OPTICAL MEASUREMENTS OF INTRACELLULAR Ca-TRANSIENTS IN VOLTAGE CLAMPED MYOCARDIAL CELLS

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Fluorescent Ca-sensitive dyes like quin-2 and fura-2 have a quantum yield and sensitivity which make them suitable for measurements of intracellular Ca activities in single enzymatically dissociated cells. Such measurements may complement the already available results obtained with auquorin and may ultimately serve to clarify the relationship between the Ca current and excitability. We decided to pursue this goal with a new technique using fura-2 and high time resolution.

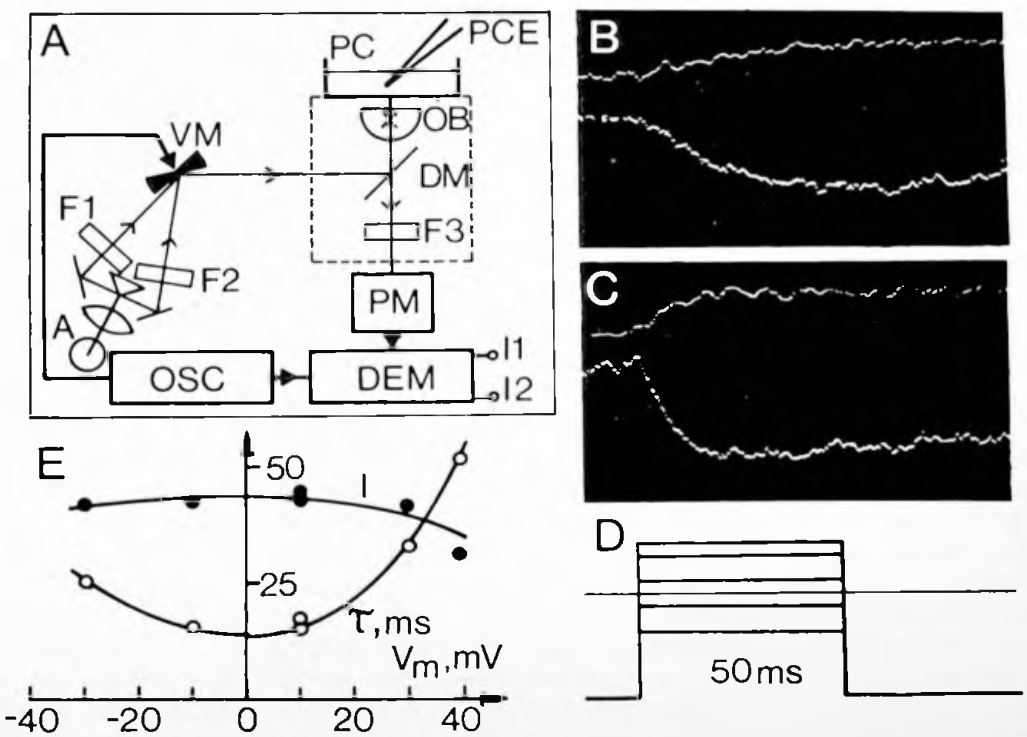
Cells from the hearts of rats and guinea pigs were transferred to a perfusion chamber (PC in Fig. 1A) on top of an inverted microscope (Rectangular box). The loading of the cells with quin-2 was studied by visual inspection and fluorescence excitation. The addition of the permeable form of the dye to the perfusate resulted in a slowly (20 min) increasing green fluorescent glow with an inhomogeneous distribution and clear delineation of subcellular organelles. A different result was obtained when the dye was added (400 uM) to the internal solution (see legend) of the patch clamp u-electrode (PCE). Gentle application of suction first established a seal and suction was then increased to break into the cell. At this point most cells went into irreversible contracture. This was probably due to the absence of an effective Ca buffer in the internal solution. Some cells, however, remained relaxed and displayed a uniform green fluorescent glow in less than a minute. It would appear that loading of the cell with dye through the pipette is required.

Figure 1A shows the special attachments which were developed for dual wavelength excitation and detection. The light beam originating from a mercury arc lamp (A) was collimated and then split in two only to be gathered again by an arrangement of mirrors. Two interference filters (F1 and F2) were used to select the wavelengths of excitation (340 nm and 400 nm) of the two halves of the beam. A vibrating mirror (VM) was used as a mechanical chopper which alternated between the two wavelengths at a frequency of 1200 Hz. From the center of the vibrating mirror the light passed into the microscope, was reflected by a dichroic mirror (DM, 420 nm) and was focused by the objective (OB) into a spot only slightly larger than the cell under examination. The fluorescent light returned through the objective was filtered and detected by a photomultiplier tube and the signal was demultiplexed (DMP) to yield signals (I1, I2) corresponding to the two wavelengths of excitation. The phaselocked amplifier which drove the mirror (OSC) served as a reference in this process.

Panel B and C of Fig. 1 show optical signals recorded at room temperature from a rat ventricular cell (+40 and +10 mV clamp potential respectively). Panel D shows the voltage clamp protocol (50 msec clamp pulses, -80 mV holding potential). The signals recorded with excitation below (340 nm), and above (400 nm), the isosbestic point give upward and downward deflections respectively, both corresponding to an increase in the intracellular Ca activity. The similar time course of the two signals

Legend

Figure 1. Optical measurements of intracellular Ca transients. The details of the figure are given in the text. Panel A shows a simplified schematic of the system used for optical recording. Heavy lines with filled in arrows are electrical signals while the light beams are shown as thin lines with open arrows. Each of the panels B and C contains recordings of the optical Ca signal below and above the isosbestic point of excitation (360 nm). Panel E is a schematic of the voltage clamp protocol. Panel E shows the voltage dependency of the magnitude and time constant of the Ca transients. (External solution (nM): NaCl 139, KCl 5.4, $CaCl_2$ 5, $MgCl_2$ 1, Na_2PO_4 0.33, Hepes 10, pH 7.4; internal solution NaCl 10, KCl 120, MgATP 5, Hepes 20, fura-2 0.4).



suggests that the signals are related to the intracellular Ca activity and not to the clearly visible contractions.

The voltage dependency of the Ca signal is plotted in panel E (I, filled circles). The magnitude of the optical Ca signal is remarkably constant in the potential range from -30 to -40 mV. Other experiments with both rat and guinea pig myocytes confirmed this finding. About 40 mV of depolarization from a holding potential of -80 mV was required to elicit a Ca transient. As seen in panels B and C the rate at which the Ca transients developed varied much more than the magnitude. The time constant was 55 msec at +40 mV (Panel B) and 16 msec at +10 mV (Panel C). These measurements and others are plotted in panel E (open circles) and yield a curve with a minimum around 0 mV. Other experiments gave time constants as low as 8 msec.

Two advantages of the technique are high time resolution and the fact that the two wavelengths of excitation are not used in two consecutive sweeps but by rapid chopping during a single sweep. This was found to be an advantage in recording spontaneous and evoked Ca transients.

In future experiments we plan to correlate the intracellular Ca transients with components of the membrane current and optical measurements of contractions.

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