, effective refractory period.

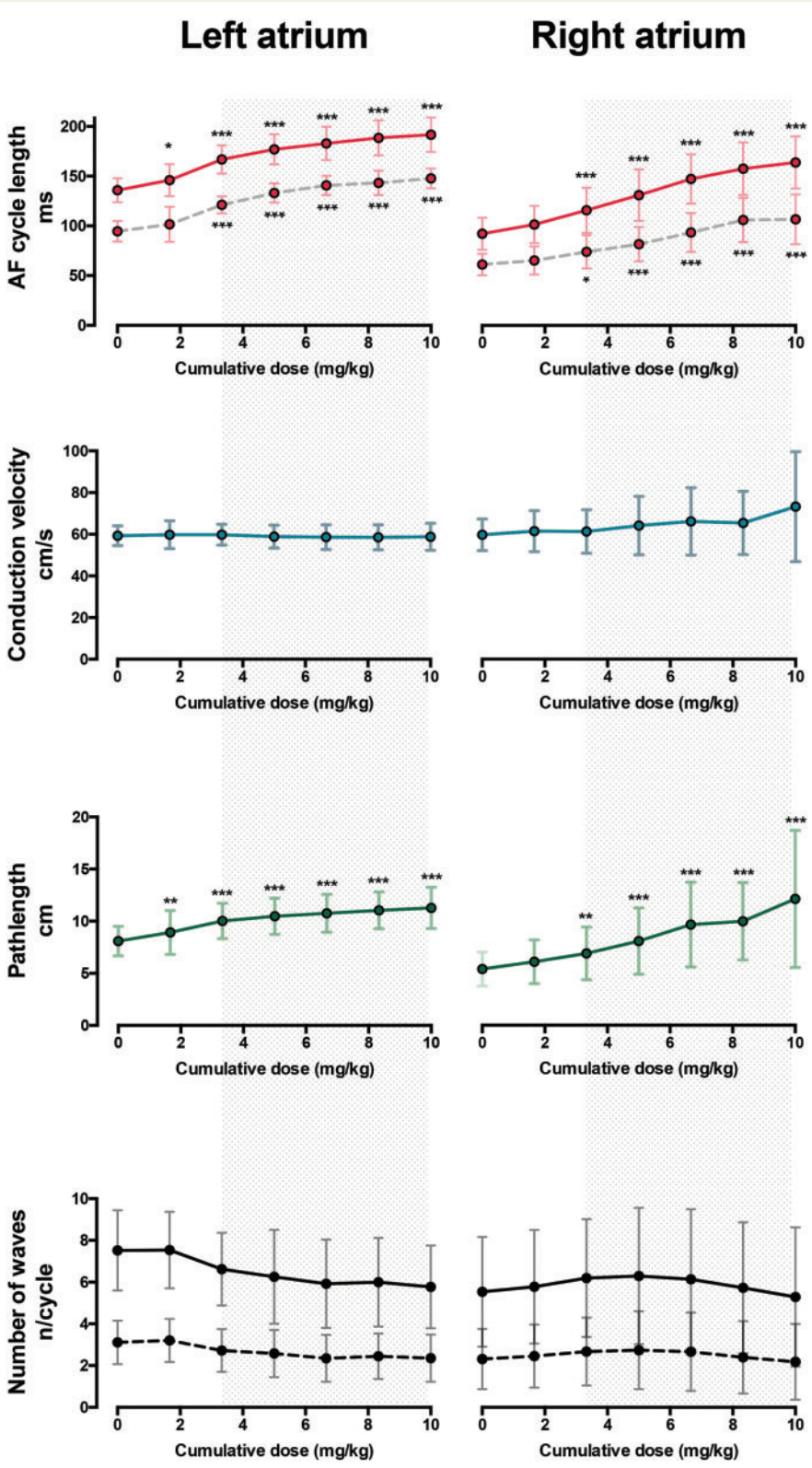

Figure 4 Electrophysiological parameters during AF at different cumulative doses of AP14145. AF cycle length (solid line) and the 5th percentile (dashed line) of the AF cycle length increased in both atria in a dose-dependent manner. In contrast to pacing, no changes in CV during AF were observed in either atrium. On the other hand, a dose-dependent increase in pathlength (CV × AF cycle length) was observed both atria. The total number of waves (solid line) and breakthrough waves (dashed lines) exhibited a trend towards a reduction in the left atrium but not in the right. AP14145 vs. baseline $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $n = 7$. AF, atrial fibrillation; CV, conduction velocity.

