

Title

Influence of pulse duration on intracellular calcium concentration for sub-10 ns pulses.

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Introduction

Calcium ions (Ca^{2+}) play a key role in a wide range of cellular processes. Intracellular calcium concentrations $[\text{Ca}^{2+}]_i$ are known to increase following nanosecond pulsed electric field (nsPEF) exposure. To date there is limited information on how nsPEF, with a duration of less than 10 ns, affect calcium levels within cells. This study aims to characterise changes in $[\text{Ca}^{2+}]_i$ following application of sub-10 ns pulses.

Methods

Changes in $[\text{Ca}^{2+}]_i$ were visualised in U-87 MG cells loaded with 0.5 μM FLUO-4, AM. Cells were imaged, in either a standard HBSS solution or a calcium free HBSS solution, by epifluorescence using a Leica DMI6000 microscope with a 63x objective.

Using two steel electrodes separated by a gap of 300 μm , with 50 Ω impedance in parallel and an nsPEF generator with 50 Ω output impedance, cells were exposed to a single pulse of 10, 8, 6, 4 or 2 ns duration, with an electric field intensity of 250 kV/cm. Change in fluorescence for each duration was averaged from 5 separate experiments with at least 3 cells per experiment.

Results

In standard HBSS solution all pulse durations caused immediate increases in $[\text{Ca}^{2+}]_i$. The amplitude of the increase was dependent on pulse duration. 2 and 4 ns pulses caused similar maximum fold increases in fluorescence of around 1.3, with fold increases of 2, 4.2 and 5.2 observed for pulses of 6, 8 and 10 ns respectively. The 2 ns pulse reached maximum fluorescence in 71 seconds after pulse application, for the other pulse durations it was reached in around 33 seconds. Within a minute of the pulse, for all durations except 2 ns, fluorescence had started to return towards baseline levels. By the end of the imaging period (2

minutes after pulse application) fluorescence levels for the 2 ns pulse were still elevated at near maximal levels.

In the calcium free HBSS solution increases in $[Ca^{2+}]_i$ were observed for all but the 2 ns pulse. The 10 ns pulse elicited the greatest increase in fluorescence (0.3 fold) with 8, 6 and 4 ns pulses all showing increases close to 0.1 fold. Maximum levels of fluorescence were observed within 2 seconds of pulse application for 10 and 8 ns pulses, with 6 and 4 ns reaching maximum within 20 seconds.

Conclusions

Whilst all of the pulse durations used were able to cause an increase in $[Ca^{2+}]_i$ the mechanisms involved appear to be different. For the 2 ns pulse the source of this calcium increase appears to be extracellular whereas for the longer pulses both intracellular calcium stores and extracellular calcium are involved. Further investigation into cell poration and the effect of sub-10 ns bipolar pulses on $[Ca^{2+}]_i$ are planned to complement this work.

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