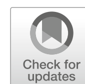

17 Enlightening Cardiac Arrhythmia with Optogenetics

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Abstract

Cardiac optogenetics offers unprecedented opportunities to study the role of different cell populations in complex biological tissues, such as the heart. To this end, light-emitting sensor proteins or light-inducible effector proteins are expressed in the target cells to either observe or steer their activity with high spatiotemporal resolution. Optogenetic tools to monitor cardiac activity include genetically encoded Ca^{2+} and voltage indicators. In addition, photo-activated ion pumps and channels are used to modulate transmembrane ionic flow and membrane voltage. In cardiac research, optogenetic approaches have been applied successfully for optical pacing, resynchronization, and defibrillation, and they have offered novel insight into cell-specific contributions to arrhythmia formation and termination, as well as simplified automatization of cardiac toxicity screening. Combining optogenetic experiments on intact myocardium with computational modelling allows one to quantitatively assess hypotheses on arrhythmia mechanisms, with the vision of developing novel, targeted approaches to prevent or terminate cardiac arrhythmias. In the following chapter, we summarize principles of optogenetic interrogations of cardiac tissue and present key experiments towards optical control of heart rhythm.

Keywords

Optogenetics · Channelrhodopsin · Optical pacing · Optical defibrillation · Cardiotoxicity screening · Computational modelling

17.1 Introduction

Successful optogenetic experiments require suitable probes, cell-population targeted gene delivery, and optical technologies for spatiotemporally defined, yet minimally invasive light application or collection. Despite the idea of selectively

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manipulating cellular activity in brain tissue being proposed as early as 1979 [1], and the discovery of light-driven ion pumps in the 1970s [2, 3], only a few optogenetic experiments were reported before the millennium. These include the pioneering work of H. Gobind Khorana expressing bovine rhodopsin in Xenopus oocytes [4] and early studies using fluorescent sensor proteins to image vesicular pH changes, intracellular Ca^{2+} concentrations, and membrane voltage dynamics [5–7]. First optogenetic manipulation of excitable cells was realized by Boris Zemelman and colleagues, co-expressing arrestin-2, rhodopsin, and the α-subunit of the corresponding heterotrimeric G protein in hippocampal neurons to increase action potential firing rate during photostimulation [8]. The breakthrough of optogenetics was the characterization of light-gated ion channels, called channelrhodopsins (ChR), at a time when methods for efficient gene transfer were available. In 2005/2006, several groups used channelrhodopsin-2 (ChR2) to drive depolarizing ion currents in neurons, thereby eliciting action potentials (AP) in vitro and in vivo [7–12]. At the same time, Tallini et al. employed the genetically encoded Ca^{2+} sensor (GCamP2) for measuring Ca^{2+} transients in vivo and for imaging Ca^{2+} signaling in the developing heart [13]. However, the use of ChR2-based tools for cardiac applications was only implemented about five years later [14, 15].

In the following, we introduce commonly applied optogenetic probes (see Figs. 17.1 and 17.2) and summarize strategies for gene targeting and optical measurements, before presenting key studies using optogenetic approaches to monitor and steer cardiac rhythm in vertebrate cells, tissues, and hearts. Furthermore, we highlight how optogenetics will foster our understanding of cardiac arrhythmias, and how we can use it for facilitated cardiotoxicity screening and for dissecting non-myocyte contributions to arrhythmia inducibility and termination.

17.2 Optogenetic Actuators in Cardiac Optogenetics

Optogenetic tools can be divided into light-activated effector proteins, also referred to as optogenetic actuators, and light-emitting sensor proteins [16]. Most commonly used actuators are based on microbial rhodopsins, small heptahelical transmembrane proteins binding all-trans retinal as co-factor for light absorption. More specifically, light-driven proton, chloride, and sodium pumps use the energy provided by visible light for the active transport of ions against the transmembrane gradient (proton and sodium pumps drive outward transport of cations, chloride pumps drive inward transport of anions). When expressed in excitable cells, light-driven pumps will thus mediate hyperpolarizing membrane currents during illumination, which may inhibit AP both in neurons and in cardiomyocytes (Fig. 17.1c). The orange-light activated, inward-directed chloride pump NpHR from Natronomonas pharaonis was first used in zebrafish hearts to induce reversible block of contractions upon global cardiac illumination. Using spatially restricted illumination patterns, it was further utilized to identify the location of pacemaker cells in the developing zebrafish heart and to optically mimic different degrees of atrioventricular conduction block [14]. NpHR-mediated hyperpolarization was also shown to silence or shorten AP in monolayers of neonatal rat ventricular myocytes [17]. Pumping protons out of the cell, the green-light-driven proton pump ArchT shows hyperpolarizing effects on the membrane potential that are comparable to NpHR effects. ArchT-mediated hyperpolarization has been exploited to silence spontaneous excitation of fibroblast–cardiomyocyte co-cultures. Interestingly, in those experiments, ArchT was expressed in fibroblasts, with the resulting hyperpolarization transmitted to cardiomyocytes by electronic coupling [18, 19]. In mouse hearts expressing ArchT in cardiomyocytes,