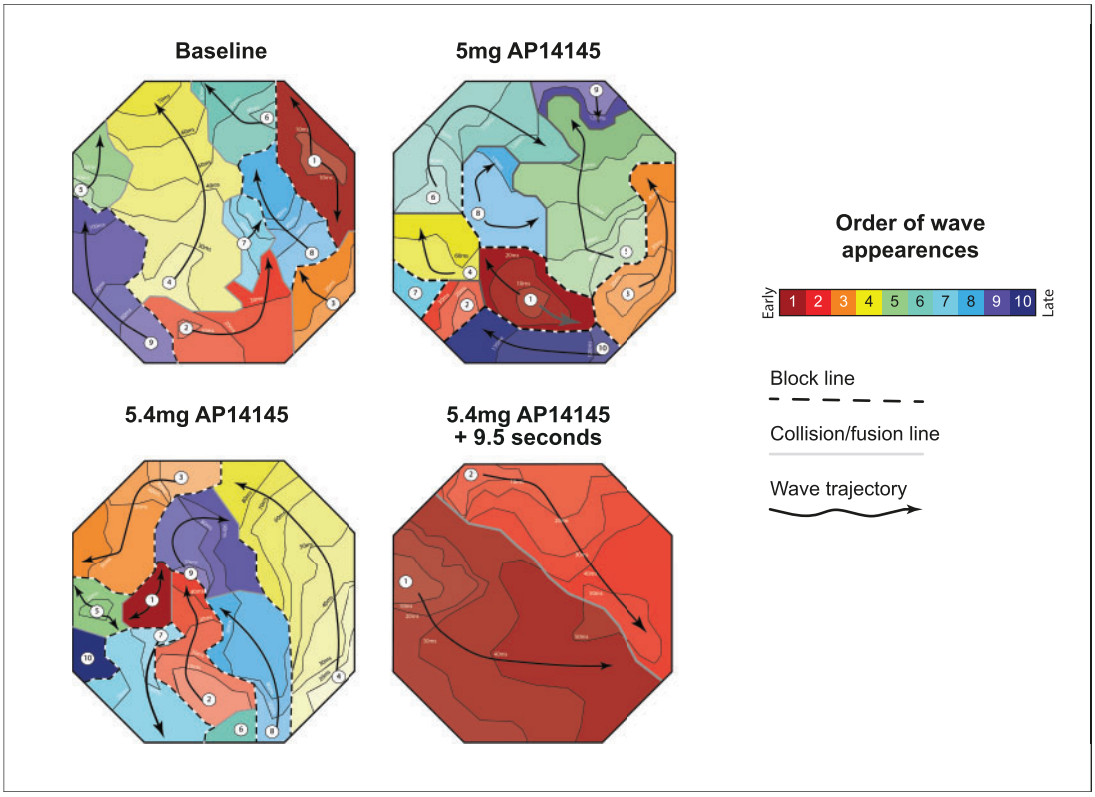

Figure 5 Snapshots of conduction patterns of fibrillation waves in the left atrium in a time frame of one AF cycle. *Upper left map, baseline.* During baseline recording, there were on average 9.4 waves per AF cycle present in this goat. Wavefronts originating at the edge and breakthrough waves had a ratio of 1:1. Wavefronts often fused while at other sites lines of conduction block appeared, for example, waves 1–4. *Upper right map, 5 mg/kg.* After 15 min of infusion, i.e. 5 mg/kg, no significant changes in pattern complexity (8.4 waves/cycle) were observed. However, at this moment, the AF cycle length had increased from 139 ms to 183 ms. *Lower left map, 5.4 mg/kg.* One minute later, during the final 10 s before AF termination, 9.4 waves/cycle were present and the AF cycle length was around 180 ms. *Lower right map, the final fibrillation cycle.* During the final cycle before termination, a rapid decline in number of fibrillation waves occurred and the AF cycle length increased further to 198 ms. AF, atrial fibrillation.

goat that terminated at a cumulative dose of ~5.4 mg. The complexity of conduction remained high (with 9–10 waves/cycle) till 10 s before AF termination. However, during the final cycles of AF, the patterns rapidly re-organized, as illustrated by the two almost synchronized colliding waves in the lower right corner.

Figure 6 depicts the temporal changes in AF parameters (AF cycle length, CV, pathlength, and number of waves) prior to cardioversion. To capture the dynamic behaviour, we analysed these parameters in windows of 500 ms. Note that the cumulative dose differs between animals, as AF terminations occurred at different time points. At 10 s before AF termination, only the AF cycle length and pathlength were significantly increased, while CV and waves were not significantly changed (Supplementary material online, *Table S1*). Few changes were observed up to 2 s before AF termination but then rapid changes occurred, exhibited by a small increase in CV and a continuous further increase in AF cycle length and pathlength. The AF cycle length in the RA, which was initially shorter, underwent the steepest prolongation, exceeding the AF cycle length observed simultaneously in the LA. This coincided with a reduction in the number of waves per cycle and AF termination. The mechanistic interpretation of these changes is dependent on the existence of such events during stable (baseline) AF. Baseline AF was analysed to identify large and abrupt fluctuations as seen just before AF termination. However, fluctuations of AF parameters to the level of AF termination were not observed at baseline, see the Supplementary material online and *Figure S2* for more details.

