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Cellular and Ionic Mechanisms Underlying Effects of Cilostazol, Milrinone and Isoproterenol to Suppress Arrhythmogenesis in an Experimental Model of Early Repolarization Syndrome

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Running title: Patocskai: Suppression of arrhythmogenesis in ERS models

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ABSTRACT

Background: Early Repolarization Syndrome (ERS) is associated with polymorphic ventricular tachycardia (PVT) and fibrillation (VF), leading to sudden cardiac death.

Objective: The present study tests the hypothesis that the I_{to} -blocking effect of phosphodiesterase-3 (PDE-3) inhibitors plays a role in reversing repolarization heterogeneities responsible for arrhythmogenesis in experimental models of ERS.

Methods: Transmembrane action potentials (AP) were simultaneously recorded from epicardial and endocardial regions of coronary-perfused canine left-ventricular (LV) wedge preparations, together with a transmural pseudo-ECG. The I_{to} -agonist NS5806 (7-15 μ M) and I_{Ca} -blocker verapamil (2-3 μ M) were used to induce an ER pattern and PVT.

Results: Following stable induction of arrhythmogenesis, the PDE-3 inhibitors cilostazol and milrinone or isoproterenol were added to the coronary perfusate. All were effective in restoring the AP dome in the LV epicardium, thus abolishing the repolarization defects responsible for phase-2-reentry (P2R) and PVT. Arrhythmic activity was suppressed in 7/8 preparations by cilostazol (10 μ M), 6/7 by milrinone (2.5 μ M) and 7/8 by isoproterenol (0.1-1 μ M). Using voltage clamp techniques applied to LV epicardial myocytes, both cilostazol (10 μ M) and milrinone (2.5 μ M) were found to reduce I_{to} by 44.4% and 40.4%, respectively, in addition to their known effects to augment I_{Ca} .

Conclusions: Our findings suggest that PDE-3 inhibitors exert an ameliorative effect in the setting of ERS by producing an inward shift in the balance of current in the early phases of the

epicardial AP via inhibition of I_{to} as well as augmentation of I_{Ca} , thus reversing the repolarization defects underlying development of P2R and VT/VF.

Key words: Phosphodiesterase-3 inhibitors, Transient outward potassium current, Pharmacology, Ventricular fibrillation, Sudden Cardiac Death, Electrophysiology, Early Repolarization

Abbreviations:

AMI: acute myocardial infarction

AP: transmembrane action potential

APD: action potential duration

ATP: adenosine-triphosphate

cAMP: cyclic adenosine-monophosphate

ECG: electrocardiogram

EDR: epicardial dispersion of repolarization

Endo: endocardium/ endocardial

Epi: epicardium/epicardial

ER: early repolarization

ERP: early repolarization pattern

ERS: early repolarization syndrome

I_{Ca} : L-type calcium current

I_{K-ATP} : ATP-sensitive potassium current

I_{Na} : cardiac voltage-gated fast sodium current

I_{to} : transient outward current

J_p : peak of the J wave (or the onset of end-QRS slur)

LV: left ventricle

P2R: phase 2 reentry

PDE-3: phosphodiesterase-3

PKA: protein kinase A (cAMP-dependent protein kinase)

(P)VT: (polymorphic) ventricular tachycardia

RV: right ventricle

SCD: sudden cardiac death

TDR: transmural dispersion of repolarization

VF: ventricular fibrillation

INTRODUCTION:

Early Repolarization Syndrome (ERS) has attracted a great deal of attention because of its association with sudden cardiac death (SCD) secondary to development of malignant arrhythmias.^{1,2} Case-control and population-based studies point to an association of ER with the development of ventricular tachycardia (VT) and fibrillation (VF) in select individuals. ER pattern (ERP) in the ECG is characterized by a J point elevation $\geq 0.1\text{mV}$ manifesting as a notch or slur on the final 50% of the downslope of the QRS (J wave).¹⁻⁵ According to the most recent consensus reports by Macfarlane et al., J point is to be measured at the peak of the end-QRS notch or the onset of the end-QRS slur, labelled as Jp. Early case-controlled studies by Haïssaguerre and co-workers, Nam and co-workers and Rosso and co-workers reported an ERP in the ECGs of 31-60 % of their patients presenting with idiopathic ventricular fibrillation⁶⁻⁸. More recent studies report association of ERP with a higher risk for development of arrhythmias during acute myocardial infarction (AMI)⁹ and therapeutic hypothermia^{10,11}.

Recent studies from our group have provided evidence in support of the hypothesis that ERS is caused by a preferential accentuation of the AP notch in the LV epicardium secondary to an outward shift in the balance of current contributing to the early phases of the action potential.^{4, 12} Higher intrinsic levels of I_{to} were shown to account for the greater sensitivity of the inferior LV wall to development of VT/VF in the setting of ERS.⁴ The outward shift in the balance of currents was attributable to mutations in genes causing a gain of function in I_{K-ATP} (*KCNJ8* and *ABCC9*) or I_{to} (*KCNE5* mutation and rare polymorphism in *DPP10*)¹³⁻¹⁷ or loss of function in I_{Ca} (*CACNA1C*, *CACNB2* and *CACNA2D1*)^{18, 19} or I_{Na} (*SCN5A* and *SCN10A*).^{20, 21} The goal of a pharmacologic approach to therapy should therefore be to produce an inward shift in the balance of current flowing during the early phases of the LV epicardial AP.

In previous studies, we demonstrated that induction of mild ER by an outward shift in the balance of current during the early phases of the AP could potentiate the effect of hypothermia to induce VT and that a pharmacologic-induced inward shift of current, using PDE-3 inhibitors or I_{to} block with quinidine, exerts an ameliorative effect.¹² The present study provides a direct test of the hypothesis that both PDE-3 inhibitors reduce I_{to} , significantly contributing to their ameliorative effect in J wave syndromes. We examine the effects of two PDE-3 inhibitors in the absence of hypothermia and contrast their actions with isoproterenol which is widely used in the clinic to suppress J wave syndrome-associated arrhythmias. We avoid using ACh in the pharmacologic model so as to exclude the potential anticholinergic effect as a contributor to their antiarrhythmic actions.

METHODS

Our ERS models were created using coronary-perfused wedge preparation isolated from the hearts of adult mongrel dogs of either sex in conformance with the Guide for Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication No 85-23, Revised 1996) and approved by the Institutional Animal Care and Use Committee.

