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Glycolysis-Respiration Relationships in a Neuroblastoma Cell Line

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Abstract

Background—Although some reciprocal glycolysis-respiration relationships are well recognized, the relationship between reduced glycolysis flux and mitochondrial respiration has not been critically characterized.

Methods—We concomitantly measured the extracellular acidification rate (ECAR) and oxygen consumption rate (OCR) of SH-SY5Y neuroblastoma cells under free and restricted glycolysis flux conditions.

Results—Under conditions of fixed energy demand ECAR and OCR values showed a reciprocal relationship. In addition to observing an expected Crabtree effect in which increasing glucose availability raised the ECAR and reduced the OCR, a novel reciprocal relationship was documented in which reducing the ECAR via glucose deprivation or glycolysis inhibition increased the OCR. Substituting galactose for glucose, which reduces net glycolysis ATP yield without blocking glycolysis flux, similarly reduced the ECAR and increased the OCR. We further determined how reduced ECAR conditions affect proteins that associate with energy sensing and energy response pathways. ERK phosphorylation, SIRT1, and HIF1 α decreased while AKT, p38, and AMPK phosphorylation increased.

Conclusions—These data document a novel intracellular glycolysis-respiration effect in which restricting glycolysis flux increases mitochondrial respiration.

General Significance—Since this effect can be used to manipulate cell bioenergetic infrastructures, this particular glycolysis-respiration effect can practically inform the development of new mitochondrial medicine approaches.

Keywords

Crabtree effect; glycolysis; mitochondria; oxidative phosphorylation; Pasteur effect; respiration

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1. Introduction

Over 150 years ago it was recognized under conditions of fixed energy demand cell glucose utilization and respiration inversely vary. For example, the Pasteur Effect describes the phenomenon in which increasing oxygen availability reduces glucose consumption [1]. The Crabtree Effect describes the phenomenon in which increasing glucose availability reduces oxygen consumption [2]. The Warburg Effect applies in tumor cells, which consume little oxygen but much glucose [3].

In prior decades mitochondrial respiration was most often quantified using isolated mitochondria. When working with isolated mitochondria, though, it is not possible to assay glycolysis fluxes. Recent technical advances now make the acquisition of whole cell oxygen consumption rate (OCR) data feasible [4]. Some platforms also simultaneously measure the whole cell extracellular acidification rate (ECAR). Because the ECAR is largely determined by lactic acid release, and lactic acid is a product of glycolysis, the ECAR approximates the glycolysis flux rate [5].

To further study OCR-ECAR relationships, we concomitantly acquired OCR and ECAR data from SH-SY5Y neuroblastoma cells maintained under free and restricted glycolysis flux conditions. We also determined how pathways and proteins that monitor and respond to cell energy states were affected by a restricted glycolysis flux. The data we generated provide insight into glycolysis-respiration relationships, as well to how cells adapt to glycolysis restriction.

2. Methods

In these experiments we used both undifferentiated and differentiated SH-SY5Y cells. The undifferentiated cells were expanded at 37°, 5% CO₂ in pyruvate-free DMEM containing 25 mM glucose (catalogue number 11965-092, Invitrogen) and supplemented with 10% fetal bovine serum (catalogue number 12203C, Sigma) and 1% of a penicillin-streptomycin stock (catalogue number 30-001-CI, Fisher Scientific). For differentiation, cells were transferred to poly-L-lysine-coated (1.5mg/ml) T75 flasks and maintained for 24 hours in DMEM supplemented with 10% FBS, 100 ug/ml pyruvate, 50 ug/ml uridine, and 1% penicillin-streptomycin. The following day the medium was changed to medium consisting of neurobasal medium, B27 supplement, 0.5 mM glutamine, 100 ug/ml pyruvate, 50 ug/ml uridine, 1% penicillin-streptomycin, and 6 nM staurosporine [6]. This medium was changed at 2–3 day intervals for a two week period. Staurosporine from a freshly prepared stock was used with each medium change.

