FUSION OF ISOLATED CARDIAC VENTRICULAR CELLS FROM RATUS RATUS

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Ion-sensitive microelectrodes have been widely used to measure intracellular ion activities in a variety of cell types (e.g. Lee, Am. J. Physiol., 241, H459-478, 1981). Associated with this technique are a number of problems, foremost among them the leakage of ions around the impalement site, which can give spurious measurements of the internal milieu. This problem is heightened in small cells, where such leakages cause irreversible cell damage (Oberleitner, Schmidt and Dietl, Proc. Natl. Acad. Sci. 83: In press, 1986). A theoretical solution to this problem is to fuse a number of these cells to form "giant" cells, so that the effect of ion leakage around the rim of the electrode is proportionately diminished. Fusion of animal cells into giant cells by incubation in polyethyleneglycol (PEG) is, in fact, a well described technique (Kohler & Milstein, Nature, 256, 495-497, 1975) and has recently been applied to renal epithelial cells to facilitate measurement of intracellular ion activities (Oberleitner, Schmidt and Dietl, Proc. Natl. Acad. Sci. 83: In press, 1986). It was therefore considered of interest to attempt to apply the technique to heart cells.

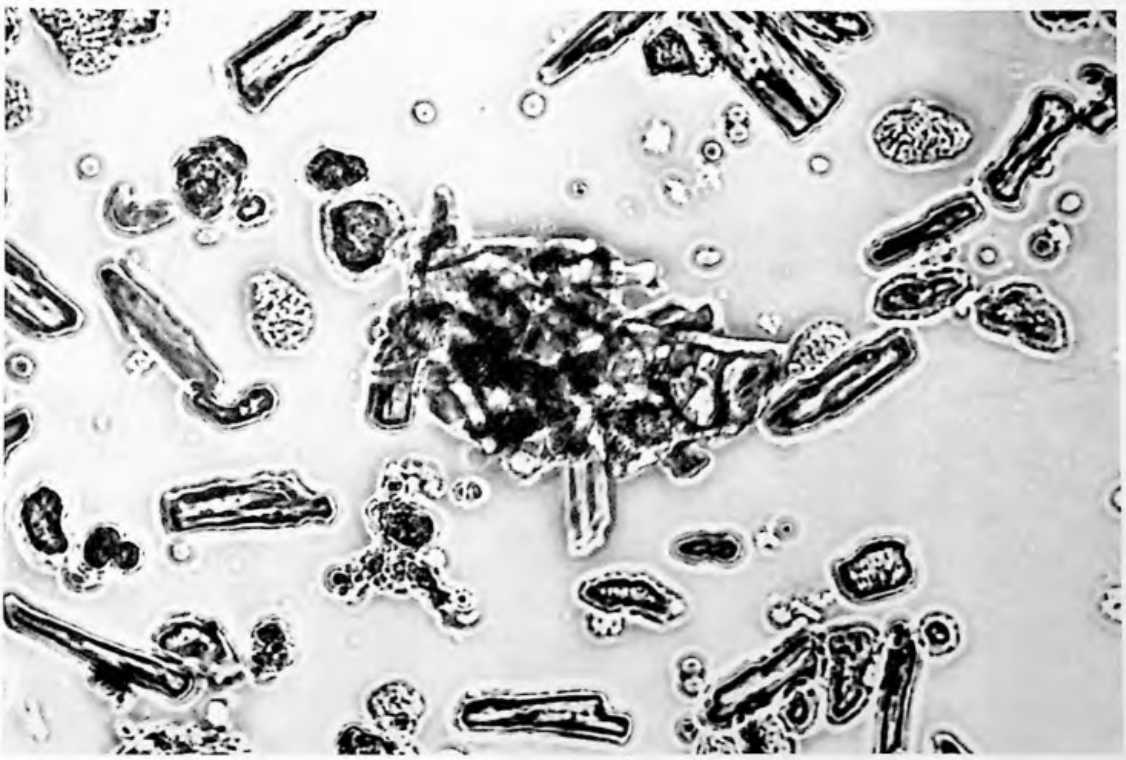
Myocardial rat cells were isolated to single cells and suspended in Tyrode solution, as described previously (Dukes et al., MDIBL, 1986). Cell aliquots (1.5 ml, approximately 10,000) were transferred to test tubes and gently mixed with one of the following 3 solutions: (a) standard solution based on Liebovitz's medium (L-15, Sigma, Catalog #L4386) with addition of 10 mM Hepes diluted to 200 mOsm and titrated to pH 7.6, (b) hypertonic solution made from standard solution by addition of 30% PEG (MW 3,500, 280 mOsm, pH 8.6), and, (c) a more hypertonic solution containing 40% PEG (300 mOsm).

