



The Bioenergetic State of a Cell Population Predicts Its Behavior

Research Area

General Cell Culture

Application

Cell Quality: Using bioenergetic rates to predict viability and cell performance

Cell Type

Murine muscle cell line (C2C12) and primary adult feline cardiomyocytes

Work Of

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Tool Box:

- Rotenone
- epinephrine

Keywords:

- Cell quality
- cardiomyocytes
- C2C12 myoblasts
- basal metabolic rates
- mitochondrial respiration
- glycolysis

Cell viability measurements are widely employed to normalize cell-based assays for biological content. Simple viability assays, however, do not provide detailed information about the functional potential or “vitality” of cells. Until recently, alternatives that are informative, non-invasive and simple to perform have not been available. With the introduction of the Seahorse XF24 analyzer, the bioenergetic state of a cell population can be easily and non-invasively measured. The resulting bioenergetic data appears to provide a useful measurement of cell quality and functional potential.

Background

Cell-based assays are typically normalized by a viable cell count obtained from a trypan blue staining procedure. It has been observed that normal cell culturing practices can produce cultures with very different basal bioenergetic states, all with the same viable cell count. Given the fundamental role of metabolism in living cells, the existence of different states raises the question of whether changes in bioenergetic state precede and/or are indicative of gross phenotypic changes such as viability. In other words, is it reasonable to expect that differences in the daily, basal bioenergetic state of a cell population would bias the outcome and/or reproducibility of assays performed that day?

In this short report, we show examples of how differences in fluxes related to basal energy metabolism correlate with observed phenotypic differences. Because XF24 assays are label free and non destructive, they are amenable to routine assessment of cell populations prior to experimentation.

Predicting changes in viability

In order to establish the predictive value of XF measurements, correlations between various bioenergetic states and gross cellular phenotypic changes were determined experimentally. To be useful as a predictor of cell stress or viability, abnormal bioenergetic changes should manifest prior to overt and irreversible changes in viability.

In Figure 1, C2C12 myoblasts were exposed to increasing doses of rotenone, a complex

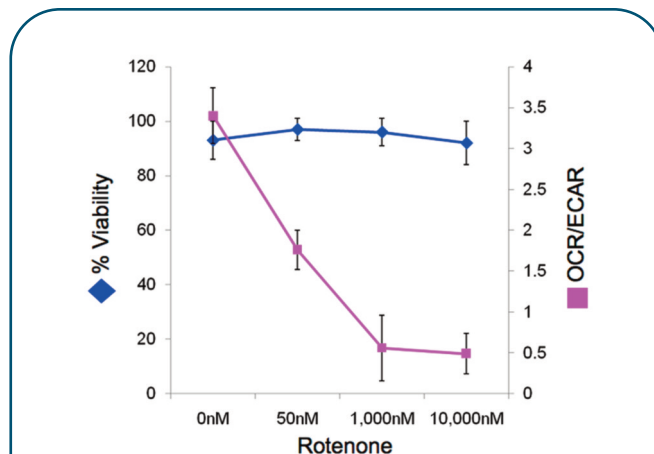


Figure 1: Bioenergetic response and viability of C2C12 myoblasts exposed to rotenone.

The muscle cell line, C2C12, was seeded in XF24 microtiter plates at 30,000 cells/well and incubated overnight. Increasing concentrations of rotenone were injected into wells and the OCR and ECAR were subsequently measured and the ratio determined (pink line). The viability (blue line) was determined by trypan blue exclusion assay using a ViCell.

Materials & Methods

Cell Culture.

Mouse muscle cell line C2C12 was obtained from ATCC and cultured in DMEM supplemented with 10% FBS, 1mM sodium pyruvate and 100 g/mL penicillin-streptomycin. Cells were seeded at 30,000 cells per well in 24-well XF plates and incubated overnight at 37°C in 10% CO₂. Primary adult feline cardiomyocytes were isolated as described in Cooper, et al. Cells were seeded at 7,500 cells/well and assayed 48 hours later.

