

Ranolazine Attenuates the Electrophysiological Effects of Myocardial Stretch in Langendorff-Perfused Rabbit Hearts

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Published online: 3 July 2015
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Abstract

Purpose Mechanical stretch is an arrhythmogenic factor found in situations of cardiac overload or dyssynchronous contraction. Ranolazine is an antianginal agent that inhibits the late Na⁺ current and has been shown to exert a protective effect against arrhythmias. The present study aims to determine whether ranolazine modifies the electrophysiological responses induced by acute mechanical stretch.

Methods The ventricular fibrillation modifications induced by acute stretch were studied in Langendorff-perfused rabbit hearts using epicardial multiple electrodes under control conditions ($n=9$) or during perfusion of the late Na⁺ current blocker ranolazine 5 μM ($n=9$). Spectral and mapping techniques were used to establish the ventricular fibrillation dominant frequency, the spectral concentration and the complexity

of myocardial activation in three situations: baseline, stretch and post-stretch.

Results Ranolazine attenuated the increase in ventricular fibrillation dominant frequency produced by stretch (23.0 vs 40.4 %) (control: baseline = 13.6 ± 2.6 Hz, stretch = 19.1 ± 3.1 Hz, $p < 0.0001$; ranolazine: baseline = 11.4 ± 1.8 Hz, stretch = 14.0 ± 2.4 Hz, $p < 0.05$ vs baseline, $p < 0.001$ vs control). During stretch, ventricular fibrillation was less complex in the ranolazine than in the control series, as evaluated by the lesser percentage of complex maps and the greater spectral concentration of ventricular fibrillation. These changes were associated to an increase in the fifth percentile of VV intervals during ventricular fibrillation (50 ± 8 vs 38 ± 5 ms, $p < 0.01$) and in the wavelength of the activation (2.4 ± 0.3 vs 1.9 ± 0.2 cm, $p < 0.001$) under ranolazine.

Conclusions The late inward Na⁺ current inhibitor ranolazine attenuates the electrophysiological effects responsible for the acceleration and increase in complexity of ventricular fibrillation produced by myocardial stretch.

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Keywords Mechanoelectric feedback · Myocardial stretch · Ranolazine · Ventricular fibrillation · Mapping

Introduction

Mechanical stretch modifies the electrophysiological and mechanical properties of myocytes. The mechanical effects consist of a rapid (immediate) and a slow (lasting minutes) increase in force [1, 2], while the electrophysiological effects, encompassed within the concept of mechanoelectric feedback [3, 4] have been implicated in the origin of cardiac arrhythmias in different situations characterized by atrial or ventricular overload or dyssynchronous contraction [4–8]. Although there are descriptions of the electrophysiological effects of

myocardial stretch [4–10] the data on the chain of events related to these effects and on their pharmacological modifications are limited. Such information could be of help in defining therapeutic strategies for avoiding cardiac arrhythmias triggered or facilitated by myocardial stretch.

Ranolazine is an antianginal agent that has been shown to exert a protective effect against cardiac arrhythmias [11–16]. Its actions include a decrease in the opening of the Na^+ channels during the action potential upstroke (peak Na^+ current) and outside the upstroke (late INa^+ current), and inhibition of the delayed rectifier potassium current (IKr) [13, 14, 17–23]. The inhibitory effect of ranolazine upon the late Na^+ current is responsible for the protective effect of the drug in relation to cellular Ca^{2+} overload.

Mechanical stretch induces an increase in Na^+ influx into myocytes and activates the Na^+/H^+ and reverse mode of the $\text{Na}^+/\text{Ca}^{2+}$ exchangers [2, 9, 10, 24], giving rise to changes in cardiac electrophysiological properties. The electrophysiological effects of acute myocardial stretching in isolated heart preparations include a shortening of the refractory periods, a decrease in the duration of the action potentials, a decrease of the wavelength of the activation process, depolarization of the resting potential, and the generation of after depolarizations [6, 7, 25–28]. The stretch-induced increase in Na^+ inflow has been related to a number of mechanisms, including stretch-activated channels that exhibit nonselective permeability to various cations including Na^+ [29, 30], mechanical activation of the Na^+/H^+ exchanger [24], or indirect Na^+/H^+ exchanger activation through autocrine/paracrine mechanisms [2]. An increase in peak Na^+ current due to an increased number of active channels has also been described [31] as an expression of mechanosensitivity of the voltage-sensitive sodium channel Nav1.5. The multichannel effects of ranolazine and the reduction of Na^+ entry into myocytes could modulate the electrophysiological effects of stretch. A reduction of Na^+ inflow may be due to action upon the ionic currents directly activated by mechanical stretch or to inhibitory action upon the late Na^+ current activated by the stretch-induced increase of intracellular Ca^{2+} concentration [32]. The effects of ranolazine upon the ventricular myocardium, at therapeutic concentrations, are mainly due to late INa and IKr inhibition. The inhibition of peak INa is weak, in contrast to what is observed at atrial level, where the antiarrhythmic effects are mainly related to blockade of the peak sodium current [13, 14, 17, 18, 22, 23].

The present study uses an experimental model involving isolated Langendorff-perfused rabbit hearts in which the analysis of the characteristics of myocardial activation during ventricular fibrillation (VF) allows us to track the time course of the electrophysiological changes induced by acute ventricular stretch [26, 27]. The study was carried out to analyze the effects of ranolazine on the electrophysiological responses induced by ventricular mechanical stretch.