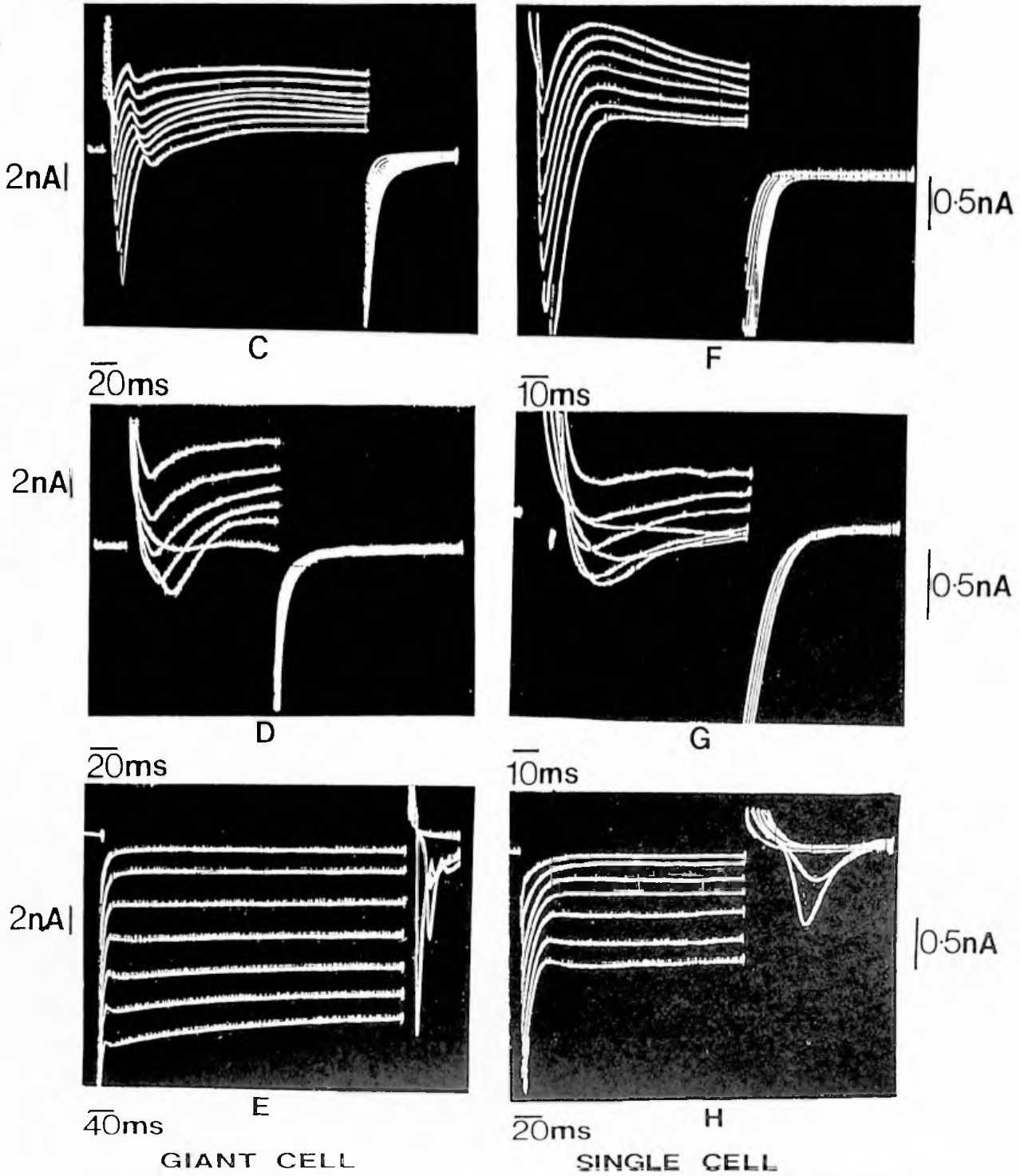
The mixtures were centrifuged for 3 minutes (100 g), and the supernatant was discarded. The remaining cells were washed once with standard solution, and were resuspended in 2 ml standard solution. The cells were incubated at room temperature (20 to 25°C) and observed periodically for 4 hours under the microscope.