Arterially perfused left ventricle wedge preparation

The methods employed were as previously described.²² Wedge preparations were excised from the lateral (25%) and the inferior wall (75%) of the left ventricle and were perfused via distal branches of the left anterior descending artery, left marginal artery or left posterior artery. Floating glass microelectrode techniques were used to simultaneously record action potentials from two epicardial (Epi1, Epi2) and one endocardial (Endo) site. Together with the action potentials, pseudo-ECG was recorded using two AgCl electrodes within the bath positioned along the transmural axis of the preparation. The epicardial microelectrodes were used to map the epicardial surface of the preparation so as to reveal heterogeneities in depolarization and/or repolarization.

Voltage-clamp measurement of I_{to}

Cardiomyocytes were isolated from the epicardium of the canine left ventricle as described previously²³. I_{to} was measured at 36.5 °C using whole-cell patch clamp techniques.⁴ I_{to} was evoked using a series of 370 ms voltage steps from -40 mV and +40 mV ms; holding potential was maintained at -80 mV and 40 ms prepulse to -20 mV was used to discharge the sodium current. Series resistance was compensated ~80% and cells with an R_s values greater than 5 M Ω were discarded from analysis. External solutions contained (in mM): 127 NaCl, 4 KCl, 10 HEPES, 1.8 CaCl₂, 1.0 MgCl₂, 10 glucose; pH=7.35 with NaOH. The pipette solution

contained (in mM): 125 Potassium aspartate, 10 KCl, 10 NaCl, 1 MgCl₂, 10 HEPES, 5 EGTA, 5 Mg₂ATP; pH = 7.2 with KOH.

AP and ECG parameters were defined and calculated as described in previous studies and briefly outlined in the online supplement¹².

Statistical analysis

Results are presented as mean \pm S.E.M. Statistical analysis was performed using paired Student's *t*-test and one-way ANOVA for repeated measurements followed by pairwise comparisons corrected using the Holm-Sidak method, as appropriate. Statistical significance was considered at $P < 0.05$.

RESULTS

Induction of ERP and VT/VF

We induced the early repolarization phenotype by adding the transient outward potassium current (I_{to}) agonist NS5806 (7-15 μ M) and the calcium channel blocker verapamil (2-3 μ M) to the coronary perfusate. This led to accentuation of the action potential notch in epicardium but not endocardium, thus leading to augmentation of the electrocardiographic J wave secondary to amplification of the transmural voltage gradients (Figures 1-3). Increased concentrations of provocative agents caused a further increase of J wave area and notch-index (Figures 1-4; Table 1), leading to all-or-none repolarization at the end of phase 1 of the Epi AP (Figures 1-4). Loss of the Epi AP dome at some sites but not others resulted in a prominent increase in epicardial dispersion of repolarization (EDR) and transmural dispersion of repolarization (TDR) (Figures 1-4; Table 1). The voltage gradient between the abbreviated Epi AP and the relatively normal Endo

AP produced a prominent ST segment elevation (Figures 1-3). A prominent APD gradient developed between sites at which the dome was maintained and where the dome was lost, thus creating a vulnerable window within epicardium as well as between epicardium and endocardium across the left ventricular wall. Propagation of the AP dome from regions at which it was maintained to regions at which it was lost, caused local re-excitation via a P2R mechanism, leading to the development of closely coupled extrasystoles and polymorphic VT/VF (Figures 1-3).

Induction of ERP was observed in all experiments. Table 1 and Figures 4 and 5 show the effect of the provocative agents to significantly increase notch-index, J wave area, EDR and TDR compared to the controls. PVT and/or VF developed in 26 of 28 LV wedge preparations, compared with 0 of 28 cases under control conditions.

Effects of cilostazol, milrinone and isoproterenol

Addition of cilostazol (10 μ M), milrinone (2.5 μ M) or isoproterenol (0.1-1 μ M) to the coronary perfusate restored the AP dome at all epicardial sites, reduced epicardial and transmural dispersion of repolarization, decreased J point and ST segment elevation and terminated all arrhythmic activity. Figures 1-3 show representative recordings of APs from LV wedge preparations obtained under baseline conditions, after NS5806 (7-15 μ M), + verapamil (2-3 μ M), + PDE-3 inhibitor (10 μ M cilostazol or 2.5 μ M milrinone) or the sympathomimetic agent isoproterenol (0.1-1 μ M) and after washout of the therapeutic compounds.

The effects of milrinone, cilostazol and isoproterenol on epicardial AP notch index, J wave area, EDR and TDR as well as APD₉₀ values for Epi1, Epi2 and Endo are summarized in Figures 4-5 and Table 1. All three agents, by virtue of their action to produce an inward shift of

balance of currents, reversed the effect of the provocative agents, restoring all electrophysiologic parameters towards normal. Cilostazol (10 μM), milrinone (2.5 μM) and isoproterenol (0.1-1 μM) restored the AP dome at all epicardial sites, thus reducing notch index, J wave area, as well as epicardial and transmural dispersion of repolarization (Figures 1-5 and Table 1).

Figures 1-3 illustrates the development of polymorphic VT following exposure to NS5806 and verapamil and the effect of cilostazol (10 μM), milrinone (2.5 μM) and isoproterenol (0.2 μM) to normalize the ECG and to terminate all arrhythmic activity. Cilostazol (10 μM) abolished VT/VF in 7 of 8 preparations, whereas milrinone (2.5 μM) abolished VT/VF in 6 out of 7 preparations, and isoproterenol terminated VT/VF in 7 of 8. In all cases, washout of the drug resulted in reappearance of arrhythmic activity (Figures 1-3).

Effect of cilostazol and milrinone on I_{to} in canine ventricular epicardial myocytes

We next evaluated the effect of cilostazol and milrinone on I_{to} in canine left ventricular epicardial myocytes using whole cell patch clamp techniques. Macroscopic outward potassium current (I_{to}) was recorded at body temperature from single cardiomyocytes using the whole cell patch-clamp technique. Figure 6A shows representative I_{to} traces recorded under control conditions (left, panel) and reduction of the current after addition of cilostazol (10 μM)(right panel). Peak I_{to} was evaluated using a square depolarization pulse to -40 mV to +40 mV applied once every 5 seconds. Figure 6B shows the effect of cilostazol (10 μM) on the I-V relationship. Cilostazol (10 μM) reduced I_{to} by 44.4 % at +40 mV (n=6, p<0.02; Figure 6C). Figure 7A shows representative I_{to} traces recorded under control conditions (left, panel) and the reduction of the current after addition of milrinone (2.5 μM)(right panel). Figure 7B shows the effect of milrinone (2.5 μM) on the I-V relationship. Milrinone (2.5 μM) reduced I_{to} by 40.4 % at +40 mV (n=8, p<0.02; Figure 7C).

