

Effect of Streptomycin on Stretch-Induced Change in Myocardial Activation During Ventricular Fibrillation

Isabel Trapero,^a Francisco J. Chorro,^{b,c} Luis Such-Miquel,^d Joaquín Cánoves,^b Álvaro Tormos,^e Francisca Pelechano,^c Laura López,^d and Luis Such^c

^aDepartamento de Enfermería, Universitat de València, Valencia, Spain

^bServicio de Cardiología, Hospital Clínico Universitario, Valencia, Spain

^cDepartamento de Medicina, Universitat de València, Valencia, Spain

^dDepartamento de Fisioterapia, Universitat de València, Valencia, Spain

^eDepartamento de Ingeniería Electrónica, Universidad Politécnica de Valencia, Valencia, Spain

The aim of this study was to determine whether the changes in myocardial activation pattern resulting from acute stretching during ventricular fibrillation can be counteracted by administering a compound that blocks receptors sensitive to stretch. The study involved 16 isolated rabbit hearts, in which refractoriness and activation frequency during ventricular fibrillation were measured before, during and after localized acute stretching of the left ventricular free wall, either without (series A, n=8) or with (series B, n=8) the presence of streptomycin, 200 μ mol. At baseline and during and after stretching, ventricular fibrillation was slower with streptomycin perfusion in series B than in series A (dominant frequency at baseline, 13[2] Hz vs 16[2] Hz, respectively; $P < .005$; dominant frequency with stretching, 14[2] Hz vs 19[3] Hz, respectively; $P < .005$). Streptomycin attenuated the electrophysiological changes produced by stretching and had a direct effect on refractoriness and activation frequency during ventricular fibrillation.

Key words: Ventricular fibrillation. Electrophysiology. Myocardial stretch. Streptomycin.

Efectos de la estreptomycin en las modificaciones de la activación miocárdica durante la fibrilación ventricular inducidas por el estiramiento

Se ha evaluado si las modificaciones en la activación miocárdica durante la fibrilación ventricular producidas por el estiramiento agudo pueden ser contrarrestadas por

un bloqueador de los receptores sensibles al estiramiento. En 16 corazones aislados de conejo, se ha analizado la refractariedad y la frecuencia de activación durante la fibrilación ventricular antes, durante y después de producir un estiramiento agudo localizado en la pared libre del ventrículo izquierdo, en ausencia (serie A, n = 8) o en presencia (serie B, n = 8) de estreptomycin 200 μ mol. Durante la perfusión de estreptomycin (serie B), tanto basalmente como durante y después del estiramiento, la fibrilación ventricular ha sido más lenta que en la serie A (frecuencia dominante basal, 13 \pm 2 Hz frente a 16 \pm 2 Hz; $p < 0,005$; frecuencia dominante estiramiento, 14 \pm 2 Hz frente a 19 \pm 3 Hz; $p < 0,005$). La estreptomycin ha atenuado las modificaciones electrofisiológicas producidas por el estiramiento y ha tenido efecto directo en la refractariedad y la frecuencia de activación durante la fibrilación ventricular.

Palabras clave: Fibrilación ventricular. Electrofisiología. Estiramiento miocárdico. Estreptomycin.

INTRODUCTION

Stretching changes the electrophysiological properties of both atrial and ventricular cardiac tissue by activating and opening stretch-sensitive channels, and causing alterations in the cellular ion currents.¹ Because there is a large variety of mechanosensitive ion channels, an agent that can block all these channels has still not been identified. The aminoglycoside antibiotic streptomycin, although it is not specific, has been reported to block stretch-sensitive channels and reduce the development of arrhythmias induced by mechanical causes.^{2,3} Myocardial activation during ventricular fibrillation (VF) depends on electrophysiological properties, and for this reason stretching also changes the characteristics of activation during arrhythmia.⁴ The mechanisms producing these changes have not been clearly established, however. The present study investigates whether stretch-induced changes in the myocardial

This study has been funded by grants from the Spanish Ministry of Health (FIS PI06/0758 and RETIC RD06/0003/0010 REDINSCOR) and the Spanish Society of Cardiology (SEC 2004).

Correspondence: Dr. F.J. Chorro Gascó.
Servicio de Cardiología. Hospital Clínico Universitario.
Avda. Blasco Ibáñez, 17. 46010 Valencia. España.
E-mail: Francisco. J. Chorro@uv.es

Received October 16, 2006.

