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LETTER TO THE EDITOR

Letter to the Editor

In their study recently published in *The Journal of Physiology*, Lipsett *et al.* (2019) attempted to explain t-tubular defects observed in pathological cardiac remodelling during heart failure (HF) as the result of reactivation of the fetal gene programme in cardiomyocytes. In fact, fetal cardiomyocytes show rudimentary t-tubular architecture, structurally similar to t-tubules of HF cardiomyocytes. The authors observed that fetal t-tubules are functional while t-tubules of HF cardiomyocytes show defective triggering of local Ca^{2+} release. This work suggests that reactivation of the fetal gene programme is partly responsible for HF remodelling but cannot completely explain t-tubule functional defects in HF cardiomyocytes.

The authors exclude the role of failure of action potential propagation in t-tubules of HF cardiomyocytes, as reported in our previous works (Sacconi *et al.* 2012; Crocini *et al.* 2014; Scardigli *et al.* 2017). In particular, Lipsett and colleagues claim that there was no difference in action potential propagation in control and HF cardiomyocytes, observed using 2D confocal scanning of action potentials. However, their results raise some concerns. In their work, about 15–20% of t-tubules in control cardiomyocytes (adult and sham-operated) did not depolarize after electrical stimulation. Failure of action potential propagation in control cardiomyocytes has never been observed in any experimental model previously employed by us, including rat cardiomyocytes. The idea that about one-fifth of the sarcolemma of each healthy cardiomyocyte is incapable of responding to electrical stimuli raises obvious questions and concerns, in particular the possibility of a systematic error in the technology used by Lipsett *et al.* Since the authors do not show action potential traces, it is difficult for readers to determine the difference between a depolarized t-tubule and a failing t-tubule. In addition, action potentials from the surface sarcolemma should have been shown as a positive internal control for cellular activation. We also wonder whether repeated measurements of action potential propagation were taken at the same site in the same cell, to exclude the possibility that 15–20% of failing tubules in control cardiomyocytes may represent a stochastic phenomenon due to low temporal resolution. The 10-ms resolution of the confocal scanning equipment might be the reason underlying the remarkably high percentage of failing t-tubules in control cardiomyocytes. As a consequence, the 7% of failing t-tubules observed in our HF cardiomyocytes (Sacconi *et al.* 2012; Crocini *et al.* 2014) may be hidden behind the methodological uncertainty of Lipsett's approach.

Lipsett and colleagues state that t-tubule dysfunction is consistent with reduced t-tubular Ca^{2+} current in HF cardiomyocytes rather than t-tubular action potential failure. In our works, the majority of t-tubules in diseased cardiomyocytes do propagate action potentials but are still associated with significantly slower local calcium release compared to healthy cardiomyocytes (Crocini *et al.* 2014, 2016; Scardigli *et al.* 2018). As calcium release depends on several factors, including the extent of the Ca^{2+} trigger (i.e. Ca^{2+} current; Bassani *et al.* 1995), we do not exclude the possibility that reduced calcium current at those functional t-tubular sites may contribute to the slower calcium release. However, lack of or reduced calcium current cannot explain failure of action potential propagation in t-tubules. Passive (electrotonic) responses of membrane potential should occur even in the absence of currents feeding the action potential itself. Passive responses are only limited by the voltage drop of the current flowing from the surface sarcolemma along the t-tubule with a characteristic *space constant* (Crocini *et al.* 2017). The space constant of t-tubules has been determined theoretically and experimentally to be of the order of 200 μm (Pásek *et al.* 2008; Kong *et al.* 2017; Scardigli *et al.* 2017). Because t-tubules are on average 25 μm long (Soeller & Cannell, 1999), the voltage drop in the cardiac action potential from the surface sarcolemma to the end of the t-tubule is only a few millivolts. In other words, the signal of a voltage probe (like the FluoVolt used by Lipsett and colleagues) should be detected even if the calcium current (or any other current) is not activated. A local drop in voltage could instead be explained by ultrastructural alterations that increase the resistivity of t-tubules (Crocini *et al.* 2017; Scardigli *et al.* 2017; Uchida & Lopatin, 2018) and could represent an additional pathological feature of HF cardiomyocytes.

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Additional information

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