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Atrial-selective prolongation of refractory period with AVE0118 is due principally to inhibition of sodium channel activity

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Abstract

AVE0118's action to prolong effective refractory period (ERP) in atria but not ventricles is thought to be due to its inhibition of I_{Kur}. However, in non-remodeled atria, AVE0118 prolongs ERP but not action potential duration (APD_{70-90}), which can be explained with inhibition of sodium, but not potassium channel current. ERP, APD, and the maximum rate of rise of the AP upstroke (V_{max}) were measured in canine isolated coronary-perfused right atrial and in superfused ventricular tissue preparations. Whole-cell patch-clamp techniques were used to measure sodium channel current (I_{Na}) in HEK293 cells stably expressing SCN5A. AVE0118 (5–10 μM) prolonged ERP ($p<0.001$), but not APD_{70} and decreased V_{max} (by 15%, 10 μM, $p<0.05$; n=10 for each). Ventricular ERP, APD_{90}, and V_{max} were not changed significantly by 10 μM AVE0118 (all p=ns; n=7). AVE0118 effectively suppressed acetylcholine-mediated persistent atrial fibrillation (AF). AVE0118 (10 μM) reduced peak current amplitude of *SCN5A*-WT current by 36.5±6.6% ($p<0.01$; n=7) and shifted half-inactivation voltage ($V_{0.5}$) of the steady- state inactivation curve from -89.9±0.5 to -96.0±0.9 mV ($p<0.01$; n=7). Our data suggest that AVE0118-induced prolongation of atrial, but not ventricular ERP, is due largely to atrial- selective depression of I_{Na}, which likely contributes to the effectiveness of AVE0118 to suppress AF.

INTRODUCTION

The notion that atrial-selective agents may suppress atrial fibrillation (AF) without the risk of induction of ventricular proarrhythmia has led to the emergence of several investigational atrial-selective pharmacological approaches for rhythm control management of AF. The most investigated and widely promoted as the most promising atrial-specific approach involves inhibition of atrial-specific $K_v1.5$-pore-forming channels, carrying the ultra-rapid delayed rectified outward potassium current (I_{Kur}).[1] Numerous agents capable of blocking I_{Kur} have been shown to prolong effective refractory period (ERP) specifically in atria.[2] ERP can be prolonged due to action potential duration (APD_{70-90}) prolongation and/or development of post- repolarization refractoriness (PRR). The former is commonly due to block of potassium channels and the latter is due to block of sodium channels. Correlation of the ERP and APD_{70-90} changes induced by I_{Kur} blockers has been poorly studied. Of note, I_{Na} blockers can produce atrial- selective ERP prolongation due to induction of PRR[3] and recent studies have revealed that I_{kur} blockers such as vernakalant and AZD1305 depress I_{Na} – mediated parameters in an atrial- selective manner.[4; 5] Vernakalant prolongs atrial ERP largely due to induction of PRR.[6]

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The main purpose of the current study was to test the hypothesis that the effect of AVE0118, a multichannel blocker inhibiting I_{Kur}, to produce an atrial selective prolongation of ERP is due largely to its atrial-selective inhibition of the sodium channel. We also used patch-clamp techniques to examine the effects of AVE0118 on I_{Na} in HEK293 cells stably expressing *SCN5A*.

METHODS

This investigation conforms to the Guide for Care and Use of Laboratory Animals published by the National Institutes of Health (The Eighth Edition of the Guide for the Care and Use of Laboratory Animals (NRC 2011)) and was approved by the Institutional Animal Care and Use Committee. Dogs weighing 20–25 kg were anticoagulated with heparin (200 IU/kg) and anesthetized with pentobarbital sodium (35 mg/kg, i.v.). Prior to surgery, the following criteria was met to insure adequate anesthesia: a lack of palpebral reflex, a lack of withdrawal from a noxious stimulus applied to the distal forelimb, a lack of breathing, and an auscultable heart rate that is no greater than 60 bpm. The chest was opened via a left thoracotomy, the heart excised, placed in a cardioplegic solution consisting of cold (4°C) Tyrode's solution containing 8.5 mM $[K^+]_o$ and transported to a dissection tray.

Experiments were performed using isolated arterially-perfused canine right atrial (RA) preparations and superfused left ventricular endocardial tissue slice preparations (1×0.5×0.1 cm). The methods used for isolation and perfusion of these preparations have been described in previous publications.[7; 8]

Unfolded RA with a rim of the right ventricle was cannulated and perfused through the ostium of the right coronary artery. Unperfused tissue was removed with a razor blade or scissors. The cut ventricular and atrial branches were ligated using silk thread. After these procedures (performed in cold cardioplegic solution, 4–8°C), the preparations were transferred to a temperature-controlled bath and arterially-perfused with Tyrode's solution by use of a roller pump. The superfused preparations, isolated using a dermatome (Davol Simon Dermatome, Cranston, RI, USA), were placed in a tissue bath (volume 5 ml, flow rate 12 ml/min) and allowed to equilibrate for at least 3 hours while superfused with oxygenated Tyrode's solution and stimulated at a basic cycle length (BCL) of 500 msec using point stimulation (rectangular stimuli 1 - 3-ms duration, 2–3 times diastolic threshold intensity). The composition of the Tyrode's solution was (in mM): NaCl 129, KCl 4, NaH_2PO_4 0.9, $NaHCO_3$ 20, $CaCl_2$ 1.8, $MgSO_4$ 0.5, and D-glucose 5.5, buffered with 95% O_2 and 5% CO_2 (37±0.5 °C, pH=7.35)