Test Compounds.

Rotenone and epinephrine were obtained from Sigma (St. Louis, MO).

Note: A more detailed protocol for the data presented in this case study can be found in Wu et al. (2007) *Am J Physiol Cell Physiol* **292:C125-136**.

References

Cooper, G. 4th, et al. (1986). Load regulation of the properties of adult feline cardiocytes. The role of substrate adhesion. *Circ. Research*, **58**:692-705

Ferrick, DA et al. (2008). Advances in measuring cellular bioenergetics using extracellular flux. *Drug Discov Today* **13**:268-274.

I inhibitor, to metabolically stress the cells. Mitochondrial respiration and glycolytic flux were determined by the XF24 and cell viability was performed using trypan blue dye exclusion on the same cells immediately after the XF measurements were made.

As expected, increasing doses of rotenone decreased mitochondrial respiration as measured by oxygen consumption rate (OCR). To compensate, the cells increased glycolytic flux as measured by extracellular acidification rate (ECAR). A consistent decline in the ratio of OCR to ECAR with increasing concentration of rotenone reflected a switch away from aerobic metabolism towards glycolysis, however, the measured viability remained constant at all concentrations during this acute exposure. However, after several hours of incubation cell viability was progressively decreased with time. The higher the concentration of rotenone the greater was the loss of cell viability. This simple experiment demonstrated that bioenergetic changes can and usually do precede more overt changes in phenotype such as loss of membrane integrity.

Basal bioenergetic rates identify “healthy” cells

A more practical utility is illustrated in Figure 2. In this experiment XF basal rates were able to clearly differentiate the functional quality of two primary adult feline cardiomyocyte preparations.

Occasional “bad” preps of cardiomyocytes can be caused by a number of variables in the procedure. In this figure, two different preparations of primary adult feline cardiomyocytes were measured for their basal rates and functional responses to the stimulant epinephrine.

Preparations containing functionally competent cardiomyocytes (shown in red) have basal OCRs of about 200-300 pmol/min and very little ECAR (0 min red circle) as indicated in Figure 2. In contrast, a second preparation (shown in blue) started out with lower than usual OCR and higher ECAR values (0 min blue box). When stimulated with epinephrine the competent cells (red) responded normally while the blue wells responded poorly. Repeated isolation of primary cardiomyocytes has consistently shown that the poor responders or “unhealthy” cardiomyocytes exhibit lower basal OCR and higher basal ECAR values. In fact, when viewed under the microscope, epinephrine stimulation of the unhealthy cells induced a single hypercontraction while the stimulated healthy cells contracted continuously for about 30 min.

Non-invasive extracellular flux measurements reflect and even predict the outcome of energy producing mechanisms in cells. XF assays can be used to optimize and monitor this energetic component of cell quality, and thereby improve the value and reliability of cell based assays.

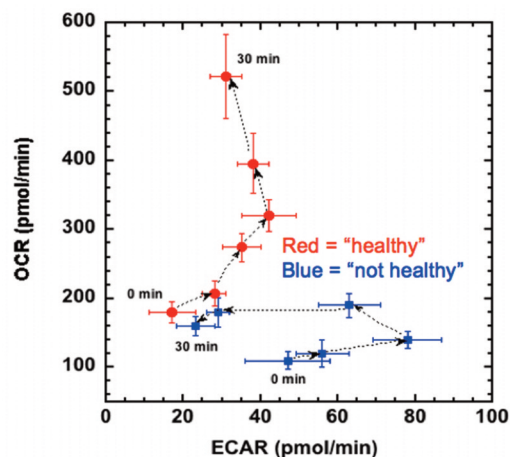


Figure 2: Basal rates and response of primary adult feline cardiomyocytes to epinephrine.

Primary cardiomyocytes were plated at 7500 cells/well and their basal rates determined (time 0). The wells were injected with 100 nM epinephrine and the OCR and ECAR were subsequently measured at 6 minute intervals for a total of 30 minutes.