Optogenetics

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Optogenetic Control of Vascular Tone with High Temporal Resolution

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Blood pressure is directly controlled by the contractile state of vascular smooth muscle cells (VSMCs) which is regulated by membrane potential-dependent Ca^{2+}-entry through Ca^{2+}-channels and Ca^{2+}-release from intracellular stores. Modulation of membrane potential in VSMCs in intact vessels can be performed by electrical field stimulation or elevation of extracellular potassium concentration, but both methods are not cell-specific and have low spatio-temporal resolution.

To overcome these limitations, we have used the light-gated cation channel channelrhodopsin2 for optogenetical control of VSMCs membrane potential. ChR2 was expressed in a7r5 VSMCs and patch clamp experiments showed light-induced inward currents. Brief light pulses with increasing light intensities led to graded depolarization and eventually initiation of action potentials. Prolonged illumination resulted in constant depolarization and elevated intracellular Ca^{2+}-concentrations.

Optogenetic stimulation of VSMCs in intact vessels was performed using transgenic mice that express ChR2 (Nat Methods. 2010;7:897-900) in VSMCs but not endothelial cells. Isometric force was analyzed in aortic rings with a wire-myograph. Illumination with blue light (475nm, 2.7mW/mm^2) reliably induced contractions with very fast on- ($<$ 800ms) and off-kinetics ($<$ 2.5s) and contraction could be maintained up to 10 min. The amplitude of contraction could be graded by variation of light intensity and reached a maximum comparable to stimulation with noradrenalin (10μM). Control vessels from wild-type mice or transgenic mice expressing EGFP did not show light-induced contractions.

Taken together ChR2 enables light-induced contraction of VSMCs with high temporal resolution and represents a powerful technique to analyze membrane-potential dependent mechanisms in VSMCs. Optogenetic stimulation allows cell-specific and localized stimulation down to single cell level and therefore can be used in the future to investigate the interplay of endothelial cells and VSMCs as well as the electrical coupling between cells within intact vessels.

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Spatiotemporal Control of Light Induced Dimerizers

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Recently, several optogenetic tools have been developed to control the biochemical activity of signaling proteins with fast temporal and subcellular spatial resolutions. Among this tools, the cryptochrome CRY2 was probed to interact with its partner, CIBN, upon blue illumination. It was then showed that this light inducible dimerizer could be used to induce signaling perturbation in cells. An important issue with the use of these new dimerizers to dissect interacting protein networks is the mapping between the light input and the induced signaling perturbation. What should be the pattern of light to shine on cell to obtain a required distribution of active proteins?

We combined TIRF microscopy with FRAP illumination to locally activate CRY2 in the cytoplasm while monitoring its recruitment to the basal plasma membrane (its partner, CIBN, being anchored to the membrane). With a simple biophysical analysis of diffusion and trapping processes grounded on experimentally measured parameters, we are able to propose simple set of rules to achieve a desired spatiotemporal distribution on the plasma membrane. In particular we showed that it is possible to establish a stable steady gradient even if the dissociation of the CRY2/CIBN complex is not light controllable. We then exploit this light induced dimerizer to control the activity of RhoGTPases with a fine spatial and temporal resolution.

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Synthesis and Characterization of a Red-Shifted, Fast Relaxing, Photoswitchable Tethered Ligand for use in Modified Glutamate Receptors