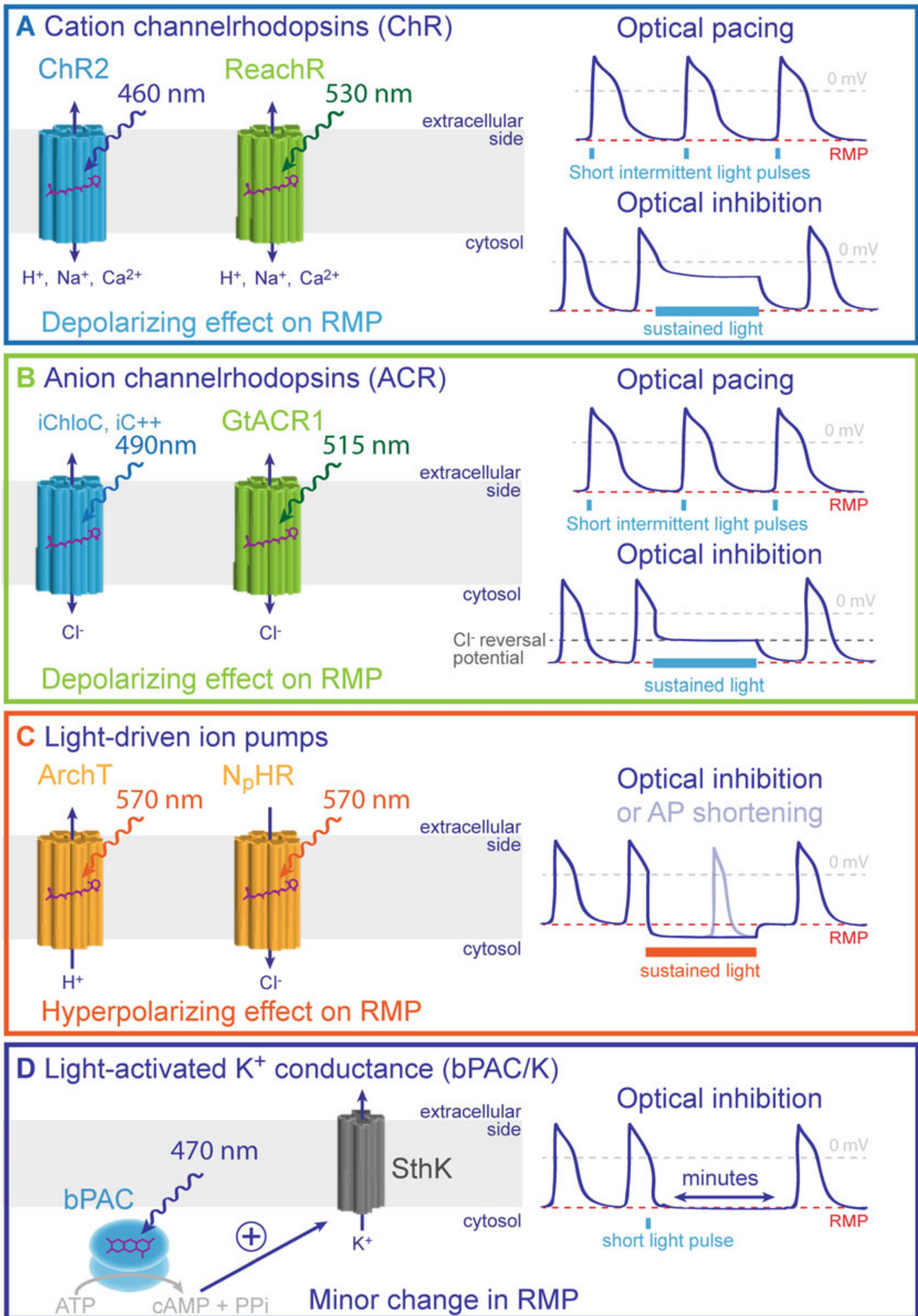

Fig. 17.1 Optogenetic actuators for membrane potential modulation and their effects in cardiomyocytes. Comparison of common optogenetic actuators and their respective effects on cardiomyocyte resting membrane potential (RMP). Wavelengths of maximal activation are indicated. Abbreviations: *ChR2* channelrhodopsin-2 from *Chlamydomonas reinhardtii, ReachR* red-activatable channelrhodopsin, *iChloC* improved chloride-conducting

hyperpolarizing currents upon green-light exposure were sufficient to terminate ventricular arrhythmia, albeit at low efficiency [20]. This could be explained either by the limited inhibitory effect of hyperpolarization per se or by the high light intensities required, as maximally one ion can be transported per absorbed photon by light-driven ion pumps.

In contrast to pumps, channelrhodopsins (ChR) are light-gated ion channels mediating passive ion flux along the electrochemical gradient upon light activation. ChR can be divided into cation-selective ChR, such as the frequently used channelrhodopsin-2 (ChR2) from *Chlamydomonas reinhardtii*, and the more recently discovered group of anion-conducting channelrhodopsins (ACR), including engineered channels (e.g. iChloC, iC++) and naturally occurring representatives from Guillardia theta (GtACR1, GtACR2) [21–23]. Cation-selective ChR conducts protons, sodium, potassium, calcium, and magnesium ions [24], resulting in depolarizing membrane currents at negative membrane potentials (reversal potential at around 0 mV). Their activation by short light pulses thus leads to short, reversible membrane depolarization of the target cells, sufficient to reliably trigger AP in cardiomyocytes (Fig. 17.1a). Prolonged illumination results in sustained depolarization, preventing repolarization to the resting membrane potential. This precludes recovery from the inactivation of fast sodium channels, thereby suppressing re-excitation of cardiomyocytes [15]. In cardiac optogenetic studies, the blue-light-activated ChR2 and the green-light-activated chimera ReachR [25] have been widely used for modulating cardiac electrophysiology, with applications ranging from cardiac pacing, resynchronization, arrhythmia termination, and drug screening, to studying the role of intracardiac neurons and interstitial non-excitable cells [26].

