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Ryanodine receptor inhibition potentiates the activity of Na channel blockers against spontaneous calcium elevations and delayed afterdepolarizations in Langendorff-perfused rabbit ventricles

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Abstract

BACKGROUND—Na channel blockers are effective in suppressing delayed afterdepolarizations (DADs) in isolated Purkinje fibers. However, in isolated mouse ventricular myocytes lacking calsequestrin, only those Na channel blockers that also inhibit type 2 ryanodine receptor channels were effective against spontaneous Ca elevation (SCaE) and DADs.

OBJECTIVE—To test the hypothesis that combined Na channel and type 2 ryanodine receptor channel blocker ((*R*)-propafenone) is more effective than a Na channel blocker (lidocaine) in suppressing SCaE and DADs in the intact rabbit ventricles.

METHODS—We compared (*R*)-propafenone (3 μ mol/L) with lidocaine (50 μ mol/L) on SCaE and DADs by using epicardial optical mapping of intracellular calcium (Ca_i) and membrane voltage in Langendorff-perfused rabbit hearts. SCaE and DADs were induced by rapid pacing trains and isoproterenol (0.3 μ mol/L) infusion. One arbitrary unit is equivalent to the Ca transient amplitude of paced beats.

RESULTS—SCaEs were observed at the cessation of rapid pacing in all hearts at baseline. (*R*)-Propafenone nearly completely inhibited DADs and SCaE (0.04 arbitrary units [95% confidence interval 0.02–0.06] vs 0.23 arbitrary units [95% confidence interval 0.18–0.28] at baseline; $n = 6$ hearts; $P < .001$). Lidocaine also significantly reduced the SCaE but was significantly ($P < .05$) less effective than (*R*)-propafenone. Both drugs increased the rise time of action potential upstroke and reduced conduction velocity to a similar extent, suggesting a significant inhibition of I_{Na} .

CONCLUSIONS—Both Na channel blockers significantly reduced tachycardia-induced SCaEs in the rabbit ventricles, but (*R*)-propafenone was significantly more effective than lidocaine. These

data suggest that type 2 ryanodine receptor inhibition potentiates the activity of Na channel blockers against SCAE and DADs in the intact hearts.

Keywords

Depolarization; Action potentials; Calcium; Antiarrhythmic agents

Introduction

Spontaneous Ca release from the sarcoplasmic reticulum (SR) is thought to underlie the delayed afterdepolarization (DAD) and triggered action potentials that can be observed in ventricular myocytes and Purkinje fibers.^{1,2} Studies in the 1980s have shown that I_{Na} blockers, such as tetrodotoxin and lidocaine, can suppress ouabain-induced DADs in isolated Purkinje fiber,³ likely by limiting intracellular Na loading during rapid heart rates.⁴ More recently, Hwang et al⁵ reported that among class I antiarrhythmic drugs, only flecainide and (*R*)-propafenone also inhibit type 2 ryanodine receptor (RyR2) channels. Propafenone is a racemic mixture of (*S*)-propafenone and (*R*)-propafenone.⁶ Both enantiomers are equally effective Na channel blockers, but (*R*)-propafenone is a significantly more potent RyR2 inhibitor than (*S*)-propafenone.⁵ (*S*)-Propafenone also blocks beta-adrenergic receptors, whereas (*R*)-propafenone does not.⁶ Consistent with RyR2-blocking effects, flecainide and (*R*)-propafenone both reduced arrhythmogenic Ca waves in cardiomyocytes isolated from calsequestrin knockout mice, a genetic model of catecholaminergic polymorphic ventricular tachycardia (CPVT). In vivo, low-dose (*R*)-propafenone prevented exercise-induced ventricular tachycardia, while lidocaine was significantly less effective. These results suggest that suppressing the spontaneous SR Ca release and arrhythmogenic Ca waves contributed to the antiarrhythmic efficacy of propafenone and flecainide in patients with CPVT.^{5,7} However, this hypothesis is supported only by studies in cells isolated from mice. Whether or not flecainide and (*R*)-propafenone suppress spontaneous SR Ca release, DAD, and triggered activity in other species and in intact cardiac muscle remains unknown. We recently reported that in intact Langendorff-perfused rabbit ventricles, isoproterenol infusion and the abrupt cessation of rapid pacing are consistently followed by spontaneous Ca elevation (SCaE) and DAD.² These hearts may also develop triggered activities from 2 different competing ventricular foci, resulting in bidirectional ventricular tachycardia.⁸ The purpose of the present study was to use this intact heart model of SCAE and DAD to test the hypothesis that (*R*)-propafenone (which blocks both RyR2 and I_{Na} but not beta-adrenergic receptor) is more effective than lidocaine (which blocks only I_{Na}) in suppressing SCAE and DAD in intact Langendorff-perfused rabbit ventricles at the cessation of rapid pacing.

