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RESEARCH LETTER



Optical Control of Cardiac Rhythm by In Vivo Photoactivation of an ERG Channel Peptide Inhibitor

Jérôme Montnach¹, Hugo Millet¹, Antoine Persello¹, Hervé Meudal¹, Stephan De Waard¹, Pietro Mesrica¹, Barbara Ribeiro¹, Jérémie Richard, Agnès Hivonnait, Agnès Tessier¹, Benjamin Lauzier¹, Flavien Charpentier¹, Matteo E. Mangoni¹, Céline Landon¹, Chris Jopling¹, Michel De Waard¹

Cardiac rhythm, conduction, and electrical activity synchronization require the coordinated action of several ion channel types that differ according to the transmural and regional specificities. Classical pharmacology affects these channels in a nonregionalized way, which explains the limited efficacy in treating focalized arrhythmias in addition to negative side-effects on nontargeted organs. Through spatio-temporal control of channel activity by light, optogenetics and photopharmacology are promising approaches to solve the selectivity and temporal issues in treating arrhythmias. While convincing reports illustrate the power of optogenetics for terminating arrhythmias,¹ this approach is irreconcilable with clinical applications because genetic modification of the heart are required to express photosensitive ion channels. In contrast, photopharmacology counteracts the negative aspects of classical pharmacology and optogenetics by restricting drug activity in a spatio-temporal manner.² As previously described, photoactivatable caged peptides are engineered by grafting a UV-photocleavable o-nitroveratryloxycarbonyl protecting group (the cage) onto an amino-acid key for channel interaction³ (Figure [A]). Caged natural peptides are advantageous because small quantities can be used in vivo owing to the high target affinity of the uncaged compounds and the preservation of the pharmacokinetics, thanks to limited peptide size modification. Here, a caged version of BeKm-1, a natural ether-a-go-go-related gene channel

peptide inhibitor, was used to regulate heart activity. Caged-BeKm-1 was produced by addition of o-nitroveratryloxycarbonyl to the lateral chain of K¹⁸ because it is key for BeKm-1/hERG (human ether-a-go-go-related gene) interaction.⁴ According to docking modeling and nuclear magnetic resonance analyses, o-nitroveratryloxycarbonyl induces severe van der Waals steric clashes with hERG and chemical rearrangements in the lateral chains of K¹⁸-surrounding amino-acids within BeKm-1 pharmacophore, all factors diminishing peptide activity. As a result, caged-BeKm-1 no longer inhibits hERG K⁺ currents in human embryonic cells (HEK293) at 0.1 to 100 nmol/L concentrations at which noncaged BeKm-1 is highly potent, while production of uncaged-BeKm-1 by 365-nm illumination restores peptide potency (noncaged BeKm-1: concentration providing 50% inhibition [IC₅₀]=1.6 nmol/L; caged-BeKm-1: IC₅₀=3847 nmol/L; uncaged-BeKm-1: IC₅₀=1.5 nmol/L; Figure [A]). In acute experiments, 100 nmol/L caged-BeKm-1 has no effect on hERG K⁺ currents, whereas a 3-minute illumination at 365-nm (45 mW/cm²) produces a gradual inhibition (Figure [B]). Photoactivation of caged-BeKm-1 was investigated on human iPS-derived cardiomyocytes, dynamically clamped at -80 mV and paced at 1000-ms cycle length. The 200 nmol/L caged-BeKm-1 has no effect on action potential duration at 90% repolarization (action potential duration₉₀: 279.1±47.2 ms versus 276.7±44.9 ms), whereas caged-BeKm-1

Key Words: arrhythmias ■ bradycardia ■ cardiomyocytes ■ peptides ■ photochemotherapy ■ potassium channels ■ zebrafish