Methods

Experimental Preparation

The present study abides with the EU Directive 2010/63/EU for animal experiments. New Zealand rabbits weighing 3–3.5 kg were used. Following pre-medication with ketamine and heparinization, the animals were sacrificed after anesthesia with sodium thiopental. Then the hearts were removed through a midsternal incision and connected to a Langendorff system for the perfusion of Tyrode solution at a pressure of 80 mmHg and a temperature of 37 ± 0.5 °C. The millimolar composition of the perfusion fluid was as follows: NaCl 130, NaHCO_3 24.2, KCl 4.7, CaCl_2 2.2, NaH_2PO_4 1.2, MgCl_2 0.6 and glucose 12. Oxygenation was carried out with a mixture of 95 % O_2 and 5 % CO_2 .

As described in previous studies of our group [26, 27, 33], an L-shaped device was inserted through the left atrium into the left ventricular cavity with the purpose of inducing mechanical stretch of a circumscribed zone of the left ventricular free wall by displacing the stem of the device. The latter consisted of a hollow tube measuring 3.5 mm in diameter, through which a stem 1.5 mm in diameter could be advanced. The distal end of the stem protruded from the L-shaped device and consisted of a circular platform measuring 7.5 mm in diameter, with which controlled stretching of a circumscribed zone of ventricle wall could be induced.

Two multiple electrodes respectively composed of 121 and 115 stainless steel electrodes (diameter=0.125 mm, interelectrode distance=1 mm) were positioned in the epicardial surface of the left ventricular free wall for recording the unipolar electrograms of the left ventricle. One of the electrodes was placed in the stretch zone in the mid-portion of the anterior wall, while the other electrode was placed in the non-stretch zone in the mid-portion of the posterior wall. Recordings and pacing techniques were similar to those used in the previously mentioned studies.

Experimental Series

Firstly, two experimental series were studied: a) a control group ($n=9$); and b) a group subjected to constant ranolazine infusion maintaining the same concentration (5 μM) throughout the experiment ($n=9$). The ranolazine concentration was within the range normally used in experimental studies involving therapeutic concentrations (2 to 8 $\mu\text{M/l}$) [13, 18–20, 23], and perfusion was initiated 15 min before the start of the electrophysiological study.

The following experimental protocol was used in each series: 30 min after placing the electrodes, VF was induced by pacing at increasing frequencies from 4 to 20 Hz, maintaining coronary perfusion during the arrhythmia. Five minutes after the induction of VF, stretch was applied by displacing the stem

of the L-shaped intraventricular device 6 mm, thereby giving rise to 12 % longitudinal increments along the vertical and horizontal axes within the stretched zone [26]. Local stretch was suppressed after 10 min, and VF recording was continued for another 5 min. In isolated rabbit hearts, end-diastolic pressures of 20 mmHg have been described with 8 % left ventricular long axis increments produced by introducing fixed volumes into the left ventricle [34]. In our model of ventricular wall stretch, the local longitudinal increments achieved could correspond (in a global stretch context) to an end-diastolic pressure of between 25 and 30 mmHg, though this estimation is not based on direct determinations.

Secondly, in two additional series the effects of increasing ranolazine concentrations (1, 5, 10 and 50 μM) on the ventricular effective refractory period (VERP) determined by the extrastimulus technique with a basic cycle length of 250 ms ($n=6$) and on the VF dominant frequency (DFR) ($n=6$) were studied by repeating the procedure for each drug concentration. The pacing threshold was determined for each concentration, and the stimulus amplitude was twice the diastolic threshold. The recovery of the drug effect was also studied after drug washout at the end of each experiment. In these series ranolazine was also

administered as an infusion, maintaining each concentration constant throughout each step and changing it 15 min before the electrophysiological determinations.

Data Analysis

Ventricular Fibrillation Spectral Analysis

The Welch method [35] was used to obtain the power spectrum of the signals recorded with each of the unipolar electrodes located in the two study zones. This analysis was made every minute before the induction of stretch, during stretch, and after the suppression of stretch (Fig. 1). The periodogram was calculated for the first 4 s of each signal (4096 points, sampling frequency 1 kHz), fragmenting the recordings into 8 segments with 50 % overlap and using Hamming's window. The mean frequency was determined with the maximum peak of power spectral density between 5 and 48 Hz (dominant frequency, DFR). Spectral concentration (SpConc) was also calculated for each channel as a percentage of the total energy contained in the interval $\text{DFR} \pm 0.5 \text{ Hz}$.