Transmembrane action potential (AP) recordings (sampling rate 41 kHz) were obtained using standard or floating glass microelectrodes (2.7 M KCl, 10–25 MΩ DC resistance). **A pseudo-electrocardiogram (ECG)** was recorded using two electrodes consisting of Ag/AgCl half cells placed in the Tyrode's solution bathing the preparation, 1.0 to 1.2 cm from opposite ends of the atrial or ventricular coronary-perfused preparations. **Effective refractory period (ERP)** was measured by delivering premature stimuli after every 10^{th} regular beat at a pacing cycle length (CL) of 500 (with 10 ms resolution; stimulation with a 2 × **diastolic threshold of excitation (DTE)** amplitude). **Post-repolarization refractoriness (PRR)** was recognized when ERP exceeded APD_{70} in atria and APD_{90} in ventricles. Note that atrial ERP is generally coincided with $APD_{70\text{-}75}$ in atria and APD_{90} in ventricles.[3; 9] **Maximum rate of rise of the AP upstroke (V_{max})**: Stable AP recordings and V_{max} measurements are difficult to obtain in vigorously contracting perfused atrial preparations and there is a significant variability in V_{max} values even at the same condition/region (shown in Fig. 1B). In coronary-perfused atria, the effect of AVE01118 on V_{max} was first determined in each atrium by averaging the largest three V_{max} values in the absence and

presence of this agent at a CL of 500 ms. Then, using these average values from each atrium, we compared V_{max} data from all atria (n=10). Due to a substantial inter-preparation variability, V_{max} values were normalized for each experiment.

Experimental Protocols

The equilibration period for the coronary-perfused atrial preparations was 30 min and for superfused ventricular slice preparations 3 hours. The concentration of AVE0118 (5.0 and 10.0 μM) was increased in a step-wise manner, with at least 20 min at each concentration before the start of recording. In an acetylcholine (ACh) -dependent AF model, we tested the effect of AVE0118 to prevent (series 1) the induction of AF as well as, in different series, the ability of these agents to terminate (series 2) persistent AF (lasting > 60 min). In cases in which the drug terminated AF, we tested if the arrhythmia could be re-induced electrically.

Electrophysiology of the Cardiac Sodium Channels in HEK293 Cell Line

The effects of AVE0118 on I_{Na} characteristics were evaluated in human embryonic kidney cell line, HEK293, stably expression SCN5A, as previously described.[10] Sodium channel characteristic were studied with whole cell patch clamp techniques, as previously described.[11] Cells were placed in a chamber for electrophysiological study (EPS; Medical Systems, Greenvale, NY). Macroscopic whole-cell I_{Na} was recorded at room temperature (22°C) using an Axopatch 200B amplifier (Molecular Devices, Inc, Sunnyvale, CA). Perfusion bath solution containing (in mmol/L) 140 NaCl, 5 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 2.8 Na Acetate, 10 HEPES, and 10 glucose (pH 7.3 with NaOH). Tetraethylammonium chloride (5 mM) was added to the buffer to block TEA-sensitive native currents. Patch clamp pipettes were pulled (1 and 2.5 MΩ) from borosilicate glass (7052; Model PP-89; Narashige, Tokoyo, Japan) and filled with a solution containing (in mM) 5 NaCl, 5 KCl, 130 CsF, 1.0 $MgCl_2$, 5 EGTA and 10 HEPES (pH 7.2 with CsOH). Steady- state availability of the sodium channel was fitted to a Boltzmann equation. Data acquisition and analysis were performed using pCLAMP programs V9.2 (Axon Instruments, Union City, CA) and ORIGIN 6.1 (Microcal Software, Northampton, MA).

Drugs

AVE0118 (a gift from Dr. Gögelein, Sanofi-Aventis , Frankfurt, Germany) was dissolved in 100% DMSO and acetylcholine (SIGMA, MO) was dissolved in distilled water as a stock of 1–10 mM at the start of each experiment.

Statistical Analysis

For data obtained from the multicellular preparations, statistical analysis was performed using paired or unpaired Student's t-test and one-way repeated measures or multiple comparison analysis of variance (ANOVA) followed by Bonferroni's test, as appropriate. Statistical differences in voltage clamp analysis were evaluated by Student's unpaired *t*-test. Data from multicellular preparations and HEK293 Cell Line are expressed as mean ± SD and mean ±SE, respectively. Significance wasassumed for $P < 0.05$; "NS" indicates non-significant changes.

RESULTS

Electrophysiologic effects of AVE0118 atrial and ventricular preparations

In atria, AVE0118 reduced V_{max} by an average of 15% at a concentration of 10 μM (CL = 500 ms, Fig. 1). AVE0118 (5–10 μM) significantly prolonged ERP in both pectinate muscle (PM) and crista terminalis (CT) regions, but abbreviated APD_{70} in CT and caused no change in APD_{70} in PM, revealing the development of PRR (Fig. 2). The early repolarization phase

in atria was significantly prolonged by AVE0118; APD_{20} in CT increased from 5±3 to 51±12 ms at a concentration of 10 μM ($p<0.001$; CL = 500 ms; n=8) (Fig. 2). AVE0118 did not significantly alter DTE (0.22±0.04 mA in control vs. 0.23±0.03 mA at 10 μM AVE0118; CL = 500 ms; n=10). The duration of P wave was significantly increased at a CL of 300 ms, but not 500 ms by 10 μM AVE0118 (Fig. 3).

In contrast to atrial preparations, ERP, APD_{90}, V_{max}, and action potential morphology were not altered significantly by AVE0118 in ventricular preparations (Fig. 4). DTE was also unaffected by AVE0118 in ventricular preparations (0.29±0.05 mA in control vs. 0.28±0.06 mA in presence of 10 μM of AVE0118; n=7; CL = 500 ms).

Block of I_{Na} by AVE-0118

Whole cell sodium current was recorded at room temperature in HEK293 cells stably expressing *SCN5A* ($Na_v1.5$). Macroscopic sodium currents (I_{Na}) elicited by 20 ms test pulses from a holding potential of −120 mV to potentials between −90 mV and +30 mV in increments of +5 mV are shown in Fig. 5A. Figure 5B shows the effect of 10 μM AVE0118 on the current- voltage (I-V) relationship for I_{Na}. AVE0118 (10 μM) caused a significant reduction of peak I_{Na} (Fig. 5C). I_{Na} elicited with pulses to −30 mV was reduced by 36.5±6.6% ($p<0.01$; n=7). Washout of AVE0118 was associated with restoration of the current.

