Optogenetic investigation of cardiac arrhythmia mechanisms

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Chapter 7

SUMMARY, CONCLUSIONS AND FUTURE PERSPECTIVES

The general introduction of this thesis, **Chapter I**, describes how electrical signals originate and propagate in a healthy heart and how disturbances of these two properties can lead to cardiac arrhythmias. Furthermore, the current anti-arrhythmic strategies are listed, while more attention is focalized on gene therapy and optogenetics. **Chapter II** describes how monolayers of neonatal rat atrial cardiomyocytes (aCMCs) are optogenetically modified with lentiviral (LV) particles encoding for the depolarizing ion channel CatCh (Ca^{2+}-permeable channelrhodopsin). In this chapter, a detailed and precise narration of different processes is presented, going from the isolation of aCMCs, the production of the LV particles, the use of such particles to optogenetically modify the aCMCs, to finally the demonstration, via optical voltage mapping, that the CatCh-expressing aCMC monolayers were responding to global blue light illumination with synchronous elicitation of an action potential. In **Chapter III**, we used the *in vitro* model described in **Chapter II**, *i.e.* CatCh-expressing aCMC monolayers, to investigate on the mechanisms of termination of rotor-guided ablation, *i.e.* a new ablation strategy that recently has emerged in clinical settings. In this study, CatCh was specifically and precisely activated by patterned illumination. Such precise control in space and time allowed us to first induce a stable rotor and secondly to study its termination by optogenetically blocking electrical activation at or near the rotor core region by using circular or linear shapes of illumination. We found that localized optogenetic targeting of rotors in atrial monolayers could both stabilize and destabilize rotors. However, termination required a line of conduction block that from the core region was reaching at least one unexcitable boundary. The beauty of such approach inspired us to continue our research and investigate on spiral wave dynamics. Indeed, in this study, which is described in **Chapter IV**, we also used CatCh-expressing aCMC monolayers and patterned illumination. Here, we describe a unique method to control spiral wave cores in time and space that we called AAD control (Attract-Anchor-Drag). This method relies on i) attraction, ii) anchoring of a spiral wave tip to a temporal heterogeneity (*i.e.* a reversible conduction block that is created close to the spiral core), and iii) dragging to a new, yet predefined location. This process allowed to move the spirals along any desired trajectory and to terminate them when dragged towards an inexcitable boundary or towards other spirals. The *in vitro* experiments were complemented by *in silico* simulations that demonstrated precise and robust spatiotemporal control over spiral wave cores in a wide range of parameters. Application of light spots of different sizes, *i.e.* different diameter (d) showed that a small-sized spot led to dragging via a cycloidal trajectory, whereas, a large spot was associated with a linear trajectory. Indeed, when the large light spots were moved at the fastest rate, the effective depolarized region resembled an elongated ellipse and caused the spiral tip to remain anchored to the boundary of the pattern, which was linear. However, at slower dragging rates, *i.e.* longer light exposure, the spiral wave executed one or more complete rotations around the region of light-induced depolarization, before being repositioned to the next illuminated spot. Hence, spots with larger size can also follow a cycloidal trajectory. Furthermore, the location of the spot of light, the location of the spiral tip, and the direction of drift played an important role in spiral wave dragging. The dragging, indeed, was observed most efficiently when the spot of light was applied sufficiently close to the location of the spiral tip and within an angular spread in the direction of drift of the spiral core. In **Chapter V**, optogenetic

termination of anatomical reentry was investigated in a more complex *in vitro* model, *i.e.* 150-µm thick slices derived from neonatal tissue ventricular slices. The slices were genetically modified with CatCh-encoding LV particles. Here, patterned illumination was used to locally activate CatCh and thereby to induce a local and reversible conduction block in the pathway of reentry. Generation of a transmural conduction block with a width of 600 mm in the re-entrant pathway always led to arrhythmia termination. In this case, the reentrant waves were not able to enter the illuminated area and reentry was therefore immediately terminated. When the conduction block did not fully obstruct the reentrant pathway the outcome was dependent on the width of the isthmus. In these cases, reentry was often terminated at the site of illumination with further narrowing of the isthmus. From a mechanistic point of view, we showed, through a set of complementary *in silico* and *in situ* experiments, that the illuminated area was characterized by an extension into the isthmus of a gradient in depolarization. The *in silico* data revealed that the membrane potential was ranging from -20 mV in the center to -30 mV at the border of the illuminated area and -70 mV in the most distal region. In line with these results, the *in situ* data showed a strong reduction (~50%) of the optical signal amplitude in the spot closest to the area of illumination, thereby suggesting the presence of a graded decrease in excitability.