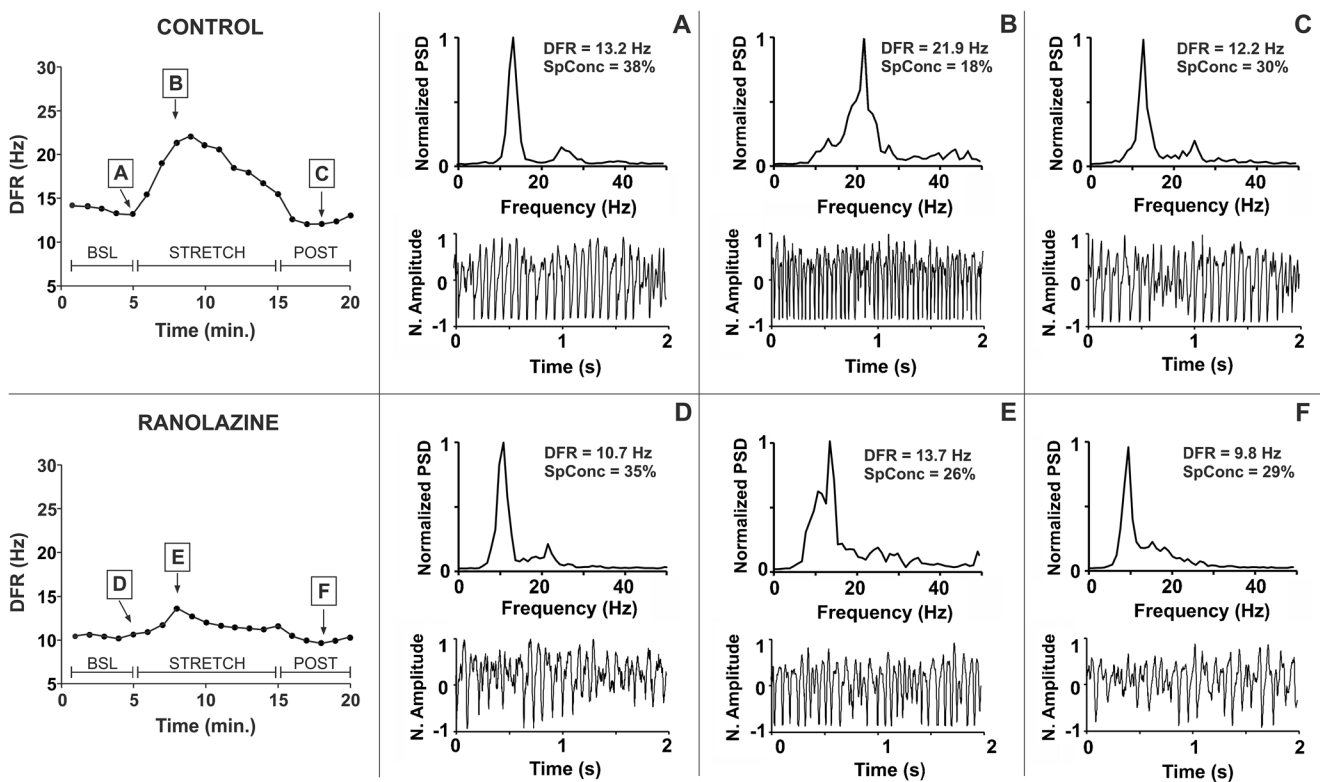


Fig. 1 Dominant frequency values during ventricular fibrillation, recorded in the stretch zone in an experiment of the control series and in an experiment of the ranolazine series during the three experimental phases: baseline, stretch and post-stretch. The figure also shows the power spectrum of the signal recorded with one of the electrodes immediately before stretch, 3 min after the start of stretch, and 3 min after stretch suppression (control: A, B, C; ranolazine: D, E, F).

Abbreviations: BSL baseline, POST post-stretch period, DFR dominant frequency, Hz hertz, N. Amplitude normalized amplitude, PSD power spectral density, SpConc spectral concentration (as %). For increased clarity, the VF recordings shown correspond to a time window of 2 s, though the spectrograms were obtained from data blocks of 4096 points at a sampling rate of 1 kHz

Ventricular Fibrillation Time-Domain Analysis

Activation times in each electrode were determined by identifying the moments of maximum negative slope of the ventricular electrograms. If the electrograms exhibited two or more deflections, then the steepest slope of the activation complex was assigned as the local activation time. The median of the consecutive activation intervals during ventricular fibrillation (VV) and the fifth percentile (P5th) were determined for each channel of the multielectrodes during time windows of 2 s in three stages: baseline (prior to stretch), during stretch (3 min after starting stretch), and post-stretch (3 min after suppressing stretch). These three time windows were chosen after performing the spectral analysis, which allowed rapid localization of the moment of maximum effect of stretching and the delay necessary for the disappearance of these effects.

Activation Maps

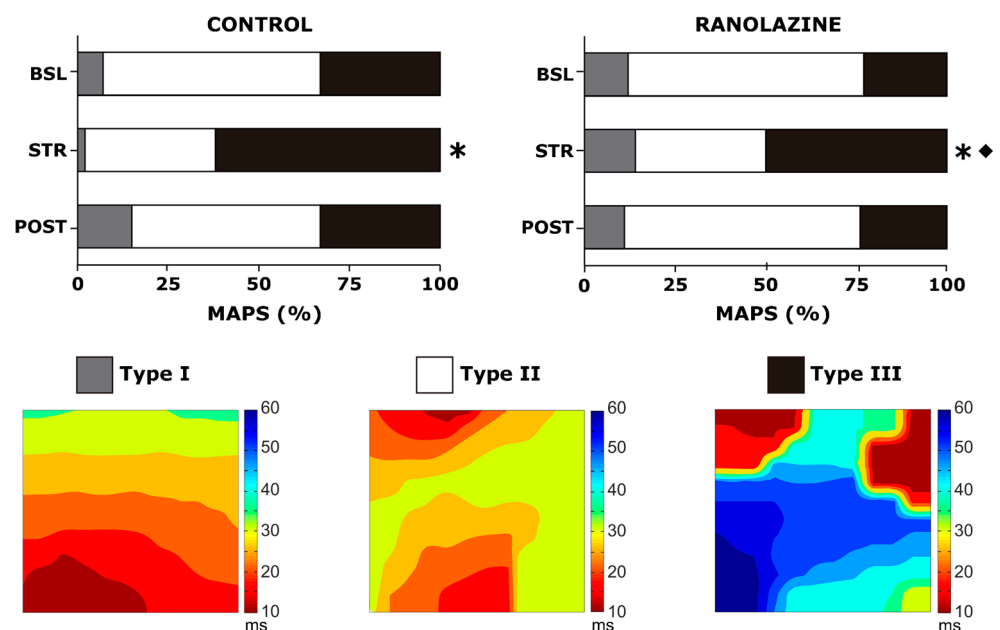
Activation maps during VF were constructed every 100 ms in the two-second time windows analyzed immediately before stretch and at the third minute of the stretch and post-stretch periods, as described in previous studies [26, 27]. Each map was classified into three categories based on its complexity: low (type I), comprising single broad wavefronts without conduction block lines or areas of slow conduction; intermediate (type II), consisting of two wavefronts or one wavefront with areas of conduction block or slow conduction; and high (type III), comprising three or more wavefronts associated with areas of slow conduction and conduction block (Fig. 2). A breakthrough