The steady-state inactivation relationship was obtained using a 500 ms prepulse to different voltages followed by a step to −20 mV (20 ms duration). AVE0118 shifted half inactivation voltage ($V_{0.5}$) to more negative potentials (from −89.9±0.5 to -96.0±0.9 mV; $p<0.01$; n=7; Fig. 6), thus further reducing the availability of sodium channels.

Anti-AF effect of AVE0118

In the presence of ACh (0.5 μM), the atrial action potential was markedly abbreviated (Fig. 7). Under these conditions, addition of AVE0118 (10 μM) significantly prolonged both APD_{70} and ERP. ERP prolonged more than APD_{70}, showing the development of PRR. With ACh alone (0.5 μM), persistent AF was inducible in 100% of coronary-perfused atria (10/10). AVE0118 (10 μM) prevented the induction of persistent ACh-mediated AF in 100% of atria (4/4). Non-sustained AF or tachycardia (<30 sec) were induced in 2 of the 4 atrial preparations. In another series of experiments, AVE0118 (10 μM) was found to terminate persistent acetylcholine-mediated AF in 6/7 atria (Fig. 7).

DISCUSSION

Our main finding is that the I_{Kur} blocker AVE0118 also importantly inhibits sodium channel activity in an atrial-selective manner, which largely accounts for the atrial selective prolongation of ERP and may contribute to anti-AF properties of AVE0118.

Atrial-selective agents for treatment of AF: I_{Kur} blockers

A major limitation of many effective anti-AF agents is the risk of induction of ventricular arrhythmias. This risk can be eliminated or diminished with the use of agents that selectively alter atrial electrophysiological parameters. Block of I_{Kur}, an atrial specific target,[1] has long been considered to be a promising atrial-specific approach for effective and safe AF therapy.[2; 12] However, enthusiasm for selective I_{Kur} blockers for AF management has diminished in recent years.[13–17]

AVE0118 is known to inhibit multiple ion channels including I_{Kur}, I_{to}, $I_{K\text{-}ACh}$, and constitutively active $I_{K\text{-}ACh}$ and to prolong atrial but not ventricular ERP.[18; 19] The atrial-

selective [20; 21] prolongation of ERP by AVE0118 has previously been attributed to inhibition of I_{Kur}. However, APD_{70-90} is abbreviated or not affected by AVE0118 in "healthy" atria and only slightly prolonged in remodeled atria.[22; 23] Cardiac ERP corresponds to APD_{70-90}. In the present study, we compared AVE-0118-induced APD and ERP changes and demonstrated that in healthy atria AVE0118 causes little change in APD_{70-90} but significantly prolongs ERP (Fig. 2). Prolongation of ERP without lengthening of APD_{70-90} is due to induction of PRR, which is due to the inhibition of peak I_{Na}, but not I_{Kur}. The ability of AVE0118 to block I_{Na} is supported by the fact that this agent reduces V_{max} in atrial preparations and reduces peak I_{Na} in heterologously- expressed sodium channels.

Atrial-selective prolongation of ERP: I_{Kur} or I_{Na} inhibition?

Many agents capable of inhibiting I_{Kur} have been shown to selectively prolong ERP in atria.[2] More recent studies have shown that block of I_{Na} can also prolong atrial ERP selectively, without altering ventricular ERP.[3; 15] Interestingly, recent studies indicate that agents with I_{Kur} blocking activity capable of producing atrial-specific/predominant ERP prolongation (such as vernakalant, AZD7009, and AZD1305) also potently inhibit peak I_{Na}.[5; 24; 25] Atrial-selective ERP prolongation with vernakalant is due to inhibition of peak I_{Na}[6] and with AZD1305 is due to block of both peak I_{Na} and I_{Kr}.[5; 26]

Several I_{Kur} blockers (vernakalant, ISQ-1 and TAEA) slow conduction in atria, but not in ventricles, [4; 27] pointing to atrial-selective I_{Na} inhibition. "Pure" inhibition of IKur with low concentrations of 4-AP, cause no or only minor changes in APD_{70-90} and as expected the changes in ERP parallel the changes in APD.[13] Of note, the potential I_{Na} blocking ability of most of the I_{Kur} blockers either has not been studied or studied under conditions that may not unmask this effect of the drug (i.e., studied in ventricular myocytes/preparations at slow pacing rates).[28] The atrial selective I_{Na} blocking effect is observed at normal and rapid, but not slow, activation rates owing to the fact that atrial-selective I_{Na} blockers possess relatively rapid unbinding kinetics from the sodium channel.[6; 15]

We determined the effect of AVE0118 on APD, V_{max}, PRR, DTE only at one pacing CL in the current study (CL = 500 ms). It is expected that at faster activation rates, sodium channel-mediated parameters (V_{max}, PRR, DTE, etc) would be altered by AVE0118 to a greater degree, as previously demonstrated for a number of atrial-selective I_{Na} blockers, including ranolazine, vernakalant, amiodarone and AZD1305.[3; 5; 6; 29] Atrial conduction time was increased by AVE0118 in a rate-dependent manner (Fig. 3) Note that at rapid activation rates (CL = 200 ms), AVE0118 statistically significantly slowed conduction velocity in atria of goat *in vivo*.[20; 30]

While AVE0118 (3–10 μM) prolongs ERP in atria of goats[20; 31] pigs[32] and dogs[33] *in vivo* as well as in coronary-perfused atria of rabbits[34] and dogs (current study), this drug (at 6 μM) produces no change in ERP in human superfused atrial preparations isolated from patients in sinus rhythm patients.[19] The difference may be species-dependent, but it can be also explained by a relatively poor pharmacological sensitivity of atrial superfused vs. perfused (or *in vivo*) preparations. A statistically significant ERP prolongation with vernakalant, ranolazine, and dl-sotalol was obtained at 3 μM in canine coronary-perfused left atrial appendage preparations,[6] but only at 30 μM in canine superfused pulmonary vein preparations.[35] The extent of prolongation of atrial ERP by the atrial-selective I_{Na} blockers ranolazine and vernakalant is strongly rate-dependent, being small or absent at 1000 ms CL and significant at ≤500 ms CL in canine atrial preparations.[6; 35] Considering that effect of AVE0118 on ERP in the human atrial preparations was recorded at a CL of 1000 ms[19] and in goat, pig, rabbit, and dog atria at much faster rates (CL = ≤400, ≤400, 200, and 500 ms,

respectively), [20; 31–34] the failure of AVE0118 to prolong ERP in human atrial preparations may be attributed to the relatively slow pacing rate.