Wavefront stability during fast pacing

As described above, complex fibrillation patterns persisted until just prior to AF termination. To explore wavefront stability as a source of complexity, we investigated wavefront propagation at short pacing cycle lengths during pacing (225, 250, and 275 ms). Wave patterns were visually inspected and classified as uniformly propagating wavefronts, wavefronts with conduction block or failure to maintain 1:1 capture. A uniformly propagating wavefront was observed at all cycle lengths at baseline (Supplementary material online, *Table S2*). In the presence of AP14145, the propagating wavefront at a pacing cycle length of 275 ms remained uniform, *Figure 7A*. However, a further reduction of the pacing cycle length led to more frequent conduction disorders and pacing failure. *Figure 7B* illustrates a case of conduction block at a pacing cycle length of 225 ms. Initially, only one entry point was present but the mapping area became activated from three sides

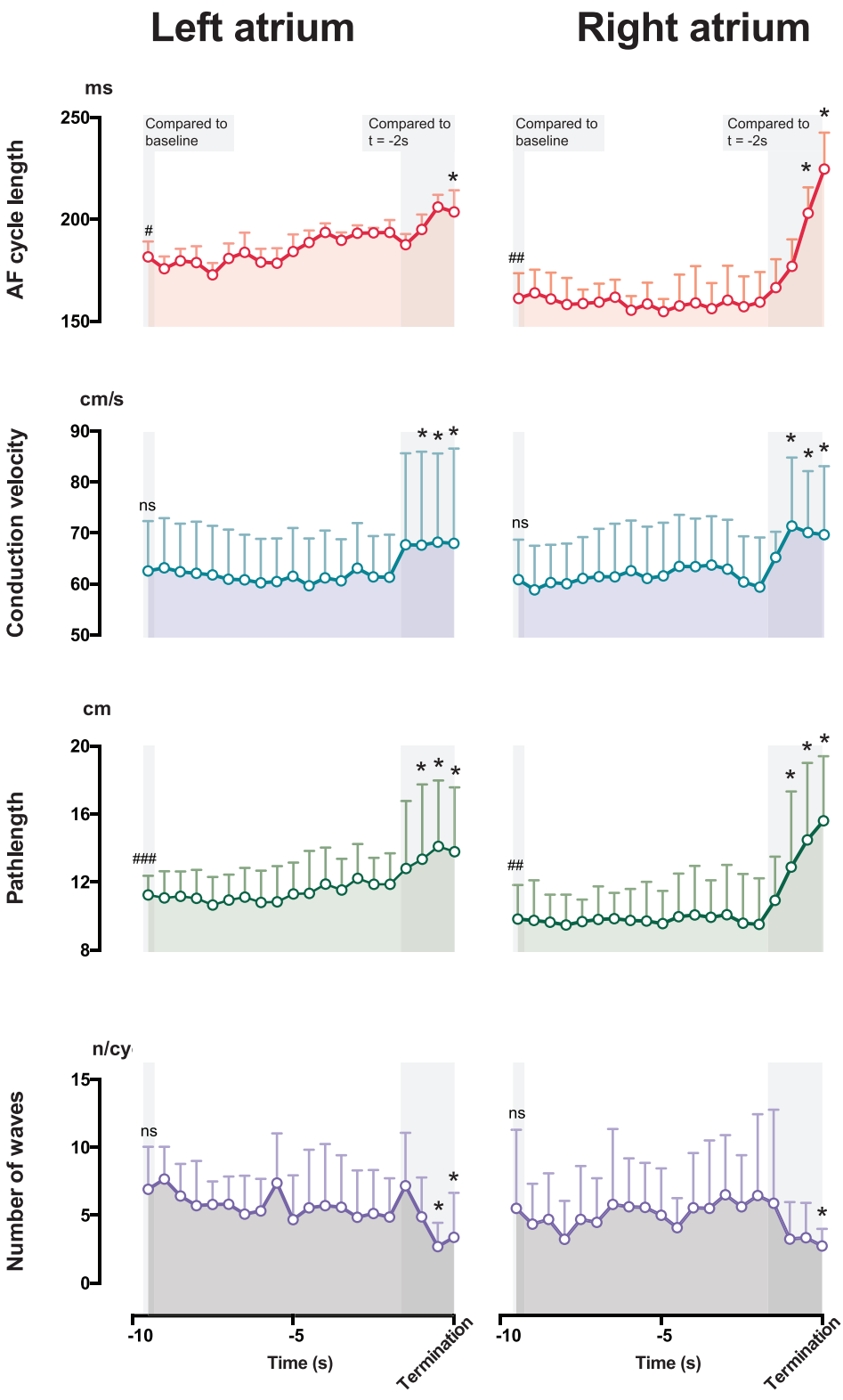

Figure 6 The temporal changes of AF properties within the last 10 s before AF termination. The data depict the first AF termination induced by AP14145 infusion. The right and left atrial data were recorded simultaneously. From top to bottom AF cycle length, CV, pathlength, and the number of waves per AF cycle. The data were analysed in non-overlapping windows of 500 ms, which generally encompassed 2–3 AF cycles. In the first 8 seconds, a slight trend towards a longer AF cycle and pathlength could be observed in the left atrium but not in the right atrium. In the final 2 s before termination, a small increase in CV in both atria was observed which was followed by a rapid increase of AF cycle length and pathlength. Somewhat later a decrease of the number of waves occurred. No significant differences between 10 and 2 seconds before termination were present but appeared 2 seconds before termination. Data are presented as mean ± SD. vs. baseline recording; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ $^{\#\#\#}P < 0.001$. vs. time point −2 s; $^{*}P < 0.05$ $n = 5$. AF, atrial fibrillation; CV, conduction velocity.