Methods

Langendorff-perfused rabbit ventricles

This study protocol was approved by the Institutional Animal Care and Use Committee of Indiana University School of Medicine and the Methodist Research Institute and conformed to the Guide for the Care and Use of Laboratory Animals. Details of this animal model have been reported elsewhere.² Briefly, New Zealand White female adult rabbits ($n = 12$) were anesthetized with Na pentobarbital (50 mg/kg). These hearts were rapidly excised and Langendorff perfused at 25–30 mL/min with oxygenated Tyrode's solution (NaCl 125 mmol/L, KCl 4.5 mmol/L, NaHCO_3 24 mmol/L, NaH_2PO_4 1.8 mmol/L, CaCl_2 1.8 mmol/L, MgCl_2 0.5 mmol/L, and glucose 5.5 mmol/L) with a pH of 7.40. We performed cryoablation of the atrioventricular (AV) junction by using a 7-cm SurgiFrost probe (CryoCath Technologies, Inc, Quebec, Canada) with the probe temperature of 135°C for 3 minutes.

During the cryoablation, the epicardium was protected by immersing the heart into warm (37°C) Tyrode's solution.

Optical mapping

The hearts were stained with Rhod-2 AM (1.48 $\mu\text{mol/L}$) for 10 minutes for Ca_i mapping and with RH237 for V_m mapping. The double-stained hearts were excited with laser light at 532 nm. The fluorescence was collected and recorded with dual-CMOS cameras (BrainVision, Tokyo, Japan) at 2 ms/frame and 100×100 pixels with a spatial resolution of $0.35 \times 0.35 \text{ mm}^2/\text{pixel}$. The fluorescence obtained through a common lens was separated with a dichroic mirror (650-nm cutoff wavelength) and directed to the respective camera with additional filtering (715-nm longpass for V_m and $580 \pm 20 \text{ nm}$ for Ca_i). Optical signals were processed with both spatial (3×3 pixels Gaussian filter) and temporal (3 frames moving average) filtering. The hearts were immobilized with 20 $\mu\text{mol/L}$ blebbistatin (Tocris, Ellisville, MO) and 5 $\mu\text{mol/L}$ cytochalasin D (Sigma, St Louis, MO) during optical mapping.

Experiment protocol

We performed rapid pacing from the right ventricular apex (150 or 200 ms, 400 beats) and induced ventricular fibrillation (VF) followed by defibrillation. In addition to rapid pacing and VF, we continuously infused isoproterenol (0.3 $\mu\text{mol/L}$) throughout the experiment. The SCaE and DADs, if any, were mapped at the cessation of rapid pacing and VF. After obtaining baseline data, we repeated the same pacing and mapping protocol with (*R*)-propafenone (3.0 $\mu\text{mol/L}$) in 6 hearts and lidocaine (50 $\mu\text{mol/L}$) in 6 hearts. In contrast to racemic propafenone or (*S*)-propafenone, (*R*)-propafenone does not inhibit beta-adrenergic receptors and therefore does not block the effects of isoproterenol. The concentrations of both drugs are within the clinical therapeutic concentration and are also comparable with that used by Hwang et al.⁵ Pseudoelectrocardiogram was monitored with 2 widely spaced bipolar electrodes.