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Nonstandard Abbreviations and Acronyms

hERG	human ether-a-go-go-related gene
IBI	interbeat interval
IC₅₀	concentration providing 50% inhibition

photoactivation induces a significant 1.3-fold increase in both action potential duration₇₀ (322.9±54.2 ms versus 256.7±46.9 ms) and action potential duration₉₀ durations (358.4±55.7 ms versus 279.1±47.2 ms). Uncaging of BeKm-1 also induces early afterdepolarization in 47% of the cells (Figure [C]) demonstrating our ability to control hERG currents by UV-illumination in several cell models. Langendorff-perfused rat hearts were used to assess hERG channel control by illumination of caged-BeKm-1 in cardiac tissue. Perfusing 250 nmol/L caged-BeKm-1 has minimal effects on interbeat intervals (IBIs), whereas a 3-minute illumination of the right atrium produces bradycardia (IBI increase: 25.7±2.9 ms for uncaged-BeKm-1 versus 8.6±2.5 ms for caged-BeKm-1 compared with IBI before caged-BeKm-1 perfusion; Figure [D]), indicating that caged-BeKm-1 is photoactivatable in adult rat heart ex vivo. Next, based on ERG blockade bradycardic effects in zebrafish,⁵ we studied how photoactivation interferes with cardiac rhythm in vivo in zebrafish larvae. About 2 nL of 50 μmol/L caged-BeKm-1 injected into larvae has no effect on IBI, whereas larvae illumination (365 nm, 3 minutes) induces an increase in IBI (648.7±75.2 ms in illuminated condition versus 379.7±13.8 ms nonilluminated one and 420.2±13.6 ms in control) and episodes of atrio-ventricular desynchrony in 7 of 11 larvae. As control, 2-hour white-light illumination of larvae injected with caged-BeKm-1 does not induce any functional effect (Figure [E]). Heart rhythm control by photopharmacology was demonstrated in anesthetized rats undergoing right mini-thoracotomy, enabling visualization and illumination of the right atrium. Intravenous injection of 350 μg/kg caged-BeKm-1 produces a minor effect on sinus rhythm with a ΔRR of 6.6±1.5 ms, while a 10-minute UV-illumination with a 1-mm diameter light guide (365 nm, 75 mW/cm²) led to a final ΔRR of 15.9±2.9 ms compared with control condition (Figure [F]). Thirty minutes after illumination, bradycardia further increased (final ΔRR of 54.2±9.3 ms versus control condition), indicating delayed in situ binding of uncaged-BeKm-1 on cardiac ERG. Within this time frame, no reversibility was observed. These results highlight that cardiac heart rhythm can be controlled experimentally in mammals by peptide-based photoactivation in vivo.

In conclusion, this report demonstrates that photopharmacology, using the caged peptide strategy, can be used for regulating cardiac electrical activity in vivo in a time-defined manner and that spatial illumination restriction dissociates the bradycardic effect from the

arrhythmic one. This technology is applicable to other channels also involved in cardiac pacemaker activity such as G protein-activated K⁺, hyperpolarization-activated cyclic nucleotide-gated, Ca_v3.1, Ca_v1.3, and Na_v1.1 channels. Several candidate peptides exist (tertiapin Q for G protein-activated K⁺ channels and huwentoxin-IV for Na_v1.1³) that can be caged. Photoactivation of natural peptides will also help defining and outlining proarrhythmic cardiac areas with regard to the distribution of a given channel type.

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ARTICLE INFORMATION

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Disclosures

M. De Waard is a founder and consultant for Smartox Biotechnology. The other authors report no conflicts.

Data Availability

The data that support the findings are available from the corresponding author on request who had full access to all the data in the study and takes responsibility for its integrity and the data analysis.