DISCUSSION and CONCLUSION:

Our results demonstrate a significant effect of both cilostazol and milrinone to block I_{to} , pointing to this as an important mechanism for their ameliorative effect in ERS, in addition to their previously demonstrated effects to augment I_{Ca} ²⁴⁻²⁶ suggesting that inhibition of I_{to} may also apply to isoproterenol (and other sympathomimetic) in addition to its boosting effect on I_{Ca} via direct stimulation of the β adrenergic receptors. Future experiments should be directed at a test of this hypothesis. It is noteworthy that cilostazol, milrinone and isoproterenol all produce positive inotropic and chronotropic effects. The elevation in heart rate would also be expected to indirectly decrease I_{to} because the current is relatively slow to recover from inactivation.

The much greater potency of milrinone is consistent with the results of previous studies reporting that the same concentration of milrinone produces a greater increase in cytosolic cAMP than cilostazol.^{27,28} possibly due to the fact that milrinone blocks both PDE-3 and PDE4.²⁸

It is noteworthy that all of these agents have the potential to enhance not only automaticity, but triggered activity as well, and thus may promote extrasystolic activity that may have unfavorable outcomes in certain cases.²⁹

Previous reports from our group have provided evidence in support of a preferential accentuation of the AP notch in LV epicardium as the basis for ERS.^{4, 12} Accentuation of the epicardial AP notch leads to accentuation of transmural gradients across the LV wall and thereby the appearance of prominent J point elevation, distinct J waves, or slurring of the descending limb of the QRS complex. Consistent with an outward shift in the balance of current, ERS has been associated with loss of function mutations in the $\alpha 1$, $\beta 2$ and $\alpha 2\delta$ subunits of the cardiac L-

type calcium channel (*CACNA1C*, *CACNB2*, and *CACNA2D1*) resulting in a reduction in I_{Ca} ^{18,30}, loss of function mutations in sodium channel genes (*SCN5A* and *SCN10A*)^{21, 31} causing a reduction in I_{Na} as well as gain of function mutations in I_{K-ATP} channel genes (*KCNJ8* and *ABCC9*) causing an increase in I_{K-ATP} . The J wave syndromes and IVF have also been associated with gain of function mutations in the transient outward potassium current (I_{to}).^{17, 32-39}

In the present study, we pharmacologically modeled the genetic defects and attendant ionic changes with the use of verapamil to block I_{Ca} and NS5806 to augment I_{to} .

NS5806-induced augmentation of I_{to} sensitized our preparations to the effects of verapamil consistent with the association of a higher density of this current in the inferior wall with a higher arrhythmic risk.⁴ Addition of verapamil further accentuates the AP notch, leading to the development of a more prominent J point and ST segment elevation. Increased concentration of these agents can then elicit all-or-none repolarization, leading to loss of the AP dome at some epicardial sites but not others, resulting in an epicardial dispersion of repolarization (EDR) (Figures 1-5; Table 1). Propagation of the AP dome from sites at which it was maintained to sites at which it was lost elicited local re-excitation via a phase 2 reentry mechanism within the left ventricular epicardium. Loss of the dome in the epicardium also creates a transmural dispersion of repolarization (TDR) giving rise to a vulnerable window across the ventricular wall which, when captured by a closely coupled extrasystole generated in the epicardium, induces VT/VF (Figures 1-3).

Early repolarization syndrome is categorized as a J wave syndrome because like Brugada syndrome its electrocardiographic and arrhythmic manifestations are associated with accentuation of J waves.^{1,3} It is therefore no surprise that cilostazol, milrinone and isoproterenol have all been found to exert an ameliorative effect in patients with Brugada syndrome and that

their mechanisms of action in the setting of Brugada syndrome is similar to their mechanisms of action in ERS.⁴⁰

STUDY LIMITATIONS

Our pharmacologic models are designed to mimic the genetic defects associated with ERS and to test the effects of ameliorative agents. To test the effects of these compounds on every single known mutation associated with the J wave syndromes is clearly beyond the scope of our (or any) study. Our pharmacological models may not perfectly mimic the effects of every mutation, but they do serve to recapitulate the net result of the ion-current imbalance created and thus provide a reasonable platform to test the effects of PDE inhibitors and β adrenergic agents.

While it would be preferable to study the effect of these agents in transgenic animal models, none at present are capable of recapitulating the arrhythmic and electrocardiographic manifestations of J wave syndromes. In a recent study, Park et al attempted to mimic Brugada syndrome phenotype in Yucatan minipigs by heterozygous expression of a nonsense mutation in SCN5A (E558X) originally identified in a child with Brugada syndrome.⁴¹ Myocytes isolated from the SCN5A^{E558X/+} pigs showed a loss of function of I_{Na} . Various conduction abnormalities were observed but not a BrS phenotype, not even after flecainide-test, because pigs lack the transient outward current and action potential notch in ventricular cardiomyocytes. These observations point to the crucial importance of I_{to} in the development of J wave syndrome phenotype. Transgenic mice are not helpful due to the fundamental differences in repolarization characteristics.

As with any study involving experimental animal models, extrapolation of the data to the clinic must be done with caution.

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Clinical perspectives:

The demonstrated ability of cilostazol and milrinone to inhibit I_{to} provides support and guidance for the development of new drugs for the management of ERS. Although most agents used in the treatment of J wave syndromes possess I_{to} -inhibitory effects (e.g., quinidine, cilostazol, bepridil), cardioselective and I_{to} -specific blockers are not available at present. Further investigation designed to address this significant gap in our antiarrhythmic armamentarium is urgently needed. Despite the similarity between canine and human electrophysiology and our ability to recapitulate the electrocardiographic and arrhythmic manifestations of ERS in this experimental model, translation of our results to humans should be approached with caution.

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Table 1: Effect of cilostazol, milrinone and isoproterenol on electrophysiological parameters in an experimental model of early repolarization syndrome.