Since the optogenetic toolbox contains not only light-gated ion channel, we exploited a different optogenetic tool, a ROS-generating protein (RGP), called miniSOG (mini singlet oxygen generator), in combination with patterned illumination to quantitatively, spatially and temporally control ROS production in monolayers of neonatal rat ventricular myocytes (NRVMs). This combination allowed to assess ROS effects on arrhythmogenicity. In **Chapter VI**, we show that microfoci of increased ROS production in myocardial monolayers can promote local disturbances in electrical impulse generation and propagation, leading to ectopic activity, functional conduction block, and reentrant arrhythmias.

In conclusion, the experiments presented in this thesis show how the simplicity of an *in vitro* model might be the key towards a better understanding of complex matters, such as mechanisms of cardiac arrhythmias initiation and termination. In all these experiments optogenetics played a crucial role. Catch was expressed in cardiac tissue allowing the possibility for i) global and local light-pacing, ii) light-dependent induction of a single spiral wave, iii) spiral wave termination when a line of block reaches from the core region to at least one unexcitable boundary, and finally iv) spiral wave dynamics control in space and time, while miniSOG was expressed to show how microfoci of increased ROS production could lead to cardiac arrhythmias by creating disturbances in electrical impulse generation and propagation.

PERSPECTIVES OF OPTOGENETIC APPLICATIONS IN CARDIAC RESEARCH

As shown throughout this thesis, the scientific research in the field of cardiology has been revolutionized by the introduction of optogenetics. In the last eight years, several research groups have shown light-excitation of cardiac myocytes. Arrenberg *et al* used light to pace zebrafish hearts expressing ChR2. They showed that brief optical stimuli were able to evoke

action potentials and therefore modify the beating frequency.[3] In the same year, similar effects were also shown *ex vivo* by pacing with light the intact hearts of ChR2(H134R) transgenic adult mice and *in vitro* by pacing the cardiomyocytes isolated from these transgenic hearts.[4] Opto-pacing was also possible when rodent cardiomyocytes were genetically modified by using viral vector technology, *i.e.* AAV for *in-ex vivo* and LV for *in vitro* applications.[4-6] In those eight years, instead, fewer scientific contributions have emerged on light-inhibition of electrical activity. Light-gated chloride pump activation, resulting in a hyperpolarizing photocurrent, was used to inhibit electrical activity in zebrafish cardiomyocytes.[3] Light-gated hyperpolarizing proton pump and natural chloride-conducting channelrhodopsin were used to suppress electrical activity in cardiac culture, by their expression in cardiac fibroblast or direct expression in cardiomyocytes, respectively.[3,7,8] Depolarizing or hyperpolarizing optogenetic tool can also be used to shape an action potential. Their activation can lead, indeed, to action potential prolongation or shortening, respectively.[9] Furthermore, recently, in addition to those aforementioned applications, the ability to optogenetically terminate tachyarrhythmias has been first shown *in vitro*[10-12] and secondly in the whole rat and mouse hearts expressing light-gated ion channels.[13-15] However, the clinical translation of such optogenetic applications might encounter various challenges and might need further development and optimization of optogenetic tools, light delivery and in gene transfer technology. A first step in understanding the mechanism and the requirements for optogenetic defibrillation in patients was done by using a patient-specific computational model of post-myocardial infarction.[13] In this study, Boyle *et al* have shown that mechanistically optogenetic termination was mediated by transmural depolarization of the myocardium that led to a temporal conduction block due to sodium channels unavailability. Regarding the requirements, they have shown that blue light attenuation through the human ventricular thick wall could be an obstacle for successful termination. Termination occurred only when a red-shifted optogenetic tool was used. Accordingly, also *in vitro* assays have shown that tissue penetration of 470-nm light is rather poor. Zaglia *et al* confirmed that the intensity of such light applied at the epicardial surface decreases by 80% when reaching areas of myocardium at a depth of 300 μm. As suggested, to improve light penetration, red-shifted optogenetic tools, such as other ChR2 variants,[15, 17] could be used and furthermore combined with, for example, elastic integumentary membranes equipped with multiple μ-LEDs[18] or implantation of injectable hardware-free μ-LEDs inside the myocardium.[19] In addition, the particular substrate of the arrhythmia, *e.g.* its composition and location in time and space, has to be considered for the selection of specific light-gated ion channels and illumination protocols.

In conclusion, the studies presented in this thesis provide novel mechanistic insight into optogenetic control of cardiac electrical functions. Thereby, these studies might contribute to a better understanding of the mechanisms of cardiac arrhythmias initiation and termination, and may lead to new, pain-free, and biology-driven strategies for cardiac arrhythmias therapies.

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