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The SK Channel Inhibitors NS8593 and UCL1684 Prevent the Development of Atrial Fibrillation via Atrial-selective Inhibition of Sodium Channel Activity

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Abstract

The mechanisms underlying atrial-selective prolongation of effective refractory period (ERP) and suppression of atrial fibrillation (AF) by NS8593 and UCL1684, small conductance calcium-activated potassium (SK) channel blockers, are poorly defined. The purpose of the study was to confirm the effectiveness of these agents to suppress AF and to probe the underlying mechanisms. Transmembrane action potentials and pseudo-ECGs were recorded from canine isolated coronary-perfused canine atrial and ventricular wedge preparations. Patch clamp techniques were used to record sodium channel current (I_{Na}) in atrial and ventricular myocytes and human embryonic kidney (HEK) cells. In both atria and ventricles, NS8593 (3–10 μ M) and UCL1684 (0.5 μ M) did not significantly alter action potential duration (APD), suggesting little to no SK channel inhibition. Both agents caused atrial-selective: 1) prolongation of ERP secondary to development of post-repolarization refractoriness [PRR], 2) reduction of V_{max} , and 3) increase of diastolic threshold of excitation (all are sodium-mediated parameters). NS8593 and UCL1684 significantly reduced I_{Na} density in HEK cells as well as in atrial but not in ventricular myocytes at physiologically relevant holding potentials. NS8593 caused a shift of steady-state inactivation to negative potentials in atrial but not ventricular cells. NS8593 and UCL1684 prevented induction of acetylcholine-mediated AF in 6/6 and 8/8 preparations, respectively. This anti-AF effect was

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associated with strong rate-dependent depression of excitability. The SK channel blockers, NS8593 and UCL1684, are effective in preventing the development of AF due to potent atrial-selective inhibition of I_{Na} , causing atrial-selective prolongation of ERP secondary to induction of PRR.

Keywords

Pharmacology; Electrophysiology; Atrial fibrillation; Antiarrhythmic drugs

INTRODUCTION

A major limitation of pharmacological approaches to suppression of atrial fibrillation (AF) is the risk of induction of ventricular pro-arrhythmia. This liability can be reduced or eliminated with the use of agents that produce atrial-selective electrophysiological effects.^{1, 2} The small conductance calcium-activated potassium (SK) channels have been suggested as prime targets for development of drugs for AF suppression because of atrial-selective expression of small-conductance Ca^{2+} -activated K^{+} channel current carried by these channels.^{3, 4} The relative contribution of the SK channel to atrial electrophysiology, however, remains poorly defined.^{4, 5} Agents that block the SK channels can cause atrial-selective prolongation of the effective refractory period (ERP) and effectively suppress AF.⁶⁻⁹ However, the mechanism underlying atrial selective ERP prolongation by SK channel blockers is poorly understood. The present study was designed to evaluate the electrophysiological effects of the SK channel blockers NS8593 and UCL1684^{6, 7, 10-12} in canine coronary-perfused atrial and ventricular preparations and to assess their effectiveness in preventing the development of AF in an acetylcholine-mediated AF model. We also determined their ability to inhibit sodium channel activity in HEK cells as well as in native canine atrial and ventricular myocytes.

METHODS

Adult mongrel dogs of either sex were used. This investigation conforms to the Guide for Care and Use of Laboratory Animals. Preparations consisting of right atrium with a rim of right ventricle (1.0–2.0 cm) were isolated from the canine hearts. The ostium of the right coronary artery was cannulated with polyethylene tubing (i.d., 1.75 mm; o.d., 2.1 mm) and the preparations were perfused with cold Tyrode's solution (12–15°C) containing 8.5 $[K^{+}]_0$. With continuous coronary perfusion, all ventricular branches of the right coronary artery were immediately clamped with metal clips (1.5×10 mm, Kent Scientific Corp., Torrington, CT, USA). The total time from excision of the heart to cannulation and perfusion of the artery was <4 min. Ventricular and atrial coronary branches were ligated using silk thread. The preparations were placed in a temperature-controlled bath (8 × 6 × 4 cm) and perfused at a rate of 8–10 ml/min using Tyrode's solution. 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, bubbled with 95% O_2 and 5% CO_2 (37±0.5 °C, pH=7.35). The perfusate was delivered to the artery by a roller pump. An air trap was used to avoid bubbles in the perfusion line.

Transmembrane action potential (AP) recordings (sampling rate 41 kHz) were obtained using 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 coronary-perfused preparations (Fig. 1). **Effective refractory period (ERP)** was measured by delivering premature stimuli after every 10th basic beat at a pacing cycle length (CL) of 500 ms. **The diastolic threshold of excitation (DTE)** was determined by increasing stimulus intensity in 0.01 mA steps starting from 0.1 mA, until a steady 1:1 activation was achieved. **The shortest S₁-S₁ pacing interval** permitting a 1:1 activation was measured at a 2xDTE, with DTE being determined at a CL of 500 ms. **Post-repolarization refractoriness (PRR)** was recognized when ERP exceeded APD₉₀ in the ventricle and APD₇₀ in atria. Under normal conditions, ventricular ERP coincided with APD₉₀, whereas atrial ERP generally coincided with APD₇₀₋₇₅.^{13, 14} In the presence of acetylcholine, atrial ERP corresponds to APD₉₀. **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 cardiac preparations.¹⁵ The effect of NS8593 and UCL1684 on V_{max} was therefore determined by selecting the largest V_{max} in the absence and presence of these agents at a CL of 500 ms in each preparation. The effect of NS8593 and UCL1684 on V_{max} was also evaluated as V_{max} changes upon acceleration from a CL of 500 to 300 ms. In vigorously beating cardiac preparations, resting membrane potential (RMP) can only be approximated. The change in RMP was approximated by comparing the largest amplitude of phase 0 of AP recorded in control and following drug administration. Significant reduction of phase 0 AP amplitude and depolarization of RMP are generally correlated.^{16, 17}

Isolation of atrial and ventricular myocytes were prepared from canine hearts using techniques described previously.¹³ Briefly, left atrial and ventricular preparations were dissected and coronary perfused first with cardioplegic solution and then with a nominally Ca²⁺-free solution (mM): NaCl 129, KCl 5.4, MgSO₄ 2.0, NaH₂PO₄ 0.9, glucose 5.5, and NaHCO₃ 20, bubbled with 95% O₂/5% CO₂ containing 0.1% BSA for a period of about 5 min. The preparations were then subjected to enzyme digestion with the nominally Ca²⁺-free solution supplemented with 0.5 mg/ml collagenase, 0.1 mg/ml protease, and 1 mg/ml BSA for 8–12 min. Then the tissues were minced and incubated in fresh buffer containing 0.5 mg/ml collagenase, 1 mg/mL BSA and agitated. The specific activities of the enzymes were as follows: Protease (Sigma P5147, 3.5 U/mg) and collagenase (Worthington, Type 2, 290U/mg). The supernatant was filtered, centrifuged at 200 rpm for 2 min, and the myocyte-containing pellet was stored in 0.5 mM Ca²⁺ HEPES buffer at room temperature.