Anion-selective channelrhodopsins predominantly conduct chloride ions under physiological conditions, thus their reversal potential is determined by the transmembrane gradient for chloride. In cardiomyocytes, ACR activation leads to membrane depolarization, suitable either for optically pacing with short light flashes or for maintaining cells at constant depolarized potentials during prolonged illumination, thereby suppressing further AP (Fig. 17.1b) [27, 28]. Despite high expression levels and large photocurrents of ACR in cardiomyocytes, their use has been restricted to proof-of-principles studies so far. Holding cardiomyocytes at their diastolic membrane potential can be achieved using light-activated K^+-conducting channel systems (Fig. 17.1d). However, currently available systems are either limited by insufficient expression levels in mammalian cells [29] or by slow off-kinetics [30], rendering them unsuitable for applications aiming at beat-by-beat control of cardiac excitation. These challenges might be overcome by the recently discovered class of natural occuring Kalium Rhodopsins, including the potent K^+- selective channel WiChR from Wobblia lunata [31, 32].

In addition to microbial rhodopsin-based tools to control the membrane potential, there is a vast range of other optogenetic actuators, including, but not limited to, light-activated G protein-coupled receptors (GPCR, Fig. 17.2a), photoactivated enzymes, and light-controlled protein interaction systems [16]. Optical control of GPCR signaling is of special interest for understanding intracellular signaling in cardiomyocytes. Suitable GPCR comprise naturally occurring light-sensitive GPCR (visual and non-visual vertebrate rhodopsins and invertebrate

Fig. 17.1 (continued) channelrhodopsin, *iC++* improved chloride-conducting chimeric channelrhodopsin, *GtACR1* anion channelrhodopsin-1 from *Guillardia theta, ArchT* proton pump from *Halorubrum sp. TP009, NpHR* halorhodopsin from *Natronomonas pharaonis, AP* action potential, *bPAC* photoactivated cyclase from *Beggiatoa, SthK* cyclic-nucleotide-gated K^+ channel from *Spirochaeta thermophile*