Approximately 80,000 undifferentiated or differentiated SH-SY5Y cells were used to seed the wells of XF cell culture microplates (Seahorse Bioscience, Billerica, MA). A standard manufacturer-recommended two-step seeding procedure was utilized. After achieving cell adherence, microplates were placed overnight in a 37°, 5% CO₂ incubator. The media used for these overnight incubations consisted of serum-free, pyruvate-free, buffered DMEM containing 25 mM glucose, 2.5 mM glucose, 0 mM glucose, or 2.5 mM galactose. On the analysis day the incubation medium for each well was aspirated, the adherent cells were washed, and cells were placed in serum-free, pyruvate-free, buffer-free DMEM; for each well the amount of glucose contained in its overnight medium was carried forward. After the switch to unbuffered media the microplates were placed in a 37°, non-CO₂ incubator for one hour before being transferred onto the microplate stage of a Seahorse XF24 flux analyzer (Seahorse).

OCR and ECAR readings were taken using a 2 minute mix, 1 minute wait, and 2 minute read cycling protocol. Various compounds were injected to alter the metabolic environment or to help determine the effects of an altered metabolic environment. These compounds included glucose, 2-deoxyglucose (2-DG), and iodoacetate. In most experiments, to distinguish mitochondrial from cell non-mitochondrial oxygen consumption we injected a mixture of rotenone and myxothiazol in order to achieve working concentrations of 1 μ M rotenone and 2 μ M myxothiazol. When specified, the non-mitochondrial OCR (the rate after rotenone/myxothiazol) was subtracted from the total OCR to provide the true mitochondrial OCR.

Several proteins that influence aerobic metabolism or are influenced by energy status were analyzed by Western blot. Primary antibodies to the following proteins were used: phospho-473 AKT (1:1000 dilution; Cell Signaling Technology); phospho-308 AKT (1:1000 dilution; Cell Signaling Technology); phospho-P38 (1:1000 dilution; Cell Signaling Technology); phospho-ERK (1:1000 dilution; Cell Signaling Technology); phospho-AMPK (1:1000 dilution; Cell Signaling Technology); PGC1 α (1:1000 dilution; Santa Cruz Biotechnology); HIF1 α (1:1000 dilution; Cell Signaling Technology); and SIRT1 (1:1000 dilution; Cell Signaling Technology). Primary antibody binding was revealed using horseradish peroxidase-conjugated secondary antibodies (1:2000 dilution; Cell Signaling Technology) and Supersignal West Femto Maximum Sensitivity Substrate (Thermo Scientific). Densitometry was performed using a ChemiDoc XRS with Quantity One software (BioRad, Hercules, CA). To ensure equivalent protein loading, blots were subsequently stained with a primary antibody to actin (1:200 dilution; Santa Cruz Biotechnology).

Cell ATP levels from cell homogenates were measured using an ATP Bioluminescent Assay Kit (Sigma-Aldrich) according to the manufacturer's instructions. This is a luminescence-based assay; luminescence values were determined using a Tecan Infinite M200 plate reader.

OCR-ECAR data are presented as absolute rates or as percent changes from a stable baseline. For comparisons of data points between cells maintained under different conditions, group means were compared by two-way Student's t-tests, with p values less than 0.05 considered significant. For comparisons of the magnitude-of-rate change that occurred following injection of metabolism-altering compounds, the mean of the percent change for each group was determined and group means were compared by two-way Student's t-tests, with p values less than 0.05 considered significant. For the immunocytochemistry studies, relative density values for each protein analyzed were summarized by average and standard error. Means were compared by two-way Student's t-tests, with p values less than or equal to 0.05 considered significant. For ATP measurements, values from individual samples belonging to a group were summarized by average and standard error. Means were compared by two-way Student's t-tests, with p values less than 0.05 considered significant.

3. Results

We confirmed the ability of our approach to detect a Crabtree effect, the phenomenon in which increasing glucose access to cells reduces mitochondrial respiration [2]. Immediately after adding 2.5 mM glucose to SH-SY5Y cells in a glucose-free medium, we observed an approximate 230% increase in the ECAR and 40% decrease in the OCR (Figure 1). When additional glucose was injected to achieve a final concentration of 10 mM, the ECAR increased again, by another 10%, and the OCR declined an additional 25%. Increasing the glucose concentration from 10 mM to 25 mM did not further modify the ECAR and OCR

flux rates. Adding rotenone and myxothiazol did not affect the ECAR, which indicates the initial glucose-induced ECAR increase was not a secondary consequence of increased mitochondrial CO₂ production. This is important to note, as CO₂ produced during the Krebs cycle can contribute to medium acidification independent of glycolysis-generated lactic acid. When we subtracted out the non-mitochondrial portion of the cell oxygen consumption, the percent OCR decrease was even greater (calculations not shown).