Data analysis

The amplitude of an SCaE was measured by an arbitrary unit (AU), where 1 AU equals the Ca_i amplitude during baseline pacing. The slope of an SCaE (AU/s) was measured at the steepest portion of Ca_i during SCaE. Ca_i map was constructed by the amplitude in Ca_i at each pixel in the mapped region. The phase 0 duration was defined as the time interval between 10% depolarization and 90% depolarization on the phase 0 of action potential. It was measured on the action potential of the last (400th) paced beat at 200-ms pacing cycle length (PCL) and was used as a measure of the Na channel-blocking effect of each drug. We chose 200-ms PCL because it is short enough to induce maximum use-dependent block of Na channel in intact ventricles⁹ but long enough to prevent the induction of VF halfway through the pacing train. The conduction velocity (CV) was also measured at that paced beat to serve as an additional measure of the Na channel-blocking effect. Continuous variables were expressed as mean and 95% confidence interval (CI). Paired Student's *t* tests were used to compare the SCaE and SCaE slopes before and after the administration of drugs. Unpaired *t* tests were used to compare the degree of SCaE between drugs. All tests were performed at a 2-tailed significance level of $P < .05$. The 95% CI of the means of relevant variables was calculated by using normal approximation. The statistics were computed with PASW Statistics 19 (IBM, Chicago, IL).

Results

Induction of ScaE

As reported by Maruyama et al,² isoproterenol infusion (0.3 $\mu\text{mol/L}$) and rapid ventricular pacing at 150- or 200-ms PCL was always followed by ScaEs in the post-pacing period (Figures 1–3). Rapid pacing repeatedly induced VF in 1 heart. ScaE was observed at the termination of pacing-induced VF in that heart (Figure 1I). The maximum amplitude and slope of ScaE and the time to ScaE averaged 0.23 AU (95% CI 0.20–0.26), 1.70 AU/s (95% CI 1.34–2.05), and 314 ms (95% CI 296–333), respectively (Table 1).

Effect of (*R*)-propafenone on ScaE

Figures 1 and 2 illustrate the effects of (*R*)-propafenone on ScaEs and DADs. In Figures 1A, 1C, 1E, and 1G and Figure 2B, ScaEs (arrows) and DADs (arrowhead) were elicited at the cessation of rapid pacing (150 ms, 400 beats) in the presence of isoproterenol (0.3 $\mu\text{mol/L}$). In Figure 1I, the ScaE (arrow) was elicited by pacing-induced VF-defibrillation episodes. In Figures 1B, 1D, 1F, 1H, and 1J and Figure 2C, the application of (*R*)-propafenone (3.0 $\mu\text{mol/L}$) nearly completely abolished the ScaE and DAD (dotted arrows). The Ca_i maps at baseline show the distribution of ScaE (green and yellow colors) in each heart (Figure 1, left column). ScaEs were almost completely suppressed by (*R*)-propafenone (Figure 1, right column and Table 1). The ScaE amplitudes were too small to allow accurate measurements of the slope of ScaE and the time to ScaE occurrence (Table 1). The earliest epicardial activation site (asterisk) of the ventricular escape rhythm (Figure 2A) after the creation of the AV block was in the left ventricular free wall (Figure 2D). During isoproterenol infusion, both ScaE (arrow) and DAD (arrowhead) were observed at the earliest activation site in the base of the right ventricle (asterisk, Figure 2E), suggesting that triggered activity occurred at a site other than the ventricular escape beat (VEB). (*R*)-Propafenone suppressed all ScaE and triggered activity (Figure 2C). The earliest activation site of the VEB (Figure 2F) returned to the same site as baseline (Figure 2D).