pattern was considered when the earliest activation was located in the area encompassed by the multiple electrodes and a centrifugal activation pattern towards the adjacent zones was seen. Conduction velocity during VF (CV) was the ratio between the distance between two electrodes positioned 5 interelectrode spaces apart in a direction perpendicular to the isochrones and the difference between their activation times (average of 3 determinations). Only maps in which both the input and output of wavefronts were identified at the edges of the activation maps were used to determine CV. The fifth percentile of the VV intervals during VF (P5th) was used as an approximation to the shortest VV intervals, and was considered as a surrogate of the functional refractory period during VF [26]. The wavelength (WL) of the activation process during VF was calculated as the product of CV and P5th.

Statistical Analysis

Continuous variables are presented as means±standard deviation (SD), and categorical variables are presented as percentages. The general linear model was used to analyze the differences in each series (differences within subjects, repetitive measurements: baseline, stretch, post-stretch) and to compare the series (control vs ranolazine, differences between subjects). Values of $p < 0.05$ were considered statistically significant. Differences between qualitative variables were analyzed using the χ^2 test. The multivariate analysis was performed using a stepwise multiple linear regression model. Data were analyzed using the statistical Package SPSS 16.0.

Fig. 2 Percentages of activation maps, classified according to their complexity (types I, II or III), observed in the stretch zone at baseline, during stretch and post-stretch in each experimental series. Abbreviations: *BSL* baseline, *STR* during stretch. Differences with respect to baseline: * $p < 0.05$; differences with respect to control: ♦ $p < 0.05$



Results

Effects of Acute Myocardial Stretch Upon VF Activation Frequency

Figure 3 show the results obtained in the first experimental series on determining DFR by spectral analysis in the stretched zone in the three experimental phases: baseline (before stretch), during stretch, and after stretch suppression. In both the controls and in the ranolazine series, DFR experienced a significant increase during stretch. However, the magnitude of the increase was significantly smaller under the action of ranolazine. The percentage increase in DFR versus baseline was 40.4 % in the control series and 23.0 % under ranolazine ($p < 0.001$ vs control). The dominant frequency at baseline was almost significantly lower in the ranolazine series ($p = 0.053$ vs control). The dominant frequency during stretch was significantly lower in the ranolazine series ($p < 0.001$ vs control). Likewise, the post-stretch differences versus the controls were significant ($p < 0.05$).

The median of the VV intervals during VF decreased significantly during stretch in both series (control: baseline = 78 ± 12 ms, stretch = 62 ± 9 ms, $p < 0.01$, post-stretch = 81 ± 9 ms; ranolazine: baseline = 91 ± 8 ms, stretch = 80 ± 7 ms, $p < 0.01$, post-stretch = 95 ± 9 ms). However, at baseline VV was significantly greater in the ranolazine series ($p < 0.02$ vs control); during stretch it showed less reduction ($p < 0.001$ vs control); and in the post-stretch phase VV was also greater than in the control series ($p < 0.01$). During stretch and post-stretch, the non-stretch zone showed no significant variations in either DFR or VV versus baseline in both series.

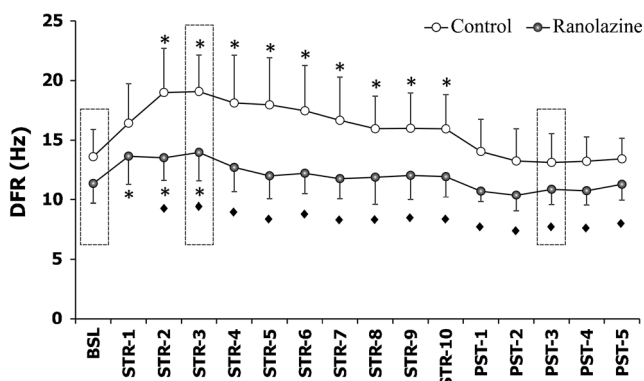


Fig. 3 Mean values (\pm standard deviation) of the dominant frequency obtained every minute in the stretched zone before the induction of stretch, during stretch and after the suppression of stretch. Abbreviations: BSL baseline, DFR dominant frequency in Hz, STR during stretch, PST post-stretch. Differences with respect to baseline: * $p < 0.05$; differences with respect to control: ♦ $p < 0.05$

Effects of Acute Myocardial Stretch Upon the Organization of VF Activation

Spectral Concentration (SpConc)

In the stretch zone, SpConc decreased significantly during stretch in the control series and showed no significant variations in the ranolazine series (Table 1). At baseline, the differences with respect to the control series did not reach statistical significance in the ranolazine series. During stretch, the values in the ranolazine series ($p < 0.01$) were significantly greater than in the control series. During and after stretch, the non-stretch zone showed no statistically significant variations versus baseline.

Activation Maps

In the control series, stretch significantly ($p < 0.0001$) modified the complexity of ventricular activation during VF, as evaluated by the percentages of map types, with increments of more complex (type III) activation maps and decrements of maps with intermediate (type II) and low complexity (type I) (Fig. 2). These variations also reached statistical significance in the ranolazine series. Prior to mechanical stretch, the differences in the percentages of map types were not significant in the control and ranolazine series. However, compared with the control series, VF activation during stretch was significantly less complex in the ranolazine series ($p < 0.01$) (Figs. 2 and 4).