Accepted for publication September 12, 2007.

activation pattern during VF are counteracted by streptomycin.

METHODS

Experiment Preparation

The experiments were performed following the regulations set down in Spanish Royal Decree 1201/2005 of October 10, regarding the use of animals for scientific purposes.

Sixteen isolated, perfused, rabbit heart preparations were used, following previously described methods.^{5,6} An L-shaped device was inserted in the left ventricular cavity to produce local stretching of the left ventricular free wall (Figure 1). Recordings were obtained with 2 multiple electrodes placed in the epicardium of the left ventricle, 1 in the area submitted to local stretching (SA) and another in an unaltered area (NSA), with 121 and 119 unipolar electrodes, respectively.

Two series of experiments were performed following the same protocol (baseline-stretching-post-stretch), the only difference being the absence (series A, n=8) or presence (series B, n=8) of streptomycin (200 μmol) in the perfusion fluid of the heart since the start of the preparation.

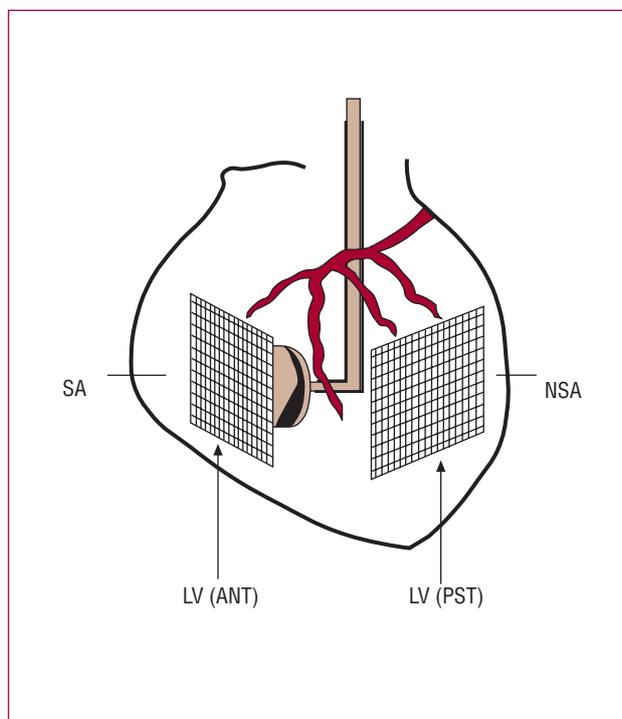


Figure 1. Schematic diagram of the device used to produce local stretching, with epicardial multielectrodes in the study areas. LV (ANT) indicates anterior left ventricle; LV (PST), posterior left ventricle; SA, stretched area; NSA, nonstretched area.

Data Analyzed

Ventricular fibrillation was induced by overstimulation, perfusion was maintained, and the following parameters were determined in both series: the dominant frequency (DFr) during VF (Welch method^{5,6}) (Figure 2), the interval between successive ventricular activations (VV interval), and the functional refractory period. These parameters were determined before (5-min period), during (10 min), and after (10 min) local ventricular wall stretching (longitudinal increase of 12%).

Statistical Calculations

The data are presented as the mean (standard deviation). Comparisons were performed using a general linear model with repeated measures, considering the phases of the experiment as a within-subject factor and the use of streptomycin or not as a between-subject factor, and applying the Bonferroni test as the post hoc test ($P < .05$, significant differences).

RESULTS

Effect of Streptomycin on Stretch-Induced DFr Changes

In series A, DFr was significantly increased in the SA ($P = .002$), but not in the NSA (general analysis, within-subject comparison test, factor phases of the experiment). Differences between the 2 heart areas were significant from minutes 2 to 6 of stretching (Figure 3).

In series B, the influence of the experiment phase factor was also significant in the SA ($P = .001$) and not in the NSA. Differences between the 2 areas were significant from minutes 2 to 4 of stretching.

Comparison between the 2 series showed that the DFr was lower in series B (streptomycin) than in series A in both areas of the heart (general analysis, between-subject differences: NSA, $P = .027$; SA, $P = .016$).

Effects of Streptomycin on Stretch-Induced VV Interval Changes during Ventricular Fibrillation

In series A, VV intervals in the SA were shorter during stretching with respect to baseline values ($P = .04$) and post-stretch values ($P = .018$), whereas in the NSA there were no significant changes (within-subject comparison, experiment phase factor) (Table 1).