Effect of lidocaine on ScaE

Figure 3 shows effects of lidocaine on ScaE. In contrast to (*R*)-propafenone, lidocaine did not nearly completely suppress the ScaE, although the amplitude and slope of ScaE were significantly decreased while the times to ScaE were significantly increased compared with baseline values before lidocaine infusion (Table 1). (*R*)-Propafenone reduced the amplitude of ScaE on average by 84.6% (95% CI 79.0–90.2), which was significantly larger than the 21.5% (95% CI 11.3–31.6) reduction produced by lidocaine ($P < .001$).

Pacing threshold

Before the application of drugs, the pacing threshold was less than 2.0 mA in all hearts studied. After (*R*)-propafenone, it was still possible to achieve 1:1 capture with 150 ms PCL for 400 beats with pacing output of 2.0 mA. However, the pacing threshold after the application of lidocaine was significantly increased to 4.5 mA (95% CI 3.6–5.4) ($P = .001$). Even with these outputs, we were not able to achieve stable 1:1 capture at 150 ms in 1 lidocaine-treated ventricle. In that ventricle, the shortest PCL was 200 ms.

Effects of (*R*)-propafenone and lidocaine on VEB recovery time

The baseline P-wave cycle length (CL) and VEB CL during AV block were 335 (95% CI 308–362) and 1030 ms (95% CI 797–1264), respectively, for all the 12 hearts studied. We defined VEB recovery time as the time interval between the 400th pacing artifact to the onset of the first postpacing VEB. The post-rapid pacing VEB intervals after the administration of (*R*)-propafenone (1585 ms [95% CI 992–2177]) and lidocaine (2103 ms

[95% CI 1993–2213]) were significantly longer than at baseline (631 ms, 95% CI 456–806, $P = .013$, and 527 ms, 95% CI 455–598, $P < .001$, respectively). To further characterize the effects of (*R*)-propafenone and lidocaine on cardiac automaticity under adrenergic stimulation, we analyzed the P-wave CL and VEB CL during AV block and isoproterenol infusion, before and after drug administration. Figure 4 shows typical pseudoelectrocardiogram tracings of a rabbit heart during AV block, after isoproterenol infusion and after adding (*R*)-propafenone during continued isoproterenol infusion. The P-wave CL and the VEB CL were shortened by isoproterenol and lengthened after adding (*R*)-propafenone. For all the 6 hearts studied, the P-wave CL was 235 ms (95% CI 202–268) and the VEB CL was 407 ms (95% CI 350–465) during isoproterenol infusion. (*R*)-Propafenone administration during continued isoproterenol infusion significantly increased P-wave CL to 256 ms (95% CI 238–274) ($P = .035$) and VEB CL to 782 ms (95% CI 425–1139) ($P = .042$). In comparison, lidocaine administration during isoproterenol infusion also significantly lengthened the VEB CL from 388 ms (95% CI 297–478) to 1131 ms (95% CI 735–1527) ($P = .005$) but did not significantly change the P-wave CL (from 228 ms [95% CI 205–251] to 245 ms [95% CI 203–287]; $P = .247$).

Effect of (*R*)-propafenone and lidocaine on the phase 0 duration and CV

Figure 5 shows effects of (*R*)-propafenone and lidocaine on the phase 0 duration and CV. The phase 0 duration and CV after the application of both drugs were significantly prolonged and decreased compared with control, respectively (all $P < .05$). These findings are consistent with Na channel–blocking effects of the drugs tested.

Discussion

The primary finding of this study is that (*R*)-propafenone and lidocaine both suppress SCaE and DADs in intact rabbit heart. However, (*R*)-propafenone was much more effective (85% reduction of SCaE amplitude), whereas the effect of lidocaine was very modest (21% reduction), even though the degree of Na channel block was similar by these 2 drugs. This result suggests that the inhibition of RyR2 is very important for drug efficacy against SCaE and DADs in the intact ventricles.