	Notch- Index	EDR (ms)	TDR (ms)	J-w- AUC_r (mV x ms)	Epi1 APD₉₀ (ms)	Epi2 APD₉₀ (ms)	Endo APD₉₀ (ms)
<i>Cilostazol</i>							
Control	199.07±44. 9	16.2±8.2	17.8±4.3	4.3±2.0	203.6±7. 8	182.8±5. 6	217.7±5. 1
NS (7-15 μM) +Ver. (2-3μM)	4863.6±669 .4*	137.9±8. 1*	111.8±13 .4*	56.6±12. 2*	252.2±7. 8†	109.4±5. 6*	237.2±14 .3¶
+ Cilostazol (10μM)	1041.7±124 .2*	11.9±3.8 *	21.3±3.8 *	15.0±4.0 *	218.4±7. 5§	205.3±4. 4*	235.6±7. 3¶
Washout Cilostazol	4809.8±590 .7*	123.3±10 .6*	106.9±11 .2*	53.0±6.2 *	252.7±10 .1§	121.8±9. 4*	237.3±11 .5¶
<i>Milrinone</i>							
Control	198.2±287	17.3±9.3	17.8±3.8	4.0±2.5	215.7±6. 9	194.8±5. 9	227.3±5. 92
NS (7-15 μM) +Ver. (2-3μM)	4732.0±764 .8*	143.0±12 .2*	110±7.9* *	68.4±19. 5*	250.1±14 .1§	104.9±8* *	230.1±4. 7¶
Milrinone (2.5μM)	1181.8±170 .1*	3.9±0.7* *	11.6±3.7 *	12.268±1 .5*	213.3±6. 1¶	212.6±6. 6*	232.6±5. 9¶
Washout Milrinone	4668.5±583 .6*	150.4±15 .1*	115.6±12 .4*	51.7±2.6 †	267.8±10 .7†	114.2±9. 23*	243.5±6. 3¶
<i>Isoproterenol</i>							
Control	176.8±19.9	13.5±5.6	14.9±5	1.6±0.4	206.5±6. 9	190.7±5. 9	213.4±4. 9
NS (7-15 μM) +Ver. (2-3 μM)	4114.7±400 .8*	123.9±11 .9*	88.9±5.2 *	46.0.0±4. 9.*	234.5±12 ¶	111.2±9. 3*	198.5±6. 3¶
+Iso. (1μM)	406.9±65.6 *	5.9±1.6* *	13.6±4* *	7.8±2.7* *	164.1±8. 3*	158.6±7. 7*	179.±7.8 ¶
Washout Iso	3628.6.±79 5.9*	87.7±25. 4*	67.1±15. 0*	39.5±8.2 *	207.9±22 .6¶	116.0±14 .3§	195.4±11 .5¶

NS=NS5806; Ver.=Verapamil; Iso=Isoproterenol; J-w-AUC_r=J wave area (normalized)

Data are presented as mean ± SEM; * P<0.001; † P<0.004; §: P<0.05; ¶ P>0.05.

n=7 for cilostazol, n=6 for milrinone, n=7 for isoproterenol(Epi1 represents the longer, Epi2 the shorter epicardial APD₉₀)

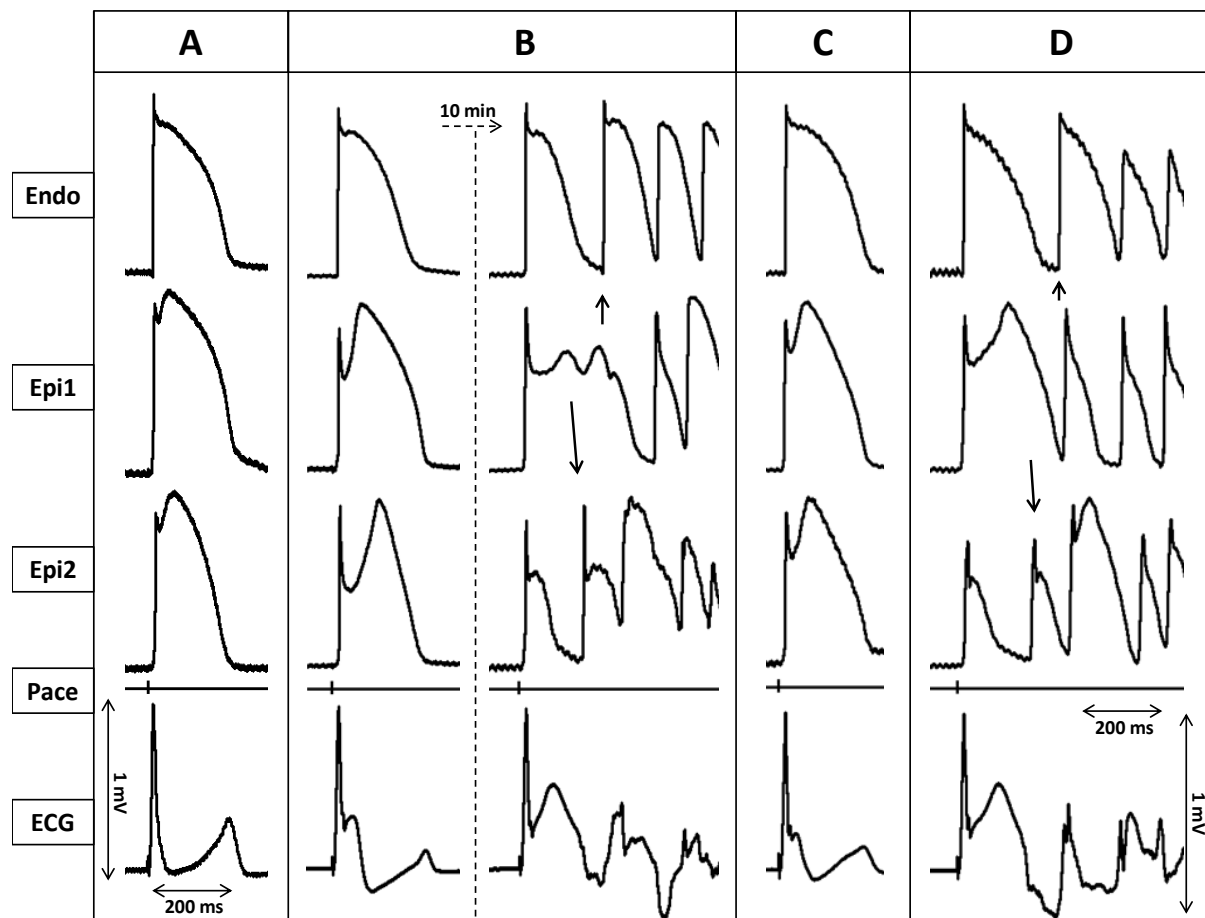


Figure 1: Ameliorative effect of **cilostazol** ($10\mu\text{M}$) in an arterially perfused canine left ventricular model of early repolarization syndrome. Each panel shows simultaneously recorded epicardial (Epi1, Epi2) and endocardial (Endo) action potentials, together with a pseudo-ECG.