In series B, VV intervals in the SA during stretching were also shorter relative to baseline ($P = .002$) and following stretching ($P = .004$) (within-subject comparison, experiment phase factor).

Comparison between the 2 series showed longer VV intervals in series B (streptomycin) than in series A in both heart areas (between-subject differences: NSA, $P = .004$; SA, $P = .001$).

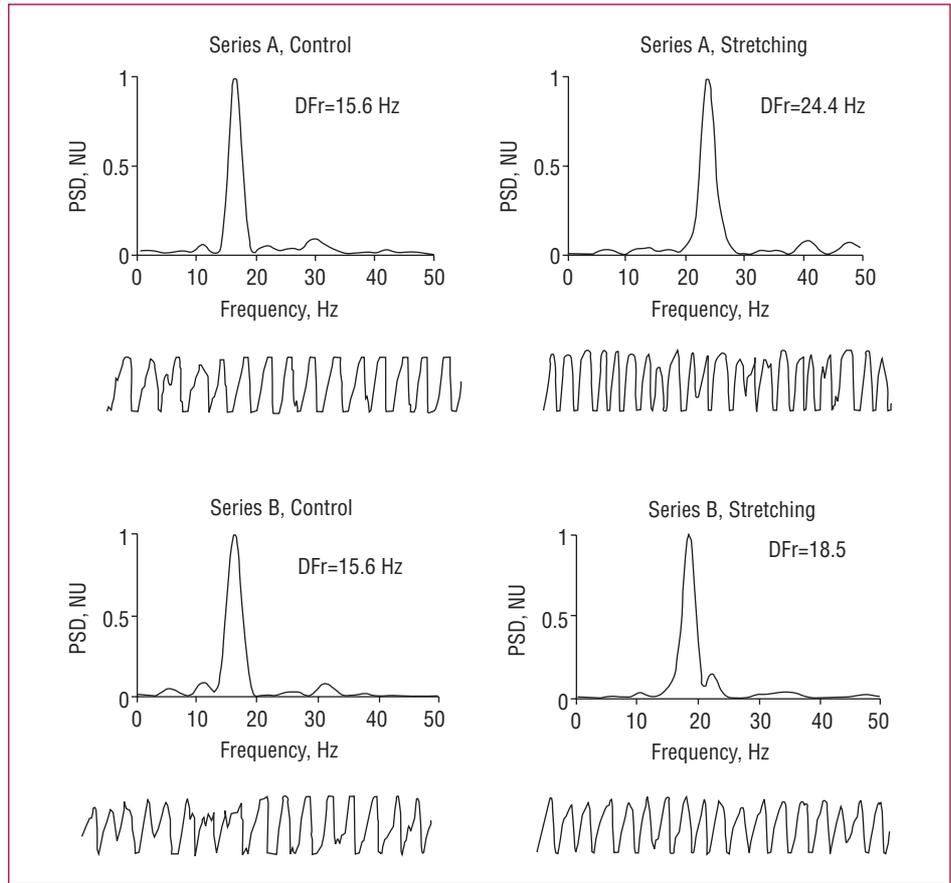


Figure 2. Power spectra of ventricular fibrillation in the altered area obtained from 1 experiment in each series at baseline and during stretching. DFr indicates dominant frequency (Hz); PSD, power spectrum density; NU, normalized units.