Having assessed how enhancing glycolysis flux affects the cell OCR, we determined how reducing glycolysis flux affects the cell OCR. This was accomplished by injecting 2-DG into SH-SY5Y cells maintained in 25 mM glucose. 2-DG prevents glycolysis by inhibiting upstream glycolysis enzymes including hexokinase and phosphoglucose isomerase, the enzyme that mediates the conversion of glucose 6-phosphate to fructose 6-phosphate [7–11]. Injecting 25 mM 2-DG into the medium induced a rapid, approximately 90% decline in the cell ECAR. This ECAR change was accompanied by an OCR increase. After correcting for non-mitochondrial oxygen consumption, the true mitochondrial OCR increased by almost 100% (Figure 2). To confirm this effect, we performed a similar experiment in which we injected 25 uM iodoacetate into the SH-SY5Y cells maintained in 25 mM glucose. Iodoacetate blocks glycolysis by inhibiting glyceraldehyde 3-phosphate dehydrogenase, which converts glyceraldehyde 3-phosphate to 1,3 bisphosphoglycerate [12]. At this iodoacetate concentration we observed a rapid 75% decrease in the ECAR, and a 35% OCR increase (Figure 3).

We placed both undifferentiated and differentiated cells under both high glucose (25 mM) and no-glucose conditions. In the undifferentiated cells, the no-glucose ECAR was only 33% of its value in 25 mM glucose, and the total OCR was 57% higher. The glucose starvation-induced OCR increase was not due to an increase in the non-mitochondrial OCR, since after subtracting out the non-mitochondrial oxygen consumption the OCR of the glucose starved cells was 72% higher than it was in the cells maintained in 25 mM glucose (Figure 4). Differentiated cells under no-glucose conditions had a lower ECAR than they did under 25 mM conditions (75% reduction) and a higher total OCR (40% increase) (Figure 5). In their differentiated state the SH-SY5Y cells may have acquired a different mitochondrial mass steady state than that of the undifferentiated cells, which would favorably reflect on the generalizability of the overall effect, but it should be noted we did not determine the actual mitochondrial mass of either differentiated or undifferentiated cells.

We evaluated the effect of galactose on SY5Y cell OCR and ECAR fluxes. Because the conversion of galactose to glucose consumes an amount of ATP equivalent to that which is directly generated by glycolysis, galactose can support a full glycolysis flux but does not permit net glycolysis ATP production [13–16]. Compared to cells maintained in 2.5 mM glucose, cells in 2.5 mM galactose and no glucose showed a 30% OCR increase. The ECAR in 2.5 mM galactose medium was only 25% of what it was in 2.5 mM glucose medium. Compared to cells in 2.5 mM glucose, cells in 2.5 mM galactose showed a five-fold increase in their OCR/ECAR ratio (Figure 6).

We evaluated the effects of glucose deprivation on proteins that are sensitive to cell bioenergetic states. Protein lysates were prepared from SH-SY5Y cells maintained in 25 mM glucose, serum-free DMEM medium for four hours, and from control SH-SY5Y cells maintained in 0 mM, serum-free DMEM for four hours. The PGC1a total protein level was unchanged (Figure 7). The glucose-deprived cells showed reduced ERK phosphorylation, total HIF1a, and total SIRT1 levels (Figure 7). AKT ser473 phosphorylation, AKT thr308 phosphorylation, p38 phosphorylation, and AMPK thr172 phosphorylation were increased (Figure 7). Consistent with the increase in AMPK phosphorylation, ATP levels were lower in glucose-deprived cells (Figure 8).

4. Discussion

An inverse relationship between glycolysis and respiratory fluxes has long been recognized to occur in two situations. First, the Pasteur Effect describes the situation in which providing oxygen to anaerobic cells, which depend on glycolysis to produce energy, increases respiration and decreases glycolysis [1]. Second, the Crabtree Effect (which we were able to demonstrate) describes the situation in which providing glucose to cells increases glycolysis and decreases respiration [2]. We now demonstrate a third inverse relationship, which is that under conditions of fixed energy demand reducing the cell glycolysis flux increases mitochondrial oxidative phosphorylation.