A number of factors are likely to contribute to the atrial selectivity of I_{Na} blockers, including a more depolarized resting membrane potential (RMP), more negative half-inactivation voltage ($V_{0.5}$), and more gradual phase 3 of the action potential in atrial cells as compared with ventricular cells (for detailed discussion see[3; 10; 36]). Rate of recovery from sodium channel block is thought to contribute to the atrial selectivity of sodium-channel blockers. Drugs, such as propafenone, which exhibit slow dissociation from the sodium channel, show little to no atrial selectivity,[37] whereas agents that dissociate rapidly, such as ranolazine, amiodarone, and vernakalant, tend to be highly atrial-selective in their inhibition of sodium channel-dependent parameters.[3; 29; 37] The unbinding kinetics of AVE0118 the sodium channel remains unknown.

Anti – AF effectiveness of AVE0118

Our demonstration of good anti-AF efficacy of AVE-0118 in coronary-perfused canine atrial preparation is consistent with the report of Blaauw et al[20] demonstrating good efficacy AVE0118 in remodeled atria of the goat *in vivo*. When atrial APD was significantly abbreviated (as after ACh), AVE-0118 prolonged both APD and ERP, the former less than the latter (Fig. 6). Prolongation of APD and ERP is likely due to block of $I_{K\text{-}ACh}$, I_{Kur}, I_{to}, and I_{Na}. The ERP prolonging effect of AVE0118 appears to be responsible for its anti-AF efficacy.

"Pure" I_{Kur} block for AF?—At concentrations that are able to effectively suppress AF, I_{Kur} blockers such as vernakalant, AVE0118, AZD1305, AZD7009, potently inhibit other currents, particularly I_{Na}, I_{to}, $I_{K\text{-}ACh}$, CA-$I_{K\text{-}ACh}$, and I_{Kr}.[5; 19; 20; 24; 26; 38] It is therefore difficult to dissect out the degree to which I_{Kur} inhibition contributes to the anti-AF effects of an agent. It is noteworthy that specific block [13;] of I_{Kur} using low concentrations of 4-AP neither prevent the induction of AF nor terminate AF. [17] Indeed, selective I_{Kur} block has been shown to abbreviate atrial APD_{90}/ERP and promote the induction of AF in atria displaying a plateau-shaped action potential morphology.[13] It is also noteworthy that loss of function mutations in *KCNA5,* the gene that encodes for the α subunit of the I_{Kur} channel, have been associated with inherited AF.[39; 40]

I_{to} inhibition is likely to contribute to the atrial-selective and anti-AF effects of AVE0118. I_{to} is larger in atrial vs. ventricular cell.[41] The predominant α-subunit of the I_{to} channel, $K_v4.3$, is expressed significantly more strongly in human atria vs. ventricles,[42] and 4-AP block of I_{to} is much more effective in atrial vs. ventricular myocytes.[43; 44]

Because the density of I_{Kur} is reduced with acceleration of pacing rate,[45] the relative contribution of I_{Kur} to atrial repolarization may be relatively small in the setting of AF. I_{Kur} density has been reported to be decreased in cells isolated from chronic AF hearts.[19; 46] While block of I_{Kur} alone may not be sufficient to suppress AF,[13; 15–17] the contribution of I_{Kur} block may [30; 36] be important in combination with inhibition of other currents (e.g., I_{to}, I_{Kr}, I_{Na}).

Study limitations

Our data were obtained from "healthy" cardiac preparations that did not manifest the structural and electrical cardiac changes commonly encountered in patients with AF. Pharmacological responses of remodeled atria may differ from those of healthy atria. Our experiments were performed in isolated atria coronary-perfused with Tyrode's solution. The

presence of autonomic influences and other factors present *in vivo* may modulate the effect of I_{Kur} inhibition, resulting in outcomes different from those observed in the present study.

While the effect of AVE0118 on I_{Na} – dependent parameters (Vmax, DTE and ERP) was measure in intact canine coronary-perfused atrial and ventricular preparations, the effect of AVE0118 on I_{Na} was measured in a heterologous expression system, HEK293 cells stably expressing *SCN5A.* The sodium channel in heart is known to co-associate with many auxiliary subunits, which may not be present in HEK293 cells. Consequently, our quantification of the effects of AVE0018 on I_{Na} may less than accurately reflect the responses of native atrial and ventricular myocytes.

Conclusions

Our data indicate that AVE0118-induced atrial-selective prolongation of ERP is due largely to the effect of the drug to produce an atrial-selective depression of I_{Na} which likely contributes to the effectiveness of AVE0118 to suppress AF. Our results provide support for the hypothesis that the atrial selectivity of multichannel blockers inhibiting I_{Kur} to prolong ERP is due largely to atrial-selective depression of I_{Na}[6; 14; 15; 36.]