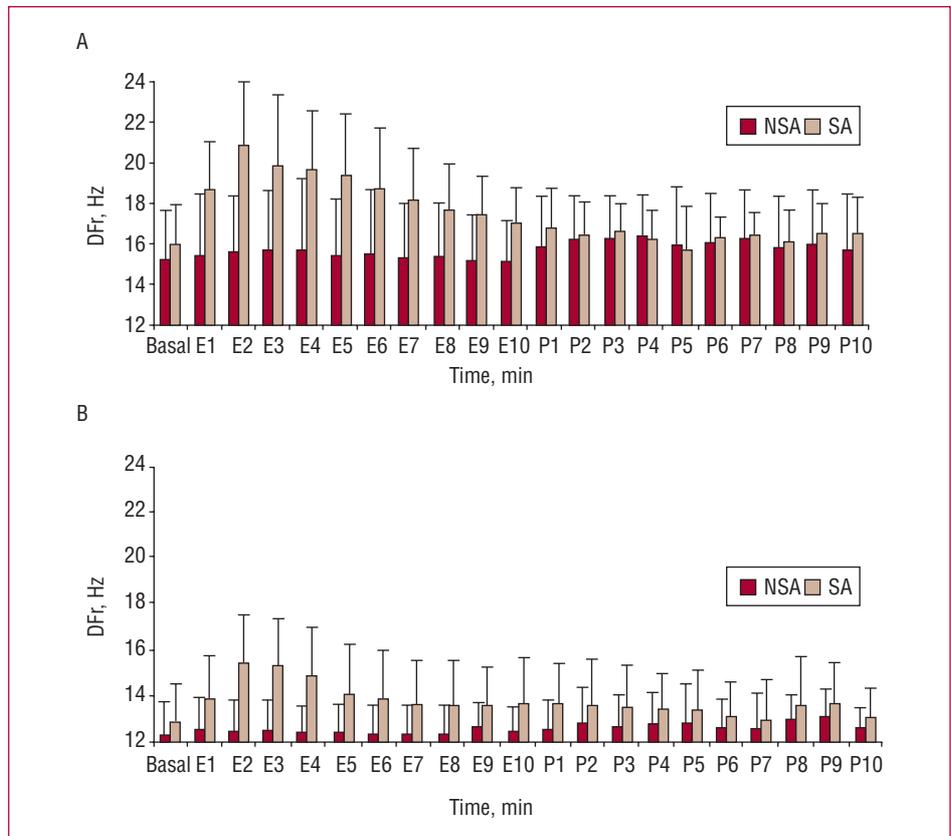


Figure 3. Dominant frequency during ventricular fibrillation (mean [standard deviation]) obtained at 1-minute intervals in the stretched and nonstretched areas of the heart in series A (above) and series B (below). E1 to E10 indicates stretching; DFr, dominant frequency (Hz); P1 to P10, post-stretch; SA, stretched area; NSA, nonstretched area.
*SA versus NSA, $P < .05$.

Effects of Streptomycin on Stretch-Induced Changes in Electrophysiological Parameters

In series A, refractory periods during VF were also shorter in the SA during stretching ($P=.026$ vs baseline and $P=.045$ vs post-stretch values), whereas no changes were seen in the NSA (Table 2).

In series B, refractory periods were decreased in the SA during stretching ($P=.01$ vs baseline $P=.001$ vs post-stretch values) and the NSA showed no variations.

With the action of streptomycin (series B), values were higher in the NSA and differences did not reach statistical significance in the SA (between-subject differences: NSA, $P=.047$; SA, $P=.075$).

DISCUSSION

The main finding of this study is that streptomycin decreased the effects of myocardial stretching during VF, and had a slowing action on VF both during and in the absence of stretching. The results were obtained with high drug concentrations, well above the therapeutic dose in humans. The concentrations used were those described to avoid the changes produced by stretching.³

Garnier et al⁷ found that streptomycin inhibited the increased intracellular calcium concentration induced by stretching in ventricular myocytes; hence the finding of reductions in these concentrations would be one of the reasons for the decrease in stretch-induced arrhythmias in the presence of streptomycin. These authors reported that streptomycin had no effect on L-type calcium channels. Moreover, Eckard et al³ did not observe the results obtained with streptomycin when using verapamil as a specific calcium blocker, thereby demonstrating that the effects of streptomycin are produced by blocking the mechanosensitive ion channels. The present study has contributed to this research with data showing the action of streptomycin on myocardium that is not subjected to any manipulation: in addition to attenuating the effects of stretching, streptomycin had a slowing effect on VF in the absence of stretching. The electrophysiological effects inherent to streptomycin may explain the VF changes observed in the present study in the control situation, in the area that was not altered by stretching.

The capability of streptomycin to inhibit triphosphate inositol production may also be implicated in its possible antiarrhythmic effect. Du et al⁸ observed that different drug compounds, among them streptomycin and gentamicin, inhibit triphosphate inositol release during reperfusion, and that intravenous gentamicin can even suppress the onset of arrhythmias. This is a parallel mechanism, in which adenosine triphosphate is likely implicated.

Bauty et al⁹ and Sung et al¹⁰ have indicated that other mechanisms in addition to activation of stretch-sensitive channels may come into play during stretching. This fact might be related to the persistence of stretch-induced

TABLE 1. Interventricular Interval Values (Mean [Standard Deviation]) During Ventricular Fibrillation, Obtained in Stretched and Nonstretched Areas of the Heart at Baseline, at 5 min of Stretching and at 10 min After Completion of Stretching in Both Experimental Series

	Baseline		During Stretching		Post-Stretch	
	NSA	SA	NSA	SA	NSA	SA
Series A, ms	65 (7)	60 (8)	66 (8)	51 (6) ^a	62 (1)	58 (4)
Series B, ms	80 (7) ^b	76 (9) ^b	78 (8) ^b	65 (2) ^{a,b}	76 (7) ^b	74 (5) ^b

Series A: no streptomycin; Series B: with streptomycin; SA: stretched area; NSA: nonstretched area.