Other investigators certainly have had opportunities to encounter this relationship, and some have indirectly referenced it. For example, Schulz et al. previously reported that chronic 2-DG ingestion by *C. elegans* increased their respiratory capacity [17], which infers that glycolysis inhibition increases respiration dependence. In HepG2 cells, a 2-DG-induced glycolysis reduction was recently noted to associate with increased oxygen consumption [18]. To our knowledge, though, our report is the first to formally and directly characterize the phenomenon through which reduced glycolysis flux increases mitochondrial oxygen consumption. Given the increasing availability of technologies that simultaneously assess respiratory and glycolysis fluxes, there is an emerging practical need for this characterization.

We demonstrated this relationship in several different ways. 2-DG interferes with glycolysis flux at relatively proximal steps, mostly at the point or just after the point that ATP is consumed to convert glucose to glucose 6-phosphate. Iodoacetate is a more downstream inhibitor that acts at a point beyond which 2 ATP have been consumed. Glucose starvation, of course, should preclude any glycolysis flux, and prevent the net cytosolic production of 2 ATP per glucose molecule. The effect of these treatments on glycolysis flux is demonstrated by a reduction in the cell ECAR, which reflects the cell's inability to produce lactic acid. It is presumed that under conditions of fixed energy demand, lost glycolysis ATP production will shift to the mitochondrial respiratory chain. Our data are consistent with this assumption.

Since the delivery of carbohydrate carbon to mitochondria is compromised by 2-DG, iodoacetate, or glucose starvation the question arises as to how mitochondrial matrix reducing equivalents are generated under these conditions. Under cell culture conditions typical of those we used, glutamine is believed to contribute to the production of mitochondrial matrix reducing equivalents [13, 19].

Galactose is converted to glucose, and can subsequently enter glycolysis. The conversion of galactose to glucose, however, requires 2 ATP and therefore when galactose serves as a carbohydrate source glycolysis by itself cannot increase cell ATP levels [15, 16]. Substituting galactose for glucose increases a cell's dependence on mitochondrial ATP production, and in our experiments using SY5Y cells this manifested as increased oxygen consumption. This finding is not surprising, as substituting galactose for glucose has been noted to increase mitochondrial oxygen consumption in other cultured cell lines [16, 20]. In extension of this basic observation, Rana et al. reported induced pluripotent stem cell-derived cardiomyocytes, when grown in galactose, increase their OCR/ECAR ratio. That study, though did not present actual ECAR data [21].

Since galactose itself should not inhibit glycolysis flux, it is interesting that galactose treatment associated not with a higher ECAR but rather a lower ECAR. We can think of several potential explanations for this. The first explanation is that due to an increased reliance on mitochondrial ATP production, pyruvate generated by the glycolysis flux was

preferentially directed away from lactic acid production and into the mitochondria, where it was used to generate reducing equivalents for oxidative phosphorylation. If so, then despite a reduction in lactic acid production the actual glycolysis flux could have declined, remained stable, or even increased. The latter two of these possibilities would imply dissociations between glycolysis flux and extracellular acidification rates can occur, a phenomenon that could complicate interpretations of OCR-ECAR relationships. The second explanation is that an increased reliance on oxidative phosphorylation increased reactive oxygen species (ROS) production and oxidative stress. This in turn should increase the cell's need for NADPH, since NADPH is needed to restore oxidized glutathione to reduced glutathione [22]. If correct, galactose-treated cells may divert galactose-derived carbon away from glycolysis and into the pentose phosphate shunt. Under this scenario, the ECAR reduction would reflect a true reduction in the glycolysis flux.

The third potential explanation is perhaps the most straightforward. Sugars enter cells via saturable transporters [23–27]. Limitations in a cell's import rate could define its maximum ECAR. We may have inadvertently demonstrated this in our Crabtree effect experiment, in which increasing the glucose concentration from 10 mM to 25 mM did not further impact the ECAR. It is worth considering, therefore, the role glucose transporters play in determining glycolysis fluxes and, by extension, ECAR values. In this respect, if galactose import is slower or less efficient than glucose import, the galactose ECAR could simply be lower for that reason.

We predicted that shifting the cell bioenergetic flux from glycolysis to respiration would impact pathways and proteins that regulate cell energy levels and redox states. An analysis of several relevant proteins, obtained under glucose starvation conditions, shows this does in fact occur. p38 is activated through phosphorylation, and under glucose deprivation conditions p38 phosphorylation increased. Since p38 is activated by oxidative stress [28], it is possible that increased ROS production due to increased mitochondrial respiration contributed to this. AMPK is sensitive to AMP/ATP levels, and when this ratio rises AMPK is activated through phosphorylation [29, 30]. We found that glucose deprivation reduced the cell ATP level, and we suspect this bioenergetic stress likely induced AMPK phosphorylation.

