# Inhibition of $I_{Kr}$ potentiates development of atrial-selective $I_{Na}$ block leading to effective suppression of atrial fibrillation

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**BACKGROUND** The availability of safe and effective drugs for the management of atrial fibrillation (AF) remains an unmet medical need.

**OBJECTIVES** The purpose of this study was to test the hypothesis that the inhibition of the rapidly activating delayed rectifier potassium current ($I_{Kr}$) greatly potentiates the development of atrial-selective sodium channel current ($I_{Na}$) block, leading to more effective suppression of AF.

**METHODS** Electrophysiological and anti-AF effects of highly selective $I_{Na}$ and $I_{Kr}$ blockers (lidocaine and E-4031) individually and in combination were determined in canine coronary-perfused atrial and ventricular preparations. Acetylcholine (1 μM) was used to induce persistent AF.

**RESULTS** Lidocaine (10 μM) caused a relatively small abbreviation of the action potential duration measured at 90% repolarization in both atria and ventricles, but caused atrial-selective prolongation of the effective refractory period owing to the induction of post-repolarization refractoriness. Lidocaine also caused modest atrial-selective depression of other $I_{Na}$-mediated parameters including excitability, maximum rate of rise of the action potential upstroke, and conduction time. E-4031 (1 μM) prolonged the action potential duration measured at 90% repolarization and effective refractory period in an atrial-predominant manner. A combination of lidocaine and E-4031 caused a greater atrial-selective depression of $I_{Na}$-mediated parameters. Persistent acetylcholine-mediated AF developed in 100% of atria under control conditions, in 80% (4 of 5) after pretreatment with lidocaine (10 μM), in 100% (4 of 4) after E-4031 (1 μM), and in only 14% (1 of 7) after the combination of lidocaine and E-4031.

**CONCLUSION** Our results provide a proof of concept that $I_{Kr}$ block greatly potentiates the effects of rapidly dissociating $I_{Na}$ blockers to depress sodium channel–dependent parameters in the canine atria but not in the ventricles, thus contributing significantly to suppression of AF.

**KEYWORDS** Electrophysiology; Pharmacology; Antiarrhythmic drugs; Lidocaine; Atrial fibrillation

**ABBREVIATIONS** **ACh** = acetylcholine; **AF** = atrial fibrillation; **AP** = action potential; **APD** = action potential duration; **$APD_{50}$, $APD_{70}$, $APD_{90}$, and $APD_{95}$** = action potential duration measured at 50, 70, 90, and 95% repolarization, respectively; **CL** = cycle length; **DI** = diastolic interval; **DTE** = diastolic threshold of excitation; **ERP** = effective refractory period; **IKr** = rapidly activating delayed rectifier potassium current; **IKur** = ultra-rapid delayed rectifier potassium current; **INa** = sodium channel current; **PRR** = post-repolarization refractoriness; **SK** = small conductance $Ca^{2+}$-activated potassium channel; **$V_{max}$** = maximum rate of rise of the action potential upstroke

(Heart Rhythm 2015;12:836–844) 

## Introduction

Atrial fibrillation (AF) is a growing clinical problem associated with increased morbidity and mortality.[1] An important limitation of currently available antiarrhythmic agents for the management of AF is the risk of induction of life-threatening ventricular arrhythmias. This limitation has generated interest in the development of atrial-selective drugs capable of prolonging the effective refractory period (ERP) in the atria but not in the ventricles.[2–4] Prolongation of the ERP is a fundamental property shared by effective anti-AF agents. Agents that inhibit sodium channel current ($I_{Na}$) with rapid dissociation kinetics have been shown to produce atrial-selective prolongation of the ERP owing to atrial-selective induction of post-repolarization refractoriness (PRR).[2,5] Block of the rapidly activating delayed rectifier potassium current ($I_{Kr}$) channels can also produce an atrial-predominant prolongation of the ERP owing to preferential prolongation of the action potential duration (APD) in the atrial vs ventricular myocardium.[6–8] Multichannel blockers

This work was supported by a grant from Gilead Sciences, the National Heart, Lung, and Blood Institute grant HL47678 (to Dr Antzelevitch), the New York State Stem Cell Science grant C026424 (to Dr Antzelevitch), and Masons of New York, Florida, Massachusetts, Connecticut, Rhode Island, Maryland, and Wisconsin. Dr Antzelevitch is a consultant to and receives research support from Gilead Sciences. Dr Belardinelli is an employee of Gilead Sciences. **Address reprint requests and correspondence:** Dr Charles Antzelevitch, Masonic Medical Research Laboratory, 2150 Bleecker St, Utica, NY 13501. E-mail address: cantzelevitch@gmail.com.

that inhibit $I_{Na}$ with rapid kinetics as well as $I_{Kr}$ (such as ranolazine and amiodarone) have been shown to cause prominent atrial-selective ERP prolongation and thus to effectively suppress the development of AF.[2,8,9] Multi-ion channel blockers have been shown to produce a greater degree of atrial selectivity in their actions to depress sodium channel–dependent parameters at rapid rates of activation. The present study is designed to provide a proof of concept that $I_{Kr}$ inhibition greatly potentiates the effects of rapidly dissociating $I_{Na}$ blockers to depress sodium channel–dependent parameters in the canine atria but not in the ventricles, thus contributing significantly to suppression of AF.

