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Original article

Experimental Study of the Effects of EIPA, Losartan, and BQ-123 on Electrophysiological Changes Induced by Myocardial Stretch

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ABSTRACT

Introduction and objectives: Mechanical response to myocardial stretch has been explained by various mechanisms, which include Na^+/H^+ exchanger activation by autocrine-paracrine system activity. Drug-induced changes were analyzed to investigate the role of these mechanisms in the electrophysiological responses to acute myocardial stretch.

Methods: Multiple epicardial electrodes and mapping techniques were used to analyze changes in ventricular fibrillation induced by acute myocardial stretch in isolated perfused rabbit hearts. Four series were studied: control (n = 9); during perfusion with the angiotensin receptor blocker losartan (1 μM, n = 8); during perfusion with the endothelin A receptor blocker BQ-123 (0.1 μM, n = 9), and during perfusion with the Na^+/H^+ exchanger inhibitor EIPA (5-[N-ethyl-N-isopropyl]-amiloride) (1 μM, n = 9).

Results: EIPA attenuated the increase in the dominant frequency of stretch-induced fibrillation (control = 40.4%; losartan = 36% [not significant]; BQ-123 = 46% [not significant]; and EIPA = 22% [$P < .001$]). During stretch, the activation maps were less complex ($P < .0001$) and the spectral concentration of the arrhythmia was greater (greater regularity) in the EIPA series: control = 18 (3%); EIPA = 26 (9%) ($P < .02$); losartan = 18 (5%) (not significant); and BQ-123 = 18 (4%) (not significant).

Conclusions: The Na^+/H^+ exchanger inhibitor EIPA attenuated the electrophysiological effects responsible for the acceleration and increased complexity of ventricular fibrillation induced by acute myocardial stretch. The angiotensin II receptor antagonist losartan and the endothelin A receptor blocker BQ-123 did not modify these effects.

Estudio experimental de los efectos de EIPA, losartán y BQ-123 sobre las modificaciones electrofisiológicas inducidas por el estiramiento miocárdico

Palabras clave:
Arritmia
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Fibrilación ventricular
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Investigación básica
Mapeo
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RESUMEN

Introducción y objetivos: Se han implicado diversos mecanismos en la respuesta mecánica al estiramiento miocárdico, que incluyen la activación del intercambiador Na^+/H^+ por acciones autocrinas y paracrinas. Se estudia la participación de estos mecanismos en las respuestas electrofisiológicas al estiramiento agudo miocárdico mediante el análisis de los cambios inducidos con fármacos.

Métodos: Se analizan las modificaciones de la fibrilación ventricular inducidas por el estiramiento agudo miocárdico en corazones de conejo aislados y perfundidos utilizando electrodos múltiples epicárdicos y técnicas cartográficas. Se estudian 4 series: control (n = 9); durante la perfusión del antagonista de los receptores de la angiotensina II, losartán (1 μM, n = 8); durante la perfusión del bloqueador del receptor de la endotelina A, BQ-123 (0,1 μM, n = 9), y durante la perfusión del inhibidor del intercambiador Na^+/H^+, EIPA *(5-[N-ethyl-N-isopropyl]-amiloride)* (1 μM, n = 9).

Resultados: EIPA atenuó el aumento de la frecuencia dominante de la fibrilación producido por el estiramiento (control = 40,4%; losartán = 36% [no significativo]; BQ-123 = 46% [no significativo], y EIPA = 22% [$p < 0,001$]). Durante el estiramiento, la complejidad de los mapas de activación fue menor en la serie con EIPA ($p < 0,0001$) y también en esta serie fue mayor la concentración espectral de la arritmia (mayor regularidad): control = 18 ± 3%; EIPA = 26 ± 9% ($p < 0,02$); losartán = 18 ± 5% (no significativo), y BQ-123 = 18 ± 4% (no significativo).

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Conclusiones: El inhibidor del intercambiador Na^+/H^+ EIPA atenúa los efectos electrofisiológicos responsables de la aceleración y del aumento de la complejidad de la fibrilación ventricular producidos por el estiramiento agudo miocárdico. Por el contrario, el antagonista de los receptores de la angiotensina II, losartán, y el del receptor A de la endotelina, BQ-123, no modifican estos efectos.

