SIMULTANEOUS MEASUREMENTS OF INTRACELLULAR Ca^{2+} TRANSIENTS AND CELL SHORTENING IN SINGLE CARDIAC MYOCYTES FROM RATUS RATUS

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Both intracellular Ca^{2+} probes and measurements of cell shortening have been used to study excitation-contraction coupling in single myocardial cells. Here we report how these two methods can be used simultaneously in the same experiment. This avoids the uncertainty inherent in the comparison of results obtained in parallel experiments.

Myocardial cells from the rat, Ratus ratus, were isolated by enzymatic dissociation and were placed in a shallow perfusion chamber (C, Figure 1) on the stage of an inverted microscope (dashed line box). The membrane current (i_m) was measured using the whole cell voltage clamp technique. The Ca^{2+}-sensitive fluorescent dye fura-2 (0.4 mM) was added to the internal solution of the patch clamp pipette (PCP).

Ultraviolet and green light were used with fura-2 to measure intracellular Ca^{2+} activity (335 nm and 410 nm excitation, 510 nm emission). Red light (>670 nm) was used to measure cell length.

The ultraviolet light came from a pulsed dual wavelength excitation module (UV), was relected by a dichroic mirror (DM_1, 430 nm, long pass), passed through the objective (OB, NA=1.3, oil, x40), and was focussed onto the cell (C). The excitation module has been previously described (Callewaert, Cleeman and Morad, PNAS 85:2009-2013, 1988) and incorporates a mercury arc lamp and vibrating mirror which is used to alternate rapidly (1200 Hz) between the two wavelengths of excitation. The green, fluorescent light passed back through the objective (OB), the dichroic mirror (DM_1), a barrier filter (B, 450 nm, long pass) and was focussed onto a moveable, adjustable aperture (A) after being reflected by a beam splitter (BS_1) which let through 20% of the light to the oculars (not shown). From the aperture the green light passed through an interference filter (F, 510 nm, 72rm band width) and was detected by a photomultiplier (PM) before it was demultiplexed to yield signals corresponding to the two wavelengths of excitation (F_{335} and F_{410}).

The incandescent light (I) used for measurement of cell length was filtered through red glass (G, 670 nm, long pass) and gave bright field illumination (collimater not shown) of the cell (C). Most of the red light from the cell was imaged onto a linear array (LA) after reflection in a dichroic mirror (DM_2, 640 nm, short pass). The linear array was read out every 4 ms and changes in cell length (L) were determined by an electronic edge detector (Nabauer and Morad, 1988).

A small fraction of the red light was passed on through the aperture (A), a lens (L) and a beam splitter (BS_2) to a CCD-camera/TV monitor (TV). The TV monitor aided both in the alignment of the cell onto the linear array by means of a rotating prism (not shown) and in the adjustment of the aperture (A) which determines the region from which fluorescent light was measured.

The glass filter (G), the interference filter (F) and the dichroic mirror (DM_2) were chosen to separate the weaker, fluorescent light from the stronger, red light.

The inset panel shows a sample record obtained from a rat ventricular myocyte. A depolarization-induced Ca^{2+} transient (F_{410}) develops rapidly to

its full value before the cell begins to shorten (L). Notice also that the twitch relaxes abruptly while the intracellular Ca^{2+} transient declines slowly. This may indicate that the activation of the myofilaments by Ca^{2+} is a highly cooperative process with a well-defined threshold.

These results demonstrate the feasibility of simultaneous optical measurements of cell length and intracellular Ca^{2+} activity.

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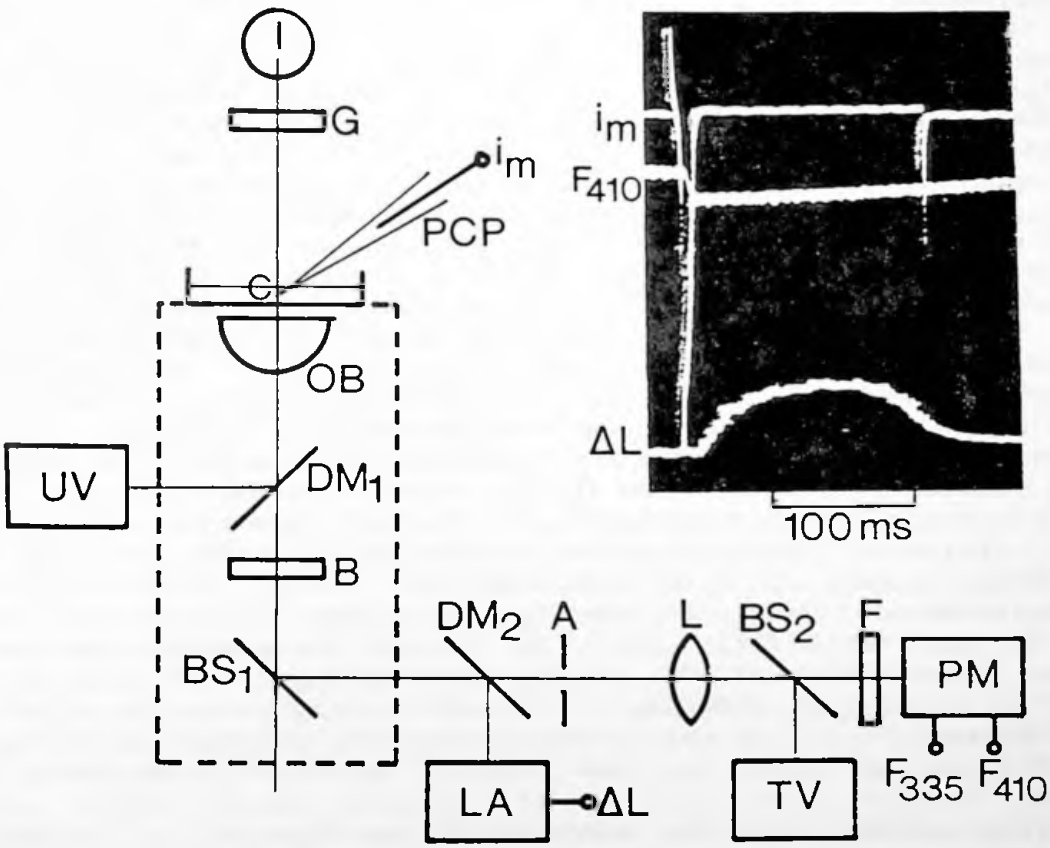

Figure 1. Simultaneous measurements of fluorescence signals (F_{335} and F_{410}) and cell shortening (L). The diagrammatic representation shows the optical axis in a thin line. For details, refer to text. The inset panel shows sample traces of the membrane current (i_m), the fura-2 fluorescence measured with excitation at 410 nm (F_{410}) and the cell shortening (L). A decrease in F_{410}) corresponds to an increase in the intracellular Ca^{2+} activity.