A: Control. **B:** Recorded 20 min and 30 min after addition of verapamil ($2\mu\text{M}$) and NS5806 ($7\mu\text{M}$) to the coronary perfusate. **C:** 15 minutes after addition of $10\mu\text{M}$ **cilostazol** to the coronary perfusate. **D:** Recorded 15 min after withdrawal of cilostazol.

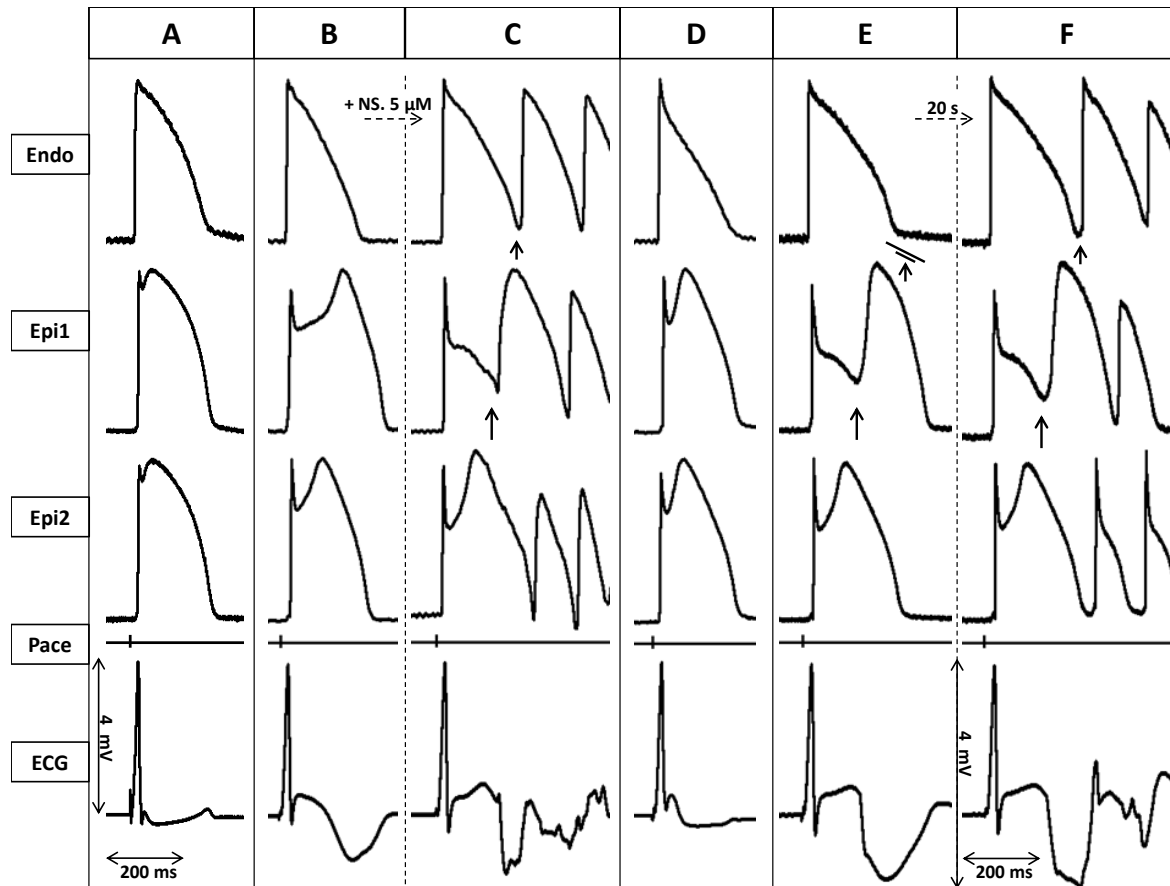


Figure 2: Ameliorative effect of **milrinone** ($2.5\mu\text{M}$) in an arterially perfused canine left ventricular model of early repolarization syndrome. Each panel shows simultaneously recorded epicardial (Epi1, Epi2) and endocardial (Endo) action potentials, together with a pseudo-ECG. **A:** Control. **B:** Traces recorded 40 min after the addition of Ca^{2+} -channel blocker verapamil ($2\mu\text{M}$) and I_{to} -agonist NS5806 ($7\mu\text{M}$). **C:** 15 minutes after raising NS5806 concentration to $12\mu\text{M}$. **D:** 10 minutes after addition of **milrinone** $2.5\mu\text{M}$ to the coronary perfusate. **E:** 20 minutes after the discontinuation of milrinone infusion. **F:** 20 seconds later.