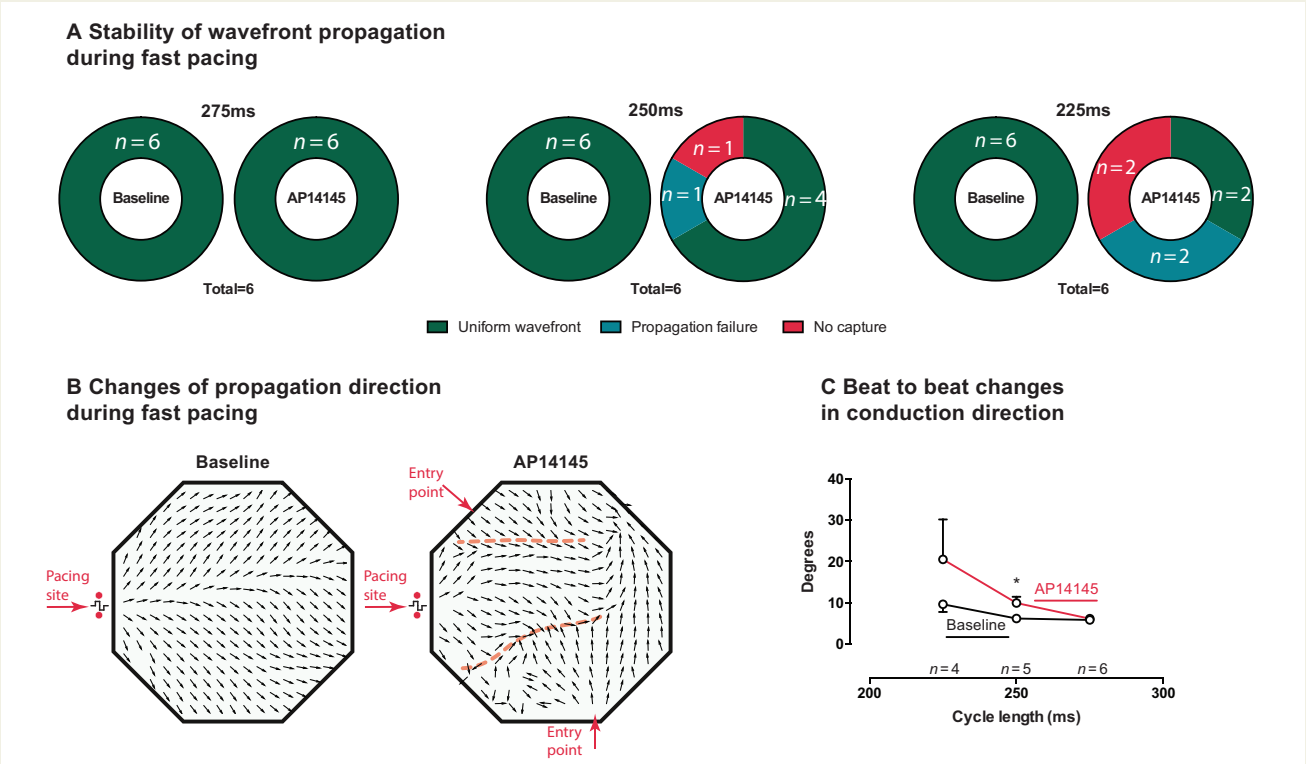

Figure 7 (*A*) Donut charts illustrate the distribution of the type of wavefronts in response to fast S1S1 pacing. Dark green slices represent homogenously and uniform conducting wavefronts, light green presents concave wavefronts or wavefronts with conduction block and red presents 1:1 conduction failure. (*B*) Two vector fields in the same goat at a pacing cycle length of 225 ms. Bipolar pacing was performed on the left side of the array, pacing electrodes are indicated by two red dots. At baseline, a homogenous wavefront can be appreciated. AP14145 led, in this case, to conduction block (red dashed line) at different sites and wavefront entry at different sites (red arrows), suggesting that the wavefront conducted along a tortuous path to enter the mapping array, despite the close proximity to the pacing site. (*C*) Temporal instability of conduction direction at three fast pacing rates. Variability of conduction direction increased when pacing cycle length was reduced and in the presence of AP14145. * vs. baseline, $P < 0.05$ $n = 6$. AF, atrial fibrillation; CV, conduction velocity.

with multiple block lines when AP14145 was present. To quantify more subtle changes in conduction, we investigated the spatial heterogeneity of conduction vectors by computing the circular variance of all conduction vectors at pacing cycle lengths of 225, 250, and 275 ms. Pacing at shorter cycle lengths led to more disperse conduction vectors (i.e. larger variance), both at baseline and in the presence of AP14145. Mixed model testing indicated an overall higher circular variance for AP14145 ($P = 0.011$) but no individual cycle lengths showed significant difference. Similarly, the temporal variability of conduction direction (*Figure* 7*C*), measured as beat-to-beat angle differences, increased with shorter cycle lengths and was significantly increased by AP14145 ($P = 0.026$).

Discussion

In this study, we investigated electrophysiological effects of the novel SK channel inhibitor AP14145. When AP14145 was infused at a rate of 20 mg/kg/h, a high success rate (five of six) of AF termination was achieved. This infusion regime did not lead to changes in ventricular electrophysiology or left ventricular pump function. During pacing, a considerable slowing of atrial conduction was induced by AP14145, coinciding with a spatiotemporal instability of wavefront propagation. During AF, AP14145 induced a profound AF cycle increase while conduction velocity was not affected. Surprisingly, no gradual reduction of AF complexity towards AF termination was observed. Instead, we report a sudden transition towards low complexity in the beats just prior to AF termination. From this study, it remains unclear what triggers the sudden change in complexity, and further research is necessary to unravel this mechanism. Our data suggest that ERP and subsequent AF cycle length prolongation are the main antiarrhythmic mechanisms of AP14145.