Cell transfection.

HEK cells were grown in DMEM with Glutamax supplemented with 10% FBS in 35 mm culture dishes and placed in a 5% CO₂ incubator at 37 °C. To assess the effect on I_{Na}, HEK cells were co-transfected using FuGeneHD (Active Motif, Carlsbad, CA.) with a 1:1 molar ratio of WT human SCN5A, and WT SCN1B ion channel genes cloned into the pcDNA3.1. In addition, enhanced green fluorescent protein cDNA-Nav1.5 was tagged for identification of transfected cells for electrophysiologic study 12–24 hours after transfection.

Experimental Protocols.

The equilibration period was 30 min. The electrophysiological parameters were recorded before (control) and after addition of NS8593 or UCL1684 to the perfusion solution. The concentration of NS8593 (3 and 10 μM) was increased in a step-wise manner, with at least 30 minutes at each concentration before the start of recording. Reported data with UCL1684 (0.5 μM) were obtained following >1 hour of exposure to the drug.

The anti-AF effect of NS8593 and UCL1684 was evaluated using an acetylcholine (ACh) - mediated AF model (1 μM). ACh permits electrical induction of persistent AF in 100% atrial preparations (by premature electrical stimulation or rapid pacing). After completion of the main protocol, ACh (1 μM) was added to the coronary perfusate in the presence of 10 μM of NS8593 and 0.5 μM of UCL1684 and induction of AF was attempted using premature electrical stimulation and rapid pacing.

Voltage clamp recordings of peak I_{Na} .

Peak I_{Na} was measured as previously described with minor modification.¹³ Experiments were performed using a MultiClamp 200B (Molecular Devices, Foster City, CA). Command voltages were delivered, and data acquired via a DigiData 1550B computer interface using pClamp9 (Molecular Devices) with data stored on a computer hard disk. Patch pipettes were pulled from borosilicate glass (1.5 mm o.d. and 1.1 mm i.d.) on a Model DMZ-Universal-Electrode-puller (Zeitz-Instruments, Germany). The electrode resistance was 1.5–2.5 $\text{M}\Omega$ when filled with the internal solution (see below). The membrane was ruptured by applying negative pressure and series resistance compensated by 75 to 80%. Whole cell current data was acquired at 20–50 kHz and filtered at 5 kHz. Cell capacitance was measured by integrating the transient charge following application of a -5mV voltage clamp step. Currents were normalized to cell capacitance and expressed as density (pA/pF). External solution contained the following (in mM): choline Cl 120, NaCl 10, Na⁺ acetate 2.8, CaCl₂ 0.5, KCl 4, MgCl₂ 1.5, CoCl₂ 1, glucose 10, HEPES 10, NaOH 5, and BaCl₂ 0.1, pH adjusted to 7.4 with NaOH/HCl. The pipette solution contained the following (mM): NaCl 15, CsF 120, MgCl₂ 1, KCl 5, HEPES 10, Na₂ATP₄, and EGTA 10, pH adjusted to 7.2 with CsOH. Recordings of I_{Na} were made at least 5 min after cell membrane rupture to minimize the effects of time-dependent negative shift of steady-state (SS) inactivation that occurs in conventional voltage clamp experiments. Whole cell currents were analyzed using the Clampfit analysis program pClamp 9 (Molecular Devices).

Drugs:

NS8593 and UCL1684 (NeuroSearch, Denmark) were dissolved in 100% DMSO as a stock of 10 and 1 mM, respectively.

Statistics:

Statistical analysis was performed using paired or unpaired t test and one-way repeated measures analysis of variance (ANOVA) followed by Bonferroni's or Student-Newman-Keuls test, as appropriate. All tissue data are expressed as mean \pm SD and single cell data as mean \pm SEM.

RESULTS:

Electrophysiological effects of NS8593 and UCL1684 in atria and ventricles

NS8593 (3–10 μM) caused marked depression of excitability in atria, but not in ventricles. At 10 μM and a CL of 500 ms, 2 of 8 atria but 0 of 5 ventricles were markedly depressed, failing to respond to stimulation in a 1:1 fashion, so that the electrophysiological parameter could not be reasonably measured in these two atria. NS8593 (3–10 μM) produced little to no change in APD_{70-90} in either atria or ventricles (Fig. 1). At a concentration of 3 μM , NS8593 prolonged ERP only in atria whereas at a concentration of 10 μM , ERP was prolonged in both atria and ventricles, but to a much greater extent in the atria (Fig. 1). The NS8593-mediated ERP prolongation was due to induction of PRR, which was much greater in atria vs. ventricles (Fig. 1). NS8593 significantly decreased V_{max} in an atrial-selective manner. However, a significant reduction of V_{max} was evident in the ventricles as well (Fig. 2). DTE and the shortest $S_1 - S_1$ pacing interval permitting 1:1 activation was also affected by NS8593 in an atrial-selective manner (Fig. 3). Of note, at an NS8593 concentration of 10 μM and CL of 300 ms, 0/8 atria but 5/5 ventricles displayed 1:1 activation (with a DTE_{ex10} determined at a CL of 500 ms), demonstrating potent rate-dependent atrial-selective effect of NS8593 on excitability. Electrophysiological effects of UCL1684 in the cardiac preparations were similar to those observed with NS8593. APD_{70-90} was not significantly altered in both atrial and ventricular preparations and ERP was prolonged in an atrial-selective manner due to development of PRR (Fig. 4). V_{max} , DTE, and the shortest S_1-S_1 were also affected exclusively or primarily in atria vs. ventricles (Fig. 4).

