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Features of the contractile characteristics of single cardiomyocytes in the myocardial sleeves of the pulmonary veins of guinea pigs

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Motivation and Aim: Myocardial sleeves of the pulmonary veins (PV) extending from the left atrium (LA) are the major source of ectopic activity causing atrial arrhythmias [1]. The structural and electrophysiological characteristics of the PV myocardium differ from those of the LA. The mechanical activity of cardiomyocytes (CM) affects cardiac excitation-contraction coupling [2], however, the mechanical function of PV CM has not been studied yet. We compared the characteristics of the actin-myosin interaction and sarcomere length (SL) dynamics in mechanically non-loaded single CM between the PV and LA in the guinea pig heart.

Methods: All experiments involving animal care and handling were conducted according to Directive 2010/63/EU of the European Parliament and approved by the Animal Care and Use Committee of the Institute of Immunology and Physiology (protocol No. 02/23 from 6 April 2023).

Single CM from the LA and PV were isolated using a combined technique of Langedorff perfusion with intra-tissue injections [3]. The characteristics of CM morphometry (length, width, and surface area) were measured on a picture of resting CM using 40× magnification with the IonOptix system (IonOptix Corporation, Milton, MA, USA) and FIJI ImageJ software (National Institutes of Health, Bethesda, MD, USA). Changes in sarcomere length (SL) during mechanically non-loaded CM contractions were recorded under steady-state conditions after 5 min of CM contractions at 1 Hz using the IonOptix system [3].

Myosin was obtained from the PV and LA tissues of guinea pigs. The sliding velocity of thin filaments over myosin was measured in an *in vitro* motility [3]. Phosphorylation levels of cardiac myosin binding protein-C (cMyBP-C), regulatory light chains of myosin (RLC), troponin T (TnT), and troponin I (TnI) were assessed using gel-electrophoresis with ProQ Diamont and SYPRO Ruby staining.

The comparisons were made using the unpaired two-tailed Student's t-test (parametric analysis) and Mann-Witney U-test (non-parametric analysis). A p-value of <0.05 was considered to indicate a significant difference between groups. Data are expressed as median and interquartile range.

Results: First, we compared LA vs. PV morphometry and contractility at the single-cell level. We found that PV CM had 1.2-fold longer length ($p = 0.0026$) and smaller width ($p = 0.0048$) than LA CM (unpaired Student's t-test), and the cell surface area was not different between the LA and PV CM ($p = 0.6868$).

Representative traces of sarcomere shortening-relengthening in contracting LA and PV CM and analyzed parameters are shown in Fig. 1A and 1B. Neither end-diastolic sarcomere length (EDSL), nor the amplitude or velocities of sarcomere shortening-relengthening in PV CM differed from those in LA CM ($p > 0.17$). In both LA and PV CM, absolute and fractional values (FS) of the amplitude of sarcomere shortening positively correlated with the maximum velocity of sarcomere shortening (v_{short}, Fig. 1C).

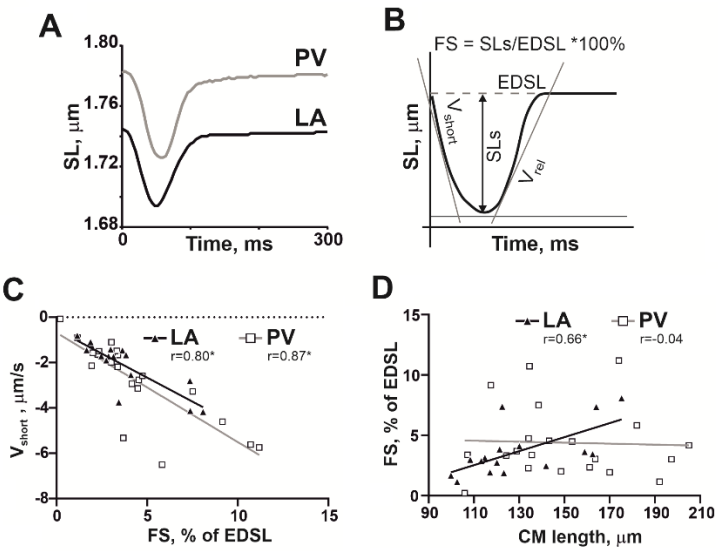

Fig. 1. (A) Representative recordings of sarcomere shortening-relengthening in contracting LA and PV CM. (B) Analyzed parameters derived from the SL change signal: SL – sarcomere length; EDSL – end-diastolic sarcomere length; v_{short} – maximum velocity of sarcomere shortening; FS = sarcomere shortening amplitude/EDSL × 100% – fractional sarcomere shortening amplitude. (C) The correlation of v_{short} with FS. (D) The correlation of FS with CM length

In LA CM, both absolute and fractional sarcomere shortening amplitude increased with an increase in CM length (Fig. 1D). In contrast, in PV CM we did not find any correlation between the characteristics of SL changes and cell morphometry (Fig. 1D).

We then studied the properties of the LA and PV myocardium at the molecular level. We compared the functional properties of myosin from the LA and PV. The sliding velocity of thin filaments at saturating Ca^{2+} concentration over myosin from the PV and LA in the *in vitro* motility assay did not differ ($p > 0.87$) that is the properties of myosin was similar. The cMyBP-C phosphorylation between the PV and LA was not different, while RLC phosphorylation was ~1.7-fold lower in the PV than in the LA ($p = 0.0065$). In the PV, TnT phosphorylation was ~1.4-fold higher ($p = 0.0130$) and TnI phosphorylation ($p = 0.0043$) was ~2.0-fold lower compared to the LA.

It was shown that RLC phosphorylation is involved in the realization of the Frank-Starling and the slow force responses in the atria [4]. Lower levels of RLC phosphorylation in the PV may result in less prominent length-dependent changes in the force of the PV than of the LA. The LA *vs.* PV differences in the levels of TnT and TnI phosphorylation may have complex effects on the contractility of PV and LA CM [5, 6]. A lower phosphorylation level of TnI may lead to the impaired length-dependent activation of myofilaments in PV CM than in LA CM.

Conclusion: The shape of cardiomyocytes is important for contractile function and depends on the strain of the myocardium [7]. In PV CM, there is no correlation between CM morphometric parameters and the amplitude of sarcomere shortening making PV CM more sensitive to volume overload than LA CM. These features, together with the electrophysiological properties of the PV myocardium, might contribute to the occurrence and development of AF.

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