No cell clusters were found among the cells exposed only to the standard solution prior to centrifugation. All these cells were normal and viable. Cells treated with 30 or 40% PEG produced clusters of cells, the yield of which varied from batch to batch. The best yields, 10 to 20 clusters per dish, were seen with exposure to 40% PEG. In time, delineation between cells in clusters decreased (Fig. 1A and B). After 3 hours of incubation at room temperature cells in clusters appeared electrically coupled, as they beat synchronously.

These giant cells were then transferred to a perfusion chamber where they were voltage clamped using the patch clamp technique. Glass pipettes, with tip diameters between 3-4 μm and resistances between 0.5-2.0 mega ohm were used to achieve seal resistances in the giga ohm range. The pipettes

Figure 1. A; appearance of giant cells. The one in the center of the field is made up of approximately 20 myocytes. C-H; comparison of ionic currents recorded from a giant (C-E) and single (F-H) cell. C & F; holding potential -80 mV, pulsed to positive potentials, in 10 mV increments from -20 to +30 mV. E & H; holding potential -60 mV, negative pulses in 10 mV increments from -70 to -130 mV. Note that the current scales are much larger for the fused cell (C, D & E) currents than their single cell counterparts (E, F & G)




2nA
C
F
0·5nA
20ms
10ms
2nA
D
G
0·5nA
20ms
10ms
2nA
E
H
0·5nA
40ms
20ms
GIANT CELL
SINGLE CELL

were filled, for the purpose of internal cell dialysis, with a KCl-based internal solution, which included 14 mM EGTA plus 1 mM $CaCl_2$ to buffer internal calcium levels at micromolar levels. The extent of the fusion process was estimated by various indirect means. All cells in a synchronously beating cluster would cease beating when the seal of the patch clamp electrode was broken and the cell interior dialyzed with EGTA. Continuity of the cytoplasm was also indicated by "balling up" of all cells in a cluster when the patch failed. Further evidence for fusion could be obtained by measuring the ionic currents in these giant cells and comparing their magnitude with those recorded from single cells. Depolarizing clamp pulses from holding potentials in the range from -80 and -40 mV activated large inward sodium and calcium currents. Unfortunately, cell clusters of six and above were too large to be effectively voltage clamped, so smaller clusters were used instead. Figs. 1C and 1D show sodium and calcium currents recorded from a giant cell composed of five cells. When these currents are compared to their single cell counterparts which had been through the same treatment protocol (Figs. 1F and G) it can be seen that the currents in the giant cell are approximately five times larger. Similarly when the size of the inward rectifier current, activated by hyperpolarizing pulses from a holding potential of -70 mV, in the same giant cell and the single cell are compared, the current in the giant cell is again approximately five times as large (Figs. 1E and H).

While the present evidence strongly suggests that cell fusion is occurring, further studies using cell dyes are required to show conclusively that cell fusion has occurred. Additionally, electrical transients (single vs. multiple time constants) might also be used to verify fusion and estimate the area of the fused cell membranes. It still, however, remains to be seen whether these cells will, as predicted, yield more reliable data when ion-sensitive microelectrodes are applied.