The maximum amplitude of atrial APs recorded in the presence of 3 and 10 μM of NS8593 and 0.5 μM of UCL1684 was 96 ± 7 , 73 ± 13 , and $94 \pm 6\%$, respectively, of those recorded under control conditions ($p=0.14$, $p<0.001$, and $p=0.09$, respectively; CL = 500 ms; $n=6-8$), consistent with the effect of these agents to block I_{Na} and suggesting the possibility that 10 μM NS8593 depolarized RMP. The maximum amplitude of APs recorded from the ventricles was not statistically significantly affected by 3 or 10 μM NS8593 or 0.5 μM UCL1684 (98 ± 6 , 94 ± 10 and $98 \pm 5\%$ of controls, respectively $n=8-10$, CL 500 ms).

Effect of NS8593 and UCL1684 to suppress atrial fibrillation

ACh alone (1 μM) significantly abbreviated atrial APD and ERP, permitting induction of persistent AF in 100% of atria (Fig. 5; Table 1). Addition of ACh in the presence of NS8593 (10 μM) or UCL1684 (0.5 μM) resulted in a much smaller abbreviation of APD and ERP than observed with ACh alone (Table 1). Both drugs maintained their ability to induce PRR in the presence of ACh. Rate-dependent excitability was also significantly depressed, as evident by prolongation of the shortest S_1-S_1 interval, so that ERP at rapid activation rates was far longer than APD (Table 1). AF could not be induced in the presence of these agents in any of the preparations tested (Table 1). Potent anti-AF action of the drugs was associated with strong rate-dependent depression of excitability, which did not permit rapid activation of the atrium (Fig. 5; Table 1).

Effects of NS8593 and UCL1684 on I_{Na}

Both agents significantly reduced I_{Na} in HEK cells, measured at HP of -80 mV (Fig. 6A). To test the hypothesis that the effect of SK channel blockers to produce atrial-selective depression of excitability is the result of inhibition of I_{Na} , we studied the direct effect of NS8593 on inhibition of I_{Na} in isolated canine atrial and ventricular myocytes at two HPs (-120 and -90 mV) using patch clamp techniques. NS8593 (10 μ M) caused a minor non-significant reduction of I_{Na} in both atrial and ventricular myocytes at a HP of -120 mV but a potent atrial- predominant decrease of I_{Na} at a HP of -90 mV (Fig. 6B). Steady-state inactivation of the sodium channels (h-curve) was not affected by NS8583 (10 μ M) in the ventricular myocytes, but was shifted to negative potentials in the atrial myocytes (Fig. 6C). As we reported previously,¹³ the h-curve of atrial cells is typically more negative than that of ventricular cells in the absence of drug (Fig. 6C).

DISCUSSION:

The principal findings of the present study are that both SK channel inhibitors studied cause electrophysiological alterations in canine cardiac single cells and multicellular preparations (i.e., reduction of I_{Na} density, negative shift of SS-inactivation, PRR induction, reduced V_{max} , and increased DTE) consistent with potent atrial-selective inhibition of sodium channel activity. Both drugs have previously been shown to inhibit SK channels^{11, 12} and these channels have previously been shown to be expressed in canine atria.¹¹ However, the absence of APD prolongation in our atrial preparations with either NS8593 or UCL1684 suggests little to no functional contribution of SK channel inhibition in canine atria under these experimental conditions. Our data demonstrate an effect of both NS8593 and UCL1684 to prevent induction of vagally-mediated AF, due to potent depression of excitability secondary to block of sodium channel activity. Collectively, our findings suggest that SK channel inhibition by NS8593 and UCL1684 does not contribute meaningfully to their potent anti-AF effects.

SK channels have been identified in the hearts of mouse, rat, rabbit, dog, and human and the Ca^{+2} -activated potassium current (I_{SK}) carried by these channels is exclusively or largely expressed in the atrium.^{6, 8, 10, 18} The selective expression of I_{SK} in the atria is thought to make these channels potential targets for effecting atrial-selective APD and ERP prolongation, thus contributing to the management of AF without adverse consequences in the ventricles.^{3, 4, 8} Some agents that block the SK channels (such as NS8553, UCL1684, ICA, ICAGEN, AP14145, and AP30663) have been shown to prolong atrial ERP and suppress AF without prolongation of QT in experimental settings.^{6, 7, 9, 19} There are however reports demonstrating a pro-AF effect induced by genetic ablation of SK channels³ or SK channel inhibition with apamin.²⁰ The mechanism of atrial-selective ERP prolongation by SK channel inhibitors is not well defined. These inhibitors may or may not prolong atrial APD.^{5, 8} Similar to the results of the present study (Figs. 1–4), previous studies have reported that NS8593, UCL1684, and ICA produce atrial-selective alterations in sodium-channel mediated parameters, i.e., reducing V_{max} , slowing conduction, and inducing PRR,^{10, 21} indicating atrial-selective inhibition of the sodium channel. However, the effect of these agents to depress sodium channel parameters were attributed to an effect of I_{SK}

inhibition to effect depolarization of RMP. A significant NS8593-induced reduction of V_{\max} in the ventricles (where SK channel are not or poorly expressed) clearly points to a direct effect of NS8593 on I_{Na} (Fig. 2). Of note, the concentration of UCL1684 used in the current study was relatively low, resulting in minor depression of sodium-channel-mediated parameters in the ventricle (Fig. 4). In the present study, we also demonstrate a direct effect of NS8593 and UCL1684 to inhibit I_{Na} using both isolated canine myocytes and HEK cells expressing the alpha and beta1 subunits of the cardiac sodium channel. Moreover, we demonstrate that I_{Na} inhibition and its atrial-selectivity are critically dependent on holding potential (HP). Inhibition of I_{Na} is clearly evident at a HP of -90 mV, but not at a HP of -120 mV (Fig. 6).