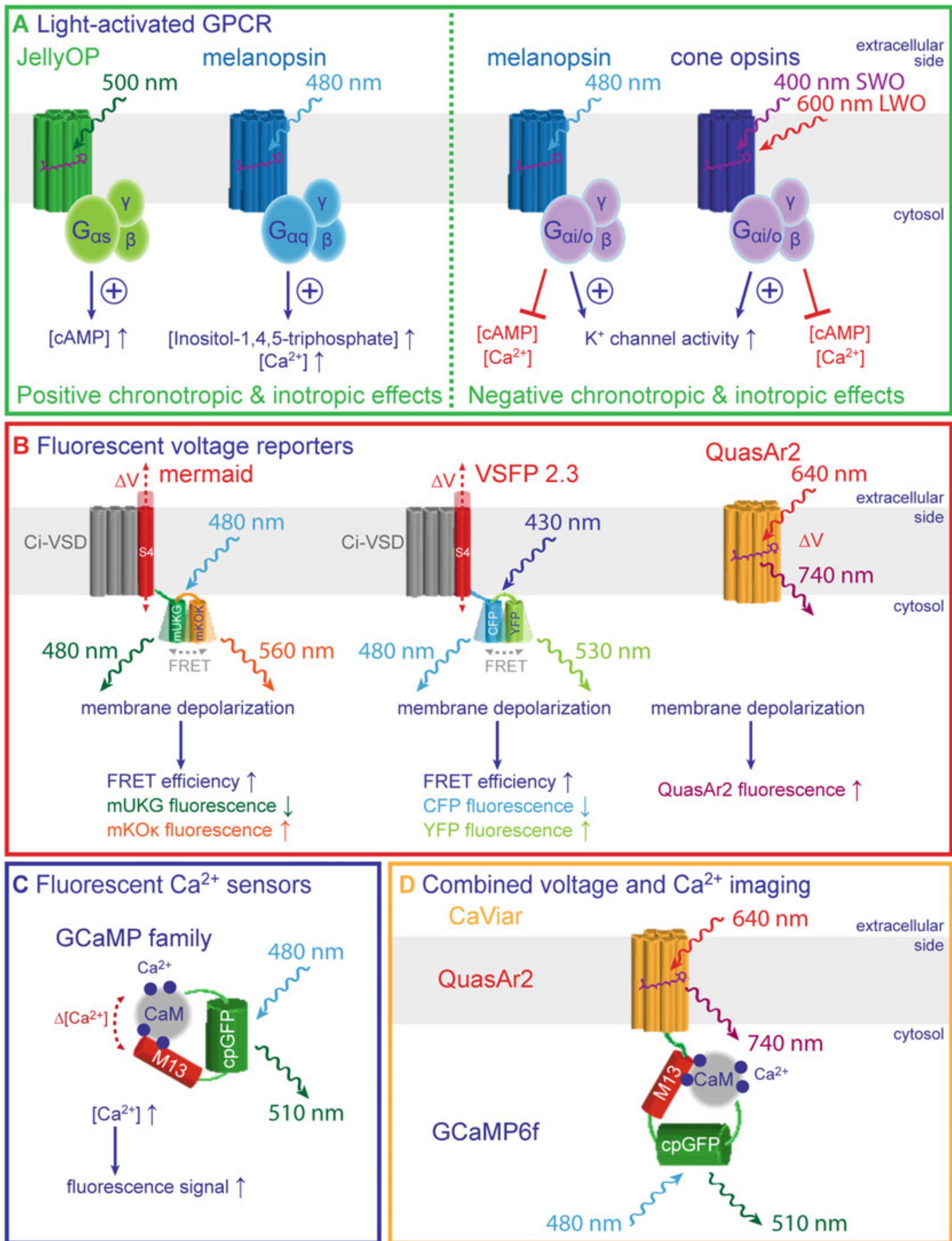

Fig. 17.2 Additional optogenetic tools: Light-activated G protein coupled-receptors (GPCR) and fluorescent reporters. (**a**) GPCR used to modulate cardiac activity include JellyOP from *Carybdea rastonii* and murine melanopsin. Of note, by binding a different G_{α} protein, melanopsin can also activate the $G_{i/o}$ signaling pathway, similar to short- and long-wavelength cone opsins (SWO and LWO). (**b**) Fluorescent voltage reporters comprise the Förster-resonance energy transfer (FRET)-based indicators mermaid and VSFP2.3, and rhodopsin-based, single-wavelength indicators such as QuasAr2. In FRET-based reporters, voltage changes are transmitted from the voltage-sensitive domain (VSD) of *Ciona intestinalis* voltage-sensing phosphatase to a FRET pair, thereby

rhodopsins) [33, 34] and custom-engineered rhodopsins referred to as opto-XR.

Light-induced activation of the invertebrate rhodopsin JellyOP allows mimicking β-adrenergic stimulation with unprecedented spatiotemporal precision within the intact heart [35]. Illumination of the right atrium of isolated murine hearts expressing JellyOP leads to an instantaneous increase in spontaneous beating rate. In contrast, illumination of the left posterior atrium at the sites of pulmonary vein insertions results in the generation of supraventricular extrasystoles. Thus, JellyOP activation can be used to look for pathway-specific arrhythmia hotspots within the intact heart. Furthermore, the use of short activating light pulses revealed different on-kinetics of the positive inotropic and lusitropic effects, with the latter being significantly faster. These experiments indicate that optogenetic approaches can facilitate the study of amplification mechanisms and temporal dynamics of G protein signaling cascades, as well as compartment-specific signaling behavior. Importantly, optogenetic control of GPCR activation can mimic physiological responses to pulsatile neurotransmitter release by repeated application of short light flashes.

In embryonic stem-cell-derived cardiomyocytes, heterologous expression of the vertebrate rhodopsin melanopsin enables light-induced G_q protein activation leading to inositol-1,4,5-trisphosphate production and elevation of intracellular Ca^{2+} concentration, thereby enhancing beating frequency of spontaneously beating embryoid bodies [36]. However, melanopsin is a promiscuous receptor also able to activate the G_i signaling cascade [37, 38]. While optogenetic GPCR for specific control of G_i pathways have been identified [39, 40], the optogenetic toolbox currently lacks probes to specifically control G_q signaling – despite the importance of this pathway for acute and chronic adaption of the heart. One possible candidate GPCR could be human Neuropsin (hOPN5), as recently proposed [41].

17.3 Fluorescent Reporters in Cardiac Optogenetics

Complementary to optogenetic actuators, fluorescent sensor proteins can be used for cell-type specific imaging of selected cellular parameters. With respect to cardiac arrhythmias, genetically encoded Ca^{2+} indicators (GECI) and genetically encoded voltage indicators (GEVI) represent the most important tools for imaging cardiac activity (Fig. 17.2b–d). GECI comprise single-fluorophore (e.g. GCaMP family) or FRET-based Ca^{2+} sensors (e.g. Cameleon) that change their fluorescence intensity and/or FRET efficiency upon Ca^{2+} binding [42]. GCaMP2 was first used to image Ca^{2+} dynamics in mouse hearts in vivo and to assess the effects of isoproterenol on diastolic and systolic Ca^{2+} levels as well as kinetics of Ca^{2+} transients in cardiomyocytes. In Langendorff-perfused hearts, GCaMP2 further enabled measurements of the conduction velocity of Ca^{2+} waves, and for combined recordings of Ca^{2+} transients and membrane voltage, the latter using the red-fluorescent voltage dye RH237 [13]. GCaMP2-based imaging also showed functional coupling between engrafted embryonic cardiomyocytes and native myocardium, preventing the risk of post-infarct arrhythmia [43]. To date, a variety of GECI with optimized properties are available, including variants with red-shifted fluorescence spectra [44, 45], fast response times [46, 47], and low-affinity indicators suitable for measuring Ca^{2+} dynamics