Contribution of I_{Na} blockade to the suppression of SCaE in intact ventricles

Among the approved antiarrhythmic drugs, flecainide, propafenone, and carvedilol appear to have significant inhibitory effects on RyR2^{5,7,10,11} and may be effective in the treatment of CPVT and other arrhythmias induced by spontaneous SR Ca release. In addition to RyR2 inhibition, flecainide and propafenone are effective I_{Na} blockers. Several reports indicate that RyR2 blockade is important for suppressing SCaE and that it contributes to the antiarrhythmic effects of flecainide.^{5,7,10–14} In variance with these studies, Liu et al¹⁵ recently found no effects of flecainide on SCaE in isolated myocytes and concluded that the antiarrhythmic activity of flecainide is primarily caused by the reduction of I_{Na} and the concomitant increase in the threshold for triggered activity. In contrast, the results of the present study demonstrate that RyR2 block is important for inhibiting SCaE in the intact heart. Our results further suggest that a third factor contributes to the antiarrhythmic efficacy: I_{Na} blockers reduce Ca_i accumulation during rapid pacing and thereby indirectly prevent postpacing SCaE. Rapid pacing can increase intracellular Na (Na_i), which slows the rate of action potential depolarization, allowing time for greater $I_{Ca,L}$ activation and enhancing the reverse mode of Na–Ca exchanger (I_{NCX}).¹⁶ It is possible that the resulting increase in Ca_i can facilitate the SR Ca accumulation, leading to large SR Ca release at the cessation of rapid pacing. A previous study by Rosen and Danilo³ documented that lidocaine and tetrodotoxin are effective in suppressing ouabain-induced DADs, especially at the cessation of rapid pacing. The latter finding can be explained by the effects of I_{Na} inhibition

on Ca_i accumulation. More recently, it has been proposed that I_{Na} inhibition underlies the mechanism by which combined therapy with dronedarone and ranolazine inhibits Ca-dependent arrhythmias from the pulmonary veins.¹⁷ In the present study, we show that I_{Na} inhibition by lidocaine prevented SCAE in the postpacing period in intact rabbit ventricles. These findings provide further insights into the antiarrhythmic actions of the I_{Na} inhibitors in conditions of Ca_i overload and sympathetic activation.

Drug dosage and antiarrhythmic activity

Clinically, active metabolites contribute importantly to propafenone's antiarrhythmic activity. For example, propafenone plasma concentrations of ~350 ng/mL (1 μM) result in a 70% inhibition of VEBs clinically, with concentrations of 1500 ng/mL (4.3 μM) required in poor metabolizers.¹⁸ Therefore, the steady-state plasma concentrations do not necessarily determine the drug efficacy. Furthermore, protein binding of propafenone is ~95%, saturable and enantiomer selective, with (*S*)-propafenone bound more than (*R*)-propafenone in humans, whereas no stereoselectivity for plasma protein binding was found in rabbits.¹⁹ Thus, while difficult to predict given that propafenone metabolism is also enantiomer selective, the free concentration of (*R*)-propafenone in humans would likely be around 10–20 ng/mL (0.03–0.06 μM) during chronic dosing. At the same time, micromolar propafenone concentrations are required to block ventricular Na currents in isolated myocytes.²⁰ A likely explanation for this discrepancy of free clinical serum concentration and Na channel-blocking effect (as evidenced by QRS widening, reduced action potential duration upstroke velocity, increased pacing threshold) is the observation that propafenone accumulates in cardiac tissue. For example, in rabbit hearts, heart tissue concentrations were > 100-fold higher than the perfusate concentrations.²¹ The same principles also apply to lidocaine, which is 70%–80% protein bound and also accumulates in cardiac tissue.¹⁸ Thus, rather than using values of human free plasma concentrations for our in vitro studies, we chose experimental drug concentrations that achieve a measurable Na channel block in the heart for both drugs (eg, reduced upstroke velocity; see Figure 5).