^a $P<.05$, SA during stretching versus SA at baseline and post-stretch.

^b $P<.05$, series B versus series A.

TABLE 2. Ventricular Functional Refractory Period Values (Mean [Standard Deviation]) During Ventricular Fibrillation Obtained in the Stretched Area and Nonstretched Area at Baseline, at 5 min of Stretching and at 10 min After Completion of Stretching in Both Experimental Series

	Baseline		During Stretching		Post-Stretch	
	NSA	SA	NSA	SA	NSA	SA
Series A, ms	41 (6)	41 (6)	42 (6)	33 (4) ^a	41 (4)	37 (3)
Series B, ms	48 (6) ^b	45 (8)	47 (8) ^b	38 (4) ^a	46 (5)	45 (5) ^b

Series A: no streptomycin; series B: with; SA: stretched area; NSA: nonstretched area.

^a $P<.05$, SA during stretching versus SA at baseline and post-stretch.

^b $P<.05$, series B versus series A.

alterations in VF, despite the use of high concentrations of streptomycin.

In conclusion, streptomycin attenuated the electrophysiological changes and alterations in myocardial activation during VF produced by acute stretching and had a direct effect on the refractoriness and activation frequency.

ACKNOWLEDGEMENTS

The authors thank César Avellaneda and Camilo Gómez for their help in preparing the electrodes and stretching device, and Rosa Cibrián for performing the statistical analysis.

REFERENCES

1. Franz MR. Stretch-activated arrhythmias. En: Zipes DP, Jalife J, editores. Cardiac Electrophysiology: From Cell to Bedside. New York: WB Saunders; 1994. p. 597-606.
2. Salmon AH, Mays JL, Dalton GR, Jones JV, Levi AJ. Effect of Streptomycin on wall-stress-induced arrhythmias in the working rat heart. Cardiovasc Res. 1997;34:493-503.

3. Eckardt L, Kirchhof P, Mönning G, Breithardt G, Borggrefe M, Haverkamp W. Modification of stretch-induced shortening of repolarization by streptomycin in the isolated rabbit heart. *J Cardiovasc Pharmacol.* 2000;36:711-21.
4. Chorro FJ, Cánoves J, Guerrero J, Mainar L, Sanchis J, Soria E, et al. Opposite effects of myocardial stretch and verapamil on the complexity of the ventricular fibrillatory pattern: An experimental study. *Pacing Clin Electrophysiol.* 2000;23:1594-603.
5. Chorro FJ, Trapero I, Guerrero J, Such LM, Cánoves J, Mainar L, et al. Modification of ventricular fibrillation activation patterns induced by local stretching. *J Cardiovasc Electrophysiol.* 2005;16:1087-96.
6. Chorro FJ, Guerrero J, Trapero I, Such-Miquel L, Mainar L, Cánoves J, et al. Análisis tiempo-frecuencia de la fibrilación ventricular. Estudio experimental. *Rev Esp Cardiol.* 2006;59:869-78.
7. Garnier F, White E, Lacapagne A, Garnier D, Le Guennec JY. Streptomycin reverse a large stretch induced increases in $[Ca^{2+}]_i$ in isolated Guinea pig ventricular myocytes. *Cardiovasc Res.* 1994; 28:1193-8.
8. Du XJ, Anderson KE, Jacobsen A, Woodcock EA, Dart AM. Suppression of ventricular arrhythmias during ischemia-reperfusion by agents inhibiting $Ins(1,4,5)P_3$ release. *Circulation.* 1995;91:2712-6.
9. Bauty D, Lab M. Heterogeneous changes of monophasic action potential induced by sustained stretch in atrium. *J Cardiovasc Electrophysiol.* 2001;12:323-9.
10. Sung D, Mills RW, Schettler J, Narayan SM, Omens JH, McCulloch AD. Ventricular filling slows epicardial conduction and increases action potential duration in an optical mapping study of the isolated rabbit heart. *J Cardiovasc Electrophysiol.* 2003;14:739-49.