It is important to recognize that the ability of the other SK channel blockers (i.e., ICA, ICAGEN, AP14145, AP30663, etc.) to inhibit the sodium channel have either not been tested or have been tested at a HP of -120 mV. Our data emphasize the need to test such agents under physiologically relevant conditions (i.e., HPs and pacing rates) in order to avoid missing their atrial-selective actions to inhibit I_{Na} . Our observation with regard to the SK channel inhibitors is reminiscent of the recent experience with I_{Kur} blockers, including vernakalant, AVE0118, AZD1305, which upon close examination proved to exert their actions to prolong ERP solely or largely via atrial-selective inhibition of sodium channel current and not as a result of inhibition of I_{Kur} .^{15, 22–24} Our findings provide yet another example of potassium channel blockers exerting their antiarrhythmic effect principally via inhibition of sodium channel activity.

I_{Na} blockers are known to abbreviate atrial APD, via inhibition of late I_{Na} ²⁵ and it is possible that a similar action on the part of NS8593 and UCL1684 might have negated the effect of SK channel inhibition to prolong atrial APD.

As discussed above, the depression of I_{Na} produced by SK channel blockers has in previous studies been attributed to drug-induced depolarization of RMP in atria.²¹ This suggestion is based largely on the observation that RMP is slightly depolarized in the presence of high concentrations of NS8553, ICAGEN, and ICA in human and rat atrial cells and tissues (by 2–4 mV).²¹ The contribution of such RMP depolarization is unlikely to provide an explanation for the strong reduction in sodium channel activity because a 2–4 mV depolarization cannot account for the 40–50% reduction of V_{\max} recorded.^{17, 21} Our demonstration using patch clamp techniques of a direct effect of NS8593 or UCL1684 to significantly produce an atrial-selective inhibition of I_{Na} (Fig. 6) can account for the atrial-selective induction of PRR and reduction of V_{\max} . It stands to reason that concomitant depolarization of RMP by whatever means is likely to accentuate the effect of the SK channel blockers to inhibit I_{Na} . Several studies have reported that inhibition of the SK channels alone does not alter RMP.^{3, 5, 8, 26} Also relevant to this issue is the observation that relatively high concentrations of I_{Na} blockers are associated with depolarization of RMP, suggesting that depolarization of RMP observed in some settings may be secondary to I_{Na} inhibition rather than SK channel inhibition.^{16, 27, 28}

Moreover, ICA at 10 μ M has been shown to be effective against AF but to only depolarize atrial RMP by 1–2 mV (non-significantly) in rat cardiac preparations.^{21, 29} Further studies

are needed to assess the contribution of SK channel inhibitors to indirect inhibition of I_{Na} via depolarization of RMP as well as the contribution of this mechanism to their anti-arrhythmic efficacy.

Atrial selective peak I_{Na} block is a typical feature of many I_{Na} blockers displaying relatively rapid unbinding kinetics, including ranolazine, amiodarone, vernakalant, dronedarone and AZD1305.^{13, 22–24, 30–32} Previous work from our group has shown that atrial selectivity of I_{Na} blockers is due to several factors including: 1) the presence of a more depolarized resting membrane potential in atria vs. ventricles; 2) a more negative half-inactivation voltage for the sodium channel in atrial vs. ventricular cells^{33–35} (both of these factors reduce the availability of sodium channels in atria more than ventricles) and 3) a more gradual phase 3 repolarization in atrial cells; this characteristic of the atrial action potential leads to progressive diminution or disappearance of the diastolic interval at rapid rates of activation, which reduces the ability of sodium channel blockers to dissociate from the sodium channel, thus leading to accumulation of sodium channel block. These distinctions between atrial and ventricular cell electrophysiology led us to suggest a novel strategy for AF suppression, namely atrial-selective sodium channel block.^{13, 33, 34} The ability of NS8593 to preferentially shift atrial $V_{0.5}$ to more negative potentials contributes to further accentuation of the atrial-selective inhibition of I_{Na} (Fig 6).

Both NS8593 and UCL1684 stunted the effect of ACh to abbreviate atrial APD. This would appear to be consistent with SK channel inhibition, but because ACh dramatically reduces intracellular calcium activity,³⁶ it is expected to reduce SK channel activity (assuming that calcium activity is reduced in the vicinity of the SK channels). Accordingly, inhibition of SK channel is unlikely to prolong APD owing to the dramatically reduced levels of intracellular calcium. The effect NS8593 and UCL1684 to blunt the ability of ACh to abbreviate APD is similar to that reported with other sodium channel blockers. Previous studies have shown that all sodium channel blockers tested at therapeutically-relevant concentrations in the coronary perfused canine right atrial preparations, including ranolazine, lidocaine, propafenone, amiodarone, AZD1305, Wenxin Keli, blunt the effect ACh to abbreviate atrial APD.^{13, 23, 30, 37, 38} Similar data have been reported with regard to the effect of flecainide and ICA to blunt the ability of carbachol to abbreviate repolarization in rat atrial tissues.²⁹ These effects may be due to the ability of I_{Na} blockers to directly inhibit I_{K-ACh} as previously reported for flecainide, propafenone, disopyramide, and pilsicainide.^{39, 40} Because the extent of PRR prolongation greatly exceeded that of APD prolongation in the presence of ACh (Table 1), it can be argued that sodium channel block by NS8593 and UCL1684 is the most important driver underlying the observed anti-arrhythmic effect of these agents.

Study limitations:

The electrophysiological actions of NS8593 and UCL1684 were studied acutely in isolated normal non-remodeled Tyrode's-perfused atrial preparations and acutely dissociated atrial and ventricular myocytes. SK channel expression is reported to be augmented in diseased ventricles (e.g., in heart failure),⁴¹ thus reducing the predominance of SK channels in atria. This however is unlikely to alter the principal conclusion of our study, i.e., that the

electrophysiological and anti-AF effects of NS8593 and UCL1684 are due to atrial selective I_{Na} inhibition. We studied the efficacy of NS8593 and UCL1684 to prevent but not to terminate AF. It is possible that SK channel inhibition by these agents may play more of a role in suppressing AF since AF is known to increase intracellular Ca^{2+} activity (including during ACh-mediated AF³⁶), which may increase the influence of SK channels on atrial repolarization and thus the efficacy of SK channel blockers to prolong atrial ERP during AF. This hypothesis remains to be tested with specific SK channel blockers. The presence of autonomic influences and other factors present *in vivo* may modulate the effect of the drugs, resulting in outcomes different from those observed in the present study.