During stretch, the percentages of maps with breakthrough patterns did not change significantly versus baseline in the control (baseline 23 %, stretch 32 %, post-stretch 26 %, ns) and ranolazine series (baseline 42 %, stretch 46 %, post-stretch 42 %, ns) - though in the ranolazine series these percentages were significantly greater than in the control series

Table 1 Ventricular fibrillation spectral concentration and electrophysiological parameters (mean \pm SD) obtained in each experimental series before, during and after stretch suppression

	SpConc (%)	P5th (ms)	CV (cm/s)	WL (cm)
CONTROL				
BSL	28 \pm 9	54 \pm 12	47.3 \pm 4.5	2.5 \pm 0.5
STR	18 \pm 3 *	38 \pm 5 *	49.3 \pm 3.0	1.9 \pm 0.2 *
POST	29 \pm 8	56 \pm 7	46.9 \pm 4.0	2.6 \pm 0.5
RANOLAZINE				
BSL	25 \pm 4	62 \pm 12	43.9 \pm 8.0	2.6 \pm 0.5
STR	23 \pm 4 ♦	50 \pm 8 * ♦	50.1 \pm 7.5	2.4 \pm 0.3 ♦
POST	28 \pm 4	65 \pm 10 ♦	44.4 \pm 7.8	2.8 \pm 0.7

Abbreviations: BSL baseline, before stretch, STR during stretch, POST post-stretch, SpConc spectral concentration, P5th fifth percentile of the VV intervals during ventricular fibrillation, CV conduction velocity during ventricular fibrillation, WL Wavelength during ventricular fibrillation * $p < 0.05$ vs baseline; ♦ $p < 0.05$ vs control series

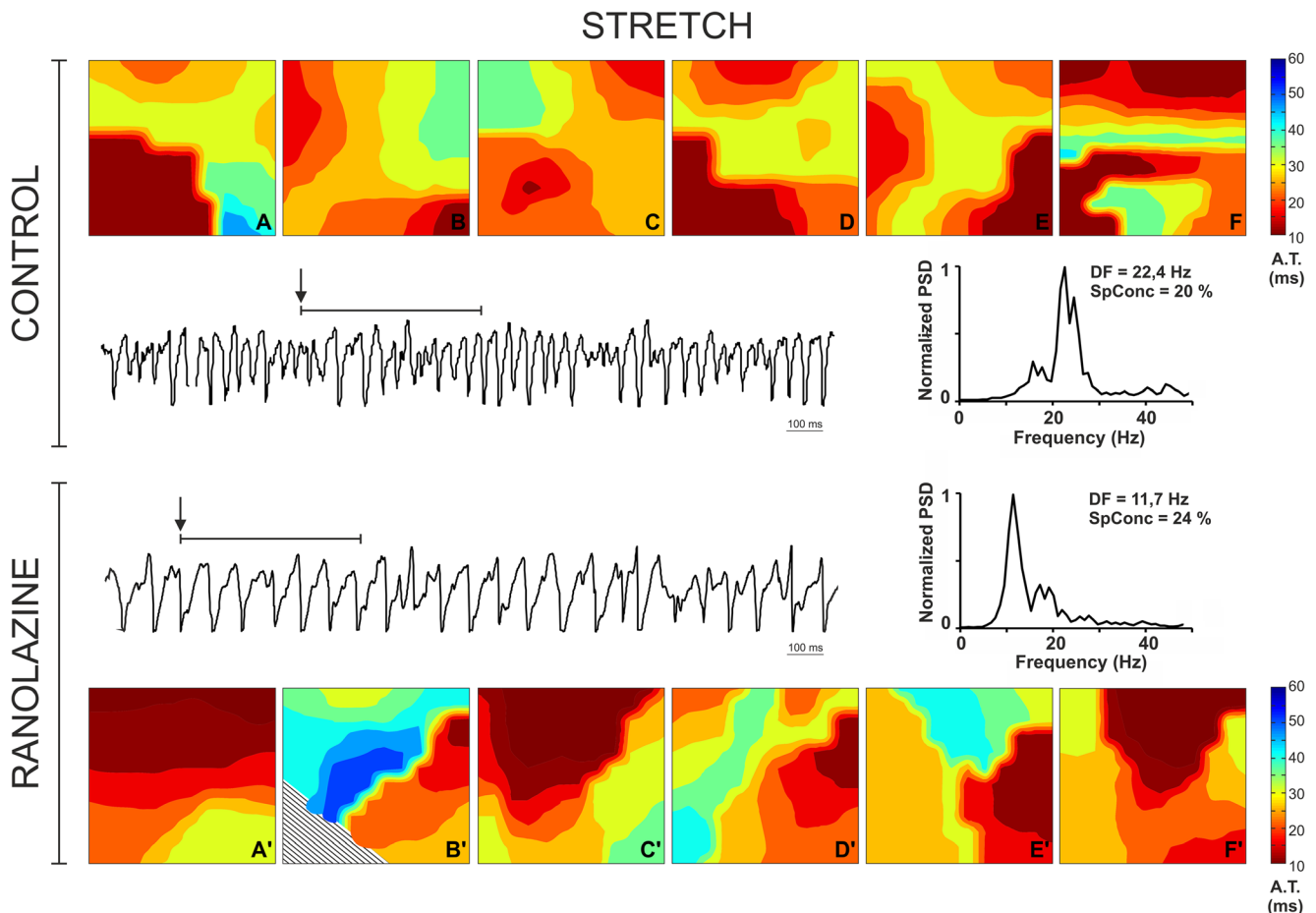


Fig. 4 Consecutive activation maps obtained during the stretch phase in an experiment of the control series and also during stretch in an experiment of the ranolazine series. Control series showed a higher percentage of the more complex activation maps, with different

simultaneous wavefronts and conduction block lines (type III). *Abbreviations:* AT activation time, DF dominant frequency, SpConc spectral concentration, PSD power spectral density

($p < 0.05$). Regarding the maps with complete reentrant patterns, no significant variations were observed during stretch in either series (control: baseline 2 %, stretch 4 %, post-stretch 1 %; ranolazine: baseline 3 %, stretch 6 %, post-stretch 3 %).

