PERSPECTIVES

Sodium channel clusters: harmonizing the cardiac conduction orchestra

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Edited by: Don Bers & Colleen Clancy

It is now widely appreciated that proteins within biological systems are organized into macromolecular complexes and that such organization is a key determinant of their function. This is due, in large part, to insights gained from molecular biology studies. We are now entering another transformative period as emerging techniques such as super-resolution microscopy shed light on the organization of proteins at the nanoscale and their local structural environment. These new structural insights invite a fundamental re-examination of how proteins behave at these scales to produce tissue and organ level function. The impact of this paradigm shift is most evident in fields such as cardiac biophysics. Indeed, the understanding that many ion channels exist in clusters, in close proximity to structural and regulatory proteins, and often surrounded by restricted intra-/extracellular clefts is transforming our understanding of cardiac electrophysiology and excitation–contraction coupling.

Ephaptic coupling in the heart

In this issue of *The Journal of Physiology*, Hichri *et al.* (2018) present a combined experimental and *in silico* investigation of how sodium channel clustering modulates the potential for ephaptic interactions between cardiac myocytes. Historically, cardiac myocytes have been viewed as being electrically coupled solely by means of gap junctions (GJs). However, theoretical studies have long suggested the possibility of ephaptic interactions between them (Sperelakis & Mann, 1977). The ephaptic hypothesis envisions sodium channels directly participating in cell-to-cell electrical coupling by mediating electrochemical transients within restricted extracellular clefts between myocytes. Long viewed as controversial due to a lack of experimental evidence, early research into this hypothesis was likely held back by ephaptic coupling being presented as a complete alternative to GJ coupling. However, *in silico* studies in recent years have suggested that both mechanisms operate in tandem in the heart (Mori *et al.* 2008), casting ephaptic coupling in a fresh light.

Additional impetus for the idea derives from the elucidation of sodium channel organization at greater resolution. Confocal immunofluorescence, with diffraction-limited resolution, revealed $Na_V1.5$ enrichment at the intercalated disk (ID) (Maier *et al.* 2004; Veeraraghavan *et al.* 2015). *In silico* studies incorporating this detail identified ID density of $Na_V1.5$ and the width of the extracellular cleft at the ID as key determinants of ephaptic conduction (Mori *et al.* 2008). However, recent studies using super-resolution techniques demonstrate that sodium channels are organized into clusters located within specific ID nanodomains, such as the perinexus (at the GJ edge) (Veeraraghavan *et al.* 2015; Veeraraghavan & Gourdie, 2016) and N-cadherin-rich regions (Leo-Macias *et al.* 2016). Furthermore, experiments suggest that selective ultrastructural changes within these nanodomains affect cardiac conduction in a manner consistent with a role for ephaptic coupling in the heart (Veeraraghavan *et al.* 2015). Thus, there is a need to understand how these aspects of ID structure modulate ephaptic effects. To date, most models have incorporated a highly simplified structure with sodium channels evenly distributed throughout the ID and suggested that the contribution of ephaptic coupling in the heart may be limited to very slow conduction when GJ coupling is severely reduced. However, Hichri and colleagues provide a very timely demonstration that ephaptic effects are enhanced by sodium channel clustering as well as the location of these clusters within the ID. Their results suggest that ephaptic effects within $Na_V1.5$-rich nanodomains located within the tortuous and complex structure of cardiomyocyte IDs may be much larger than previously thought. In short, their results point to a potentially much greater role for ephaptic coupling in the heart, perhaps even extending to normal physiology.

The nanoscale machinery of cardiac biophysics

In the broader context, the work by Hichri *et al.* is part of a paradigm shift based on the understanding that proteins and their ultrastructural milieu constitute nanomachines that are the fundamental functional units of cardiac biophysics. While we are only beginning to understand the involvement of nanodomains in impulse propagation, their role of in cardiac calcium cycling has been recognized for a number of years. Interestingly, however, emerging research is identifying neuronal sodium channel isoforms as previously unanticipated, albeit functionally key, components of calcium cycling nanodomains at the dyadic cleft (Veeraraghavan *et al.* 2017). Thus, the functional implications of nanodomain organization – ion channel clustering and ultrastructure – extend well beyond the role of ephaptic coupling in cardiac conduction. Rather, they hold truly fundamental implications given the emergence of nanodomains containing ion channels and transporters as fundamental functional units of cardiac biophysics, and by extension, of pathophysiological processes such as arrhythmias.

In the context of cardiac disease, dysregulation of ephaptic nanodomains may constitute a nanoscale substrate for conduction defects and re-entrant arrhythmias. Likewise, arrhythmogenic aberrant calcium release often results from dyadic nanodomain dysfunction involving calcium leak from the sarcoplasmic reticulum and abnormal sodium entry. And, ephaptic coupling may contribute to the translation of abnormal impulses generated in the working myocardium into premature beats by lowering the source–sink

 DOI: 10.1113/JP275632

The Journal of Physiology

mismatch barrier associated with electrotonic propagation. Thus, understanding the nanoscale biophysical mechanisms of arrhythmias may prove crucial and, perhaps, explain some of the complexity in the relationship between genotype and phenotype in this context (Pazoki *et al.* 2013). More importantly, recapitulating nanodomain structural features involved in masking the arrhythmogenic phenotypic impact of mutations could represent a powerful new strategy for antiarrhythmic therapy.

Conclusion

In summary, Hichri *et al.* present innovative experimental and modelling results that advance our understanding of unique biophysical phenomena that occur at the nanoscale with important implications for organ-level function and, therefore, for healthcare.

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Additional information

Competing interests

The authors report no conflicts of interest.

Author contributions

Both authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.