Abbreviations

DF: dominant frequency
P5: 5th percentile of the consecutive activation intervals during ventricular fibrillation
SC: spectral concentration
VF: ventricular fibrillation
VV: median of the consecutive activation intervals during ventricular fibrillation

INTRODUCTION

The mechanical response of myocytes to stretch has been explained by various mechanisms, which include the local release of angiotensin II and endothelin, Na^+/H^+ exchanger activation, increased Na^+ influx, Na^+/Ca^{2+} exchanger reverse mode activation, and increased Ca^{2+} transients.[1,2] There is little information on the role of these mechanisms in the electrophysiological responses to myocardial stretch (electromechanical feedback) or on pharmacological modifications of the proarrhythmic effects of stretch.[3–10]

Inhibition of the Na^+/H^+ or Na^+/Ca^{2+} exchangers decreases the slow inotropic response to stretch and the magnitude of Ca^{2+} transients.[1,2,11–14] In turn, in relation to electromechanical feedback, the Na^+/Ca^{2+} exchanger inhibitor, KB-R7943, reduces the electrophysiological changes induced by stretch.[5] However, it remains unknown whether these changes are also reduced by the inhibition of the Na^+/H^+ exchanger or by blocking the effects of substances that may be involved in its activation after myocardial stretch, such as angiotensin II or endothelin.[1,15]

An experimental model was used to obtain more information on the mechanisms involved in the electrophysiological responses to myocardial stretch and its pharmacological modifications. The characteristics of myocardial activation during ventricular fibrillation (VF) can be analyzed to determine the time course of changes in electrophysiological myocardial properties caused by acute myocardial stretch applied in the left ventricular free wall.[5,16,17] The objectives of this study were: *a)* to determine whether the inhibition of the Na^+/H^+ exchanger, whose activation during stretch is a prior step to Na^+/Ca^{2+} reverse mode activation, also blocks or attenuates electrophysiological responses to stretch, and *b)* to determine whether the inhibition of angiotensin II type I receptors or endothelin A receptors, whose activation is thought to intervene in the mechanical response of myocytes to stretch, also modifies the manifestations of electromechanical feedback in the experimental model.

METHODS

Experimental Preparation

This study fulfilled the recommendations of the European Union directive 2010/63/EU on animal experimentation. New Zealand rabbits were premedicated with ketamine, administered heparin, and killed with sodium thiopental. After the heart was removed, the aorta was cannulated using a Langendorff system to perfuse oxygenated Tyrode at 80 mmHg and 37 (0.5) °C.

As described in previous studies,[5,16–18] a device was placed in the left ventricular cavity via the atrium to induce stretch in a specific area of the ventricular wall. Two multiple electrodes comprising 121 and 115 stainless steel unipolar electrodes (interelectrode distance = 1 mm) were positioned in the epicardium of the anterior wall (stretch zone) and the rear wall (nonstretch zone) (Figure 1). Recordings and stimulation techniques were similar to those described in the cited studies.

Experimental Series

Four series were studied: *a)* control (n = 9); *b)* during perfusion with the angiotensin receptor blocker losartan (1 μM, n = 8); *c)* during perfusion with the endothelin A receptor blocker BQ-123 (0.1 μM, n = 9); and *d)* during perfusion with the Na^+/H^+ exchanger inhibitor EIPA (5-[N-ethyl-N-isopropyl]-amiloride) (1 μM, n = 9). The concentration of these substances was within the ranges used in experimental studies,[15,19–22] and perfusion was started 15 min before electrophysiological study.

In each series, 30 min after placing the electrodes, VF was induced by stimulation at increasing frequencies while coronary perfusion was maintained. Five minutes after VF was induced, stretch was applied at longitudinal increments of 12% in the vertical and horizontal axes of the modified zone.[16] Local stretch was suppressed after 10 min.

Data Analysis

Ventricular Fibrillation Spectral Analysis

The Welch method[23] was used to obtain the power spectrum of the signals recorded with each unipolar electrode located in the 2 study sites. The spectral analysis was performed each minute before stretch induction, during stretch, and after stretch suppression (Figure 1). The spectrum corresponded to the first 4 s of each record (4096 points, sampling rate = 1 kHz) The dominant frequency (DF) in each electrode was obtained by determining the maximum value of the power spectral density. In addition, spectral concentration (SC) was calculated as the percentage of total energy within the DF range (0.5 Hz).

Time-domain Analysis

The methodology described above[5,16–18] was used to determine local activation times in each electrode. The median of the consecutive activation intervals during VF (VV) and the 5th percentile (P5) in each electrode were determined during 2-s time windows at baseline, 3 min after stretch induction, and 3 min after stretch suppression. These 3 time windows were chosen after performing the spectral analysis, thus enabling the rapid determination of the moment of maximum effect during stretch and the time interval until these effects disappeared.