Safety and selectivity of the SK channel inhibitor AP14145

Expression of SK channels has been reported for a variety of organs, including the heart and central nervous system (CNS).[7] An SK channel inhibitor that does not pass the blood–brain barrier could potentially avoid CNS-related side effect. In the present work, we studied the newly developed SK channel inhibitor AP14145, which has been presented as a possible atrial-selective potassium channel inhibitor that exhibits an equipotent profile on different subtypes of SK

channels in HEK cells.[7] The administration of AP14145 did not lead to acute CNS effects in mice,[7] but moderate tremors were observed in anaesthetized pigs.[9] In this study, we could not observe tremors as muscle relaxants were an integral component of the anaesthesia. However, pilot pharmacokinetic studies in the awake goats revealed mild tremors after an intravenous bolus of 5 mg/kg of AP14145.

SK channel selectivity of AP14145 has been evidenced by a minor inhibitory effect on hERG (K_v11.1) and lack of effect on Na_v1.5 measured at a frequency of 1 Hz.[4] Atrial ERP prolongation without a prolongation of the ventricular ERP or QT interval has indeed been observed in different species with this small molecule compound.[4,9,10] Safety profiling revealed no ventricular proarrhythmic effects.[9] In line with these observations, we did not observe QRS or QT prolongation nor ventricular arrhythmias, suggesting a high degree of atrial selectivity. Also, no haemodynamic instability was observed.

SK channels, effective refractory period, and conduction velocity

Early studies indicated that SK channels play a role in the late phase 3 repolarization of the cardiomyocyte. In a variety of species, and this study, demonstrated an action potential and ERP prolonging effects of SK channel inhibition.[2–4] Treatment with SK channel inhibition results in shorter AF paroxysms and termination of sustained AF.[3,4] SK channel inhibition was reported by Skibsbye *et al.*[2] to also decrease dV/dt_{max} and action potential amplitude which was suggested to be caused by the observed minor depolarization of the resting membrane potential. These observations were reported for two mechanistically different SK channel inhibitors, the negative allosteric modulator NS8593 and the direct pore inhibitor ICAGEN. Both drugs have a relatively high selectivity for SK2 and SK3, suggesting that SK inhibition generally leads to an indirect reduction of excitability which may be interpreted as a functional class I effect.[2] However, Burashnikov *et al.*[11] recently demonstrated that NS8593 also contains a direct and atrial specific sodium current inhibiting property.