Importance of RyR2 blockade

To control for the effect of I_{Na} inhibition, lidocaine was used in our experiments at a 16.6-fold higher concentrations (50 μM) than (*R*)-propafenone (3 μM), given that lidocaine is used clinically at 10- to 15-fold higher serum concentrations than is propafenone.¹⁸ At these concentrations, both Na blockers significantly reduced tachycardia-induced SCaEs in the rabbit ventricles, but (*R*)-propafenone was significantly more effective than lidocaine in suppressing SCAE. These findings are consistent with a previous study that showed that propafenone was more effective than lidocaine in suppressing ventricular arrhythmias in a canine model²² and that (*R*)-propafenone had the greatest effects on spontaneous Ca waves among Na channel blockers.^{5,23} The report by Hwang et al⁵ also showed the (*R*)-propafenone is highly effective in preventing ventricular tachycardia in mice or human patients with CPVT, a disease associated with RyR2 mutation that causes abnormal spontaneous Ca release from the SR during adrenergic stimulation.^{24–26} Our results suggest that (*R*)-propafenone is highly effective in suppressing postpacing SCAE not only because it inhibits I_{Na} and prevents Ca_i accumulation but also because it inhibits RyR2 to prevent spontaneous SR Ca releases. The latter antiarrhythmic property is not shared by lidocaine.

(*R*)-Propafenone and automaticity

Kato et al²⁷ documented that propafenone (racemic mixture of (*S*)-propafenone and (*R*)-propafenone) suppresses Purkinje fiber automaticity. It also suppresses sinus node automaticity in the presence of complete autonomic blockade. In the present study, we found that (*R*)-propafenone lengthens the P-wave and VEB CL and prolonged postpacing VEB recovery time during isoproterenol infusion. Multiple recent studies show that beta-

adrenergic stimulation accelerates the sinoatrial node and the AV node primarily by activation of the Ca clock, which depends on both SR Ca sequestration through Ca-ATP pump and spontaneous SR Ca release through RyR2.^{28–31} Because (*R*)-propafenone significantly suppresses the SCAE in intact ventricles, it is possible that (*R*)-propafenone also suppresses the SCAE in sinus node and Purkinje fibers, hence reducing their firing rate and prolonging the P-wave and VEB CL. However, lidocaine had a similar effect as (*R*)-propafenone on VEB CL, suggesting that I_{Na} inhibition alone may be sufficient to suppress VEB generation. More experiments in the sinus node or Purkinje fibers will have to be done in future to test the relative contribution of these mechanisms.

Clinical implication

A major clinical implication of the present study is that the Na channel block alone only has modest efficacy for inhibiting SCAE and DAD in the intact ventricle. These effects may be attributable to the prevention of Ca_i accumulation during tachycardia and isoproterenol infusion. In contrast, (*R*)-propafenone, which inhibits both the Na channel and the RyR2, is much more effective than lidocaine in suppressing postpacing SCAE and DADs. These findings suggest that (*R*)-propafenone may be a highly effective antiarrhythmic drug for arrhythmias related to Ca_i accumulation and sympathetic stimulation.

Study limitation

We used blebbistatin (20 μ mol/L) and cytochalasin D (5 μ mol/L) to suppress cardiac contraction during isoproterenol infusion. Whether or not these electromechanical uncouplers have affected the results of this study remain unknown.

Conclusions

Both Na channel blockers significantly reduced tachycardia-induced SCAEs in the rabbit ventricles, but (*R*)-propafenone was significantly more effective than lidocaine. These data suggest that type 2 ryanodine receptor inhibition potentiates the activity of Na channel blockers against SCAE and DADs in the intact hearts.

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ABBREVIATIONS

AV	atrioventricular
CI	confidence interval
CL	cycle length
CPVT	catecholaminergic polymorphic ventricular tachycardia
CV	conduction velocity
DAD	delayed after-depolarization
PCL	pacing cycle length
RyR2	type 2 ryanodine receptor

ScaE	spontaneous Ca elevation
SR	sarcoplasmic reticulum
VEB	ventricular escape beat
VF	ventricular fibrillation

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Figure 1.

