PERSPECTIVES

What grows together, goes together: assessing variability in cardiomyocyte function

Diederik W. D. Kuster
Amsterdam UMC, Vrije Universiteit Amsterdam, Physiology, Amsterdam Cardiovascular Sciences, Amsterdam, The Netherlands

Email: d.kuster@vumc.nl

Edited by: Don Bers & Bjorn Knollmann

Pumping out blood and subsequent filling during each heart beat is achieved by the synchronized contraction and relaxation of millions of cardiomyocytes. Studying adult cardiomyocytes requires them to be isolated from the heart. Anyone who has ever seen a dish of isolated adult cardiomyocytes will have noticed the incredible variation in cardiomyocyte shape and size. However, not much is known about the variation in contractile function of individual cardiomyocytes.

Contraction in cardiomyocytes is initiated by Ca^{2+} entry into the cell through the L-type Ca^{2+} channel, which opens in reaction to membrane depolarization. This initial Ca^{2+} influx triggers a second and much larger Ca^{2+} release from the sarcoplasmic reticulum into the cytosol. Cytosolic Ca^{2+} binds to troponin C, initiating a set of conformational changes that enable myosin to interact with actin. Once myosin and actin interact, myosin undergoes the power stroke that causes force production/cell shortening. Relaxation occurs when cytosolic $[Ca^{2+}]$ is reduced by pumping Ca^{2+} back into the SR by SERCA2. The amount of force production is primarily dependent on the amount of calcium that is released, while relaxation is dependent on the speed of calcium reuptake and the sensitivity of the myofilaments to calcium (i.e. how much force is produced for a given amount of calcium). Increasing contraction and relaxation occurs for instance during exercise where sympathetic system activation increases calcium release, accelerates calcium reuptake and reduces myofilament calcium sensitivity. This is mediated through protein kinase A (PKA)-dependent phosphorylation of, among others, phospholamban (phosphorylation removes its inhibiting role on SERCA2 Ca^{2+} pumping) and cardiac troponin I (phosphorylation decreases myofilament calcium sensitivity). Calcium release and reuptake as well as contraction and relaxation can be assessed by single cardiomyocyte contractile measurements.

The number of contractile measurements that can be performed is limited because they require manual cell finding and focusing, and therefore most studies measure 10–20 cardiomyocytes per condition. This method is useful to determine averages, but is not well suited to assess variability.

In this issue of *The Journal of Physiology*, Clark and Campbell used a computer-controlled movable stage to increase the throughput of contractile measurements to hundreds of cardiomyocyte from each rat heart (Clark & Campbell, 2019). By recording different experimental parameters such as temperature and time of pacing for each cell, they could exclude these factors as sources of variability. This is important as the extent to which these experimental sources impact contractile parameters has recently been shown (Sikkel *et al.* 2017). Clark and Campbell tried to establish if variability in contractile properties diminishes if cardiomyocytes are isolated from a smaller area (i.e. if cells that are spatially close are more similar than cells that are further apart). They identified that cells isolated from a volume of ≤ 7 mm^3 had the smallest degree of variability. It is unclear if this volume corresponds to any functional region of the heart. A volume of 7 mm^3 could contain up to 28,000 cardiomyocytes (cell volume of an adult rat cardiomyocyte is 25,000 μm^3; Bensley *et al.* 2016), which does not directly correspond to any known macroscopic structure in the heart. Another more plausible explanation for the reduced variability is that local factors such as strain patterns and paracrine signaling events (i.e. the microenvironment) have an effect on the contractile phenotype. Elucidating which factors contribute to this microenvironment and testing if the volume of 7 mm^3 holds true in species with different heart sizes would provide insight into local regulation of cardiac contractility.

Variability in functional properties within the heart has been studied before, but this has mainly focused on detergent-permeabilized cardiomyocyte function and on transmural differences. For instance length-dependent activation (LDA) of cardiomyocytes is higher in endocardial cardiomyocytes than in epicardial cells across species (Cazorla & Lacampagne, 2011). It was also observed that in rat permeabilized cardiomyocytes, two populations of cardiomyocytes exist: one with a strong LDA response and one with a weak response (Hanft & McDonald, 2010). After incubations with PKA these differences disappear, implying that the initial variability in LDA is driven by inherent differences in PKA phosphorylation level.

Changes in PKA-dependent phosphorylation as a source of variability of contractile properties were also investigated in the current study (Clark & Campbell, 2019). The authors performed the following elegant experiment to study the mechanism of the variability in relaxation time. First contractile function was measured. From this analysis, they selected a population of fast and a population of slow relaxing cells, which were then separately collected using an automated aspirating pipette system. In these two groups they assessed the degree of cardiac troponin I phosphorylation. They found that cTnI phosphorylation at Ser23/24 (PKA target site) was lower in the slower relaxing cells. It should be noted that the phosphorylation state of the isolated cardiomyocytes does not fully reflect the state of the cells in the heart *in vivo*, because we have seen previously that during isolation dephosphorylation of cardiac troponin I and phospholamban takes place (Najafi *et al.* 2016). But the current findings together with experiments in permeabilized cardiomyocytes (Hanft & McDonald, 2010), in which this dephosphorylation during isolation does not occur, do imply an important role for PKA phosphorylation level in modulating cell-to-cell functional variability.

Studies into cellular heterogeneity have taken flight since the advent of techniques such as single cell sequencing and proteomics. This study has shown the feasibility of coupling functional to molecular analysis almost on a cell-to-cell basis. Expanding this and coupling functional measurements to single cell omics analyses would uncover a whole

 DOI: 10.1113/JP277287

The Journal of Physiology

new layer of understanding in cardiac pathophysiology.

References

Bensley JG, De Matteo R, Harding R & Black MJ (2016). Three-dimensional direct measurement of cardiomyocyte volume, nuclearity, and ploidy in thick histological sections. *Sci Rep* **6**, 23756.

Cazorla O & Lacampagne A (2011). Regional variation in myofilament length-dependent activation. *Pflugers Arch* **462**, 15–28.

Clark JA & Campbell SG (2019). Diverse relaxation rates exist among rat cardiomyocytes isolated from a single myocardial region. *J Physiol* **597**, 711–722.

Hanft LM & McDonald KS (2010). Length dependence of force generation exhibit similarities between rat cardiac myocytes and skeletal muscle fibres. *J Physiol* **588**, 2891–2903.

Najafi A, Sequeira V, Helmes M, Bollen IA, Goebel M, Regan JA, Carrier L, Kuster DW & Van Der Velden J (2016). Selective phosphorylation of PKA targets after beta-adrenergic receptor stimulation impairs myofilament function in Mybpc3-targeted HCM mouse model. *Cardiovasc Res* **110**, 200–214.

Sikkel MB, Francis DP, Howard J, Gordon F, Rowlands C, Peters NS, Lyon AR, Harding SE & MacLeod KT (2017). Hierarchical statistical techniques are necessary to draw reliable conclusions from analysis of isolated cardiomyocyte studies. *Cardiovasc Res* **113**, 1743–1752.

Additional information

Competing interests

None declared.

Author contributions

Sole author.

Funding

I acknowledge support from the Netherlands Cardiovascular Research Initiative – an initiative with support of the Dutch Heart Foundation, CVON: The Netherlands Cardio-Vascular Research Committee, CVON2014-40 DOSIS.