In this study, SK inhibition by AP14145 led to profound slowing of atrial CV and this was not coincided with QRS duration. AP14145 was reported not to affect I_{Na} at 1 Hz[4] but a direct I_{Na} inhibition could have occurred at higher frequencies, or at more depolarized holding potentials. From these experiments, we cannot unravel the underlying mechanism for the functional class I effect we observed. Interestingly, CV during AF remained unaffected although AF cycle length prolonged profoundly. Drug-induced AF cycle length prolongation is associated with a widening of the temporal excitable gap.[12] Therefore, it is likely that Class III AAD treatment leads to longer sodium channel recovery times resulting in an increase of CV. Indeed, treatment of AF by the class III drug d-Sotalol increased both cycle length and CV during AF in the goat.[12] Conduction velocity in the presence of AP14145 was slower than to be expected based on the AF cycle length prolongation, assuming that the widening of the temporal excitable gap applies to AP14145 too. We argue that functionally a small Class I effect was present during AF which may have contributed to the cardioversion potential of AP14145. The potential for a Class I effect of SK inhibition is also further underscored by a series of experiments in Langendorff-perfused guinea pig hearts where it was shown that sub-efficacious doses of sodium channel blockers (flecainide and ranolazine) potentiate the antiarrhythmic effects of SK channel inhibition.[6] Possibly, such synergistic potential may apply to AP14145 as well.

Atrial fibrillation cycle length prolongation is the first step to AP14145-induced AF termination

In a tachy-paced pig model of AF, Diness *et al.*[4] demonstrated a high potency of AP14145 to treat AF. This was demonstrated by the ability of AP14145 to terminate AF at a stage of remodelling where clinical dosages of vernakalant could no longer terminate AF. In this study, AP14145 terminated persistent AF in 83% of the goats within 10–30 min at an infusion rate of 20/mg/kg/h.

Short AF cycle lengths and atrial fibrosis are thought to be one of the main contributors to conduction block, complex fibrillation patterns and AF stability.[13] Rotor dynamics and arrhythmia stability are also modulated by the cycle length.[14] This was also reported by Schuessler *et al.*,[15] who demonstrated in canine atria that the shortening of the AF cycle length alone is sufficient to increase AF stability, wave-break and fibrillatory complexity. The most consistent effect of antiarrhythmic drugs, despite their wide variety of targets, is the dose-dependent increase of the AF cycle length.[16] In this study, AP14145 also strongly increased ERP that was translated to a substantial increase of AF cycle length and pathlength. We consider these effects as the main mechanism for antiarrhythmic effect of AP14145.

Using contact mapping, Wang and Nattel[17] showed that a drug-induced prolongation of AF cycle length was indeed associated with fewer propagating wavefronts. Remarkably, in this study, complex conduction patterns persisted until the final seconds before AF termination. Our findings may suggest that AP14145 affects fibrillatory conduction in such a way that it becomes more prone to termination without decreasing complexity. The process of termination itself is characterized by the observed rapid change in the whole electrophysiological profile (i.e. cycle length, CV, pathlength, and ultimately complexity of propagation patterns). From this study, it remains unclear what triggers the sudden change in behaviour, and further research is necessary to unravel this mechanism.

No organization of conduction patterns occurred despite a significant prolongation in atrial fibrillation cycle length

The combination of AF cycle length prolongation and persistence of complex patterns is an intriguing observation. It is conceivable that SK inhibition by AP14145 in this study produced both antiarrhythmic effects (APD prolongation and reduced excitability) and some proarrhythmic effects. Although this and other studies found inhibition of SK channels to be antiarrhythmic,[3,4] an important biological role for SK channels has been proposed as it contributes to the repolarization reserve[18] and inhibition may therefore have proarrhythmic effects.

One proarrhythmic mechanism of SK channel block is thought to be based on the tight link between free cytosolic Ca^{2+} concentration and the SK channel conductance. SK channel would thereby counteract spontaneous Ca^{2+} release events from the sarcoplasmic reticulum and suppress focal activity.[19] Yet, investigations of SK channel

inhibition in models with ventricular substrates for arrhythmias, ischaemia and pacing induced heart failure, demonstrated fewer premature ventricular contractions and spontaneous episodes of ventricular tachycardia or fibrillation.[20] In the context of AF more frequent focal activity, observed as BT patterns, would obviously contribute to a higher complexity. However, our data did not give an indication of an increased incidence of BT patterns nor an altered distribution of BT sites.

