

Real-time analysis of mitochondrial and glycolytic metabolic pathways using the **Bionas Discovery™ 2500 system**

INTRODUCTION

Increasing evidence demonstrates the involvement of mitochondrial dysfunction in numerous diseases, like diabetes, obesity, cancer, and neurodegenerative as well as cardiovascular diseases. In general, two cellular processes are known to generate ATP in the cell, namely glycolysis and mitochondrial respiration. Therefore, eukaryotic cells take in carbohydrates (e.g. glucose) which are then broken down in the cytoplasm and mitochondria. The breakdown products of glycolysis are lactate and carbon dioxide, which contribute to extracellular acidification. The main objective of cell respiration is to generate energy in the form of ATP a metabolic process which mainly occurs in the mitochondria. Dysfunction of the mitochondrial respiratory chain results in a disturbance of the production of ATP. By generating profiles of oxygen consumption and extracellular acidification which both determine the acute rates of the cellular energy metabolism, the multi-parametric sensor chip allows the monitoring of the action of substances and their cytotoxic effects including potential regeneration. In addition, cell impedance/adhesion measurement detects alterations in the adhesion and morphology of the cells.

MATERIALS & METHODS

Cell culture. CHO and V79 cells were purchased from Cell Line Service (Germany) and cultured in DMEM as a basal medium. For measurement, the **Bionas® running medium** (w/o sodium bicarbonate) was used.

Test Compounds.

Sodium fluoride (NaF), sodium azide (NaN₃), potassium cyanide (KCN) and cytochalasin B were purchased from Sigma Aldrich (Germany).

Analysis in the Bionas Discovery™ 2500 system. During measurement, fresh medium is provided through an automated fluidic perfusion system following a stop and go cycle of 4 minutes each. Breakdown products like lactate and CO₂ and the oxygen consumption of the cells result in a change of pH and oxygen content in the medium measured during the stop phase of the pump cycle. The base lines for the three parameters is achieved by applying running medium (RM) for 2-4h. In the following pump phase the "used" medium is substituted with fresh medium of a predefined pH and oxygen content. In the experimental set up described here, test compounds were applied for 2-4h followed by 1h regeneration with RM.

Inhibition of glycolysis

Figure 2 and 3 show the results during the treatment of cells with NaF and cytochalasin B, both inhibitors of glycolysis. NaF (Fig. 2) inhibition is evidenced by a reduction of the acidification rate during NaF treatment. Supplying the cells with medium without NaF resulted in a complete regeneration. The glucose transport through the membrane as well as the actin polymerisation of cells is inhibited by cytochalasin B (Fig. 3). The changes of the metabolic activity, as a consequence of substance exposure, are monitored in real-time during the course of the experiment.

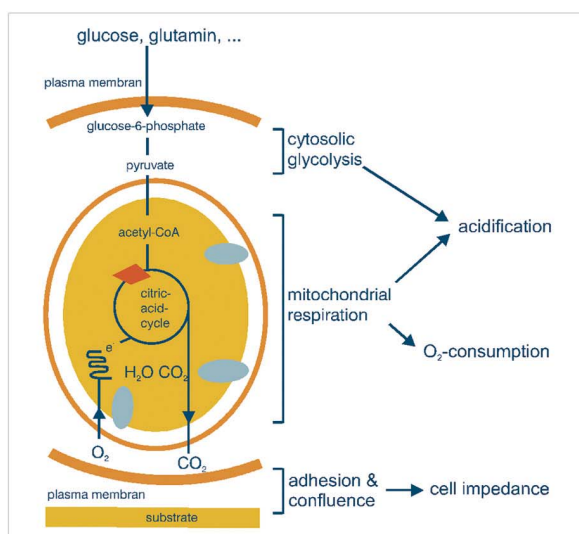
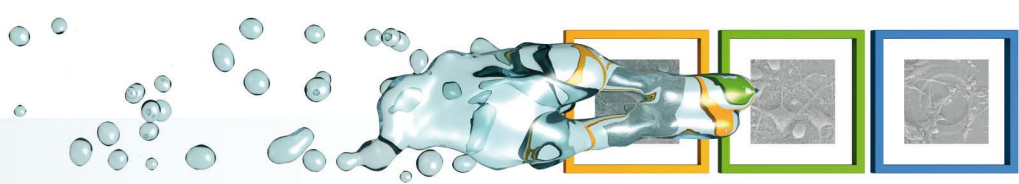


Fig. 1: Scheme representing cell metabolism and physiological parameters measured with the **Bionas Discovery™ 2500 system**.





