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Letter to the Editor · Open Access ·

Variability in local action potential durations, dispersion of repolarization and wavelength restitution in aged wild-type and Scn5a$^{+/-}$ mouse hearts modeling human Brugada syndrome

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Brugada syndrome is a primary arrhythmia syndrome characterized by loss-of-function mutations in the *SCN5A* gene, which encodes for the cardiac Na^+ channel. In affected individuals, the risk of developing malignant ventricular arrhythmias and sudden cardiac death are increased.[1] Two leading theories, the depolarization and repolarization hypotheses, have been put forward to explain the underlying electrophysiological mechanisms[2] with the use of mouse models providing much insight into this controversial area. The monophasic action potential (MAP) recording technique has been extensively used to examine electrophysiology at the whole heart level.[3] This letter attempts to provide a brief overview to illustrate the importance of understanding the limitations of experimental methods and the need to appraise experimental data.

A secondary examination and analysis of published data from several studies in mice revealed several major methodological and statistical flaws, leading us to conclude that the conclusions are unconvincing. Firstly, Martin, *et al*.[4] reported apparent spatial heterogeneities action potential durations at 90% repolarization (APD_{90}) and effective refractory periods (ERPs) in mouse *Scn5a*$^{+/-}$ hearts modelling Brugada syndrome. The authors obtained MAP recordings from the epicardium or endocardium of the left ventricle (LV) or right ventricle (RV) in wild-type and *Scn5a*$^{+/-}$ hearts using a S1S2 pacing protocol. This involves the delivery of successively premature S2 stimuli after a train of regular S1 stimuli to provoke arrhythmias. Comparisons of this study with previous two other studies from the same group in 2007 and 2011 yielded significant variations in APD_{90} values.[4–6] For LV measurements, wild-type hearts showed epicardial APD_{90} values from 39 ms to 55 ms and endocardial APD_{90} values from 50 ms to 54 ms. In *Scn5a*$^{+/-}$ hearts, epicardium APD_{90} ranged from 40 ms to 46 ms, and endocardium APD_{90} ranged from 43 ms to 52 ms. For RV measurements, wild-type hearts epicardial APD_{90} values ranged from 33 ms to 43 ms, and endocardial APD_{90} values ranged from 43 ms to 47 ms. In *Scn5a*$^{+/-}$ hearts, epicardium APD_{90} ranged from 29 ms to 38 ms whereas endocardial APD_{90} values ranged from 43 ms to 47 ms. Thus, from the same cardiac region (e.g., RV epicardium), a large range of APD_{90} values is observed. These differences can be attributed to operator-dependent effects and the intrinsic property of the MAP technique.

Secondly, activation latencies were derived from MAP recordings made with the stimulating and recording electrodes 5 mm or 10 mm apart in the Martin, *et al*.[4] study. The study made no mention on the method used to measure this distance. A constant distance between the stimulating and recording electrodes is required for the comparisons to be meaningful. Given the short distance, how did the authors ensure that the electrodes were not moved during the course of the experiments? Reported values of activation latencies were around 15 ms. Surely, an ever slight movement in the electrodes is expect to further alter these latencies.

Thirdly, as reported in our editorial elsewhere,[7] there were numerous inconsistencies and contradictions in the several studies published by the same group. Their scientific reporting was also misleading and inconsistent. For example, the Martin, *et al*.[8] study demonstrated "In wild-type hearts, flecainide shortened the APD values in all 4 ventricular regions investigated, but doing so significantly only for RV epicardium". The aim of this paper was to illustrate specifi-

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cally that RV changes were greater than those in the LV. As we noted earlier,[7] their wording suggested "no significant shortenings in APD values were observed in the LV epicardium, LV endocardium or RV endocardium". Yet, experiments from the same group showed that "flecainide not only shortened APD_{90} significantly but also shortened APD_{70} and APD_{50} significantly in the LV epicardium, and shortened only APD_{90} and APD_{70} but not APD_{50} significantly in the LV endocardium".[6] Therefore only two conclusions can be made: either the data were inconsistent, or the authors in their 2016 paper specifically exaggerated RV abnormalities in relation to those in the LV, whereas in fact little difference between RV and LV existed. Together with several other contradictions, critical analysis of these published data therefore casts doubt on their reliability.

Fourthly, Matthews, *et al*.[9] reported that wavelength (λ) restitution predicted the onset of APD alternans using a dynamic pacing protocol that progressively increased the rate of regular pacing delivered to the hearts. λ is defined as conduction velocity (CV) × ERP. Their analysis of λ was based on numerous assumptions. For example, the reciprocal of activation latency (θ') was used to approximate CV and APD was used to approximate ERP, in order to calculate λ'. However, it was the earlier work of these authors who demonstrated the following relationships: APD > ERP in wild-type hearts and APD < ERP in *Scn5a*$^{+/-}$ hearts.[10] Together with the unreliable method by which activation latencies and CV were obtained, how can any calculated value of λ be meaningful not only within the same group but also between the wild-type and *Scn5a*$^{+/-}$ groups?

In conclusion, mouse models have advanced our understanding of the mechanisms underlying cardiac arrhythmogenesis,[11] and provided much insights for translational application for arrhythmic risk prediction.[12–14] Specifically in Brugada syndrome, there is no doubt that both depolarization and repolarization abnormalities contribute to arrhythmic substrate, and that the single conduction-repolarization parameter, λ, is likely to be central in determining arrhythmogenicity.[15] However, using the publications as an example, we demonstrate the need to be able to critically analyze published data and to recognize limitations in experimental methodology.

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