A proarrhythmic mechanism at tissue level was observed in an optical mapping study in canine left atria. The authors showed higher degrees of action potential dispersion, alternans, and the propensity towards wave-break during S1S1 pacing when SK current was inhibited by apamin.[21] Similar effects were also observed in the rabbit ventricle under hypokalaemic conditions, leading to more wavefronts (phase singularities) during ventricular fibrillation.[18] In this study, a higher spatiotemporal instability and propensity towards wave-break and propagation failure during fast pacing rates were observed in the presence of AP14145. The direct mechanism for this instability of wavefront propagation could not be investigated in this study. It is conceivable that the mechanism leading wavefront instability contributed to the maintained AF complexity and may play a critical role in the sudden transition towards re-organization and AF termination.

Limitations

This study is limited by a number of aspects. First, SK channel expression levels in the goat are unknown. However, SK channel expression is reported for a wide variety of species, e.g. rat,[20] dogs,[3] pigs,[4] and humans.[2] Considering this and the atrial-specific electrophysiological effects, we believe it is highly likely that our observations are caused atrial SK-channel inhibition. Second, the *in vivo* approach of high-density mapping did not allow for action potential recording. This limits our understanding on the contribution of the Class I effect on the ERP prolongation and during AF itself. Third, the sample size was relatively small. This may have affected the statistical power to detect differences for some parameters. Fourth, mapping the atria with the two mapping arrays provides coverage of ~50% of the atrial surface. The data are discussed on the assumption that mapping of the free walls is representative for AF behaviour over the entire atria. However, if AF mechanisms that could drive AF, and maintain complexity, occurred outside the field of view it could have remained undetected. Finally, no pharmacokinetic data were included to this study. Results can therefore not be directly correlated to plasma levels.

Conclusions

AP14145 provided a successful therapy for the termination of persistent AF in the goat. Its antiarrhythmic mechanism during AF is mainly based on ERP and AF cycle length prolongation. A Class I effect was present in the atria, but not in the ventricles, during pacing and to a smaller extent also during AF. These electrophysiological effects did not lead to a gradual but a sudden organization of conduction. The mechanisms of these sudden changes need to be elucidated in further studies.

Supplementary material

Supplementary material is available at *Europace* online.

Funding

This work was supported by the ITN Network AFibTrainNet, No. 675351 and the Netherlands Heart Foundation (CVON2014-09, RACE V Reappraisal of Atrial Fibrillation: Interaction between hyperCoagulability, Electrical remodelling, and Vascular Destabilisation in the Progression of AF).

Conflict of interest: *Acesion Pharma, Copenhagen, Denmark,* has developed *AP1415*. U.S.S. is a co-founder and is shareholder of *Acesion Pharma.* J.D. and B.B. have warrants and are employed by *Acesion Pharma.* U.S. is a cofounder and shareholder of YourRhythmics BV and received funding from Roche Diagnostics and EP Solutions.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

References

1. Brandes A, Crijns HJGM, Rienstra M, Kirchhof P, Grove EL, Pedersen KB *et al.* Cardioversion of atrial fibrillation and atrial flutter revisited: current evidence and practical guidance for a common procedure. *Europace* 2020;**22**:1149–61.
2. Skibsbye L, Poulet C, Diness JG, Bentzen BH, Yuan L, Kappert U *et al.* Small-conductance calcium-activated potassium (SK) channels contribute to action potential repolarization in human atria. *Cardiovasc Res* 2014;**103**:156–67.
3. Qi X-Y, Diness JG, Brundel BJJM, Zhou X-B, Naud P, Wu C-T *et al.* Role of small-conductance calcium-activated potassium channels in atrial electrophysiology and fibrillation in the dog. *Circulation* 2014;**129**:430–40.
4. Diness JG, Skibsbye L, Simó-Vicens R, Santos JL, Lundegaard P, Citerni C *et al.* Termination of vernakalant-resistant atrial fibrillation by inhibition of small-conductance Ca2+-activated K+ channels in pigs. *Circ Arrhythm Electrophysiol* 2017;**10**:e005125.
5. Skibsbye L, Bengaard AK, Uldum-Nielsen AM, Boddum K, Christ T, Jespersen T. Inhibition of small conductance calcium-activated potassium (SK) channels prevents arrhythmias in rat atria during β-adrenergic and muscarinic receptor activation. *Front Physiol* 2018;**9**:1–13.
6. Kirchhoff JE, Diness JG, Sheykhzade M, Grunnet M, Jespersen T. Synergistic antiarrhythmic effect of combining inhibition of Ca^{2+}-activated K^+ (SK) channels and voltage-gated Na^+ channels in an isolated heart model of atrial fibrillation. *Heart Rhythm* 2015;**12**:409–418.
7. Simó-Vicens R, Kirchhoff JE, Dolce B, Abildgaard L, Speerschneider T, Sørensen US *et al.* A new negative allosteric modulator, AP14145, for the study of small conductance calcium-activated potassium (KCa 2) channels. *Br J Pharmacol* 2017;**174**:4396–408.
8. Zeemering S, Maesen B, Nijs J, Lau DH, Granier M, Verheule S *et al.* Automated quantification of atrial fibrillation complexity by probabilistic electrogram analysis and fibrillation wave reconstruction. *Conf Proc IEEE Eng Med Biol Soc* 2012;**2012**:6357–60.
9. Lubberding AF, Sattler SM, Grunnet M, Sørensen US, Tfelt-Hansen J, Jespersen T. Arrhythmia development during inhibition of small-conductance calcium-activated potassium channels in acute myocardial infarction in a porcine model. *Europace* 2019;**21**:1584–93.
10. Kirchhoff JE, Skarsfeldt MA, Muthukumarasamy KM, Simó-Vicens R, Bomholtz SH, Abildgaard L *et al.* The KCa2 channel inhibitor AP14145, but not dofetilide or ondansetron, provides functional atrial selectivity in guinea pig hearts. *Front Pharmacol* 2019;**10**:668.
11. Burashnikov A, Barajas-Martinez H, Hu D, Robinson VM, Grunnet M, Antzelevitch C. The small conductance calcium-activated potassium channel inhibitors NS8593 and UCL1684 prevent the development of atrial fibrillation through atrial-selective inhibition of sodium channel activity. *J Cardiovasc Pharmacol* 2020;**76**:164–72.
12. Wijffels MC, Dorland R, Mast F, Allessie MA. Widening of the excitable gap during pharmacological cardioversion of atrial fibrillation in the goat: effects of cibenzoline, hydroquinidine, flecainide, and d-sotalol. *Circulation* 2000;**102**:260–7.
13. Haïssaguerre M, Lim K-T, Jacquemet V, Rotter M, Dang L, Hocini M *et al.* Atrial fibrillatory cycle length: computer simulation and potential clinical importance. *Europace* 2007;**9**:vi64–70.