Some of our results, though, were perhaps less predictable. HIF1 α is reported to enhance glycolysis [31], and we had originally predicted that under glucose starvation conditions HIF1 α activity might increase in an attempt to enhance glycolysis. Our data show the opposite occurs, and suggest that when glycolysis is not possible because no glucose is present cells do not invest in trying to maintain a glycolysis flux. We believe further studies to critically assess this possibility are indicated, since recognizing the ways in which bioenergetic fluxes reciprocally influence the pathways, genes, and proteins that evolved to control them should prove informative. Such efforts would build upon and extend previously reported studies of the mitochondrial retrograde response [32]. Also, we had predicted forcing cells to rely on aerobic ATP production would increase PGC1 α and SIRT1 activity [33]. Our data showed no change in the PGC1 α protein level and an actual decrease in SIRT1 protein, and therefore do not support this prediction. These responses may extrapolate to other models, or simply reflect our use of a tumor cell line. Studies utilizing other experimental models could help address this question.

ERK plays a role in cell cycling [34, 35]. Since glycolysis-derived carbon intermediates are used to synthesize lipids and proteins required for cell growth, preventing glycolysis would predictably slow cell cycling. Decreased ERK phosphorylation may represent either a cause or consequence of arrested cell cycling. On the other hand, AKT, which also promotes cell growth [36], showed increased phosphorylation. AKT phosphorylation suggests AKT

activity increased. AKT, though, has multiple functions and its phosphorylation can be accomplished through different kinases that are induced by different types of stimuli. It is not clear to us why AKT phosphorylation increased with glucose starvation, although we speculate it constitutes part of an overall stress response. In any event, the Ras-ERK and PI3K-mTOR pathways that use ERK and AKT as effector molecules are integrated, and cross-inhibition and cross-activation interactions have both been described [37, 38]. While these pathways are classically considered to sense extracellular cues, they additionally are influenced by bioenergetic flux-related phenomena and mechanisms through which reduced glycolysis flux affects Ras-ERK and PI3K-mTOR relationships warrant further study. The mTOR-based target of rapamycin complex 2 (TORC2) is also influenced by energy-sensitive physiologies, and since TORC2 itself phosphorylates AKT its role in glucose starvation-induced AKT phosphorylation requires consideration [39, 40].

Given the Pasteur effect, Crabtree effect, and the effect we now describe (in which reducing glycolysis flux or glycolysis flux-derived ATP production associates with an OCR increase), under in vitro conditions in which energy demand is not being directly manipulated it is relatively easy to demonstrate inverse ECAR-OCR relationships. It may be the case that under conditions of fixed energy demand, cells attempt to maintain a constant rate of overall ATP production. We wish to emphasize, though, that maintaining a constant ATP level may not be the only determinant of ECAR-OCR relationships. For example, as discussed above our galactose data raise the question of whether increased oxidative phosphorylation, by increasing ROS production, may divert glucose from glycolysis to the pentose phosphate shunt pathway.

Our findings have translational implications and applications. A spectrum of diseases show altered or perturbed bioenergetic flux changes [41]. In these diseases, fixing bioenergetic flux perturbations could constitute a reasonable therapeutic approach [42]. In this respect, understanding how various bioenergetic flux manipulations affect overall bioenergetic relationships will facilitate rational therapeutic design.

As we only evaluated SH-SY5Y cells, a commonly used neuronal cell model, we cannot say how closely phenomena observed in these studies generalize to other cell types. Other cell types may behave quite differently. Also, while the data we now present will prove useful to those utilizing technologies that simultaneously quantify respiratory and glycolysis fluxes, this report is not intended to serve as an experimental design-oriented “methods” paper. Rather, our intent here is to formally characterize an under-appreciated bioenergetic relationship which we hope could form the basis of a novel mitochondrial medicine approach. The goal of this approach is to induce aerobically failing cells to enhance their aerobic infrastructures by preventing their over-reliance on the anaerobic alternative. As efforts to exploit this phenomenon for clinical purposes proceed, though, it is important to keep in mind that some tissues contain bioenergetically heterogeneous cell populations. The brain contains neurons, which rely on respiration, and glia, which favor glycolysis [43]. This relationship is reminiscent of muscle, which consists of highly aerobic red muscle and the more anaerobic white muscle. In such cases, enhancing respiration at the expense of glycolysis could have unintended consequences. Potential mitochondrial medicine approaches, including the one we now propose, will need to account for this possibility.