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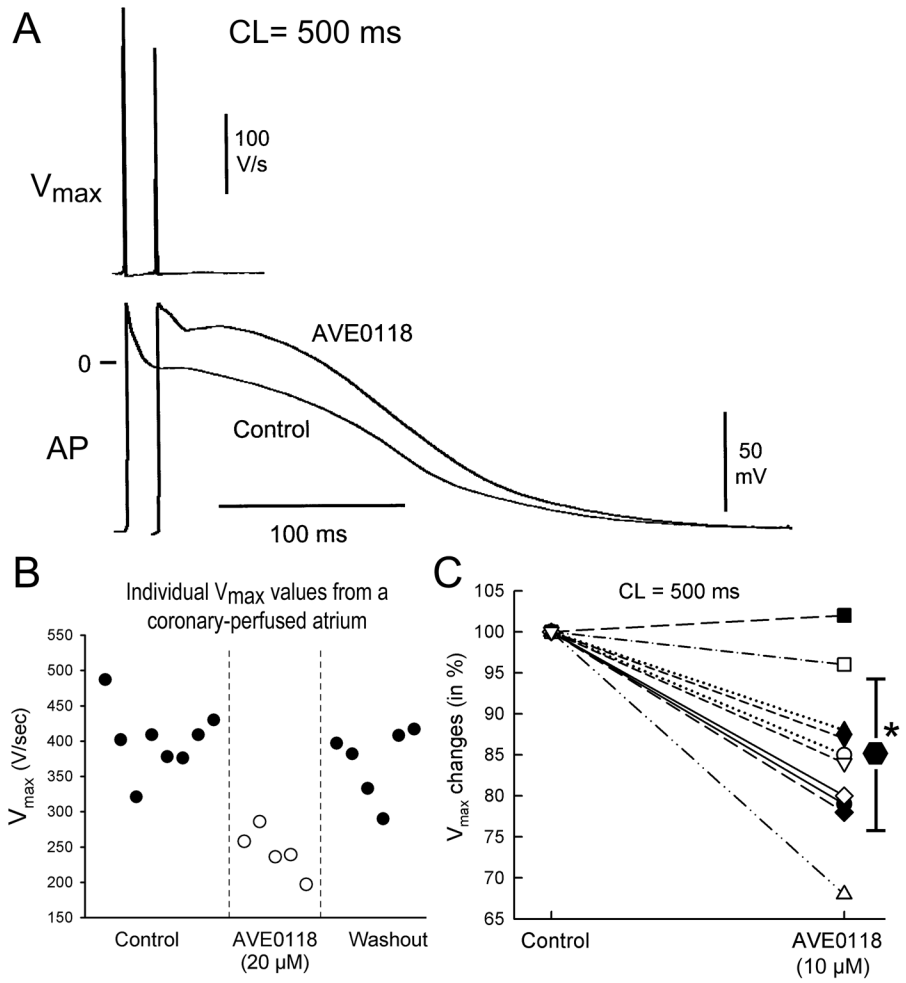

Figure 1. AVE0118 decreases the maximum rate of rise of the action potential upstroke (V_{max}) in canine coronary perfused right atria

A: Representative action potentials and corresponding V_{max} values recorded under control conditions and following addition of AVE0118 (10 μM). **B**: Individual V_{max} values from an atrial preparation recorded under control conditions, after 20 μM AVE0118 and after washout of the drug. **C**: AVE0118 (10 μM) - induced reduction of V_{max} as a % of baseline values (n=10). * -$p<0.05$ vs. control.

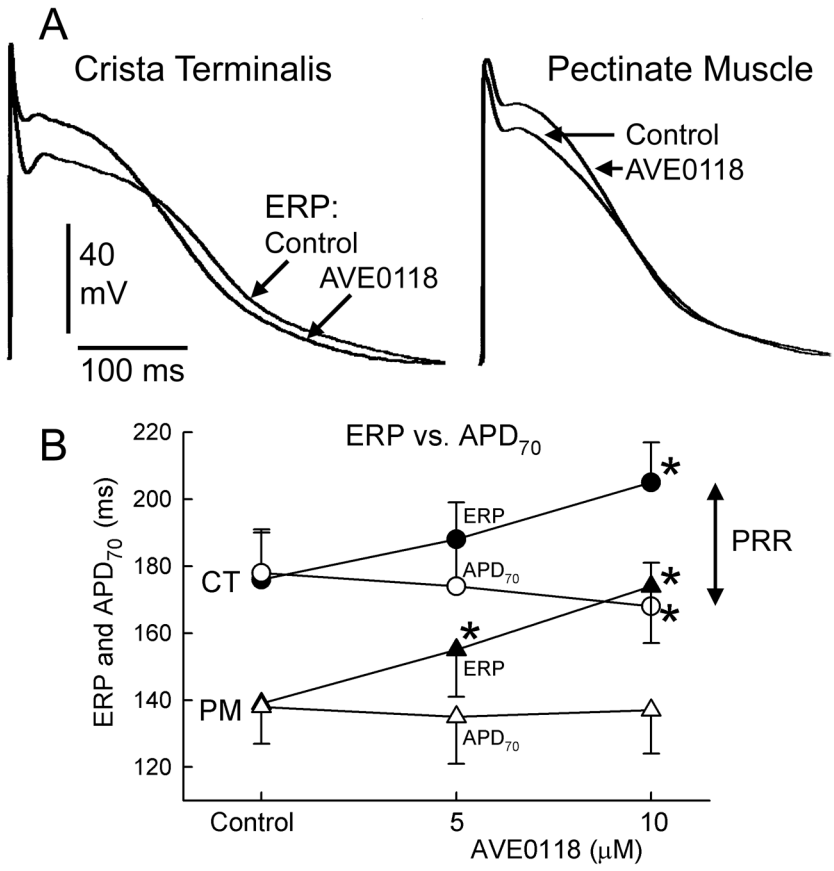

Figure 2.