14. Laksman Z, Wauchop M, Lin E, Protze S, Lee J, Yang W *et al.* Modeling atrial fibrillation using human embryonic stem cell-derived atrial tissue. *Sci Rep* 2017;**7**:1–11.
15. Schuessler RB, Grayson TM, Bromberg BI, Cox JL, Boineau JP. Cholinergically mediated tachyarrhythmias induced by a single extrastimulus in the isolated canine right atrium. *Circ Res* 1992;**71**:1254–67.
16. Workman AJ, Smith GL, Rankin AC. Mechanisms of termination and prevention of atrial fibrillation by drug therapy. *Pharmacol Ther* 2011;**131**:221–41.
17. Wang Z, Fermini B, Nattel S. Mechanism of flecainide's rate-dependent actions on action potential duration in canine atrial tissue. *J Pharmacol Exp Ther* 1993; **267**:575–81.
18. Chan Y-H, Tsai W-C, Ko J-S, Yin D, Chang P-C, Rubart M *et al.* Small-conductance calcium-activated potassium current is activated during hypokalemia and masks short-term cardiac memory induced by ventricular pacing. *Circulation* 2015;**132**:1377–86.
19. Terentyev D, Rochira JA, Terentyeva R, Roder K, Koren G, Li W. Sarcoplasmic reticulum Ca^{2+} release is both necessary and sufficient for SK channel activation in ventricular myocytes. *AJP Heart Circul Physiol* 2014;**306**:H738–46.
20. Gui L, Bao Z, Jia Y, Qin X, Cheng ZJ, Zhu J *et al.* Ventricular tachyarrhythmias in rats with acute myocardial infarction involves activation of small-conductance Ca2+-activated K+ channels. *AJP Heart Circul Physiol* 2013;**304**:H118–30.
21. Hsueh C-H, Chang P-C, Hsieh Y-C, Reher T, Chen P-S, Lin S-F. Proarrhythmic effect of blocking the small conductance calcium activated potassium channel in isolated canine left atrium. *Heart Rhythm* 2013;**10**:891–8.

IMAGES IN ELECTROPHYSIOLOGY

doi:10.1093/europace/euab165
Online publish-ahead-of-print 11 August 2021

Peri-tricuspid atrial tachycardia late after heart transplantation

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Electrophysiological study was performed 20 years after orthotopic biatrial heart transplantation and demonstrated a regular atrial tachycardia with 2:1 conduction. Three-dimensional electroanatomical map (*Panel A*) identified recipient atrium (I), suture line (II, *Panel B*), and donor atrium (III). Entrainment manoeuvres showed perfect post-pacing intervals at the proximal coronary sinus, cavotricuspid isthmus (CTI), and along the suture line (*Panel C*). Catheter ablation of the CTI resulted into tachycardia termination (*Panel D* and red asterisk). However, crista terminalis presented non-excitable in the recipient atrium, resulting in tachycardia activation along the anterior suture line suggesting peri-tricuspid atrial tachycardia mimicking atrial flutter.