REFERENCES

- Bruegmann T, Boyle PM, Vogt CC, Karathanos TV, Arevalo HJ, Fleischmann BK, Trayanova NA, Sasse P. Optogenetic defibrillation terminates ventricular arrhythmia in mouse hearts and human simulations. *J Clin Invest*. 2016;126:3894–3904. doi: 10.1172/JCI88950
- Velema WA, Szymanski W, Feringa BL. Photopharmacology: beyond proof of principle. *J Am Chem Soc*. 2014;136:2178–2191. doi: 10.1021/ja413063e
- Montnach J, Blomer LA, Lopez L, Filipis L, Meudal H, Lafoux A, Nicolas S, Chu D, Caumes C, Beroud R, et al. In vivo spatiotemporal control of voltage-gated ion channels by using photoactivatable peptidic toxins. *Nat Commun*. 2022;13:417. doi: 10.1038/s41467-022-27974-w
- Korolkova YV, Bocharov EV, Angelo K, Maslennikov IV, Grinenko OV, Lipkin AV, Nosyreva ED, Pluzhnikov KA, Olesen SP, Arseniev AS, et al. New binding site on common molecular scaffold provides hERG channel specificity of scorpion toxin BeKm-1. *J Biol Chem*. 2002;277:43104–43109. doi: 10.1074/jbc.M204083200
- Milan DJ, Peterson TA, Ruskin JN, Peterson RT, MacRae CA. Drugs that induce repolarization abnormalities cause bradycardia in zebrafish. *Circulation*. 2003;107:1355–1358. doi: 10.1161/01.cir.0000061912.88753.87