AVE0118 causes little change in APD_{70} but causes a significant prolongation of ERP in canine atria, leading to development of post-repolarization refractoriness (PRR). In atria, ERP corresponds to APD at 70–75% of repolarization. Shown are: superimposed action potential tracings recorded before and after AVE0118 in crista terminalis (CT) and pectinate muscle (PM) regions (A) and summary data of APD_{70} and ERP (B). * - $p<0.05$ vs. control. CL = 500 ms. n=10

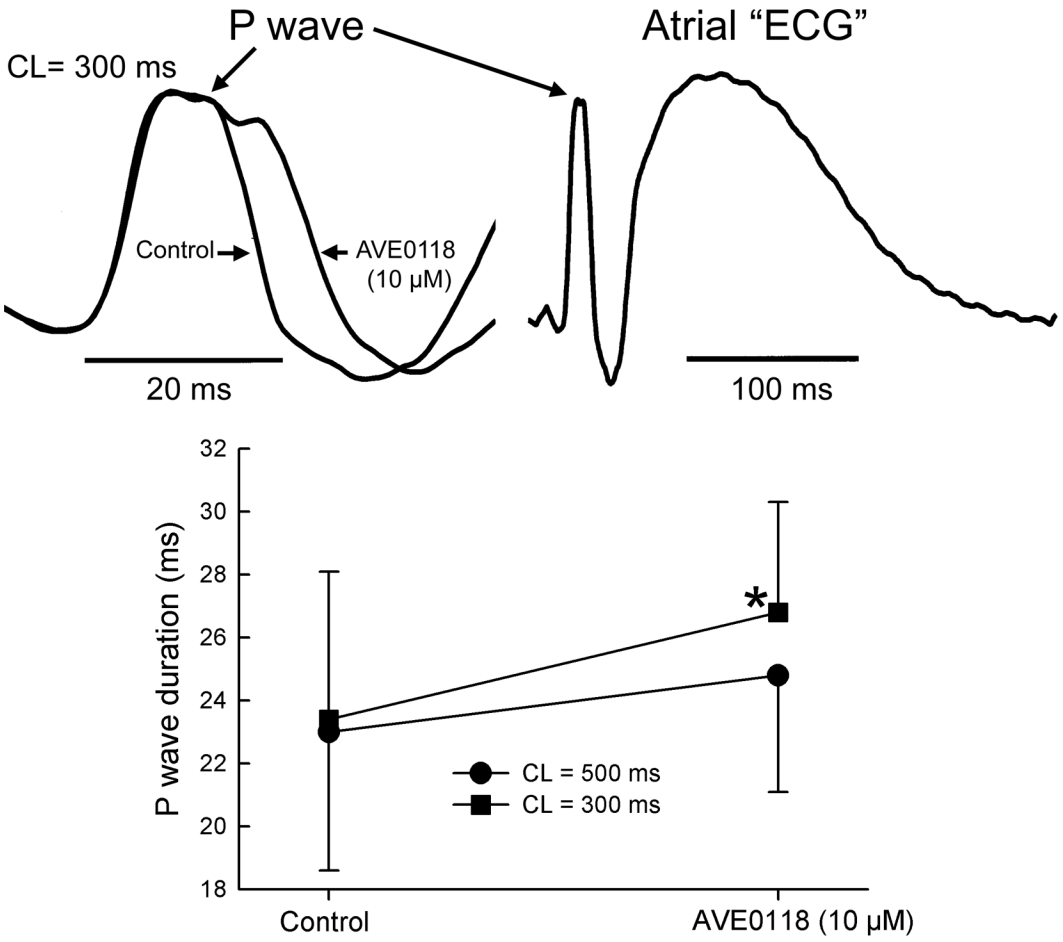

Figure 3.
AVE0118 significantly increased the duration of the atrial P wave complex (a measure of atrial conduction) at a pacing CL of 300 ms in coronary-perfused atrial preparations.
* - $p<0.05$ vs. control. n=5 for each.

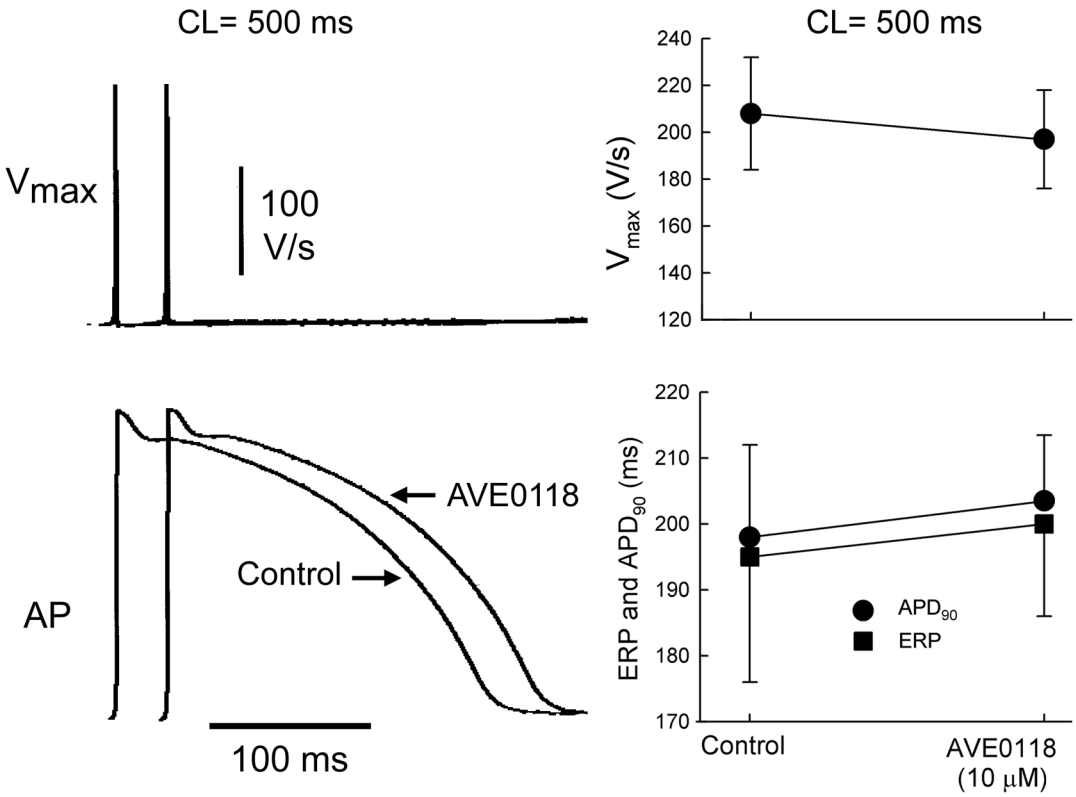

Figure 4. AVE0118 fails to significantly alter V_{max}, APD, and ERP in canine left ventricular superfused endocardial tissue slices

Left panels: action potentials recordings and corresponding V_{max} values. Right panels: Summary data for V_{max}, ERP and APD_{90}. n=7. CL = 500 ms.