CONCLUSION

NS8593 and UCL1684 are very effective in preventing the development of AF in our ACh models of AF. The electrophysiological alterations induced by NS8593 and UCL1684 in canine cardiac preparations and isolated cells suggest that these compounds exert their electrophysiological and anti-AF effects predominantly or exclusively via atrial-selective inhibition of the sodium channel. These conclusions pertain to the specific drugs and conditions studied and cannot be directly applied to other conditions or other SK channel blockers. Our findings, coupled with available data, suggest that NS8593 and UCL1684 are effective for the management of AF and lack any pro-arrhythmic liability in the ventricle because of their atrial-selectivity.

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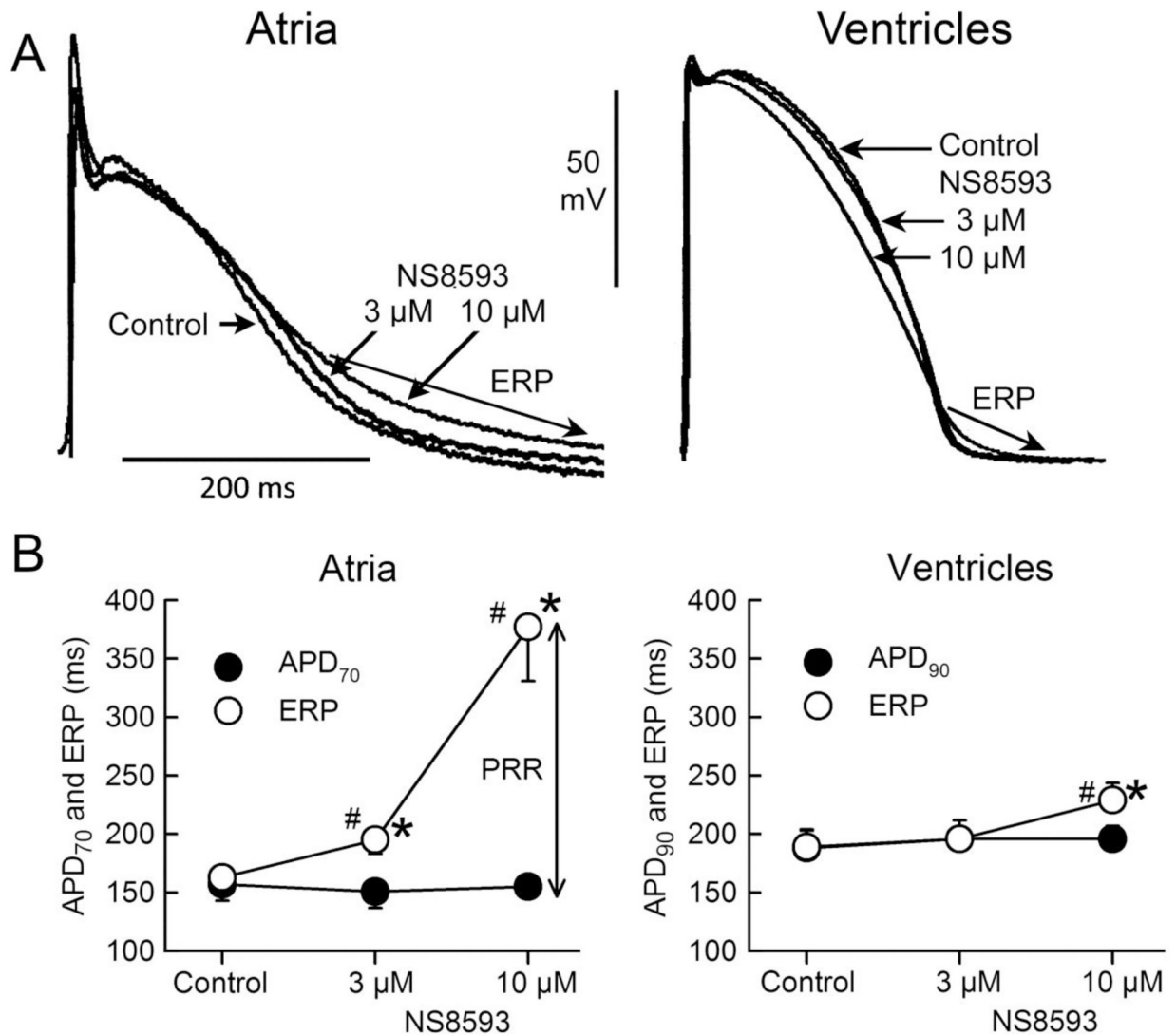


Figure 1. NS8593 produces atrial-selective prolongation of effective refractory period (ERP) due to development of post-repolarization refractoriness (PRR). Little change is observed in action potential duration (APD) in either atria or ventricles.

A: Atrial and ventricular superimposed action potentials recorded under control conditions and following addition of 3 and 10 μ M NS8593 to the coronary perfusate.

B: Summary data of the effect of NS8593 to alter APD and ERP in atria and ventricles. Note that ERP corresponds to APD₇₀₋₇₅ in atria and to APD₉₀ in ventricles. N=6 for atria and 8 for ventricles because 2 out of the 8 atria were either very depressed or inexcitable in the presence of 10 μ M NS8593. CL = 500 ms. * p<0.05 vs. control. # - p<0.05 vs. respective APD₇₀ value.

