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Cardiac sympathetic innervation and arrhythmogenesis

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The autonomic nervous system (ANS) plays an essential role in regulating many aspects of cardiac physiology including modulation of chronotropy, dromotropy, inotropy and lusitropy (Gardner *et al.* 2016; Zaglia & Mongillo, 2017; Shivkumar, 2019). Dysregulation of the cardiac ANS, therefore, is involved in the pathogenesis of various cardiovascular diseases from hypertension, heart failure and myocardial infarction to ventricular arrhythmia. Since sympathetic activation is believed to contribute to ventricular arrhythmogenesis, neuromodulation therapies for ventricular arrhythmia have targeted cardiac sympathetic innervation at different sites – β-blockers to inhibit β-adrenergic receptors, thoracic epidural anaesthesia to induce sympathetic block at the T1–T4 levels, and stellate ganglion blockade with surgical or percutaneous approaches (Shivkumar, 2019). However, despite substantial progress in our understanding of the physiological function of the cardiac ANS, the dynamics and regulation of intercellular communication between cardiac sympathetic neurons (SNs) and cardiomyocytes remain poorly understood.

Recent work from the group of Drs Zaglia and Mongillo at the University of Padova has provided novel insight into the molecular mechanisms by which cardiac SNs interact with and modulate ventricular cardiomyocytes (Zaglia & Mongillo, 2017). During acute distress, stimulation of cardiac SNs induces the so called 'fight-or-flight' response, which is associated with positive inotropic and chronotropic effects via activation of β-adrenergic receptors. Cardiac sympathetic innervation also provides basal and constitutive regulation of cardiomyocyte size and trophic signalling at resting conditions. Indeed, chemical sympathectomy experiments showed that denervation of cardiac SNs led to a significant reduction in the cardiac mass and cardiomyocyte size by affecting the β2-adrenergic receptor-mediated suppression of the proteolysis/ubiquitin pathway (Zaglia *et al.* 2013). Direct intercellular communication in co-cultured SNs and cardiomyocytes has been investigated *in vitro* by studying live cell cAMP signalling in SN-sensitized cardiomyocytes in response to neuronal depolarization. The cAMP signalling elicited by β-adrenergic receptor activation is likely confined to a diffusion-limited domain at the intercellular contact site, reminiscent of the characteristics of the neuromuscular junction. Furthermore, spatially selective activation of cardiac SNs *in vivo* with optogenetics to modulate sinoatrial node function suggests direct coupling of SNs and effector cells, supporting the hypothesis of locally released noradrenaline at a junctional intercellular domain (Prando *et al.* 2018).

In a recent article in *The Journal of Physiology*, to further delineate the physiological coupling between cardiac SNs and cardiomyocytes, Pianca *et al.* (2019) performed well-designed experiments to map the topology of cardiac SN distribution and examine the relationship between SN innervation and transmural heterogeneity in cardiomyocyte size with correlation to specific intercellular neuro-cardiac junctions. Firstly, they showed that in the *in vitro* co-culture system that was maintained for 15 days, cardiomyocytes innervated by SNs displayed a significant 1.4-fold increase in sizes compared with non-innervated counterparts (cardiomyocyte area, innervated: 1197.03 $\pm$ 84.46 *vs.* non-innervated: 844.47 $\pm$ 59.38 μm^2), consistent with the prior observation that cardiac SNs elicit trophic inputs on innervated cardiomyocytes (Zaglia *et al.* 2013). In the transmural (short-axis) analysis of adult rodent hearts quantified by the number of tyrosine hydroxylase-positive fibres per cardiomyocyte, the subepicardium (EPI) layer contained three times denser SN innervation than the subendocardium (ENDO) layer, which correlated with larger cardiomyocyte cross-sectional areas in the EPI cardiomyocytes compared with the ENDO cardiomyocytes. In contrast, there was no significant difference in the longitudinal axis in terms of cardiomyocyte length.

Secondly, to overcome the limitation of thin-sliced tissue samples in a two-dimensional structure, the three-dimensional SN network in the intact anatomical context was stained by an anti-tyrosine hydroxylase antibody and was visualized by using the whole-mount two-photon immunofluorescence characterization of tissue-clarified murine myocardial blocks. Nearly all cardiomyocytes across the left ventricular wall were innervated and more SN processes per cardiomyocyte were detected in the EPI region compared with the ENDO region (1.69 ± 0.10 *vs.* 1.24 ± 0.08, respectively). A similar complex myocardial innervation network with highly arborized, regularly distributed varicosities was also found in a post-mortem human heart.

