Fluorescence detection of Ca^{2+} (free calcium) in muscle tissue

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The use of fluorescent probes in cell physiology has emerged as indispensable tool in the analysis of cell functioning over the past years. A typical example is the detection of the transient increase in the cytoplasmic/myoplasmic free calcium concentration ($\Delta[Ca^{2+}]$) as the intermediate signaling event of the excitation-contraction coupling.

Experimental setup

WPI offers a fiber optic based Biofluorometer (SI-BF-100) for physiological research. The instrument features three exchangeable high-power LED modules as excitation source and two highly sensitive photomultipliers, allowing the detection of **weak** fluorescent signals. Excitation light is guided from the SI-BF-100 light output to the tissue/cell sample and emission light from the tissue/cell sample to the photomultiplier (PMTs) using optical fibers, so-called *liquid lightguides* (LLGs) or small tissue probes (*dipping probe*). Such an optical instrumentation allows direct and simple connection using either fiber probes to a microscope set-up or adjacent to a tissue bath (e.g. SI-MT-L or SI-HTB2).

The ratiometric dye Fura-8™ was selected for the detection of $\Delta[Ca^{2+}]$ in heart muscle tissue slices using a ratiometric fluorescent measurement technique, i.e. *dual excitation/single emission* mode option to cancel out possible effects of uneven loading, inhomogeneous distribution of fluorescence indicator in the cells or indicator bleaching in the detection of $\Delta[Ca^{2+}]$ in the muscle tissue. Fura-8™ was excited with High-Power LEDs at 365nm and 410nm wavelengths and the emission was detected at 535nm.

Results and discussion

The present study addressed to examine changes in free calcium concentration with a microscope set-up and in an organ bath.

Representative results from human left ventricular slices in the microscope set-up shows a clear increase in the $\Delta[Ca^{2+}]$ when the slices were electrically stimulated to obtain a single twitch contraction. Qualitatively, fluorescence data form the SI-BF-100 detection were equivalent to those detected by an imaging system, while data-processing allows for further sharpening of the detected signal, using a 50Hz low-pass filter.

The murine myocardium samples were set-up in a horizontal tissue bath and a microscope. In both conditions, a clear increase in the $\Delta[Ca^{2+}]$ was observed. Thanks to the higher time resolution of the SI-BF-100 (sampling rate of 1,000 Hz), it was possible to detected $\Delta[Ca^{2+}]$ at 3Hz electrical stimulation with a good spatial resolution, even when filtered at 50Hz.

Conclusion

The SI-BF-100 system is an accurate tool for the detection of Ca^{2+} transients in human and murine myocardium slices. The research was carried out successfully using a microscope set-up and an organ bath. The SI-BF-100 system performs well with excitation light from the three different LED-based excitation sources starting at 365nm wavelength. Speeds of up to 1,000 Hz can be measured from the two PMT-based fiber coupled detector inputs.