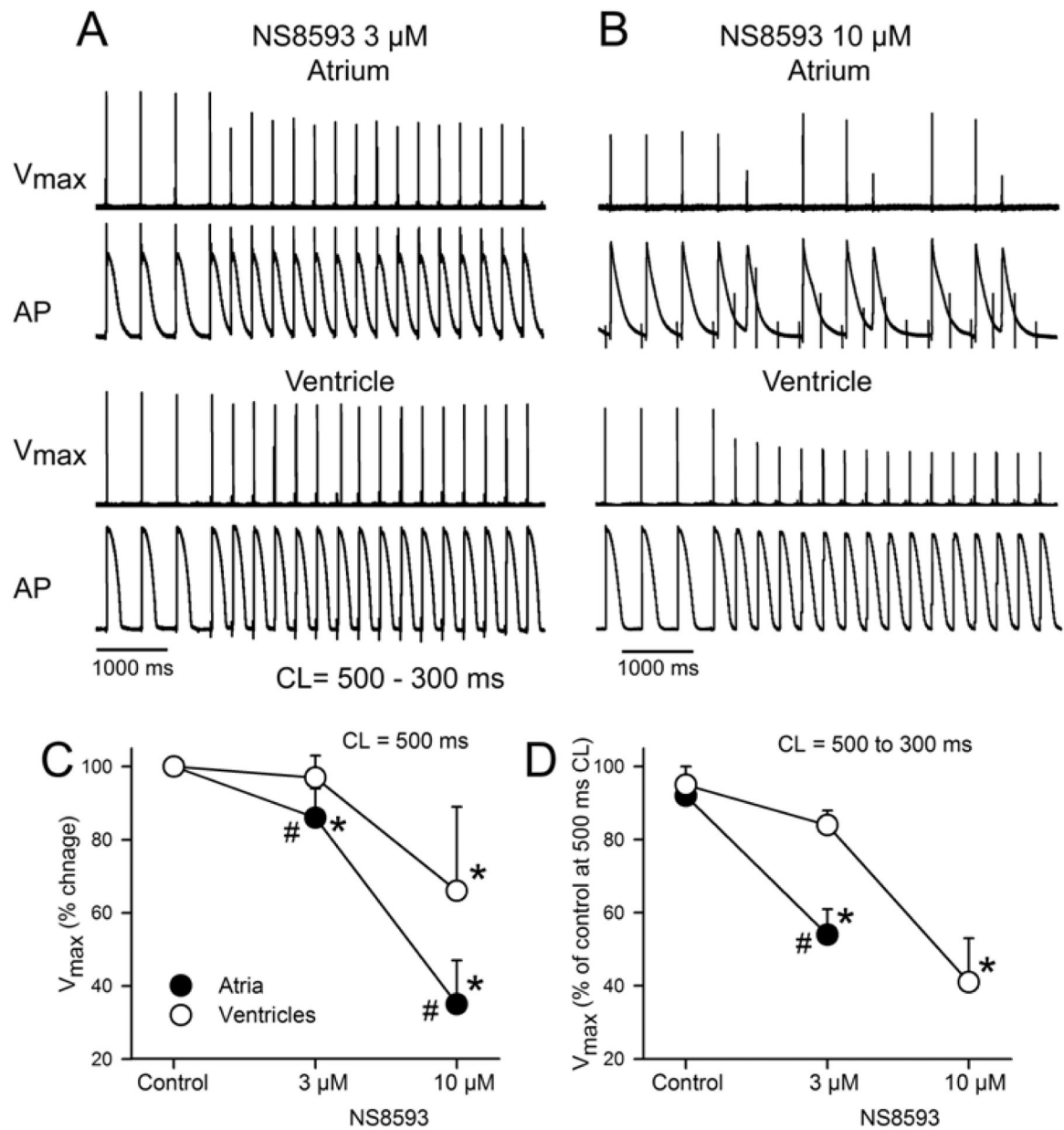


Figure 2. Atrial-selective rate-dependent reduction of maximum rate of rise of the action potential (AP) upstroke (V_{max}) and depression of excitability by NS8593.

A and **B**: Action potentials and corresponding V_{max} values recorded from atria and ventricles during acceleration of pacing rate from a CL of 500 to 300 ms following exposure to 3 or 10 μM of NS8593. Failure of 1:1 activation was observed in 100% of atrial preparations at a CL of 300 ms in the presence of 10 μM NS8593. **C**: Composite data of V_{max} of atria and ventricles at a CL of 500 ms expressed as % of control. **D**: Composite data of V_{max} of atrial and ventricular APs following acceleration from a CL of 500 to 300 ms expressed as % of V_{max} value at a CL of 500 ms in Controls. $N=6-8$. * - $p<0.05$ vs. respective control. # - $p<0.05$ vs. respective ventricular values.

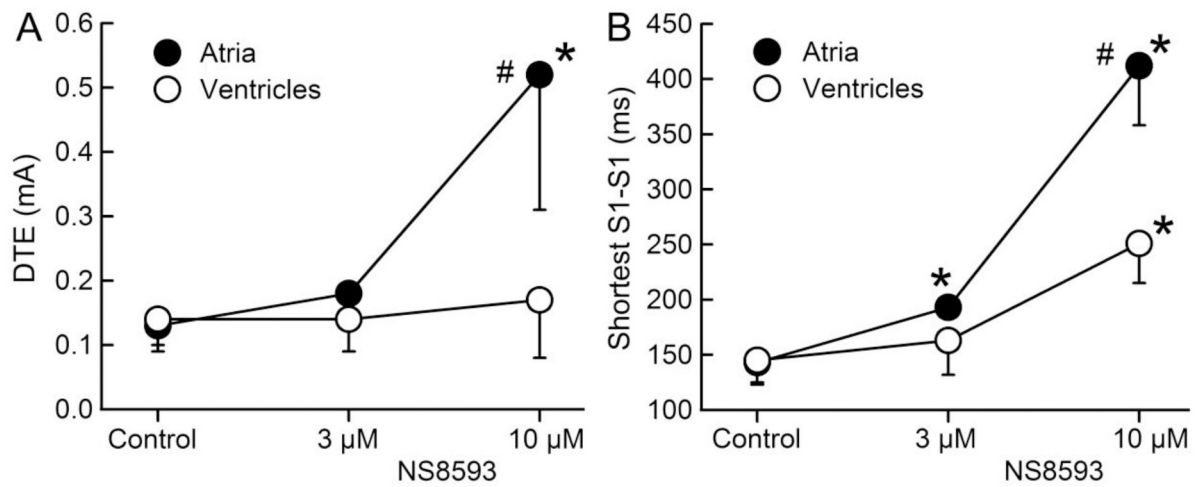


Figure 3. NS8593 increases diastolic threshold of excitation (DTE) and prolongs the shortest $S_1 - S_1$ interval permitting 1:1 activation much more in atria than in ventricles. * $p < 0.05$ vs. control. # - $p < 0.05$ vs. respective ventricular value. $N=6-8$. DTE was measured at a CL of 500 ms.