Next, to decipher the direct *in vivo* effects of cardiac SNs on cardiomyocyte size, Pianca *et al.* showed that when the cardiac SN innervation gradually reached completion around the third postnatal week at P21, the ratio between the cross-sectional areas of EPI and ENDO cardiomyocytes was also progressively increased through the postnatal period from the baseline ratio $\sim$1 at P1 when SN innervation was absent (Pianca *et al.* 2019). This SN innervation effect on cardiomyocyte size was abolished by pharmacological sympathectomy to disrupt early cardiac SN innervation, causing cardiac atrophy. Similarly, pharmacological neuronal ablation in adult mice, but not caloric restriction, resulted in diminished transmural heterogeneity in cardiomyocyte size. In addition, since cardiac SN-induced trophic input relies on the β2-adrenergic receptor–ubiquitin ligase MuRF-1 signalling axis, modulation of the signalling axis with either pharmacological

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The Journal of Physiology

inhibition (atrophic effect) or over-stimulation (hypertrophic effect) of β2-adrenergic receptors, or gene knockout of *MuRF1*, resulted in cardiac remodelling and abolished transmural differences without cardiac SN innervation disruption.

Finally, because SN denervation causes a more dramatic change in the EPI myocardium to diminish the transmural differences, *MuRF1* expression level and activity were investigated after chemical denervation. Indeed, *MuRIF1* expression level was significantly higher in the EPI region than in the ENDO region after denervation. MuRF1-induced ubiquitination of cardiac troponin I was also increased in the EPI myocardium. These findings indicate that transmural heterogeneity of cardiomyocyte size mediated by the distinct cardiac SN distribution across the ventricular wall is associated with local regulation of the cardiomyocyte proteolytic machinery.

Taken together, Pianca *et al.* have provided novel physiological aspects of the effect of differential cardiac SN innervation on ventricular structural remodelling at the molecular, cellular and organ levels (Pianca *et al.* 2019). How can these novel findings be translated into our understanding of pathophysiological mechanisms of ventricular arrhythmia? In heart transplant patients, SN denervation occurs immediately following surgical interruption of the SN network. Sympathetic reinnervation, if occurring later on, appears to be incomplete and displays heterogeneous patterns on the ventricular wall. Theoretically, interruption of SN innervation would lead to impaired transmural heterogeneity of cardiomyocyte size because of loss of trophic signalling through cardiac SNs. Partial and heterogeneous restoration of sympathetic innervation might cause variations across the ventricular surface area and transmural regions in the ventricle, which, in turn, could exaggerate the arrhythmogenic response to stimuli such as catecholamines.

As shown in Fig. 2*A* of Pianca *et al.* (2019), Kv4.2 is differentially expressed between EPI and ENDO myocardium. Could SN denervation and loss of trophic signalling also affect ion channel expression besides reducing cardiomyocyte size? The answer is probably yes as demonstrated in prior cardiac K^+ channel studies. Regional variations in sympathetic dysinnervation through either hyperinnervation or denervation likely contribute to serious ventricular arrhythmia and sudden cardiac death in myocardial infarction and heart failure (Gardner *et al.* 2016; Shivkumar, 2019). β-Blockers remain the first-line anti-arrhythmic therapy for ventricular arrhythmia, especially in patients with heart failure with reduced ejection fraction and polymorphic ventricular tachycardia after myocardial infarction (Gardner *et al.* 2016; Al-Khatib *et al.* 2018).

In a ventricular tachycardia/fibrillation storm refractory to anti-arrhythmic medications and catheter ablation, cardiac SN denervation by the transient chemical approach (thoracic epidural anaesthesia) or permanent surgical approach (stellate ganglionectomy) has been used to manage these life-threatening conditions (Shivkumar, 2019). Due to technical difficulties and limited data available from clinical studies (Al-Khatib *et al.* 2018), cardiac SN denervation has not been widely applied in clinical settings. The basic science behind SN denervation is also not comprehensive given little is known about how the ANS is remodelled during the initiation and progression of ventricular arrhythmia. The elegant studies undertaken by Pianca *et al.* (2019), together with their prior reports on dynamic intercellular communication between cardiac SN innervation and cardiomyocytes (Zaglia *et al.* 2013; Zaglia & Mongillo, 2017; Prando *et al.* 2018), will no doubt help further advance our understanding and ability to determine the molecular mechanisms of sympathetic remodelling-induced ventricular arrhythmia and identify potential new therapeutic targets.

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Additional information

Competing interests

None to declare.

Author contributions

Both authors have read and approved the final version of this manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Keywords

Arrhythmogenesis, Cardiomyocytes, Proteolysis, Sympathetic nerve, Ventricular arrhythmia