Effects of Acute Myocardial Stretch Upon the Electrophysiological Parameters During VF

Table 1 shows the fifth percentile of the VV intervals obtained in control and ranolazine series in each of the experimental phases. This parameter decreased significantly during stretch. At baseline, P5th was slightly but non-significantly greater in the ranolazine series. During stretch, P5th was significantly greater than in the control series ($p < 0.01$). Regarding CV during VF, the variations under stretch versus the baseline values failed to reach statistical significance in either series. Both at baseline and during stretch and post-stretch, the differences between the ranolazine and control series were not

statistically significant. Regarding WL during VF, this parameter decreased significantly during stretch in the control series, but in ranolazine series the decrease was not significant. On comparing both series during stretch, WL was significantly greater in the ranolazine series ($p < 0.001$).

Table 2 shows the regression lines obtained on relating the complexity of VF to DFR and the electrophysiological parameters P5th, CV and WL. The degree of complexity was expressed as a quantitative variable using the formula: Complexity index = (number of type I maps \times 0.1 + number of type II maps \times 1 + number of type III maps \times 2) / total number of activation maps [36]. The coefficients were significant in the case of DFR, P5th (Fig. 5) and WL, but not in the case of CV. Likewise, DFR showed a significant correlation to P5th and WL. On performing the multiple linear regression analysis taking the index of complexity as dependent variable and the electrophysiological parameters as independent variables, the model only identified P5th as independent variable.

Table 2 Regression analysis obtained on relating VF complexity or DFR to the electrophysiological parameters

	r	Constant	Coefficient	p	see
Complexity vs:					
DFR	0.52	0.64	0.046	0.0001	0.27
P5th	-0.68	2.15	-0.016	0.0001	0.22
CV	0.25	0.91	0.009	0.079	0.22
WL	-0.43	1.75	-0.176	0.002	0.21
DFR vs:					
P5th	-0.83	26.21	-0.230	0.0001	1.99
CV	0.26	6.86	0.150	0.075	3.41
WL	-0.65	24.17	-4.197	0.0001	2.69

Abbreviations: DFR ventricular fibrillation dominant frequency, P5th fifth percentile of the VV intervals during ventricular fibrillation, CV conduction velocity during ventricular fibrillation, WL wavelength during ventricular fibrillation, r regression coefficient, see standard error of estimate

Effect of Increasing Ranolazine Concentrations Upon VF Dominant Frequency and Ventricular Refractoriness

Figure 6a shows the VF DFR in the control group and for each ranolazine concentration. Both without and during stretch, DFR significantly decreased with respect to control during the perfusion of ranolazine 5 μM and 10 μM , and returned to control values after ranolazine washout. During 50 μM ranolazine perfusion, VF was not inducible in any experiment. Stretch significantly increased DFR with respect to pre-stretch values under control conditions and during the perfusion of 1 μM and 5 μM ranolazine concentrations, but not with 10 μM . The magnitude of the stretch-induced increment in DFR was seen to decrease on increasing the ranolazine concentrations (control: 37.5 %, 1 μM : 23 %, 5 μM : 20 %, 10 μM : 14 %, and after drug washout: 35 %).

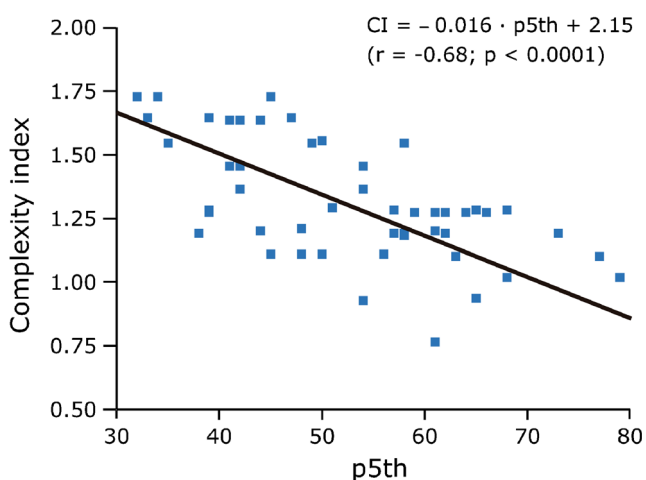


Fig. 5 Regression straight line obtained on relating ventricular fibrillation complexity and the fifth percentile of VV intervals during ventricular fibrillation. *Abbreviations:* CI complexity index, p5th fifth percentile of VV intervals during ventricular fibrillation (in ms); r regression coefficient

10 μM : 14 %, and after drug washout: 35 %). Figure 6b shows VERP determined by the extrastimulus technique both before and during stretch, under control conditions and for each ranolazine concentration. This parameter increased significantly with respect to control during the perfusion of ranolazine 50 μM , and returned to control values after ranolazine washout. Stretch significantly reduced VERP with respect to pre-stretch values under control conditions and during the perfusion of 1 μM and 5 μM ranolazine concentrations. The variations in VERP produced by stretch were not statistically significant for 10 μM and 50 μM ranolazine concentrations. During stretch, VERP was significantly higher than in control at 5 μM , 10 μM and 50 μM concentrations. After ranolazine washout, the reduction of VERP during stretch was again statistically significant.