## Methods

This investigation conforms to the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health (NIH Publication No. 85-23, Revised 1996) and was approved by the Animal Care and Use Committee of the Masonic Medical Research Laboratory.

Mongrel dogs ($\geq$1 year old) were anticoagulated with heparin and anesthetized with pentobarbital (intravenously an initial dose of 30–35 mg/kg, and if needed, an additional dose of 15–20 mg/kg). After the total loss of corneal reflex, 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. Preparations consisting of the whole right atrium with a rim of the right ventricle (1–1.5 cm) were isolated from the canine hearts. The ostium of the right coronary artery was cannulated with polyethylene tubing (inner diameter 1.75 mm; outer diameter 2.1 mm), and the preparations were perfused with cold Tyrode's solution (12–15ºC) containing 8.5 mM $[K^+]_o$. With continuous coronary perfusion, all ventricular and atrial cut 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 with the Tyrode's solution. The composition of the Tyrode's solution was (in mM) as follows: 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°C ± 0.5°C; pH 7.35). The perfusate was delivered to the artery via a roller pump, and 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 direct current resistance) connected to a high input impedance amplification system (World Precision Instruments, Sarasota, FL). The signals were amplified, digitized, and analyzed (Spike2, Cambridge Electronic Design, Cambridge, England). A pseudo-electrocardiogram was recorded using 2 electrodes consisting of AgCl half cells attached to Tyrode's solution–filled tapered polyethylene electrodes that were placed in the bath solution 1.0–1.2 cm from the opposite ends of the preparations. The diastolic threshold of excitation (DTE) was determined by increasing stimulus intensity in 0.01-mA steps. ERP was measured by delivering premature stimuli after every 10th regular beat at pacing cycle lengths (CLs) of 500 ms (with 5–10 ms resolution; stimulation intensity was 2× DTE). The shortest $S_1$-$S_1$ interval permitting 1:1 activation was measured by progressively shortening the CL, starting from a CL of 500 ms (at an intensity of 2× DTE determined at a CL of 500 ms). PRR was recognized when ERP exceeded APD measured at 90% repolarization ($APD_{90}$) in the ventricle and $APD_{70}$ in the atrium. Note that ventricular ERP coincided with $APD_{90}$, whereas atrial ERP generally coincided with $APD_{70–75}$.[2] Diastolic interval (DI) was measured as the difference between the pacing CL and $APD_{95}$. Stable AP recordings could not be readily obtained in the vigorously contracting coronary-perfused preparations. A large variability in maximum rate of rise of the AP upstroke ($V_{max}$) measurements is normally encountered at any given condition, primarily owing to variability in the amplitude of phase 0 of the AP that strongly determines $V_{max}$ values.[10] The largest recorded $V_{max}$ values per experimental condition were taken for statistical comparison. Because of a substantial inter-preparation variability, $V_{max}$ values were normalized for each experiment and then averaged. Changes in conduction time in the ventricles and atria were assessed by measuring the duration of the electrocardiographic QRS and P waves, respectively (at 50% of the total amplitude).

### Experimental protocols

The equilibration period for the preparations was 30 minutes. Recording were obtained at a pacing CL of 500 ms. Rate-dependent changes in $V_{max}$ were measured from the 20th beat after acceleration from a CL of 500–300 ms (during stable impalements). Ventricular data were obtained from the endocardial base region and atrial data from the endocardial pectinate muscle. The preparations were exposed to 10 μM lidocaine, 1 μM E-4031 (after 30-minute washout of lidocaine), and the combination of these agents for a period of at least 20 minutes. At these concentrations, lidocaine and E-4031 are selective for block of $I_{Na}$ and $I_{Kr}$, respectively.[11,12]

To assess the anti-AF potential of lidocaine (10 μM), E-4031 (1 μM), and their combination, we used an acetylcholine (ACh) (1.0 μM)–mediated AF model. The effects of monotherapy with high concentrations of lidocaine (30 μM) and E-4031 (5 μM) were investigated as well. In the presence of ACh, premature electrical stimulation or rapid pacing (CL 50–80 ms) induces persistent AF in 100% of canine coronary-perfused right atrial preparations (defined as AF persisting for $>$2 hours).[2]

### Drugs

Lidocaine and ACh (both Sigma, St. Louis, MO) and E-4031 (Eisai Co., Ltd., Tokyo, Japan) were dissolved in distilled water in a stock solution of 1–30 mM.