Fig. 17.2 (continued) increasing FRET efficiency upon membrane depolarization. Mermaid employs mUKG and mKOκ, VSFP2.3 uses cyan and yellow fluorescent protein (CFP and YFP) as FRET donors and acceptors, respectively. (**c**) Genetically encoded Ca^{2+} sensors of the GCamP family are single-wavelength indicators, wherein Ca^{2+}-induced changes in the interaction of the myosin light chain kinase fragment (M13) and calmodulin (CaM) change fluorescence intensity of a circular permutated green fluorescent protein (cpGFP). (**d**) CaViar can be used for simultaneous imaging of Ca^{2+} and voltage dynamics

in intracellular organelles with elevated Ca^{2+} levels such as the sarcoplasmic reticulum [48–50].

GEVI include single-wavelength and ratiometric fluorescent indicators that enable imaging cell-type specific membrane voltage dynamics in real-time [51, 52]. Being expressed under the control of the cardiomyocyte-specific myosin light chain 2 promoter, the ratiometric GEVI mermaid, for example, was used for non-invasive imaging of cardiac activity in embryonic zebrafish hearts, revealing altered cardiac excitation patterns in the presence of the hERG channel blocker astemizole [53]. Similarly, cardiomyocyte-targeted expression of the voltage-sensitive fluorescent protein VSFP2.3 was applied for measuring optical cardiograms, both during sinus rhythm and ventricular tachyarrhythmia in intact mouse hearts [54]. When targeted to cardiac non-myocytes VSFP2.3 was further used to explore electrotonic coupling from cardiomyocytes to non-myocytes in the scar border zone of murine hearts [55]. Additionally, GEVI facilitated rapid phenotyping of stem-cell-derived cardiomyocytes [52, 56]. Improved GEVI comprise variants exhibiting minimized photobleaching and optimized performance for 2-photon imaging, as well as near-IR fluorescent sensors [51, 57], but their utility for cardiac application still needs to be demonstrated.

17.4 Targeted Transgene Delivery

After selecting the appropriate molecular tools for an optogenetic experiment, they need to be targeted to the specific cell population of interest. The four main strategies for targeted transgene delivery to cardiac cells are (1) the generation of knock-in animals expressing the gene of interest under a cell-type specific promoter, (2) the use of recombinatorial animal models (mainly mice) where a cell-type specific driver line (e.g. expressing Cre recombinase) is cross-bred with a driver-dependent line coding for the probe (e.g. Cre-dependent line containing a floxed or flexed transgene) [58–63], (3) viral delivery of genes of interest [64–66], and (4) injection of cells expressing the respective optogenetic probe [67]. The technical details and challenges of the individual methods have been discussed earlier [68, 69]. We would like to strengthen the point that the model generation itself is one of the essential steps towards meaningful optogenetic experiments, which requires thorough controls to exclude off-target expression [70, 71] and side-effects such as cardiotoxicity [72, 73] and immunogenicity [74].

17.5 Ex Vivo Optical Stimulation and Readouts

Another challenge is suitable light delivery and collection for spatiotemporally precise photostimulation and optical readouts of cardiac activity. In principle, for ex vivo experiments, light delivery via conventional light sources for fluorescence microscopy such as shutter-controlled halogen or mercury lamps with suitable bandpass filters, or standard LEDs in combination with microscope/macroscope optics for spatial focusing provide sufficiently high light intensities for optical probe activation. Patterned illumination can be achieved with different optical approaches, e.g. using digital micromirror devices (DMD) from projectors [14, 75], or by rapid scanning of focused excitation light with acousto-optical deflectors [76]. Combining optical stimulation with simultaneous readouts of electrical activity, e.g. via optical mapping, allows one to establish closed-loop systems for real-time adaptation of optical stimulation patterns to observed electrical activation maps. Potential applications for all-optical systems include fast light-controlled restoration of normal electrical activity in hearts showing AV block, and optical termination of ventricular arrhythmias [77, 78].

17.6 Optogenetic Approaches for Controlling Heart Rate and Rhythm

Commonly used devices for heart rhythm control are artificial electrical pacemakers (atrial,

ventricular, or dual-chamber pacemakers) and implantable cardioverter defibrillators (ICD), which rely on the application of electrical shocks by electrodes for triggering cardiac excitation and defibrillation, respectively. Pacemakers, on the one hand, have proven extremely useful for long-term maintenance of cardiac activity and come with the advantage that electrical pulses usually remain unnoticeable to patients. ICD shocks, on the other hand, use approximately one thousand times more energy, leading to non-specific tissue excitation of myocardium, nerves, and skeletal muscle. This is associated with adverse effects, including severe pain, chronic anxiety, and structural tissue remodeling [79, 80]. In contrast, optogenetic approaches enable depolarization or hyperpolarization of spatially defined subsets of cardiomyocytes, with minimal effects on other cardiac cell populations. Optogenetic-based systems for external heart rhythm control may thus, in the long term, provide more specific, pain-free, and effective alternatives for heart rhythm management in patients.

