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Load dependency in force–length relations in isolated single cardiomyocytes

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ABSTRACT

The previously reported pressure–volume (PV) relationship in frog hearts shows that end-systolic PV relation (ESPVR) is load dependent, whereas ESPVR in canine hearts is load independent. To study intrinsic cardiac mechanics in detail, it is desirable to study mechanics in a single isolated cardiomyocyte that is free from interstitial connective tissue. Previous single cell mechanics studies used a pair of carbon fibers (CF) attached to the upper surface of opposite cell ends to stretch cells. These studies showed that end-systolic force–length (FL) relation (ESFLR) is load independent. However, the range of applicable mechanical load using the conventional technique is limited because of weak cell-CF attachment. Therefore, the behavior of ESFLR in single cells under physiologically possible conditions of greater load is not yet well known. To cover wider loading range, we contrived a new method to hold cell-ends more firmly using two pairs of CF attached to both upper and bottom surfaces of cells. The new method allowed stretching cells to 2.2 μm or more in end-diastolic sarcomere length. ESFLR virtually behaves in a load independent manner only with end-diastolic sarcomere length less than 1.95 μm. It exhibited clear load dependency with higher preload, especially with low afterload conditions. Instantaneous cellular elastance curves showed that decreasing afterload enhanced relaxation and slowed time to peak elastance, as previously reported. A simulation study of a mathematical model with detailed description of thin filament activation suggested that velocity dependent thin filament inactivation is crucial for the observed load dependent behaviors and previously reported afterload dependent change in Ca^{2+} transient shape.

1. Introduction

1.1. General background

The heart is a mechanically functioning blood pump whose mechanical properties can be described by left ventricular (LV) pressure (LVP) and LV volume (LVV). Therefore, many cardiac mechanics studies focusing on pressure–volume (PV) relation have been conducted. In the late 19th century, Otto Frank studied PV relations in isolated frog hearts and obtained PV diagrams such as that shown in Fig. 1A (Frank, 1990). The shallow concave curve at the bottom shows the end-diastolic PV relation (EDPVR), and the steep convex curves illustrate end-systolic PV relations (ESPVR). Interestingly, the steepness of ESPVR is greatly affected by afterload conditions. ESPVR in isovolumic contraction (highest afterload; top curve) is the steepest, and that in isobaric condition (lowest afterload, middle solid curve) is more moderate. The ESPVR of normal "working" contractions (dashed line) depends on the end-diastolic state of the heart and can be described by a line between the corresponding points on isovolumic and isobaric ESPVR. Although there is an afterload dependency of ESPVR, one can generalize that the higher the preload (higher end-diastolic LVV), the greater the mechanical work, within a physiologically possible range of preloads.

Suga and Sagawa investigated mammalian cardiac LV pump behavior by analyzing the PV diagram in detail (Sagawa, 1981; Suga and Sagawa, 1974; Suga et al., 1973). Suga et al. reported that, in the absence of changes in contractility, ESPVR is independent of variations in pre- and afterload in excised cross-circulated canine hearts: ESPVR maxima follow a single, linear relation. Suga found that all isochronal points of the PV relation are also located on a

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single line, which is located between the ESPVR and EDPVR curves (Fig. 1B). During each heartbeat, the slope of the isochronal connection line initially becomes steeper (during contraction), and after reaching a maximum at the end-systolic point, returns to end-diastolic levels (during relaxation). The slope of the isochronal connection line describes the instantaneous PV ratio, that is, the ventricular elastance. This concept is called the "time-varying elastance model," which describes a ventricle as an elastic pouch whose instantaneous elastance can be determined using the instantaneous PV ratio. In this context, the shallow EDPVR indicates that the end-diastolic heart behaves as a soft "balloon," whereas it behaves as a more rigid balloon at end-systole, as shown by the far steeper ESPVR. The slope of ESPVR indicates the maximum elastance of the ventricle, called E_{max} (Suga and Sagawa, 1974; Suga et al., 1973).

1.2. Specific background

As mentioned above, the unique concept of the time-varying elastance model is that the ESPVR is independent of preload and afterload within a physiologically possible range of load conditions. Strictly speaking, however, instantaneous ventricular pressure can be more or less reduced from isovolumic pressure, when the velocity of ejection is increased (Baan and Van der Velde, 1988; Leach et al., 1980; Suga et al., 1980). In papillary muscle preparation, similar force reduction has been observed in force–length (FL) relations (Hisano and Cooper, 1987). One of the factors that may explain the pressure/force reduction during shortening is viscoelastic resistance of the extracellular matrix like connective tissue. Other factors are shortening-dependent change in Ca^{2+} and crossbridge dynamics (shortening deactivation) (Backx and Ter Keurs, 1993; Janssen and de Tombe, 1997; Kentish and Wrzosek, 1998; Lab et al., 1984; Yasuda et al., 2003).