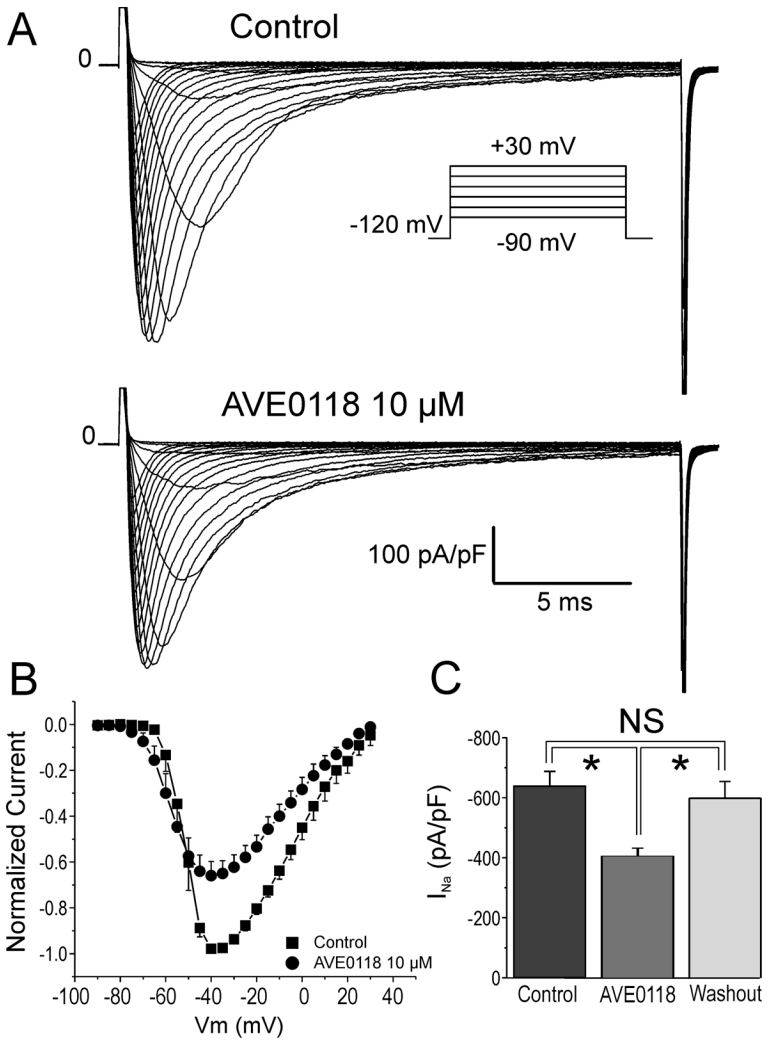

Figure 5. Effect of AVE0118 on sodium channel current (I_{Na}) in HEK293 cells stably expressing *SCN5A*.

A: I_{Na} elicited by 20 ms test pulses from a holding potential of -120 mV to potentials between -90 mV and +30 mV, in increments of 5 mV (inset). **B:** Current voltage (I-V) relationship for I_{Na} in the absence and presence of 10 μM AVE0118. Peak currents were normalized to the maximum current recorded under control conditions and following application of AVE0118. **C:** Effect of 10 μM AVE0118 to decrease ^{I}Na. I_{Na} density was reduced by 36.5±6.6% (*- $p<0.01$; n=7) at -30 mV. Washout of AVE0118 is associated with restoration of the current. Mean ± SEM (n=7).