17.6.1 Optical Pacing

Depolarizing ChR2-expressing myocytes by short blue-light pulses reliably triggers AP, allowing atrial and ventricular optical pacing in intact hearts from transgenic or virally transduced animals [14, 15, 64, 66]. Furthermore, dual- and multi-site optical stimulation effectively synchronizes ventricular activation, indicating the feasibility of light-driven cardiac resynchronization therapy [66]. Cell-type-specific optogenetic depolarization was also used to assess the number of activated cardiomyocytes or Purkinje cells required for inducing focal ectopic beats, finding that simultaneous depolarization of at least 1300–1800 working cardiomyocytes or 90–160 Purkinje fibers was necessary to trigger extrabeats in murine hearts [58]. Finally, ChR2 activation in human induced pluripotent stem cells in 3D-engineered heart tissue allowed for intermittent tachypacing over the duration of several weeks, providing novel insights into electrical and mechanical remodeling in ventricular and atrial tachycardia, as well as into long-term effects of optogenetic interventions [81, 82].

17.6.2 Optical Defibrillation

Using optogenetic approaches for terminating either atrial or ventricular tachyarrhythmias does not only improve our mechanistic understanding of cardioversion but may also facilitate the development of strategies towards optimized defibrillation therapy, e.g. by testing different locations, geometries, and light levels in a reversible and minimally invasive manner. So far, this relied on ChR2- or ReachR-based depolarization of cardiomyocytes by longer light pulses (hundreds of milliseconds to seconds), thereby suppressing effective repolarization, extending the period of Na^+ channel inactivation and thus inhibiting re-excitation. Bruegmann et al. showed effective termination of ventricular tachycardia by ChR2 activation via blue light application to the anterior ventricular epicardium of Langendorff-perfused mouse hearts, both for healthy hearts and following myocardial infarction [79]. Crocini et al. [76] used a similar experimental approach to terminate induced ventricular tachycardia in ChR2-expressing mouse hearts and showed that a three-barrier pattern of ventricular illumination—based on prior knowledge of the location and geometry of the underlying re-entry pathway—was equally potent to block VT as unfocussed light application on the left ventricular surface, albeit at much lower total irradiation energy (~ 4% of energy). In contrast, single-barrier or point stimulation was insufficient to effectively block arrhythmias in their study [76]. Successful optical defibrillation was also demonstrated on rat hearts expressing the green-light-activated ChR chimera ReaChR [65]. Follow-up studies have since demonstrated the feasibility of optical defibrillation also for terminating atrial fibrillation [83, 84].

Based on experimental studies varying the timing, location and/or intensity of optical stimulation in combination with in silico models, several mechanisms have been suggested for

effective optogenetic cardioversion. These include ChR-mediated depolarization and conduction block into illuminated tissue volumes [79]. In this case, transmural depolarization to keep Na^+ channels refractory seems to be mandatory [79, 85]. Alternatively, filling the "excitable gap" can be achieved by pacing the excitable region between wavefronts, promoting the extinction of self-sustained re-entry. While the energy requirements are much lower for this mechanism, filling the excitable gap either relies on spatiotemporal information on rotating wavefronts or requires global illumination of the entire ventricular surface (which obliterates the energetic advantage) [86]. Optogenetic tissue depolarization is also associated with prolonged action potential duration (APD), which has been proposed as a distinct mechanism for terminating VT [65]. Finally, a recent study suggested that low-intensity illumination, leading to sub-threshold depolarization, may be effective for steering rotors towards more depolarized tissue areas, which could be used to terminate cardiac arrhythmias [87]. Interestingly, optogenetic termination of VT is also possible using the light-driven proton pump ArchT, although at lower efficacy compared to depolarizing ChR, potentially via an increased electrical sink mechanism [20]. Light-gated K^+ channels [29, 30] could serve as potent tools for arrhythmia termination if current amplitudes were sufficiently large to counteract the large Na^+ inward currents associated with the propagating wave of excitation, thereby preventing electrical activation and conduction in illuminated tissue areas.

Combining monitoring of heart rhythm and cardiac conduction with optical arrhythmia termination offers the opportunity for automated, real-time correction of rhythm disturbances. Following this idea, Burton et al. implemented light-controlled reversal of spiral wave chirality based on dye-free imaging of contractions in cardiomyocyte monolayers [78]. Scardigli et al. developed a closed-loop system comprising a high-speed sCMOS camera for optical mapping of membrane voltage and a DMD-based projector for optical stimulation, which allowed them to correct for atrioventricular node delay and to optically simulate re-entrant tachycardia [77]. A hybrid bioelectric system with automated ECG-based optogenetic stimulation of the right-atrial epicardial surface was used by Nyns et al. and was shown to be effective in terminating atrial tachyarrhythmias in rats in vivo [84].

The studies described here demonstrate the feasibility of optical rhythm control in select animal models. Further advances in optogenetic proteins (e.g. red-light activated channels with high light sensitivity), methods for safe and targeted gene delivery (ensuring long-lasting, stable protein expression), and software/hardware for optical stimulation (including biocompatible miniature LEDs for photostimulation) will be needed before these concepts may be transferrable to patients in the future [73].