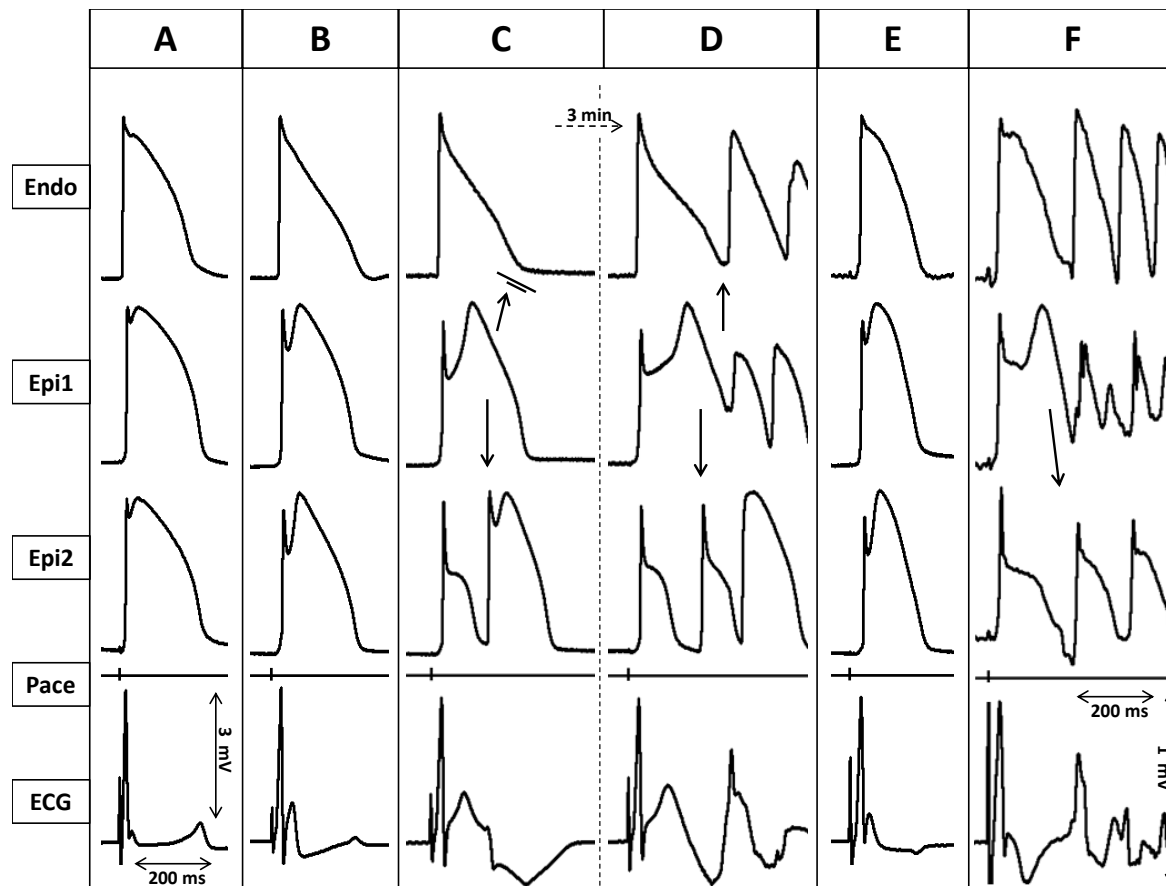


Figure 3: Ameliorative effects of **isoproterenol** ($0.2 \mu\text{M}$) in an arterially perfused canine left ventricular model of early repolarization syndrome. Simultaneously recorded epicardial (Epi1, Epi2) and endocardial (Endo) action potentials, together with pseudo-ECG positioned in the transmural axis. **A:** Control. **B:** 20 min after addition of the I_{to} agonist NS5806 ($12 \mu\text{M}$) to the coronary perfusate. **C:** After 16 min exposure to verapamil ($2 \mu\text{M}$), in addition to NS5806 ($12 \mu\text{M}$). **D:** 3 minutes later. **E:** 5 min after the start of **isoproterenol** ($0.2 \mu\text{M}$) infusion. **F:** 5 min after the discontinuation of isoproterenol infusion.

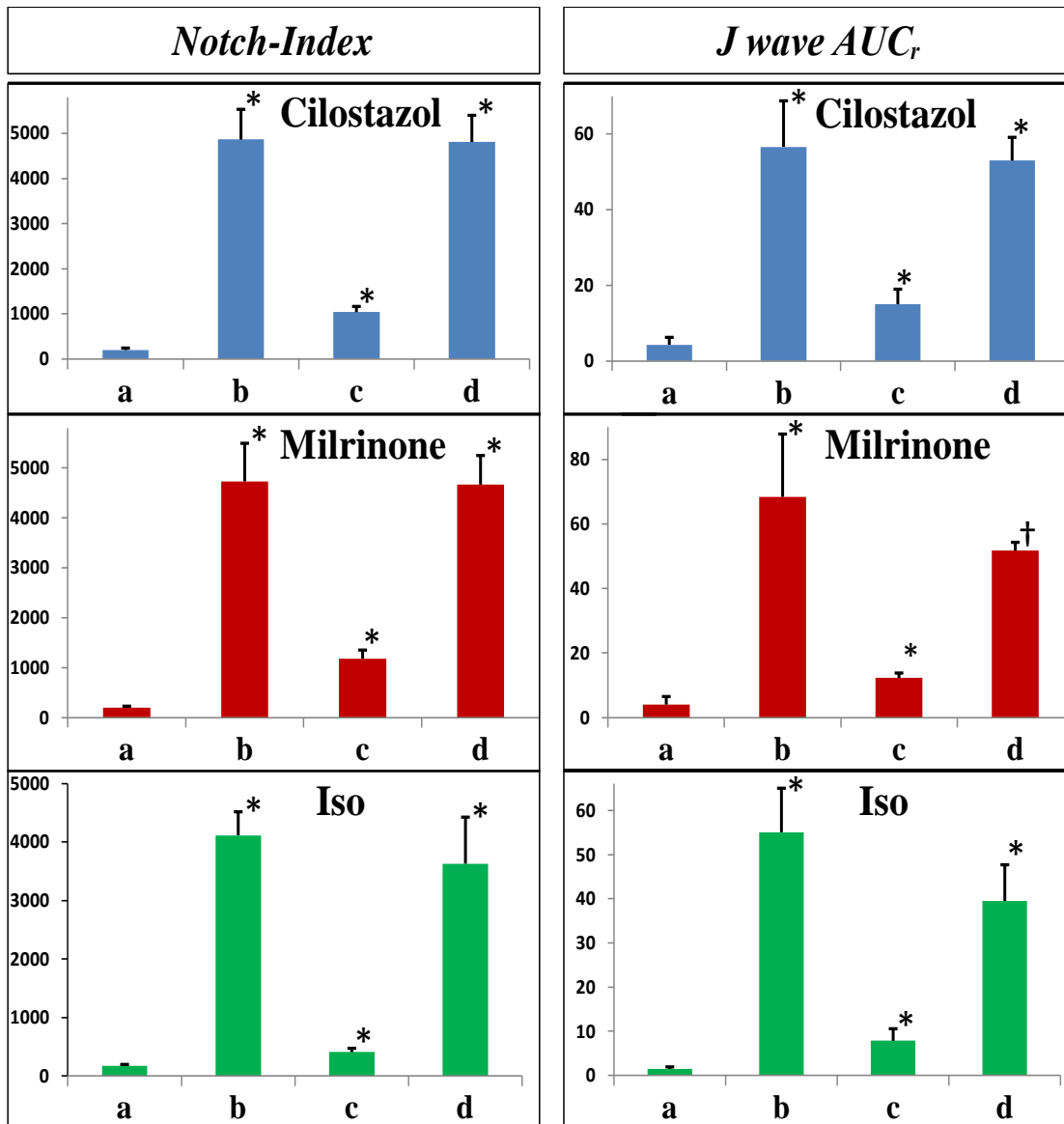


Figure 4: Notch-index (left) and normalized J wave area (right) at each experimental step.

In each panel: a. Control; b. Recorded after application of the I_{to} agonist NS5806 and I_{Ca} antagonist verapamil; c. Recorded after addition of cilostazol 10 μ M (top row), milrinone 2.5 μ M (middle row), or isoproterenol 1 μ M (bottom row); d. Recorded after wash-out of drugs. Data are presented as mean \pm SEM. Significance is shown relative to previous treatment: * $P < 0.001$; † $P < 0.004$. (n = 7 for cilostazol; n = 6 for milrinone; n = 7 for isoproterenol).