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ABBREVIATIONS

AMPK	AMP-activated protein kinase
2-DG	2-deoxyglucose
ECAR	extracellular acidification rate
ERK	extracellular signal-regulated kinase
HIF1a	hypoxia inducible factor 1 alpha
IA	iodoacetate
mTOR	mammalian target of rapamycin
OCR	oxygen consumption rate
PGC1a	peroxisome proliferator activated receptor gamma coactivator 1 alpha
SIRT1	sirtuin (silent mating type information regulation 2 homolog) 1
TORC2	target of rapamycin complex 2

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HIGHLIGHTS

1. Inhibiting glycolysis flux increases mitochondrial respiration
2. Reducing net glycolytic ATP production increases mitochondrial respiration
3. These studies define a specific, reciprocal glycolysis-respiration relationship
4. This reciprocal relationship can be used to manipulate bioenergetics infrastructures

Figure 1. Adding glucose to glucose-deprived SH-SY5Y cells induces a Crabtree effect

The change in the ECAR is shown as a percent change from baseline (A), and the absolute ECAR values under the different glucose conditions are shown in (B). The change in the OCR is shown as a percent change from baseline (C), and the absolute OCR values under the different glucose conditions are shown in (D). Rot=rotenone, myx=myxothiazol.

Figure 2. In addition to reducing the ECAR, adding 2-DG to SH-SY5Y cells induces an OCR increase

The change in the ECAR is shown as a percent change from baseline (A), and the absolute ECAR values before and after 2-DG are shown in (B). The change in the OCR is shown as a percent change from baseline (C), and the absolute, true mitochondrial OCR values after the different injections are shown in (D). To demonstrate system stability, for the first injection half the wells received 25 mM 2-DG, while the other half of the wells received a control injection (25 mM glucose, same medium as the medium in the wells). After the 2-DG injection, the OCR was significantly increased relative to the pre-injection baseline of those wells, and also to the wells that had received the control injection. Blue line=2-DG treated cells, red line=control-treated cells.

Figure 3. In addition to reducing the ECAR, adding iodoacetate to SH-SY5Y cells induces an OCR increase

The change in the ECAR is shown as a percent change from baseline (A), and the absolute ECAR values before and after iodoacetate are shown in (B). The change in the OCR is shown as a percent change from baseline (C), and the absolute, true mitochondrial OCR values after the different injections are shown in (D). To demonstrate system stability, for the first injection half the wells received 25 μM iodoacetate, while the other half of the wells received a control injection (2.5 mM glucose, same medium as the medium in the wells). After the iodoacetate injection, the OCR was significantly increased relative to the pre-injection baseline of those wells, and also to the wells that had received the control injection. Blue line=iodoacetate-treated cells, red line=control-treated cells. IA=iodoacetate.

Figure 4. In addition to reducing the ECAR, glucose starvation of undifferentiated SH-SY5Y cells induces an OCR increase

The difference in the absolute ECAR values from cells in 25 mM and 0 mM glucose is shown in (A). The difference in the absolute total and mitochondrial OCR values are shown in (B) and (C).

Figure 5. In addition to reducing the ECAR, glucose starvation of differentiated SH-SY5Y cells induces an OCR increase

The difference in the absolute ECAR values from cells in 25 mM and 0 mM glucose is shown in (A). The difference in the total mitochondrial OCR values is shown in (B).

Figure 6. Galactose effects on SH-SY5Y cell OCR and ECAR

Cells in galactose medium had a higher OCR than cells in a comparable amount of glucose (A), as well as a lower ECAR (B). The OCR/ECAR ratio, accordingly, was higher for the cells in the galactose medium (C).

Figure 7. Effect of glucose deprivation on proteins regulated by cell bioenergetic fluxes
After four hours, PGC1 α levels were unchanged (A), ERK phosphorylation, HIF1 α , and SIRT1 decreased (B–D), and AKT phosphorylation, p38, and AMPK increased (E–H). These experiments were conducted in serum-free medium. NS=not significant.

Figure 8. Relative ATP levels from SH-SY5Y cells maintained in 25 mM glucose and 0 mM glucose
ATP levels are reduced in glucose-starved cells.