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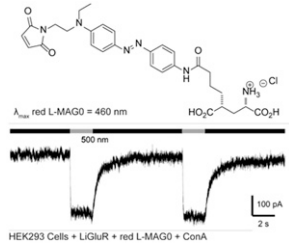
A two-component chemical and biological approach based on a Photoswitchable Tethered Ligand (PTL) and a modified ionotropic glutamate receptor (LiGluR) was recently developed. The PTL Maleimide-Azobenzene-Glutamate (MAG) takes advantage of the photochemistry of azobenzene, which changes its shape depending on the irradiation wavelength used. When MAG covalently binds via its maleimide end to an engineered cysteine residue near the ligand binding domain of LiGluR, it allows for the precise control of channel gating, as light isomerizes the azobenzene and docks or removes the glutamate from the receptor binding site. The photochemical properties of MAG offer opportunity for shifting the activating wavelength from UV light (380 nm), which is scattered by tissue and therefore penetrates poorly. We have synthesized a red-shifted MAG (460 nm peak absorption) and used it on LiGluR in whole-cell patch clamp experiments in HEK293 cells. The red-shifted MAG functions as state-dependent tethered agonist, which can be activated with a broad spectrum of visible light and thermally relaxes to inactivity.



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Optogenetic G_q Signaling in Cardiomyocytes

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Stimulation of G_q-protein coupled receptors activates phospholipase C (PLC) which produces inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol. The G_q-signaling cascade is involved in many fundamental cellular processes including cardiac pacemaking and arrhythmogenesis.

G_q-proteins can be activated by agonists with low spatio-temporal precision. To overcome this limitation, we use melanopsin, a directly light-sensitive G_q-coupled receptor (Qiu et al., Nature 2005; 433:745-9).

In melanopsin-expressing HEK293 cells cytosolic Ca^{2+}-transients could be induced by brief light-pulses and biochemical analyses revealed IP_3-production upon illumination.

Embryonic stem cells with stable expression of melanopsin under control of the chicken β-actin promoter showed repetitive light-induced Ca^{2+}-transients. Cardiomyocytes were differentiated from these embryonic stem cells by generation of spontaneously contracting embryoid bodies (EBs). Immunofluorescence staining revealed clear melanopsin expression in α-actinin-positive cardiomyocytes.

Brief illumination (60 s) of spontaneously contracting EBs caused an acceleration of beating rate. A slight and delayed increase (to 117 ± 3% of control frequency, n=12) was observed at low light-intensities of 9.1 nW/mm^2. In contrast stimulation with 166.7 nW/mm^2 led to an instantaneous acceleration of frequency to 301 ± 19% with subsequent decline to a constant plateau of 211 ± 13% (n=11). Similar to dose-response-curves of receptor agonists, the acceleration of frequency showed a sigmoid dependence on light-intensity. Addition of the PLC-blocker U-73122 or the IP_3-receptor-blocker 2-aminoethoxydiphenyl borate attenuated the light-induced acceleration of beating. Interestingly we observed a higher rate of arrhythmic contractions during and after light-stimulation of melanopsin.

The effect of local G_q-activation was analyzed using micro-electrode arrays to identify the leading pacemaker site in EBs. Stimulation of a small (Ø180 μm) region with focused light led to pacemaker activity or arrhythmias in the illuminated area indicating the importance of G_q-signaling for pacemaking.

In summary optogenetic G_q-activation in cardiomyocytes using melanopsin induces pacemaking and arrhythmia and will enable the investigation of G_q-signaling with high spatio-temporal precision.

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Optimizing of Local Nano-Particle Heating for Thermo-Magnetic Stimulation of Cells

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We have developed thermo-magnetic stimulation of cells by coupling radio-frequency magnetic field heating of supraparamagnetic nanoparticles to temperature sensitive ion channels. Optimizing the thermomagnetic heating requires nanoparticles with increased heating power, high, short field pulses, and better understanding of the molecular scale heat transfer from nanoparticles to the surrounding fluid. We are developing supraparamagnetic nanoparticles with increased magnetic moment and heating capacity. Several sizes, materials and core-shell geometries were synthesized. The nanoparticles were made water-soluble by either encasing them in silica or by polymer coating. To optimize the efficiency of these nanoparticles in activating the TRPV1 channels, we label channel-expressing cells with nanoparticles and compare the efficiency of various field frequencies and strengths, as well as pulse durations. To study the molecular scale heat transfer from nanoparticles to