EFFECT OF GLYCOLYSIS AND GLUCOSE TRANSPORT INHIBITORS

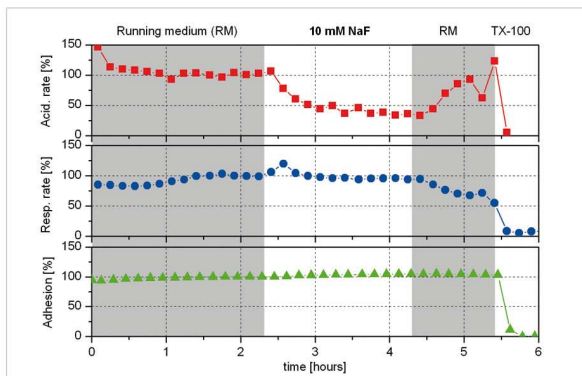


Fig. 2: Real-time measurement of acidification and respiration rates and impedance/adhesion of CHO cells treated with NaF.

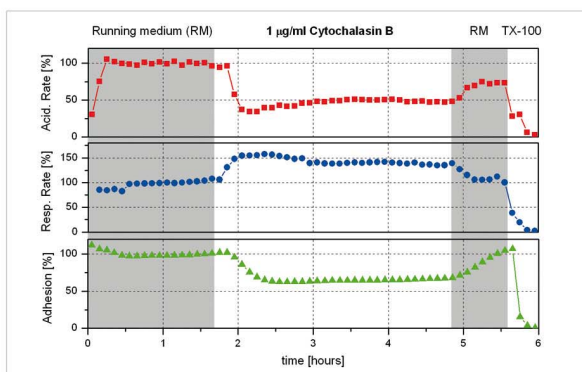


Fig. 3: Real-time measurement of acidification and respiration rates and impedance/adhesion of V79 cells treated with cytochalasin B.

Concurrently, the respiration rate is increased, which indicated a compensation of reduced ATP production. Cell impedance/adhesion is also clearly reduced. All three parameters show an almost complete regeneration after removal of cytochalasin B after 3h.

Inhibition of mitochondrial respiration

Figure 4 and 5 represent the results of the treatment of CHO cells with 0.1 mM KCN and 2.5 mM NaN_3 which both block cytochrome-C oxidase and thereby inhibit the respiratory chain. Applying KCN (Fig. 4) rapidly reduced the respiration rate by 50% while the acidification rate was increased by 25%. Cell impedance/adhesion was only marginally influenced. After the withdrawal of KCN a complete regeneration was seen. The application of NaN_3 (Fig. 5) also severely reduced the respiratory rates and showed a 100% recovery, but acidification and impedance/adhesion were not influenced at all.

EFFECT OF RESPIRATORY CHAIN INHIBITORS

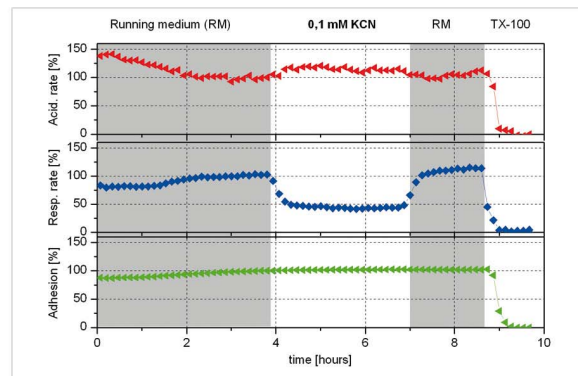


Fig. 4: Real-time measurement of acidification and respiration rates and impedance/adhesion of CHO cells treated with KCN.

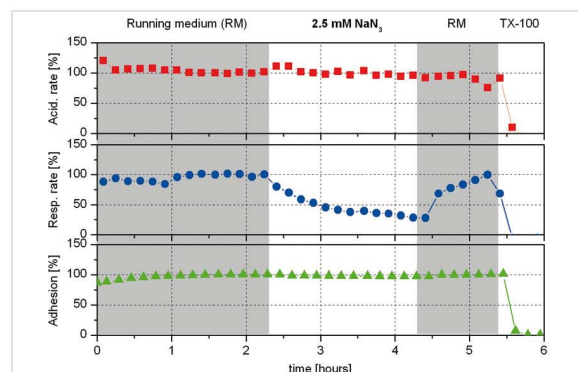


Fig. 5: Real-time measurement of acidification and respiration rates and impedance/adhesion of CHO cells treated with NaN_3 .

CONCLUSION

By the inhibition of single metabolic pathways, we illustrate sensitivity and specificity of the signals detected by shifts between mitochondrial respiration and glycolysis. The **Bionas Discovery™ 2500 system** is the only commercially available device capable of analyzing the combination of three parameters to investigate mitochondrial and cellular toxicity in cell-based functional studies.

KEYWORDS

Mitotoxicity, inhibition, ATP, mitochondrial respiration, glycolysis, regeneration

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