

PB-94 [14:30]

Visualisation of an nsPEF induced calcium wave using the genetically encoded calcium indicator GCaMP in U87 human glioblastoma cells

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Keywords: *In vitro*, Pulsed, Completed (published)

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This study looks at the application of genetically encoded calcium indicators (GECIs), to investigate the spatiotemporal nature of nsPEF-induced calcium signals, using fluorescent live cell imaging. GECIs can be targeted to different cellular locations and, by using a plasma membrane expressed GECI, we demonstrate calcium entry into the cell as a wave, initiated at the cathode side, following nsPEF treatment. This wave was masked by commercially available chemical, cytosolic, calcium indicators.

Introduction

Calcium ions (Ca^{2+}) play a key role in a wide range of cellular processes, with elevation of intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) being a significant signalling event. An increase in $[\text{Ca}^{2+}]_i$

following application of nanosecond pulsed electric fields (nsPEF) has been widely reported[1].

To date all nsPEF induced changes in $[Ca^{2+}]_i$, measured using live cell imaging, have used chemical calcium indicators, that are generally cytosolic, including FLUO-4[2–4], Fura-2[5–7] and Calcium Green[8–14]. Genetically encoded calcium indicators (GECIs), such as GCaMP[15], provide an alternative to chemical indicators and they have yet to be used to investigate the nsPEF calcium response. They present several advantages over chemical indicators, including their use straight from the incubator, removing any negative effects of a room temperature indicator loading protocol. Being easy to target to specific locations within the cell, they could allow a more in depth investigation of the nsPEF response on particular compartments.

In this study we demonstrate the application of GECIs to investigate the nsPEF-induced calcium response. We compare the plasma membrane targeted GCaMP5-G and a cytosolically expressed GCaMP6-S in nsPEF studies with two commonly used cytosolic chemical calcium indicators (Fura Red and FLUO-4). With GCaMP5-G we show a wave of calcium entry into the cell following nsPEF treatment, which is not observed with any of the cytosolic indicators, highlighting the potential interest of being able to target GECIs to specific regions of the cell especially when no chemical alternative is available.

Methods

Changes in $[Ca^{2+}]_i$ were visualised in U87 cells that either expressed a GECI (GCaMP5-G or GCaMP6-S), or were loaded with a chemical indicator (1.25 μ M Fura Red, AM, 0.5 μ M FLUO-4, AM or 1 μ M of FFP-18). Cells were imaged, in a standard HBSS solution, by epifluorescence using a Leica DMI6000 microscope with a 100x objective. Fluorescent excitation was provided by a Spectra 7 light engine (Lumencor). Emitted light was filtered and captured on an electron-multiplying charge-coupled device camera (EMCCD; Photometrics Evolve 512, Roper) with 512 x 512 pixels.

Using two steel electrodes separated by a gap of 1.2 mm, with 50 Ω impedance in parallel[16] and an nsPEF generator (FPG 10-1NM-T, FID Technology, Germany) with 50 Ω output impedance, cells were exposed to 10 ns pulses, with an electric field intensity of 44 kV/cm.

Results

When 100, 10 ns pulses were applied at 10 Hz all three of the cytosolic calcium indicators (FLUO-4, Fura Red and GCaMP6-S) showed a global, uniform increase in intracellular calcium. The membrane bound GCaMP5-G however showed a spatially focused increase in fluorescence that initiated at the cathode before spreading as a wave across the cell (see figure 1). The speed (in μ m/s \pm SE) that this wave travelled across the cell was dependent on the frequency of pulse application: 100P 100 Hz: 7.23 ± 0.89 (n = 4); 100P 10 Hz: 2.65 ± 0.38 (n = 4); 100P 1 Hz: 0.40 ± 0.04 (n = 4). The wave was not propagated by calcium induced calcium release, but could be recreated in a cell loaded with a membrane bound chemical indicator.

Discussion and conclusion

Whilst synthetic calcium indicators are easy to use, we show in this study an advantage of using GECIs for studying nsPEF calcium responses. By targeting the GECI to the plasma membrane we were able to demonstrate the influx of extracellular calcium in a spatiotemporal wave, which was masked when cytosolic indicators were used. As all calcium indicators are essentially calcium buffers, they have a perturbing effect on the underlying calcium signal and modelling experiments have shown that mobile buffers are able to eliminate spatial calcium waves, whereas immobile buffers have little effect[17]. Targeting GECIs to other locations within the cell, for example intracellular calcium stores, could be used to further understand the calcium response to nsPEF.

Acknowledgement

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 737164 SUMCASTEC. Our research was conducted in the scope of LEA-EBAM, a European Associated Laboratory entitled, "Pulsed Electric Fields

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Figures