Discussion

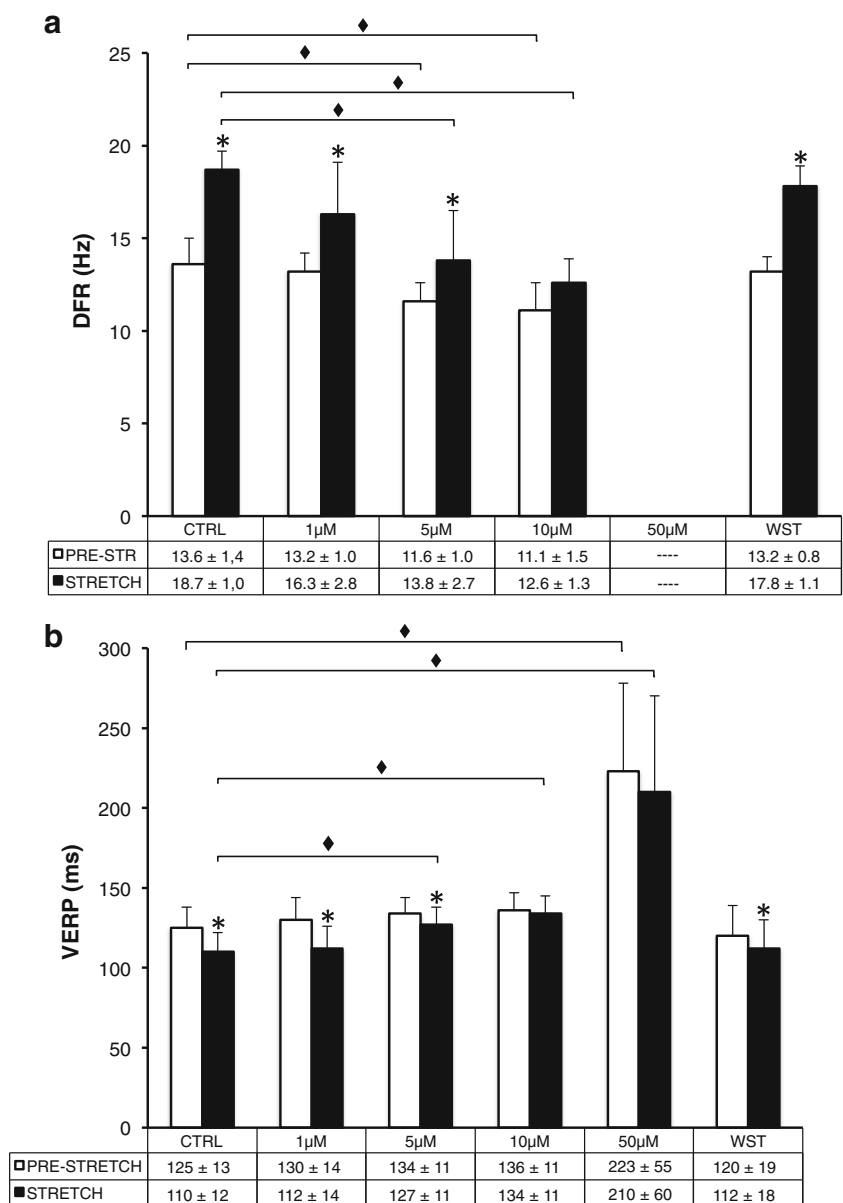
The main finding of this study is that ranolazine attenuates the increases in VF activation frequency and complexity induced by acute myocardial stretch. This effect is associated to an attenuated decrease in refractoriness and wavelength of the activation process during stretch.

Effects of Ranolazine on VF Activation Patterns

During baseline recordings we observed a slowing effect on myocardial activation during VF by ranolazine. The median of the VV intervals during VF was greater in the ranolazine series, and this result was consistent with the observations on using the spectral techniques, which showed an almost significant slow frequency in the ranolazine series. This effect was not accompanied by a reduction in the complexity of the VF activation patterns, as evaluated by the types of activation maps during the arrhythmia and by the spectral concentration. The baseline percentages of map types were not significantly different in both series, and the spectral concentration around the maximum value of the power spectrum was similar. An increase in this latter parameter would indicate more regular activation during the arrhythmia, as has been observed under the action of several antiarrhythmic drugs [27].

Ranolazine has multichannel actions, and at therapeutic concentrations (2–8 $\mu\text{M/l}$) the effects in ventricular myocardium are mainly due to late I_{Na} and I_{Kr} inhibition [13, 14, 17, 18, 22, 23]. The potency varies in different animal species. In this respect, the half maximal inhibitory concentration (IC_{50}) for late I_{Na} is between 5.9 and 16.5 $\mu\text{M/l}$, versus 11.5–14.4 $\mu\text{M/l}$ for I_{Kr} [13, 18, 20, 37]. The inhibition of peak I_{Na} is weak in ventricular cells (IC_{50} between 135 and 428 $\mu\text{M/l}$), but this action is potent in atrial cells [14, 22]. However, the IC_{50} values for the inhibition of peak I_{Na} decreases at fast rates and in depolarized cardiac tissues. At high