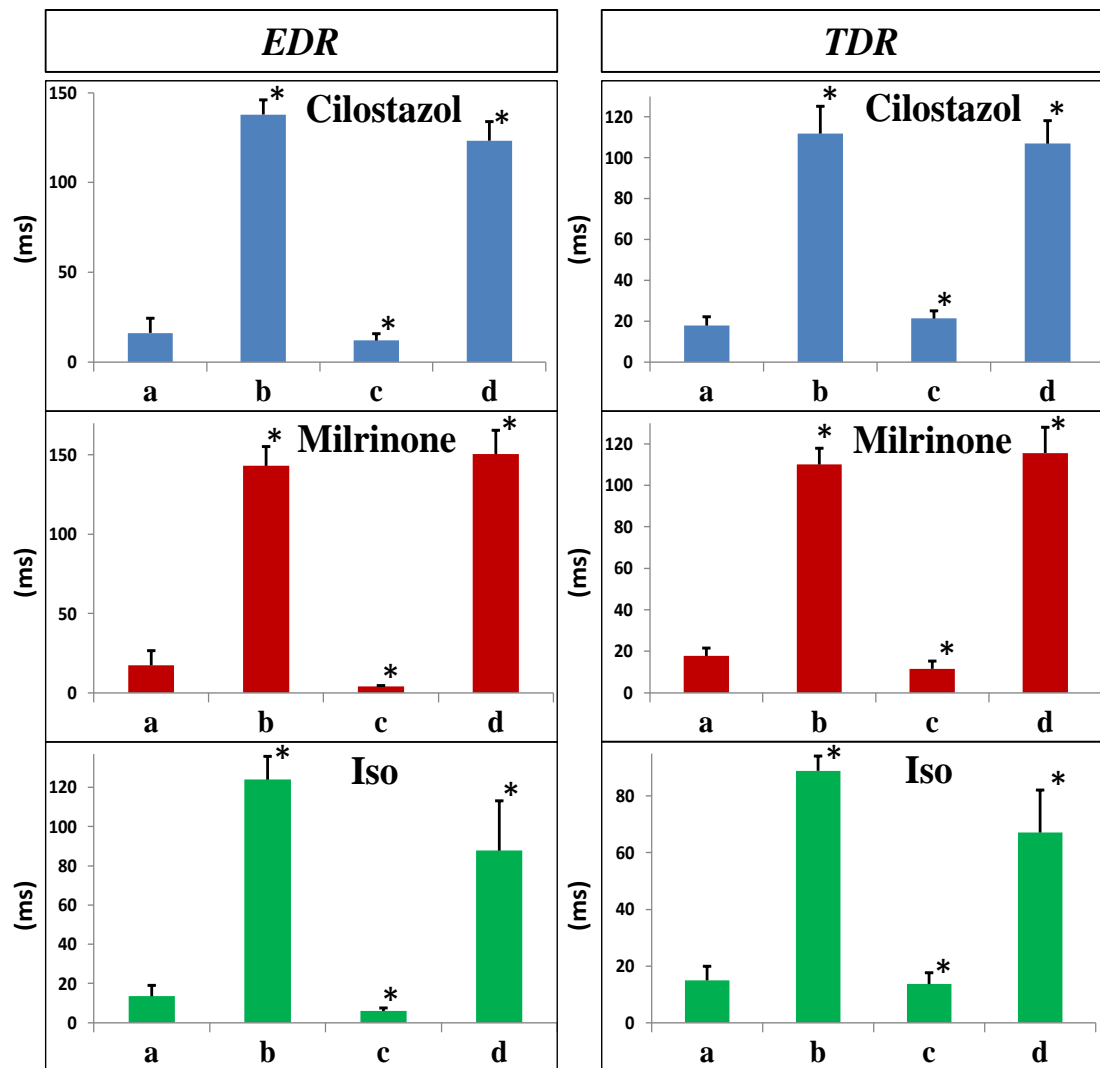


Figure 5: Epicardial (EDR, left) and transmural (TDR, right) dispersion of repolarization at each experimental step. In each panel: a. Control; b. Recorded after application of the I_{to} agonist NS5806 and I_{Ca} antagonist verapamil; c. Recorded after addition of cilostazol 10 μ M (top row), milrinone 2.5 μ M (middle row), or isoproterenol 1 μ M (bottom row); d. Recorded after wash-out of drugs. Data are presented as mean \pm SEM. Significance is shown relative to previous treatment: * $P < 0.001$ ($n = 7$ for cilostazol; $n = 6$ for milrinone; $n = 7$ for isoproterenol).