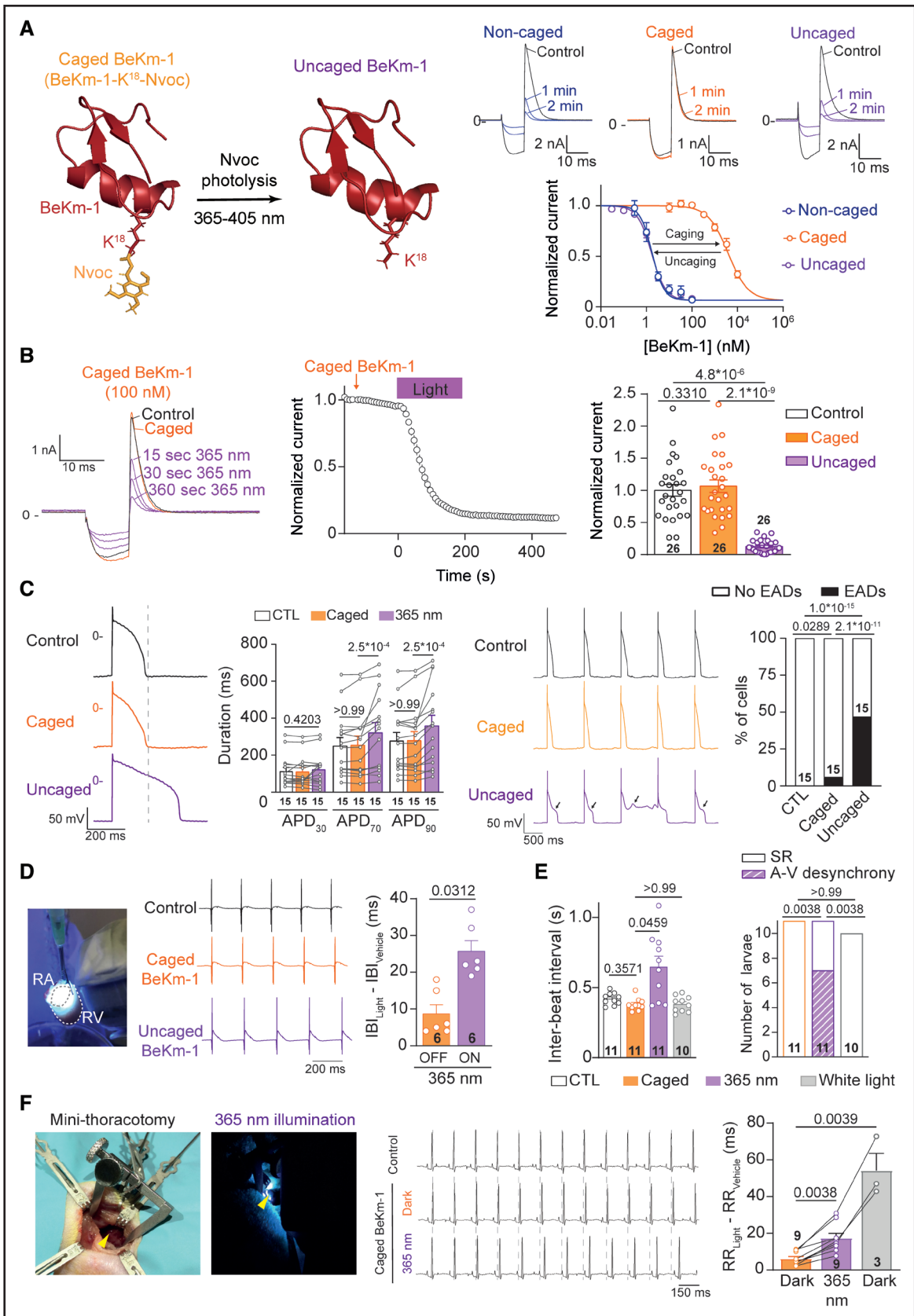


Figure. Cardiac optical control of ether-a-go-go-related gene (ERG) using photoactivatable BeKm-1 analogue.
A, Left: Schematic of the BeKm-1 uncaging process. **Right:** hERG (human ether-a-go-go-related gene) current recordings in human embryonic cells (HEK293) illustrating kinetics of current inhibition by incubation with 100 nmol/L noncaged (blue, n=46), caged- (orange, n=94) and uncaged-BeKm-1 (purple, n=180 cells). Average dose-response curves for hERG current inhibition. **B, Left:** hERG current recordings (*Continued*)

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Figure Continued. in control (black), after 100 nmol/L caged-BeKm-1 (orange) and after various illumination times (purple). **Middle:** Average normalized time course of hERG current before, during, and following light application (n=26 cells). **Right:** Quantification of current inhibition induced by illumination at steady-state. **C, Left:** Traces and quantification of action potential duration (APD) at 30%, 70%, and 90% (APD_{30} , APD_{70} , and APD_{90}) repolarization from IK_1 -clamped human induced pluripotent stem cells (hiPS)-derived cardiomyocytes in control condition (CTL), after perfusion with 200 nmol/L of caged-BeKm-1 (caged) and after illumination (365 nm). **Right:** Occurrence of early afterdepolarizations ([EADs]; arrows) after BeKm-1 uncaging in hiPS-derived cardiomyocytes and percentages of cells displaying EADs. **D, Left:** Right atrium-focused illumination area in perfused rat heart. **Middle:** multielectrode array (MEA) recordings from the right atrium in control (black), after perfusion of 250 nmol/L caged-BeKm-1 (orange) and after uncaging (purple). **Right:** Quantification of light-induced increase in interbeat interval (IBI). **E,** Quantification of IBI in zebrafish larvae after injection of 50 μ mol/L of caged-BeKm-1 (orange), or after additional 3-min 365-nm (purple) or 2-hours white-light illumination (gray). Quantification of atrio-ventricular (A-V) desynchrony in the same larvae. **F, Left:** Illustration of the mini-thoracotomy and the illumination of the right atrium (arrow) in anesthetized rat. **Middle:** Electrocardiograms in control condition, after intravenous injection of 350 μ g/kg caged-BeKm-1 (orange), after 10 minutes of illumination (purple) and additional 30 minutes after end of illumination (gray). **Right:** Quantification of light-induced increase in RR interval in anesthetized rats. Data are presented as mean \pm SEM, and the number of samples are indicated. Statistical analyses were performed with Prism8 (GraphPad Software Inc.). *P* values are indicated and obtained by Friedman repeated measure ANOVA with Dunn's multiple comparison tests (**B, C left**, and **E left**), Fisher exact test (**C right** and **E right**), and Wilcoxon test (**D** and **F**). SR indicates sinus rhythm.