17.7 Using Optogenetics for Cardiotoxicity Screening

Cardiac toxicity and, in particular, drug-induced long QT syndrome are major reasons why newly developed drugs fail to enter or are withdrawn from the market. To avoid life-threatening side effects, as well as the related financial consequences for the pharmaceutical industry, new approaches providing more accuracy and predictive power are required during preclinical drug development [88]. Drug-induced cardiotoxicity is often related to inhibiting Kv11.1 channels, reducing hERG currents, and prolonging APD. Moreover, drugs may also modulate Nav1.5 channels. Inhibition of Nav1.5 channels can induce Brugada-like syndromes, whereas activation of late sodium currents may cause AP prolongation similar to LQT3 syndrome [89–91].

Classically, cardiotoxicity screening of cardiac syncytia relies on spontaneous beating (limited to slow beating rates) or electrical field stimulation (associated with potential stimulation artefacts and/or side effects on stimulated cells) for rate control. In contrast, all-optical approaches provide the unique opportunity for precise external pacing, simultaneous recordings of membrane potential or Ca^{2+} levels, and measurements of

myocyte contractility [17, 92, 93]. This further comes with the advantage of potential automatization [94], even if spectral separation can be difficult (i.e. one needs to separate light used for ChR2 and dye excitation from emission light by fluorescent proteins and dyes) [95]. OptoDyce is a platform combining ChR2-mediated stimulation and synthetic dyes for voltage and Ca^{2+} imaging with dye-free video tracking of contractions of cardiomyocyte monolayers. OptoDyce uses short light flashes for optical pacing and temporally separated (intermitted) optical recordings to avoid cross-contamination of the different light signals [92]. In subsequent work, the same group described a quantitative all-optical assay of intercellular connectivity (i.e., OptoGap) [96]; this study also used computational modelling, as discussed in the next section, to show that it may be feasible to scale up this approach to intact human hearts, even after accounting for light attenuation effects. Dempsey et al. used a co-culture of two different, spatially separated transgenic cardiomyocyte lines differentiated from human-induced pluripotent stem cells (a ChR line for optical pacing and a reporter line expressing CaViar; Fig. 17.2d) [93]. Spectral separation was also achieved by employing infrared-compatible dyes for voltage and Ca^{2+} imaging [97]. Alternatively, optical pacing was combined with the recording of electrical field potentials [98]. Global illumination results in near-synchronous activation of all cardiomyocytes, allowing spatial averaging of measured field potentials, as shown with averaging from 60 electrodes within an array [99] and later using one larger electrode [100], thereby simplifying high-throughput screening. Importantly, studies employing optogenetics for cardiotoxicity screening neither reported side effects from overexpression of ChR2, nor acute effects on myocyte electrophysiology. Furthermore, no drug effect on ChR photoactivation has been reported so far. However, chronic optogenetic tachypacing has been shown to be arrhythmogenic by itself [81, 82]. Taken together, combined optogenetic rate control and imaging of cardiomyocyte behaviour provide a unique opportunity for high-throughput cardiotoxicity screening.

17.8 Computational Modelling and Simulation of Cardiac Optogenetics

Multi-scale simulations of cardiac electrophysiology have emerged as a means for investigating mechanistic underpinnings of arrhythmia perpetuation, and for exploring new therapeutic approaches in realistic image-based human atrial or ventricular models [101]. Thanks to the development of robust mathematical representations of light-, voltage-, and time-dependent responses of microbial rhodopsins (e.g., ChR2-H134R) to light stimulation [102], it is possible to simulate optogenetic manipulation of the heart [103, 104]. This involves modifications of existing computational approaches at the cell, tissue, and organ scale to account for the expression of photo-sensitive channels and pumps, spatial distribution of light-sensitized cells resulting from viral gene delivery, and attenuation of light applied to the myocardial surface due to photon scattering and absorption.

Simulations have been used to explore the theoretical possibility of deploying optogenetics in the hearts of larger mammals (e.g., rabbits, dogs, and humans [103]), which thus far cannot be tested in an experimental setting. This work provided proof-of-concept evidence for the feasibility of light-based arrhythmia termination in human atrial [105, 106] and ventricular [79, 107] models. A prominent take-home message has been that the significant attenuation of blue light in the thicker-walled chambers of larger species is expected to be a major hindrance to the translation of approaches from smaller animals, leading to the prediction that optogenetic actuators with red-shifted action spectra would be necessary. Another important finding is that spatially targeted illumination of critical areas to disrupt re-entrant circuits could dramatically reduce the energy needs for optogenetic defibrillation [79, 107], confirming experimental findings in smaller hearts [83]. More recent work has assessed the use of ACR to disrupt re-entrant arrhythmia in human hearts, showing that utilizing these high-efficiency chloride

conducting channels may lower the energy required for defibrillation by 2–3 orders of magnitude [108].

A second application of computational cardiac optogenetics research is the use of simulations to enhance the understanding of data from wet lab preparations like tandem cell units [67, 103] or monolayers of cardiomyocytes co-cultured with light-sensitized donor cells [109], or to validate and explore the expansion of methodologies like the OptoGap approach described in the prior section [96]. Likewise, this type of modelling work has been employed to explore completely new applications of optogenetics like the use of subthreshold light-induced depolarization to steer re-entrant drivers towards non-conductive boundaries, ultimately resulting in arrhythmia termination [87]. Notably, while much of the recent interest in computational modelling of cardiac optogenetics has focused on actuator opsins (e.g., ChR2, GtACR1), similar tools have also been developed to realistically simulate light emission from dyes used extensively in optical mapping experiments [110–112].

