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STATE-OF-THE-ART REVIEW

Cardiac Optogenetics: 2018

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ABSTRACT

Cardiac optogenetics is an emergent research area involving the delivery of light-sensitive proteins (opsins) to excitable heart tissue to enable optical modulation of cardiac electrical function. Optogenetic stimulation has many noteworthy advantages over conventional electrical methods, including selective electrophysiological modulation in specifically targeted cell subpopulations, high-resolution spatiotemporal control via patterned illumination, and use of different opsins to elicit inward or outward transmembrane current. This review summarizes developments achieved since the inception of cardiac optogenetics research, which has spanned nearly a decade. The authors first provide an overview of recent methodological advances in opsin engineering, light sensitization of cardiac tissue, strategies for illuminating the heart, and frameworks for simulating optogenetics in realistic computational models of patient hearts. They then review recent cardiac optogenetics applications, including: 1) all-optical, high-throughput, contactless assays for quantification of electrophysiological properties; 2) optogenetic perturbation of cardiac tissue to unveil mechanistic insights on the initiation, perpetuation, and termination of arrhythmia; and 3) disruptive translational innovations such as light-based pacemaking and defibrillation. (J Am Coll Cardiol EP 2018;■:■–■) © 2018 by the American College of Cardiology Foundation.

Optogenetics is a neologism first introduced in 2006 (1) to describe an emerging biomedical approach in which excitable cells are rendered photosensitive via heterologous expression of specific microbial opsins, thereby enabling the use of light to manipulate transmembrane potential. The naming of this technology followed a phase of rapid advancement (2002 to 2006), during which Nagel et al. (2,3) cloned channelrhodopsin-1 (ChR1) and ChR2 (light-gated ion channels produced by green algae) and multiple groups proved the feasibility of using light to elicit action potentials (APs) in ChR2-expressing mammalian neurons (4–6). By 2010, the use of optogenetics to enable selective control of specific cell populations became widespread in neuroscience and the approach was named *Nature Methods*' "Method of the Year" (7). Contemporaneously, the first studies describing cardiac applications of optogenetics were published by Bruegmann et al. (8), who demonstrated that light could be used to pace the hearts of transgenic mice in vivo, and Arrenberg et al. (9), who showed that pacemaker activity in the zebrafish heart could be modulated via optical stimulation of specialized pacemaker regions. Additionally, Jia et al. (10) produced light-sensitized cardiomyocyte monolayers suitable for in vitro electrophysiology experiments by coculturing exogenous ChR2-rich human embryonic kidney (HEK293) cells with unmodified cardiac cells and Abilez et al. (11) derived ChR2-expressing cardiomyocyte-like cells from human embryonic stem cells photosensitized by lentiviral vectors. These advances were complemented by the development of a comprehensive framework for realistically simulating optogenetics in multiscale computational models of human hearts (12), providing a rich infrastructure capable of

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ABBREVIATIONS AND ACRONYMS

AAV = adeno-associated virus

AP = action potential

APD = action potential duration

ChR1 = channelrhodopsin-1

ChR2 = channelrhodopsin-2

HB = His bundle

VT = ventricular tachycardia

virtually probing a wide gamut of possible applications and identifying potential limitations.

These studies marked the advent of a promising new research area: cardiac optogenetics. A landmark 2013 review by Entcheva (13) summarized 6 key distinguishing features that make this technology potentially transformative in the realm of electrophysiology research: simplicity of opsin expression, minimal interference with endogenous cardiomyocyte function, feasibility of targeting specific cell types for selective expression, high spatial and temporal precision of manipulation, versatility of opsin function (i.e., excitatory or inhibitory modulation), and modest energy requirements. In the intervening years, a second generation of original research studies in cardiac optogenetics has shown how these features can be exploited to advance electrophysiology research along several trajectories. As summarized in the **Central Illustration**, the current paper aims to review these recent developments. First, we describe methodological advances, ranging from novel opsin variants and strategies for light sensitization to schemes for delivering light stimuli to the working heart. Second, we review in vitro applications of optogenetics, either as a means to perform contactless, high-throughput assays or as an enabling tool to better understand arrhythmia mechanisms by manipulating cell behavior. Finally, we discuss studies that have explored the possibility of translational applications of cardiac optogenetics, including light-based pacing and defibrillation.

Notably, because the scope of this review is limited to optogenetic actuators, as described previously, we do not discuss genetically encoded proteins that emit light in response to cell-scale changes (in membrane voltage, pH, and so on), which are sometimes described as optogenetic sensors. Recent applications of this approach include attempts to selectively induce expression of genetically encoded voltage indicators in cardiac fibroblasts to assess whether the latter cells couple electrically with working myocytes in vivo (14). As explained by Yu et al. (15), results from studies involving optogenetic sensor can be challenging to interpret because of issues with promoter promiscuity (e.g., the WT1 promoter used in the latter study has also been shown to drive expression in myocytes) (16). Nonetheless, this methodology has the potential to enable experimental approaches that were once thought impossible. Interested readers can consult a recent in-depth review of optogenetic sensors (17).

METHODOLOGICAL ADVANCES

NOVEL OPSIN VARIANTS. A major research thrust has been modification of ChR2 to produce variants with properties that could enable new optogenetic applications. An early example was the work of Nagel et al. (18) on the high-conductance ChR2-H134R variant. Recent developments include variants with enhanced Ca^{2+} permeability and light sensitivity (CatCh) (19), augmented expression and photocurrent (ChR2-XXL) (20), and red-shifted absorption spectra to facilitate deeper penetration in biological tissue (ReaChR) (21). These are all relevant to cardiac optogenetics, and attempt to address the need for opsins that can facilitate light-based excitation within the myocardial wall, which is relatively opaque due to photon scattering and absorption effects.

In addition to tools that facilitate stimulation (e.g., ChR2), a separate category of opsins exists that can be used to suppress the propensity for AP initiation. For example, archaea-derived light-sensitive ion pumps have been used to suppress spontaneous activity in zebrafish hearts (9) or shorten cardiomyocyte APs in vitro (22). Recently, Govorunova et al. (23) showed that light pulses reliably suppressed spontaneous beating of monolayers expressing light-gated chloride-conducting ion channels (GtACR1/2). The light intensities used (~10 to 100 $\mu W/mm^2$) were orders of magnitude lower than what was required to produce similar behavior with light-sensitive pumps, suggesting that organ-scale excitation suppression may be feasible with GtACR in larger hearts.

LIGHT SENSITIZATION OF CARDIAC TISSUE. An important aspect of cardiac optogenetics research is the means by which light sensitivity is inscribed in heart cells. Recent progress in this area suggests that robust cardiac expression of ChR2 and other opsins can be induced safely and reliably. Independent research groups have shown, in both mice (24) and rats (25), that high rates of cardiac ChR2 expression (up to 90%) can be achieved via a single systemic injection of adeno-associated virus (AAV) expressing ChR2 under the chicken β-actin promoter (**Figures 1A and 1B**). Bruegmann et al. (26) reported that optogenetic stimulation of mouse hearts photosensitized via this approach remained viable >1-year post-injection. Importantly, Vogt et al. (24) also showed that AAV-chicken β-actin-ChR2 injection led to highly selective cardiac delivery with minimal off-target expression in skeletal muscle and the diaphragm. These findings are relevant in the context of possible future translational applications, because they suggest that in vivo light sensitization of the human