Regulatory volume decrease in isolated cardiomyocytes: differences between freshly isolated and cultured cells?

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Changes in cardiomyocyte volume can have profound effects on contractile function and have been suggested to contribute to the pathophysiology of ischemia/reperfusion injury. There is extensive evidence that many different cell types regulate their volume in response to hypo-osmotic-induced cell swelling, employing a series of signaling pathways to promote extrusion of intracellular osmolytes and to facilitate the movement of water out of the cell (regulatory volume decrease, RVD). Reports of cell volume regulation in isolated cardiomyocytes have varied depending on the model used. Cultured chick cardiomyocytes consistently show RVD, in contrast to conflicting reports in freshly isolated, adult mammalian cardiomyocytes. The aims of this study were:

1. to validate a novel method utilising the IonOptix video edge-detection system for the continuous monitoring of cell width as an index of cell volume;
2. to establish whether freshly isolated mouse ventricular myocytes can activate an RVD in response to hypo-osmotic superfusion;
3. to compare the response of freshly isolated and cultured adult mouse ventricular myocytes to hypo-osmotic-induced cell swelling.

Male C57Bl/6 mice were anaesthetized (sodium pentobarbitone, i.p. 200 mg/kg with sodium heparin, 200 IU/kg), and the hearts excised and Langendorff perfused. Cardiomyocytes were isolated through enzymatic dispersion with collagenase (0.34 mg/ml) and trypsin (0.14 mg/ml) and directly pipetted onto glass cover slips in the superfusion chamber. Cardiomyocytes were superfused (1.5 ml/min, 37˚C) with an isosmotic, low Na solution supplemented with mannitol (~309 mOsmol/l) for 5 mins, then switched to a hypo-osmotic, low Na solution (no mannitol, ~217 mOsmol/l) for a further 10 mins. Changes in cell dimensions were measured simultaneously with both manual two-dimensional (2D) tracings of video images (length, width, area, 1 min intervals) and video edge detection (width only). A similar hypo-osmotic stress was subsequently used to compare RVD in freshly isolated and cultured cardiomyocytes (24hr culture on laminin-coated cover-slips).

2D tracings of hypo-osmotic-induced swelling in freshly isolated cardiomyocytes revealed substantial increases in cell width (111.3 ± 0.8% of pre-hypo-osmotic basal width at 10 min, $n = 5$) and area (111.5 ± 1.0%, $n = 5$), but not length (99.4 ± 0.4%, $n = 5$), with *no* evidence of RVD. Comparison of cell width measurements between the 2D tracings and the video-edge detection recordings showed a linear relationship. Further studies in freshly isolated and cultured cardiomyocytes identified occurrence of RVD in 2 out of 7 cultured cardiomyocytes, and complete absence of RVD in freshly isolated cells ($n = 7$).

These studies validate the use of the IonOptix video edge-detection system as a simple, continuous method for measuring small changes in cardiomyocyte width, as an index of cell volume. Freshly isolated mouse ventricular myocytes do not show any evidence of RVD in response to hypo-osmotic superfusion while cultured cells showed a limited response. Assuming cardiac myocytes *in vivo* are capable of mounting an RVD, this suggests the enzymatic isolation process perturbs the myocyte's ability to mount an RVD *in vitro* and this may be partially restored by short-term culture.