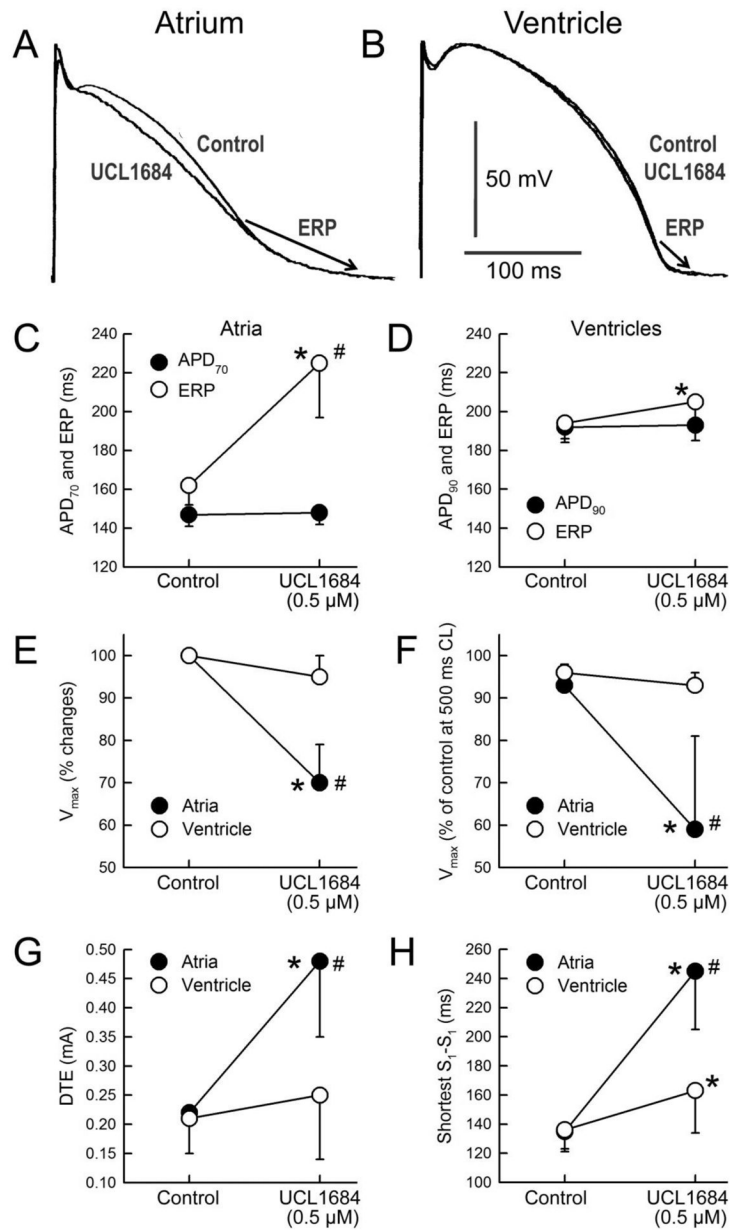


Figure 4. Atrial selective electrophysiological effects of UCL1684 in the induction of PRR (A-D), reduction of V_{max} (E-F), increase in DTE (G), and prolongation of the shortest S_1 - S_1 interval permitting 1:1 activation (H). Composite data of V_{max} of atrial and ventricular APs following acceleration from a CL of 500 to 300 ms expressed as % of V_{max} value recorded at a CL of 500 ms in Controls (F). * $p < 0.05$ vs. control. # - $p < 0.05$ vs. respective ventricular value. $N=7-10$. Data presented in C, D, E, and G were obtained at a pacing CL = 500 ms.