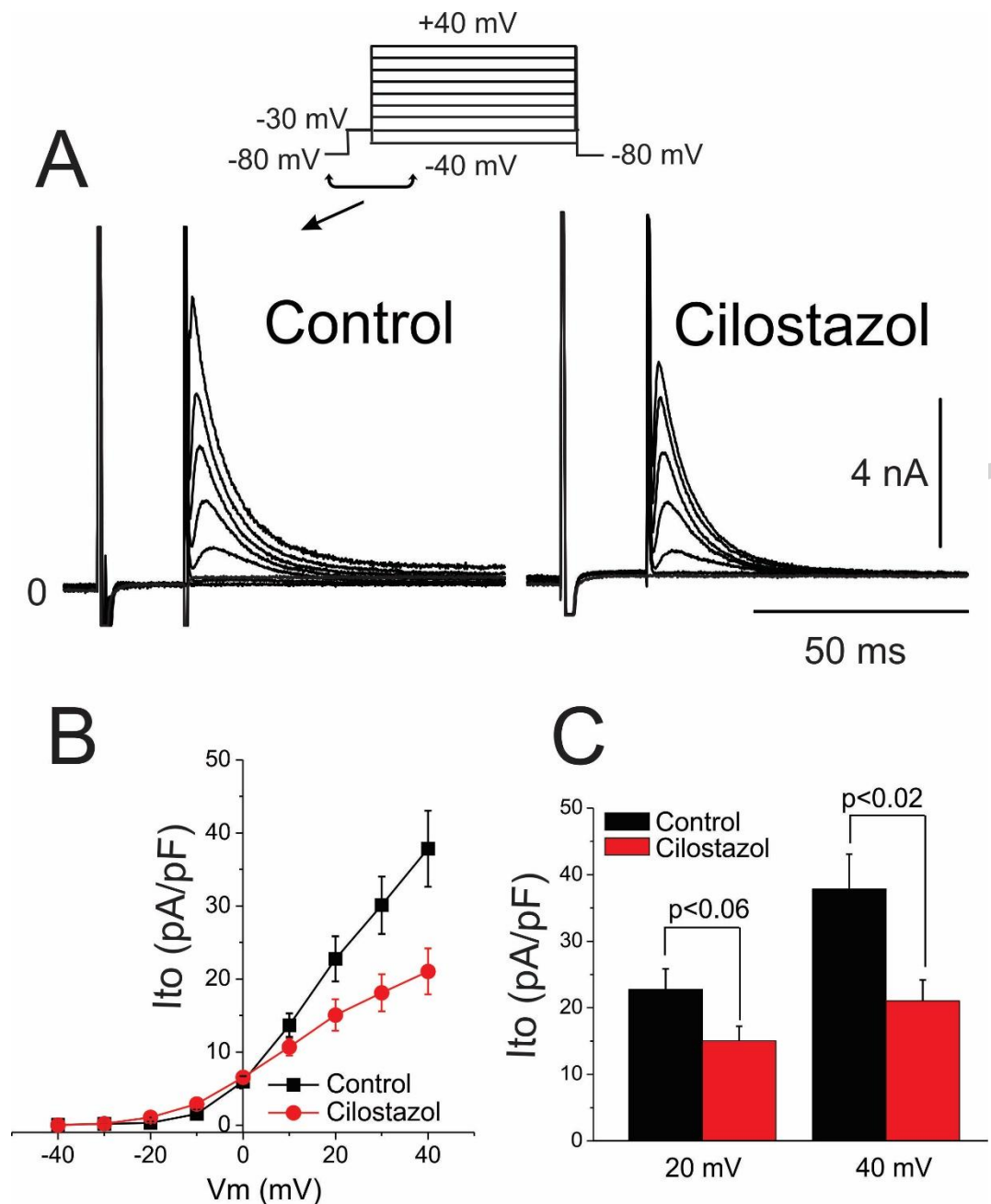


Figure 6: Cilostazol inhibition of transient outward potassium current (I_{to}). **A.** Representative macroscopic I_{to} current traces for control and cilostazol (10 μ M). **B.** Current-Voltage (I-V) relationship for I_{to} density in control and in response to 10 μ M cilostazol ($n=6$ for each). **C.** Bar graph showing I_{to} at following a step to 20 and 40 mV in presence and absence of cilostazol (10 μ M). Mean \pm SEM.

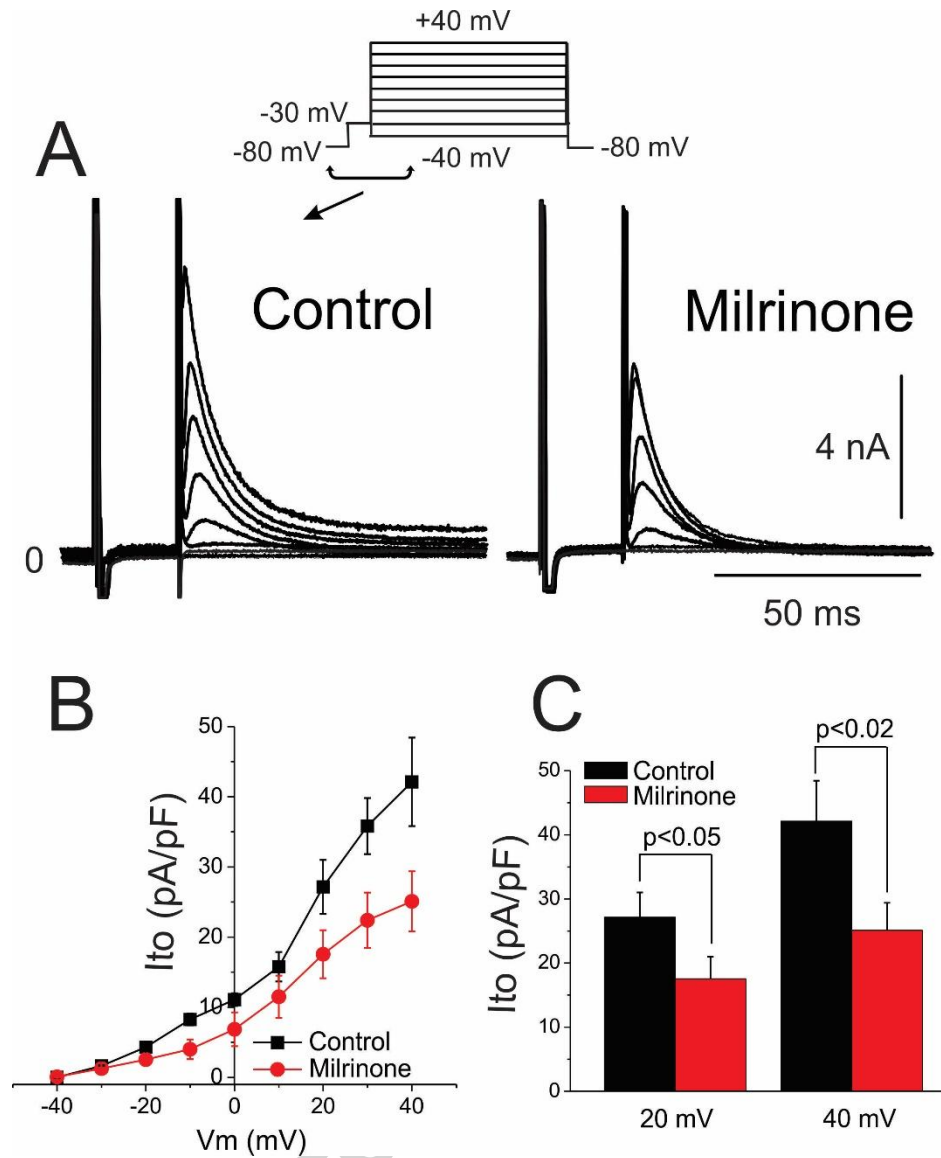


Figure 7: Milrinone inhibition of transient outward potassium current (I_{to}). **A.** Representative macroscopic I_{to} current traces for control and milrinone (2.5 μ M). **B.** Current-Voltage (I-V) relationship for I_{to} density in control and in response to 2.5 μ M milrinone (n=8 for each). **C.** Bar graph showing I_{to} at following a step to 20 and 40 mV in presence and absence of milrinone (2.5 μ M). Mean \pm SEM.