Fig. 6 a) Mean values (\pm standard deviation) of the dominant frequency during ventricular fibrillation, recorded under control conditions and at increasing ranolazine concentrations, both before and during stretch. *Abbreviations:* CTRL control, STR stretch, DFR dominant frequency in Hz, WST after drug washout. Differences between stretch and pre-stretch: * $p < 0.05$; differences with respect to control $\blacklozenge p < 0.05$. **b)** Mean values (\pm standard deviation) of the effective refractory period obtained with the extrastimulus technique under control conditions and at increasing ranolazine concentrations, both before and during stretch. *Abbreviations:* CTRL control, STR stretch, DFR dominant frequency in Hz, WST after drug washout. Differences between stretch and pre-stretch: * $p < 0.05$; differences with respect to control $\blacklozenge p < 0.05$



concentrations it also exerts inhibitory effects upon the L-type calcium current [13, 18, 37]. Ranolazine increases the ventricular refractory period in rabbits [12] and pigs [21]. The inhibition of IKr prolongs refractoriness, and on the other hand it has been reported that the effect on refractoriness is greater than on prolongation of the QT interval or action potential duration; for this reason there is an increase in post-repolarization refractoriness related to a prolongation of the recovery of Na⁺ channels [12]. In the present study we observed a non-significant trend ($p=0.1$) towards greater values of the surrogate marker for refractory period during VF (P5th) in the first ranolazine series, and a significant inverse correlation between DFR and this parameter has been recorded. In order to analyze the effects of ranolazine upon VERP obtained by the extrastimulus technique, this parameter was directly

measured in the additional series under increasing ranolazine concentrations. A trend to increase this parameter was seen, and differences were statistically significant for the 50 μ M concentration. This effect disappeared after drug washout. The great VERP increment observed at this concentration explains the impossibility in inducing VF at this drug concentration.

Effects of Stretch Under the Action of Ranolazine

In previous studies we found acute stretch of the ventricular myocardium to accelerate VF and increase the complexity of the arrhythmia [26, 27, 33]. The analysis of the DFR time changes in our model has revealed that the maximum effect occurs around 3 min after starting stretch, and that this effect

then diminishes but remains significant after 10 min. Tracking of the electrophysiological modifications during acute myocardial stretch is difficult when using conventional electrophysiological techniques such as the extrastimulus test and pacing at increasing frequencies, while analysis of the changes in the FV activation patterns allows us to visualize these modifications [26]. On using this experimental model, we have observed the modifying influence of several drugs upon the electrophysiological effects of myocardial stretch [27]. In the present work, as in previous studies, we found that stretch increased the VF dominant frequency and the complexity of the VF activation maps in the control series. It also produced a decrease in the spectral concentration of the VF recordings, indicating a more disorganized activation during the arrhythmia. Stretch also diminished the surrogate marker of refractoriness P5th and the WL of the activation process during the arrhythmia.

The stretch induced changes were attenuated under the action of ranolazine. Dominant frequency increments under stretch were smaller, and this attenuating effect was more pronounced on increasing the ranolazine concentrations. Upon analyzing the observed effects at therapeutic concentrations (5 μM), during stretch, P5th decreased 27 % in the control series, while the reduction in the ranolazine series was 19 %. On the other hand, WL during stretch decreased significantly in the control series, while in the ranolazine series the variation versus baseline failed to reach statistical significance. In turn, on comparing both series during stretch, WL was found to be significantly greater in the ranolazine series.

A factor that may be related to the effects of ranolazine during stretch is the action of the drug upon the stretch-induced modulation of Nav 1.5. The mechanosensitivity of Nav 1.5 consists of accelerated kinetics, an increased peak current, and stabilization of inactivation [38, 39]. However, modulation of the mechanosensitivity of Nav 1.5 has been demonstrated at high ranolazine concentrations (50 $\mu\text{M/l}$), though it has been argued that the drug effects depend on the magnitude of stretch and on the ranolazine membrane concentration [38].

On the other hand, the effects of ranolazine upon the Na^+ current could counter the increase in cellular Na^+ originated by stretch and reverse mode activation of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger that leads to Ca^{2+} overload. Calcium overload favors the appearance of after depolarizations and also modifies the slope of the action potential duration restitution curve [40] - favoring wave breaks and more disorganized activation patterns, as occurs during stretch. The inhibition of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger reduces the electrophysiological effects of mechanical stretch, as we have observed in a previous study using a similar experimental model [27]. Lastly, another possible mechanism implicated in the effects of ranolazine could be related to its binding affinity for beta-adrenergic receptors and its beta-adrenergic antagonist activity [19, 41, 42]. In an earlier study we found beta-adrenergic block with propranolol

to decrease the ventricular fibrillation accelerating effect of acute mechanical stretch and also reduce the increase in activation complexity produced by stretch [27]. However, it must be taken into account that the concentrations at which ranolazine blocks beta-adrenergic receptors are quite high.

Clinical Implications

Mechanoelectric feedback has been implicated in proarrhythmic situations, since stretch promotes the triggering of arrhythmias and increases the electrophysiological heterogeneity of myocardial tissue [5–8, 28]. Although clinical trials on the antiarrhythmic effects of ranolazine are limited, the drug has been reported to decrease non-sustained tachycardias in the context of acute coronary syndrome without ST-segment elevation [11]. In DAI carriers reduces ventricular tachycardia burden and ICD shocks [15], and appears effective for symptomatic ventricular arrhythmia reduction [43]. The present study contributes information on the possible role of ranolazine in reducing the arrhythmogenic effects of myocardial stretch. Its inhibitory effects upon mechanoelectric feedback, activated under conditions of overload or dyssynchronous ventricular contraction, could be related to the protective effect of the drug.

Limitations

The results obtained depend on the characteristics and conditions of the experimental preparation used in acute stretch application. The effects of stretch can give rise to different manifestations in chronic preparations and in in situ heart preparations, due among other factors to the existence of associated neurohumoral reflexes. On the other hand, the possible existence of interspecies differences must be taken into account in extrapolating the results obtained.

Conclusions

In the experimental model used, the late inward Na^+ current inhibitor ranolazine attenuates the electrophysiological effects responsible for the acceleration and increase in complexity of VF produced by acute myocardial stretch.

Acknowledgments Funding: This work was supported by Spain's Ministry of Economy and Competitiveness (Carlos III Health Institute) Grants FIS PS09/02417, FIS PI12/00407 and RETIC "RIC" RD12/0042/0048, and by *Generalitat Valenciana* Grant PROMETEO 2010/093.

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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