To study intrinsic properties of cardiac mechanics in detail, it is desirable to study mechanics in single isolated cardiomyocyte that is free from viscoelastic resistance of the extracellular matrix. Interestingly, previous studies on FL relations in single cells demonstrated that end-systolic FL relation (ESFLR) is load independent in guinea pig and even frog cardiomyocytes (Iribe et al., 2007; Parikh et al., 1993). Given these findings, one might conclude that the intrinsic myocardial mechanical property is load independent. However, the contractile profile of individual cells, characterized by their elastance, shows clear load dependency (slower time-to-peak elastance, faster decay in low afterload conditions compared to high afterload) (Iribe et al., 2007). Previous studies also showed that the shape of Ca^{2+} transient is load dependent (Backx and Ter Keurs, 1993; Janssen and de Tombe, 1997; Kentish and Wrzosek, 1998; Lab et al., 1984; Yasuda et al., 2003). Regarding findings in single cell preparations, Yasuda et al. reported that the isotonic Ca^{2+} transient is higher than that of isometric contractions initially, and then the two transients cross each other in the early decay period, so that the isometric Ca^{2+} transient is higher than that of isotonic contractions for the rest of the decay period (Yasuda et al., 2003).

Why did load dependent Ca^{2+} transient and elastance profiles yield load independent ESFLR (maximum elastance) in previous single cell studies? One possibility we need to consider is that the applied preload range was not wide enough to cover loading conditions in which load dependent behavior of ESFLR can be observed, if it indeed does exist. Even in the PV relation for a frog's heart, ESPVR of isotonic and isometric contraction are close with small LV volume (Fig. 1A) (Frank, 1990). In a ferret papillary muscle study, ESFLR in shortening contraction from L_{max} does not reach the ESFLR in isometric contraction, whereas ESFLR in shortening contraction from lower preload does reach that in isometric contraction (Hisano and Cooper, 1987). In our previous single cell study, diastolic sarcomere length (SL) was elongated up to approximately 2.0 μm using a carbon fiber technique (Iribe et al., 2007), whereas the diastolic SL with L_{max} in most papillary muscle studies is approximately 2.2–2.3 μm (Page, 1974). To confirm the inherent mechanical properties of single cardiomyocytes, cells must be stretched to this range of length.

To study single cell mechanics, we have been using a pair of piezo translator (PZT)-positioned carbon fibers (CF) attached to the upper surface of opposite cell ends to stretch isolated cardiomyocytes (Iribe et al., 2007). However, the CF attachment force in this conventional method is limited; therefore, it is difficult to stretch cardiomyocytes beyond 2.0 μm in SL. In the present study, to overcome this limitation, we develop a novel cell gripping technique that allows attaching two CFs to both upper and lower cell surfaces to improve cell holding and apply more stretching force. Using this new technique, we investigate load dependency/independency in single cell mechanics under a wider range of mechanical loading conditions than was previously possible. Additionally, mathematical model simulations are performed to examine the possible mechanisms underlying observed load dependency/independency.

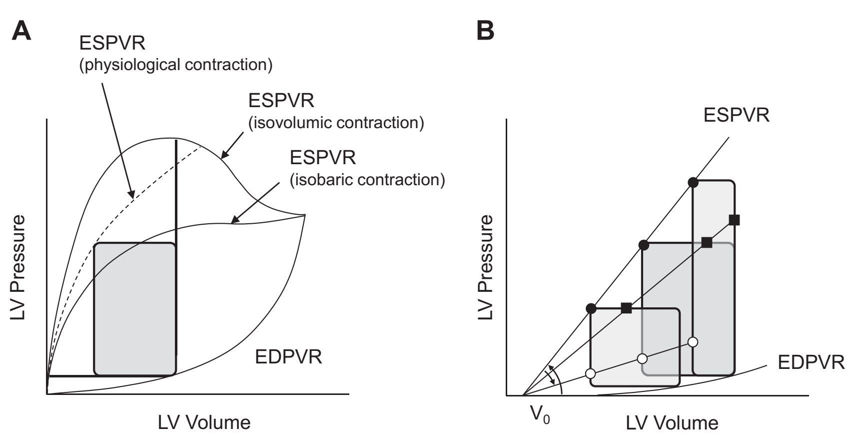

Fig. 1. Schematic diagram of pressure–volume loop in different species. A: frog heart. End-systolic pressure–volume (PV) relations (ESPVR) are affected by afterload conditions. B: canine heart. ESPVR is a load independent single linear line. Different markers (open and solid circles and squares) indicate different isochronal points. The slope of the isochronal connection line increases in the systole and decreases in the diastole (time-varying elastance model).

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