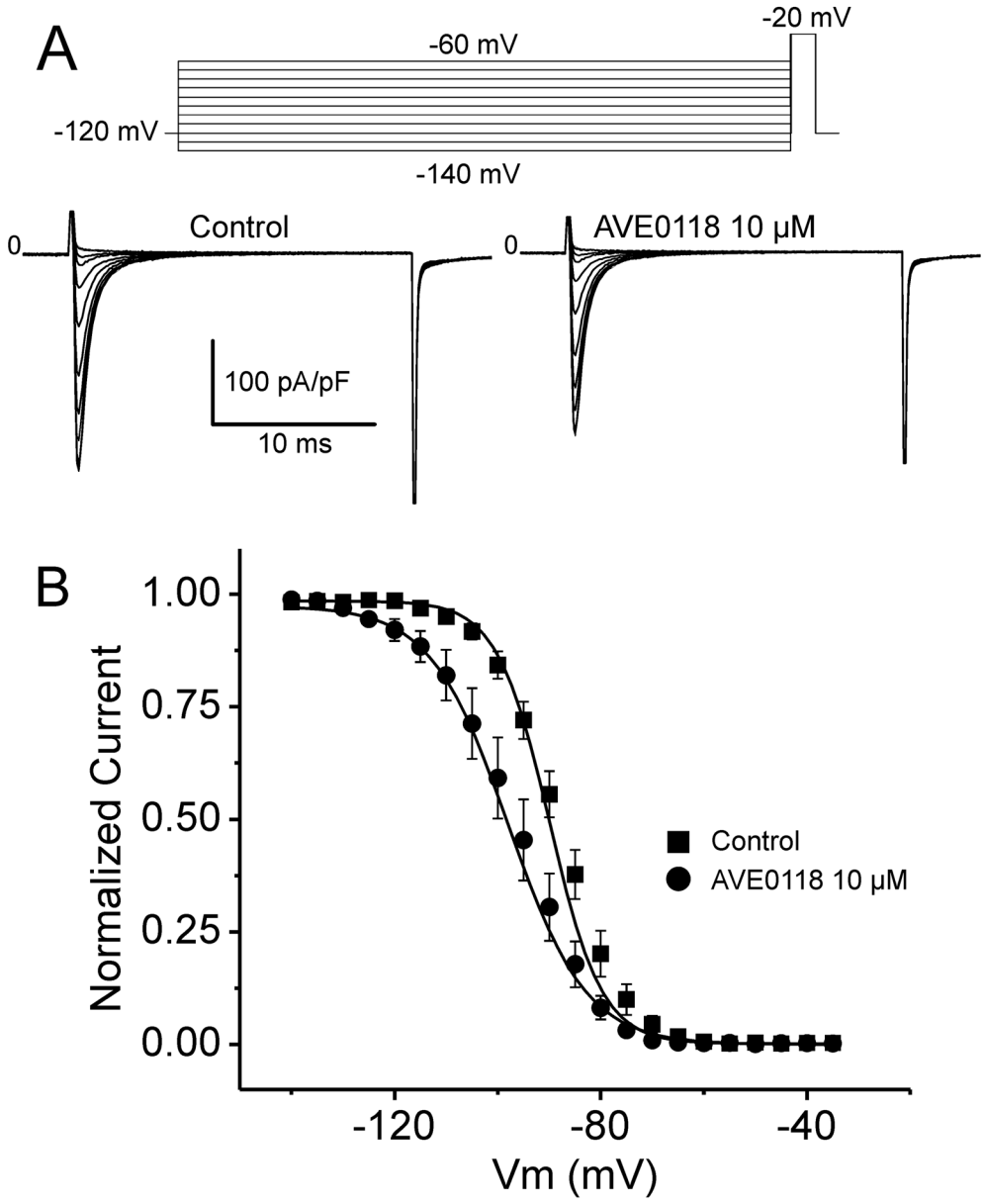

Figure 6. Effect of AVE0118 on Steady-state Inactivation of Cardiac Sodium Channels in HEK293 cells

A: Representative $Na_v1.5$ current traces recorded before and after 10 μM AVE0118. Currents were recorded using the protocol pictured in the inset. **B:** Peak $Na_v1.5$ current was normalized to the maximum current recorded under control conditions or following 10 μM AVE0118 (-89.0 ± 0.55 mV vs. -95.96 ± 0.55 mV, respectively). Steady-state inactivation is plotted as a function of conditioning potential and fitted to a Boltzmann distribution. AVE0118 induces a significant shift in mid-inactivation voltage ($V_{1/2}$; $p<0.01$). Mean ± SEM (n=7).

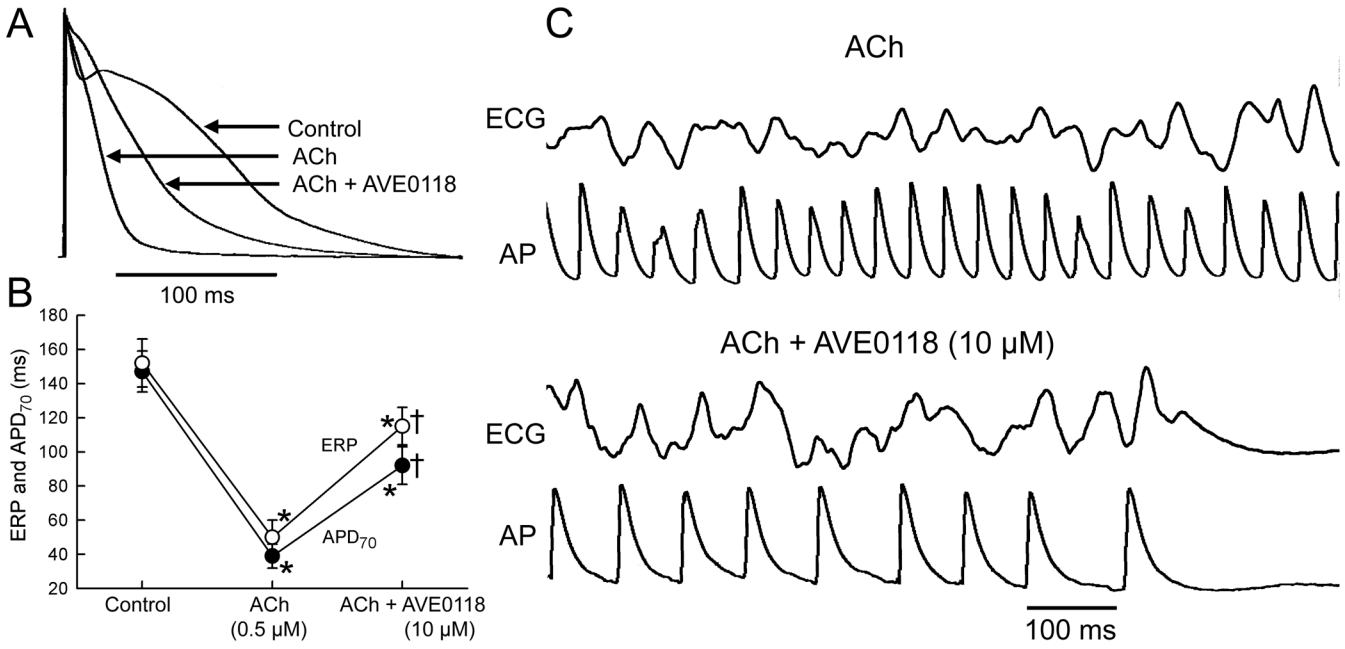

Figure 7. AVE0118 prolongs atrial APD and ERP in the presence of acetylcholine (ACh) and effectively terminates ACh-mediated AF

A: Superimposed action potentials (AP) recorded in control, in the presence of ACh, and ACh plus AVE0118. **B:** Plot depicts average changes in APD_{70} and ERP. All data are from pectinate muscle stimulated at a CL of 500 ms. * - $p<0.05$ vs. control. † $p<0.05$ vs. ACh. n=5–10. **C:** Shown are ECG and AP tracings recorded during persistent AF in the presence of ACh alone and following the addition of AVE0118, which terminated the arrhythmia on the 12^{th} minute.