Effects of (*R*)-propafenone on SCAE. The black and red lines indicate optical tracing of V_m and Ca_i , respectively, from the sites labeled by an asterisk in Ca_i maps. **A, C, E, and G:** SCAE (arrows) and DAD (arrowhead) were elicited at the cessation of rapid pacing. **I:** SCAE (arrow) was elicited after defibrillating PIVF. **B, D, F, H, and J:** The application of (*R*)-propafenone ($3.0 \mu\text{mol/L}$) abolished the SCAE and DAD (dotted arrows). In the Ca_i maps, SCAE (green and yellow colors) was present at baseline but not after the application of (*R*)-propafenone. AU = arbitrary unit; DAD = delayed afterdepolarizations; PIVF = pacing-induced ventricular fibrillation; SCAE = spontaneous Ca elevation; VEB = ventricular escape beat.

Figure 2.

Effect of (*R*)-propafenone on SCAE (arrows), DAD (arrowheads), and triggered activity. Optical tracings of **A**, **B**, and **C** were obtained at the earliest activation site (white asterisk) in the activation map of **D**, **E**, and **F**, respectively. The application of (*R*)-propafenone abolished SCAE, DAD, and DAD-mediated triggered activity (**C**). AV = atrioventricular; DAD = delayed afterdepolarizations; SCAE = spontaneous Ca elevation; VEB = ventricular escape beat.

Figure 3.

Effects of lidocaine on SCAE. Each optical tracing was recorded from the site labeled by an asterisk in the corresponding Ca_i map. **A, C, and E:** SCAE (arrows) and DAD (arrowheads) were elicited at the cessation of rapid pacing (150 or 200 ms, 400 beats) under isoproterenol infusion ($0.3 \mu\text{mol/L}$). **B, D, and F:** The application of lidocaine did not abolish the SCAE (dotted arrows) but effectively suppressed VEBs. In the Ca_i maps, SCAE (green or yellow colors) under isoproterenol infusion was also observed after the application of lidocaine. AU = arbitrary unit; DAD = delayed afterdepolarizations; SCAE = spontaneous Ca elevation; VEB = ventricular escape beat.

Figure 4.

Effects of (*R*)-propafenone on the cycle length (CL) of ventricular escape beats (VEBs, arrows) and P waves (asterisks) on the pECG. **A:** After the creation of AV block, pECG showed the dissociation between P wave and VEB. **B:** The acceleration of both VEB and sinus rate after isoproterenol infusion (0.3 μmol/L). **C:** After the application of (*R*)-propafenone (3.0 μmol/L), VEB and P-wave CL lengthened in spite of continuous isoproterenol infusion. AV = atrioventricular; pECG = pseudoelectrocardiogram; VEB = ventricular escape beat.

Figure 5.

Effects of drugs on the duration of phase 0 and conduction velocity (CV). **A** and **B**: The black tracings show V_m tracings during isoproterenol (Iso) infusion, before drug administration, and the red lines show the V_m tracings after the administration of (*R*)-propafenone (R-propa) and lidocaine (Lido). **C** and **D**: The activation map represents the CV during isoproterenol infusion and after drug administration. The duration of phase 0 and CV after the administration of (*R*)-propafenone and lidocaine were significantly prolonged and decreased compared with control, respectively. * $P < .05$.

Table 1

Effects of drug on SCaE

	Amplitude of SCaE (AU)		Slope of SCaE (AU/s)		Time to SCaE (ms)	
	Iso	After drug	P	Iso	After drug	P
(R)-Propafenone (n = 6)	0.23 (0.18–0.28)	0.04 (0.02–0.06)	<.001	1.86 (1.35–2.37)	312 (278–348)	
Lidocaine (n = 6)	0.24 (0.18–0.30)	0.19 (0.15–0.22)	.010	1.45 (0.88–2.02)	316 (288–363)	.039
Total (n = 12)	0.24 (0.20–0.27)			1.64 (1.30–1.98)	314 (296–333)	

All data are expressed as mean and 95% confidence interval.

AU = arbitrary unit; Iso = during baseline isoproterenol infusion; SCaE = spontaneous Ca elevation.