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EDITORIAL

Fiat lux in understanding cardiac pacing, resynchronization and signalling by way of optogenetics

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This editorial refers to 'Optogenetic activation of G_q signalling modulates pacemaker activity of cardiomyocytes' by T. Beiert *et al.*, pp. 507–516, this issue.

This editorial refers to 'Modulation of cardiac tissue electrophysiological properties with light-sensitive proteins' by U. Nussinovitch *et al.*, 2014;102:176–187.

Multiple cell types and complex signalling cascades control the generation and regularity of the heartbeat. Understanding their concerted operation *in vivo* is a challenge that necessitates new technical tools. Optogenetics[1,2] uses genetically encoded light-sensitive proteins to both actuate and/or sense fast biological processes in cells, tissues, and in behaving animals.[3] The use of light and light-sensitive biological elements, i.e. the 'optical' aspect of this approach, enables remote, spatio-temporally precise probing and control; the 'genetic' addressing confers cell specificity. While the utility of this technology has been fully embraced in neuroscience research, its extension to the cardiovascular field has been rather slow.[4–8] Two recent papers in this journal—by Beiert *et al.*[9] and by Nussinovitch *et al.*[10]—further contribute to the budding field of cardiac optogenetics.

Most current optogenetic applications deal with direct perturbation of membrane potential, i.e. excitation or inhibition of electrical activity through the expression of light-sensitive ion channels and pumps (opsins), providing inward/excitatory or outward/repolarizing currents. These opsins can be introduced into the heart through development of transgenic cell lines and animals,[4,5] direct viral delivery in cardiomyocytes[11] or by cell delivery.[7] In the latter case, dedicated donor cells, that may not be excitable (e.g. fibroblasts) carry the opsin of interest and, upon electrical coupling to excitable cells (cardiomyocytes), render cardiac tissue light-sensitive and enable optical pacing and control—a concept we termed a tandem-cell-unit (TCU) approach to optogenetics.[7] We have shown that it is possible to optically excite adult ventricular cardiomyocytes and to optically pace cardiac syncytium using such Channelrhodopsin (ChR2)-expressing HEK293 donor cells.[7]

Now, Nussinovitch *et al.* further corroborate such a cell delivery approach (and the applicability of the TCU concept) to optical cardiac stimulation by using different ChR2-donor cells—NIH-3T3 embryonic fibroblast cell line—coupled to either neonatal rat cardiomyocytes or to human embryonic stem cell-derived cardiomyocytes *in vitro*. In this new study, the authors used multielectrode recordings to confirm optical pacing of cardiac syncytium for both disperse and localized distribution of the donor cells. As expected, multisite light delivery combined with dispersed opsin-expressing cells yielded shortening of activation times compared with single-site electrical pacing, i.e. resulted in global cardiac pacing and resynchronization.

Caution should be used in extrapolating these model-system results to cardiac fibroblasts and myocytes in the native heart; both cell lines (HEK293 in Jia *et al.*[7] and NIH-3T3 in Nussinovitch *et al.*[10]) share very similar I–V characteristics and input impedance with each other, but both are electrophysiologically different from primary cardiac fibroblasts with more hyperpolarized resting membrane potential and a shallower I–V relationship.[12] Importantly, the electrical coupling (and the gap junctional expression) of the cell lines used in these two studies are likely different from those in primary cardiac fibroblasts *in vivo*, thus the TCU-driven optogenetic stimulation may work slightly differently, but it may instead inspire new ways to probe the coupling between cardiac fibroblasts and cardiomyocytes *in vivo*. The existence and modulation of such electrical coupling has been a point of contention, with scarce experimental evidence[13] but of much interest to the cardiac community.[12] Finally, Nussinovitch *et al.* discuss the potential use of the method for cardiac resynchronization therapy. However, spatially distributed light delivery in the heart is extremely challenging; a feasible solution will likely involve targeting key structures of the conduction system, as suggested computationally[14] or alternative methods using implantable miniaturized electronics that allow distributed low-power electrical or optical stimulation.[15]

Beyond the direct manipulation of membrane voltage by excitatory or inhibitory microbial opsins, recently optogenetic tools have been expanded to provide cell-specific, precise dynamic manipulation of cell signalling,[16,17] and/or feedback control of gene expression by light-induced transcriptional effectors.[18] Beiert *et al.*[9] present a new

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technique to optically perturb $G\alpha_q$ signalling in cardiomyocytes, comparable with treatment with endothelin-1, but more precise and versatile. This optogenetic method involves the genetic expression of melanopsin, a light-sensitive G-protein coupled receptor (GPCR), and the use of very low levels of blue light for stimulation. The authors demonstrate perturbation of intracellular Ca^{2+} (likely via $G\alpha_q$-IP3-mediated Ca^{2+} 'puffs') in HEK293 and HL-1 cells and related optical modulation of pacemaking in ES-derived cardiomyocytes, including an increase in irregularity (arrhythmogenic effects in the latter) after light stimulation. For cautious interpretation of the results, one has to keep in mind that, as a GPCR non-native to the heart, melanopsin may exert yet unknown effects beyond classic $G\alpha_q$ signalling. Furthermore, the exquisite light sensitivity of the method, desirable for potential *in vivo* use, may, ironically, present practical problems (in keeping dark control conditions), especially when coupled with optical sensing *in vitro*.

Overall, this study offers a new and important tool for dissecting cardiac $G\alpha_q$ signalling with high selectivity, spatiotemporal resolution, and ability for repeated and/or feedback-controlled continuous manipulation *in vitro* and *in vivo*. Existing techniques to perturb $G\alpha_q$ signalling in the heart, such as biochemical stimulation, lack temporal, spatial resolution, and selectivity; related photolysis of caged IP3, while a very direct and fast targeting method, is terminal (non-repeatable) and impractical *in vivo*.

In addition to modulating fast events, i.e. the beat-to-beat Ca^{2+} (and pacing), demonstrated here,[9] this optogenetic technique can be applied to probe, in a dynamic manner, the distinct contribution of $G\alpha_q$ signalling to slower, transcription events in the heart via the calcineurin/NFAT pathways.[17] Pathological conditions of interest include abnormal fibroblast proliferation in the atria and cardiac hypertrophy, in general; both of these can, theoretically, be induced in a cell/region-specific, dynamic and potentially reversible way by light, using this method. Finally, because of the selectivity and the spatial resolution of optogenetics, the technique has the potential to be not only cell-type specific, but also to be applied subcellularly by proper light focusing *in vitro*, to dissect $G\alpha_q$ signalling in structures of interest,[19] such as the nuclear envelope or the T-tubule portion of the plasma membrane of ventricular myocytes, for example. The proposed method is applicable not only to cardiomyocytes, but also to other key cell types in the cardiovascular system, including smooth muscle and endothelial cells.

Both of the proof-of-principle studies discussed here explore new ways to optically actuate and control cardiac activity; they illustrate the power of optical manipulation over traditional electrical and chemical means of perturbing cardiac function. The translation of these ideas *in vivo* would require further work, most notably, the identification of proper promoters for distinct cardiac cells (cells in the conduction system, ventricular or atrial myocytes, and cardiac fibroblasts), suitable *in vivo* methods of gene or cell delivery, as well as optical access to the areas of interest.[20] Nevertheless, as technical tools, these new optogenetic approaches hold great promise to elucidate cell-specific contributions to cardiac signalling, pacemaking and arrhythmogenesis in the intact heart. Indeed, fiat lux—let there be light—in these exciting cardiovascular quests!

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