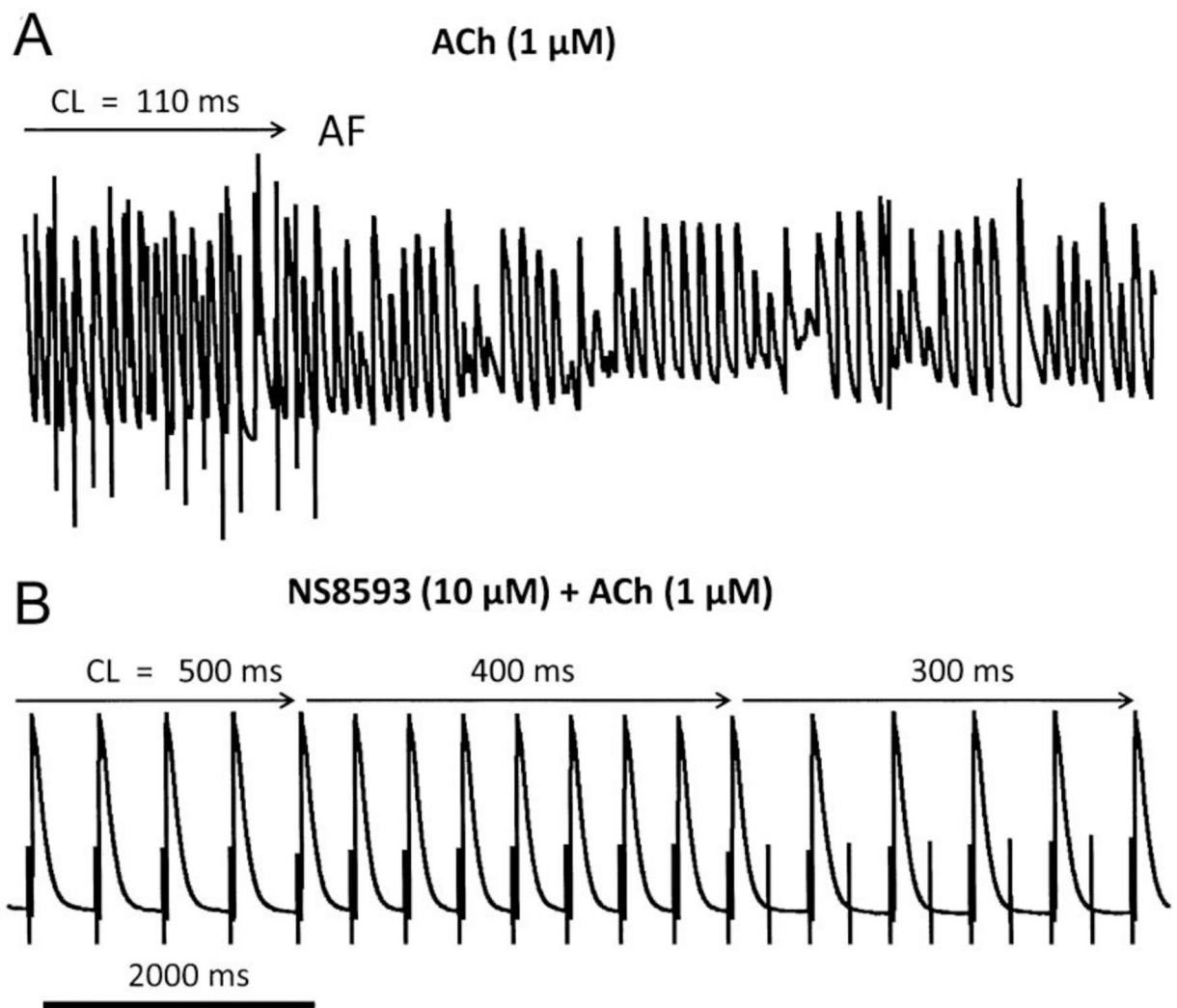


Figure 5. NS8593 (10 μ M) prevents the induction of acetylcholine (ACh) - mediated atrial fibrillation largely by depressing excitability. Shown are action potential tracings. **A:** Rapid pacing induced AF in the presence of ACh alone. **B:** Rapid pacing fails to induce AF in the presence of NS8593 and ACh due to significant rate-dependent depression of excitability, manifested as a failure of 1:1 activation.

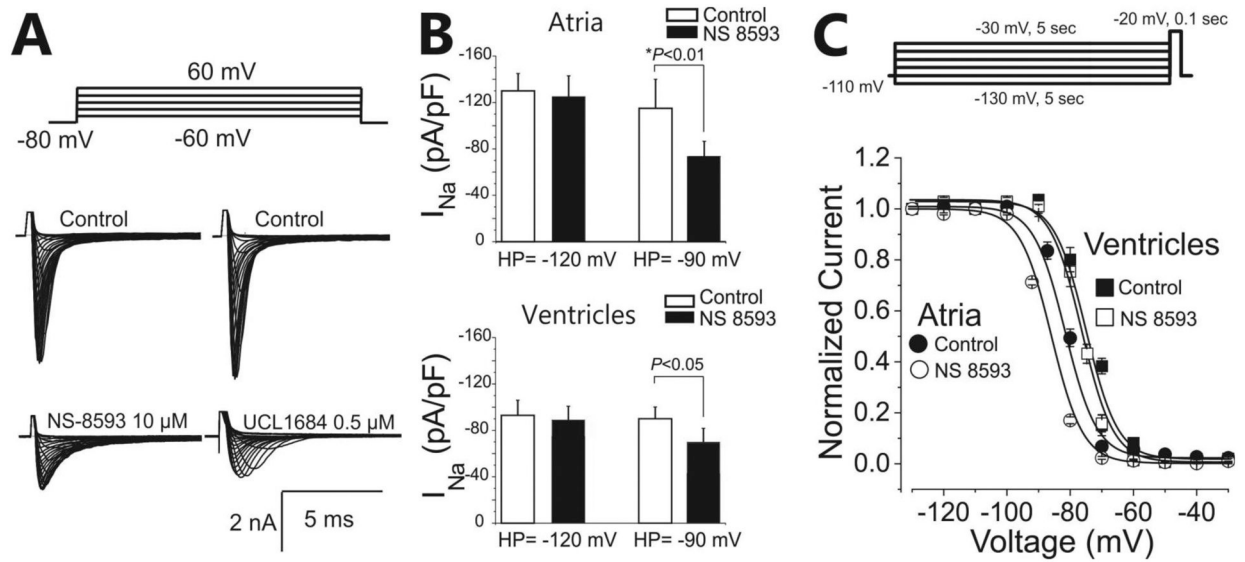


Figure 6. UCL1684 and NS8593 produce direct atrial-selective inhibition of sodium channel current (I_{Na}) and shift steady-state inactivation of the cardiac sodium channels.

A: Representative I_{Na} recordings from a Nav1.5-WT plus NavB1-WT expressed in HEK cells in presence of UCL1684. **B:** Effect of NS8593 (10 μ M) effect on sodium channel current density at test potential of -20 mV in canine atrial and ventricular myocytes recorded at holding potentials (HP) of -120 mV and -90 mV. **C.** Effect of NS8593 (10 μ M) on steady-state inactivation of the sodium channels in atrial and ventricular cardiac myocytes.

Table 1.

Effects of ACh on atrial electrophysiological parameters in the presence of NS8593 and UCL1684 and on induction of persistent AF in isolated canine coronary-perfused right atria.

	APD ₉₀ (ms)	APD ₇₀ (ms)	ERP (ms)	PRR (ms)	Shortest S ₁ -S ₁ (ms)	Induction of AF
Control	204±11	153±9	158±17	4±7	129±8	0% (0/10)
ACh (1 μM)	51±8	48±7	61±8	10±8	65±14	100% (10/10)
NS8593 (10 μM) + ACh (1 μM)	115±11 *	104±10 *	332±56 †#	217±56 †	371±76 †	0% (0/6)
UCL1684 (0.5 μM) + ACh (1 μM)	94±12 *	87±11 *	177±64 †#	83±51 †	205±52 †	0% (0/8)

Data were obtained at a cycle length (CL) of 500 ms (n=6 for each).

* <0.05 vs. acetylcholine alone (ACh, 1.0 μM).

† - p<0.001 vs. acetylcholine alone.

- p<0.001 vs. APD₉₀.

Post-repolarization refractoriness (PRR) was defined as the difference between ERP and APD₉₀ in the presence of ACh, and between ERP and APD₇₀ in control. ACh was added in the presence of the SK channel blockers. The APD and ERP values for ACh alone are historical data collected in similar preparations in previous studies.