Optogenetic investigation of cardiac arrhythmia mechanisms

Feola, I.

Appendix

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RESPONSE BY FEOLA *ET AL* TO LETTER REGARDING ARTICLE, "LOCALIZED OPTOGENETIC TARGETING OF ROTORS IN ATRIAL CARDIOMYOCYTE MONOLAYERS"

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IN RESPONSE

We thank Houston *et al* for their interest in our study. In their letter, they raise the question whether the rotors and accompanied spiral waves observed in our study represent microreentrant circuits anchored to lines of conduction block/slowing (*i.e.* anatomical reentry), instead of reentrant activity around an unexcited, yet excitable core region (*i.e.* functional reentry). Their comment is based on a movie published on their website, showing high-resolution mapping, in an HL-1 culture, of reentrant activity that seems anchored to microregions of conduction abnormalities. Although appraisal of these data is difficult without a detailed description of methods and results, we still would like to add our thoughts about the distinction between functional and anatomical reentry on a cellular level. Heterogeneities, such as gradients in excitability, refractoriness, and nonhomogeneous distribution of cardiac fibroblasts may have a destabilizing effect on functional reentry by contributing to drift, meandering, and breakup of spiral waves.[1,2] Furthermore, reentrant activity may undergo alternating transitions between functional and anatomical reentry by pinning to or unpinning from an anatomical obstruction.[3–6] These transitions depend on several factors (*e.g.* size of the obstacle and tissue excitability)[5,6] and highlight the difficulty to determine which type of reentry underlies arrhythmic activity at a given location and time. To deal with the complex dynamic nature of reentrant activity, we used confluent monolayers of optogenetically modified neonatal rat atrial cardiomyocytes and patterned illumination to induce and target a single stable rotor. The resolution of optical voltage mapping allowed us to visualize electrical activity in the entire monolayer and to investigate the effects of rotor targeting by light-controlled induction of conduction blocks once a rotor had been established at a predefined location through light-based cross-field stimulation.[7]

Although any involvement of microreentry cannot be ruled out completely, we found evidence that rotor stability was related to the absence of the aforementioned heterogeneities. Our cultures showed uniform and fast activation with little dispersion in action potential duration on 1-Hz electric stimulation. Moreover, by changing the timing and location of the second light stimulus, stable rotors at different, yet predefined locations could be induced. Finally, we found spiral wave drifting by creating a line of block away from the core region, followed by post-illumination rotor stabilization. Such drifting was also evident in other cases where light-induced conduction block did not cause effective reentry termination. The reentrant wave described by Houston *et al* was able to follow a path of block, despite the poor excitability and coupling of the medium, because of the permanent expression of the oncogenic SV40-LT-ag (simian virus 40–large T-antigen) in HL-1 cells (*e.g.* conduction velocity of 4.1±0.1 cm/s)[8,9] and the sharp corners (*e.g.* U-turns) in the trajectory. These conditions would normally favor unpinning and subsequent transition into functional reentry, sparking curiosity about the underlying biophysical mechanism(s).[3,4]

Clinically, it remains to be determined whether and how a strict distinction between functional and anatomical reentry, on a cellular level, could further improve the treatment of reentry-driven arrhythmias.[10] We encourage the authors to publish an in-depth study on microreentry dynamics in HL-1 cultures.

DISCLOSURES

None.

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