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Review

Relevance of cardiomyocyte mechano-electric coupling to stretch-induced arrhythmias: Optical voltage/calcium measurement in mechanically stimulated cells, tissues and organs

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ABSTRACT

Stretch-induced arrhythmias are multi-scale phenomena in which alterations in channel activities and/or calcium handling lead to the organ level derangement of the heart rhythm. To understand how cellular mechano-electric coupling (MEC) leads to stretch-induced arrhythmias at the organ level, we developed stretching devices and optical voltage/calcium measurement techniques optimized to each cardiac level. This review introduces these experimental techniques of (1) optical voltage measurement coupled with a carbon-fiber technique for single isolated cardiomyocytes, (2) optical voltage mapping combined with motion tracking technique for myocardial tissue/whole heart preparations and (3) real-time calcium imaging coupled with a laser optical trap technique for cardiomyocytes. Following the overview of each methodology, results are presented. We conclude that individual MEC in cardiomyocytes can be heterogeneous at the ventricular level, especially when moderate amplitude mechanical stretches are applied to the heart, and that this heterogeneous MEC can evoke focal excitation that develops into re-entrant arrhythmias.

Contents

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1. Introduction

Alterations in the mechanical state of the heart can affect its electrophysiological behavior (Kohl et al., 2011; Ravens, 2003; Taggart and Lab, 2008). This mechano-electric coupling (MEC) is considered to play an important role in cardiac rhythm abnormalities, especially in disease states such as old myocardial infarction and chronic heart failure, in which ventricular walls are subjected to abnormal hemodynamic loading stresses (Aimond et al., 1999; Janse, 2004; Tomaselli and Marban, 1999). Sudden mechanical impact to the heart can also disarrange the heart rhythm even in healthy subjects, which in some instances leads to sudden cardiac death (Link, 2012). However, this phenomenon (termed *commotio cordis*) is rare, and evidence linking mechanical impact to fatal arrhythmias is scarce.

The mechanisms underlying MEC relate to the activation of ion channels by mechanical stretch (Sachs, 2010, 2011). Because such stretch-activated channels (SACs) have long been suspected as important contributors to MEC, there are many reports on characterization of these channels (Bett and Sachs, 2000; Craelius et al., 1988; Kohl et al., 1998; Niu and Sachs, 2003; Ward et al., 2008). In particular, considering the physiological loading conditions for each myocyte in the ventricular wall, numerous studies have been performed in isolated cardiomyocytes in response to axial stretching to elucidate the properties of SACs (Belus and White, 2003; Iribe et al., 2010; Kamkin et al., 2003; Riemer and Tung, 2003; Sasaki et al., 1992; Zeng et al., 2000). However, single channel recording in the stretch-imposed ventricular myocytes demands technical proficiency because of the difficulty in maintaining stable attachment of the glass electrode during the stretch. These restrictions also impair the ability to study the dynamic properties of cellular MEC induced by transient mechanical stretches.

Recent studies have revealed that axial stretching of cardiomyocytes enhances Ca^{2+} spark/wave rate via mechanisms dependent on microtubule-mediated modulation (Iribe et al., 2009), NADPH2 oxidase (NOX2) activation (Prosser et al., 2011) and neuronal nitric oxide synthase (nNOS) activation (Jian et al., 2014) of the ryanodine receptor. On the one hand, stretch acutely increases the affinity of troponin C to Ca^{2+} (Allen and Kentish, 1988), so that when stretched tissue is released there is a surge in intracellular Ca^{2+}, which can lead to Ca^{2+} waves (ter Keurs et al., 2008). These spontaneous Ca^{2+} releases are regarded as an important substrate for triggered arrhythmias and delayed after depolarizations (Fujiwara et al., 2008), though whether SACs and/or Ca^{2+} cycling contributes to MEC-related arrhythmias is unclear.

To clarify how MEC contributes to ventricular arrhythmias, various studies have been performed with cardiac tissues and whole heart preparations (Fasciano and Tung, 1999; Franz et al., 1992; Hansen et al., 1990; Parker et al., 2001). However, many of these reports are limited owing to lack of experimental methodologies for recording the electrical and mechanical activity simultaneously and with high spatiotemporal resolution. For instance, optical mapping (Efimov et al., 2004; Herron et al., 2012), a technique used to examine spatiotemporal electrical behavior in the heart, often necessitates either physically or pharmacologically constraining the heart contraction, which limits its utility for MEC research.

Herein, we review our techniques that we developed to resolve these various methodological issues in isolated cardiac myocytes (Section 2) and myocardial tissues (Section 3). Next, we discuss a possible scenario for the link between cellular MEC and fatal arrhythmias (Section 4). Furthermore, we briefly introduce our ongoing studies using an optical trap technique to elucidate the mechanisms of stretch-mediated Ca^{2+} handling (Section 5), and then provide a final conclusion (Section 6).

2. Cardiomyocyte study

There are only a small number of studies reporting the characteristics of cardiomyocytes MEC upon axial stretching. Considering the dynamic changes in stress and strain that an individual cardiomyocyte experiences in the beating heart, it is important to examine the responses to dynamic stretching on single cardiomyocytes. In this section, we introduce our methodology for examining single cardiomyocytes, in which we combine a carbon-fiber technique with optical voltage measurement for assessing cellular MEC upon dynamic stretching.

2.1. Carbon-fiber technique for single cardiomyocyte stretching

Single adult cardiomyocytes have been widely used to relate subcellular molecular events to functional characteristics of the heart. The simple geometry of a myocyte offers substantial advantages over multicellular preparations due to the fairly homogeneous strain distribution and exogenous factors (Palmer et al., 1996; Yasuda et al., 2001). However, they are notoriously difficult to manipulate and maintain because of the irritability and fragility of the sarcolemma. A number of techniques have been proposed to overcome these limitations. For example, Kamkin applied local stretches to ventricular myocytes by pulling with a glass stylus and patch-pipette (Kamkin et al., 2000), while Zeng and Riemer used a pair of suction pipettes to pull the myocyte from each end (Riemer and Tung, 2003; Zeng et al., 2000). A totally different technique was introduced by Le Guennec (Le Guennec et al., 1990) in which a pair of thin carbon fibers was attached to the myocyte surface, likely because of electrostatic forces between the fibers and the surface (Garnier, 1994). This technique was later modified with the use of graphite-reinforced carbon (GRC; Tsukuba Material Information Laboratory Ltd, Tsukuba, Japan) fiber (Sugiura et al., 2006) and/or biocompatible adhesive (MyoTak; IonOptix, Milton, MA, USA; or World Precision Instruments Inc., Sarasota, FL, USA) (Khairallah et al., 2012; Prosser et al., 2011) to enable firmer attachment between the fibers and the cellular surface, and was used for the dynamic stretching of single cardiomyocytes (Nishimura et al., 2006a, 2006b; Seo et al., 2014).

We typically use a pair of carbon fibers and attach them to each end of a cardiomyocyte to clamp it under a microscope (Sugiura et al., 2006). One fiber is rigid to serve as a mechanical anchor, while the other fiber is compliant and controlled quickly and digitally by a connected piezoelectric transducer (P-841.40; Physik Instrumente, Karlsruhe, Germany). The magnitude of the bending motion of the compliant carbon fiber is monitored by a fast digital dimensioning CCD camera (IonOptix, Milton, MA, USA) (Fig. 1A) to calculate the contractile force by multiplying by the fiber stiffness. The attachment of the carbon fibers to the myocyte surface results