17.9 Cardiac Optogenetics beyond Cardiomyocytes

Taking advantage of the possibility to selectively target individual cell populations of interest, optogenetic approaches have been extended to cardiac non-myocytes, including cardiac interstitial cells, and intracardiac sympathetic and parasympathetic neurons. While interstitial cells (e.g. fibroblasts and resident immune cells) have traditionally been viewed as electrical insulators, there has been long-standing evidence that cardiac fibroblasts can be electrotonically coupled to cardiomyocytes in vitro, acting as an electrical load and/or interlinking otherwise unconnected cardiomyocytes [113]. Based on fibroblast-specific activation of either ChR2, ArchT, or eNpHR, several optogenetic studies have confirmed electrotonic coupling between (myo) fibroblasts and cardiomyocytes in diverse in vitro systems, with direct effects on cardiomyocyte excitability, AP properties, and conduction velocity [18, 67, 114–116]. In 2016, Quinn et al. showed that fibroblasts expressing VSFP2.3 follow rhythmic depolarizations of cardiomyocytes in scar border zone tissue following ventricular cryoinjury in Langendorff-perfused whole hearts [55]. Similar coupling was subsequently observed by Rubart et al. studying myofibroblasts after myocardial infarction [117]. In 2017, Hulsmans et al. described Cx43-based gap junctions between cardiomyocytes and tissue-resident macrophages and demonstrated that ChR2-mediated macrophage depolarization could facilitate AV node conduction at high atrial stimulation rates [63]. These studies exemplify how cell-type-specific optogenetic manipulation and observation can be applied for unravelling cellular functions that are difficult to assess with classic electrophysiological techniques such as patch-clamp measurements or dye-based optical mapping of membrane voltage.

Another exciting avenue of cardiac optogenetics is the selective photostimulation of intracardiac neurons. Wengrowski et al. established a mouse model expressing ChR2 in murine catecholaminergic sympathetic neurons. In Langendorff-perfused hearts, photostimulation increased both heart rate and developed force of contraction and led to a significant shortening of the AP plateau phase. Moreover, the optogenetic release of norepinephrine increases both the incidence and severity of ventricular arrhythmias following burst pacing [59]. Based on experiments using optogenetic stimulation of sympathetic neurons and FRET-based imaging of cAMP levels, Prando et al. concluded that neurotransmission from sympathetic neurons to cardiomyocytes occurs within spatially (diffusion)-restricted intermembrane domains [60]. In line with the observed chronotropic effects of sympathetic nerve activation, reversible neuronal hyperpolarization by ArchT activation in the left stellate ganglion decreases the amplitude and frequency of nerve activity. This resulted in reduced systolic blood pressure increase upon neuronal stimulation, longer APD and effective refractory periods, decreased heart rate variability, and suppression of ischemia-induced ventricular arrhythmias in an in vivo study using virally transduced beagles [118].

Moreno et al. targeted ChR2 to choline acetyltransferase-expressing neurons; hence, blue-light mediated ChR2 activation induced acetylcholine release from cardiac parasympathetic neurons. Accordingly, illumination slowed the sinus rate and delayed atrioventricular node conduction [119]. When using ChR2-mediated activation of cholinergic neurons of the inferior pulmonary vein ganglionated plexus, Rajendran et al. also observed a light-dependent decrease in the heart rate; however, in their experimental setting, no change in AV node conduction was seen, suggesting that neuronal fibers originating from this plexus may pass the AV node without forming synapses. Photostimulation at higher frequencies further induced ectopic atrial activation and asystole. Finally, the study showed differential effects of optogenetic vs. electrical stimulation of the vagus nerve, enabling authors to dissect the effects of efferent and afferent vagal nerve activation [120]. Taken together, optogenetic interrogation of intracardiac neurons thus represents an elegant method to elucidate the output of defined neuronal subsets on myocyte activity from single-cell to whole-heart level, as selective photostimulation enables superior specificity compared to electrical activation that cannot discriminate well between different neuronal classes, and that may also directly stimulate neighboring myocytes.

17.10 Conclusions

We have summarized the experimental approaches in cardiac optogenetics and how they can be used for studying cardiac electrophysiology and intercellular communication. Moreover, we have highlighted key applications of optical heart rhythm control and cardiotoxicity screening. Computational modelling facilitates the interpretation of experimental data from laboratory-based optogenetic studies and data integration across scales and species.

As cardiac optogenetics opens up a host of new opportunities to tackle basic research questions at present, it may pave the way for cell-type specific anti-arrhythmic therapies in the future. However, proof-of-concept studies are still limited to small animals and major hurdles have to be taken before transfer from bench to patient is realistic, with efficient and safe gene transfer, development of implantable light sources, and prevention of an immune response representing some of the most important challenges. The intrinsic technical advantages of optogenetics compared to pharmacological and unspecific electrical stimulation have already provided important insights into cardiac arrhythmia mechanisms—and we envision further exciting insights to come.

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