A Computational Metabolic Model of the NG108-15 cell for High Content Drug Screening with Electrophysiological Readout

Aditya Reddy Kolli[1,2], Frank Sommerhage[2], Peter Molnar[1,6], Jonathan E. Hood[4], Jerry J. Jenkins[4,5], Faraz Hussain[3], Arup K. Ghosh[3], Sumit Kumar Jha[3], James J. Hickman[1,2,3*]

Email: AK: AdityaReddy.Kolli@ucf.edu, FS: Frank.Sommerhage@ucf.edu, PM: pmolnar@pminfonet.com, JH: jeh@cfdrc.com, JJ: jjenkins@hudsonalpha.org, FH: fhussain@eecs.ucf.edu, AG: akghosh@eecs.ucf.edu, SJ: jha@eecs.ucf.edu, JH: jhickman@ucf.edu*

ABSTRACT

Computational Systems Modeling could play a significant role in improving and speeding up of the drug development process. By the incorporation of cellular modeling into a High Information Content Drug Screening platform the information content of the pharmacological test could be significantly increased through a deeper understanding of cellular pathways and signaling mechanisms. Unfortunately, many of the cellular signaling pathways in the cells are yet to be explored. Moreover, which is an even larger problem, their integration into a functional signaling network at the whole cell level is almost unknown or untested. Thus, there is an urgent need to develop a data-driven functional whole-cell model which enables the correlation of biochemical and physiological experimental results at the whole cell level with partial information available for the metabolic and signal transduction pathways of the cell. We have built a whole-cell model of NG108-15 cells and validated some of the underlying cellular metabolic and signal transduction networks with a series of detailed experiments in order to predict cellular responses to a wide variety of extracellular stimuli. This validated assay system will be an important tool for the identification of cellular changes and activation of signal transduction pathways based on changes of electrophysiological properties and responses of the cell and would have a high impact on drug screening and toxicity evaluation at the cell-system level.

Categories and Subject Descriptors

D.2.2 [Coding Tools and Techniques]: Top-down programming; I.6 [Simulation and Modeling]: Applications, Modeling methodologies; J.3 [Life and Medical Sciences] Biology and genetics

General Terms

Design, Experimentation, Measurement and Verification.

Keywords

High-Content Screening, Hodgkin-Huxley model, NG108-15, Cellular metabolism, Glycolysis.

ACM-BCB'12, October 7-10, 2012, Orlando, FL, USA ACM 978-1-4503-1670-5/12/10.

1. INTRODUCTION

Computational Systems Modeling has the potential to provide greater insights into the dynamics of a single cell and provides a framework for the interpretation of experimental data. Moreover, it has the potential to create and organize the vast amount of experimental data into now available knowledge databases. The importance of these databases and computational tools is increasing as many of the drug side effects and interactions are being unaccounted at the drug discovery stage. Acceleration of the drug discovery process is the primary interest for pharmaceutical companies, because it is expensive, time consuming and labor intensive. By integrating mathematical modeling and high-content drug screening the information content of the tests could be increased which would improve the drug discovery process[1].

We have developed a data-driven computational model of NG108-15 cell. This cell line, a rat neuroblastoma x mouse glioma hybrid, is a good candidate to study the effects of drugs and to identify intracellular biochemical pathways, because these cells do not form synapses, are easy to culture and show neuron-like electrophysiological properties. The current model developed in our lab incorporates the major metabolic pathways: Glycolysis, Pentose phosphate, Krebs cycle, Oxidative phosphorylation and Electron transport chain. Moreover, this metabolic model is coupled with a Hodgkin-Huxley-type mathematical model of the cell membrane to enable the prediction of changes in cellular physiology based on changes in membrane electrical properties.

[1] NanoScience Technology Center, University of Central Florida, Orlando, FL, USA 32816

[2] Department of Chemistry, University of Central Florida, Orlando, FL, USA 32816

[3] Department of Electrical Engineering and Computer Science, University of Central Florida, Orlando, FL, USA 32816

[4] CFD Research Corporation, 215 Wynn Drive, Huntsville, AL, USA 35805

[5] HudsonAlpha Institute for Biotechnology, 601 Genome way, Huntsville, AL, USA 35805

[6] Department of Zoology, University of West Hungary, Szombathely, H-9700, Hungary

* Corresponding author

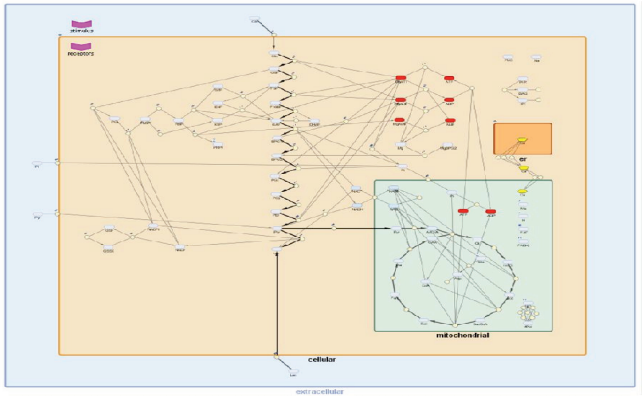

Figure: 1 Representative Model of NG108-15 cellular Metabolism.

2. MATERIALS AND METHODS

2.1 NG 108-15 Cell Culture

NG108-15 cells were cultured according to published protocols [1]. Briefly, the cell stock was grown in a T-75 flask in 90% Dulbecco's modified Eagle's medium (DMEM, GIBCO) supplemented with 10% fetal bovine serum and HAT (GIBCO, 100×) at 37 °C with 10% CO_2. Differentiation of the cells was induced by plating them in a serum-free defined medium (DMEM + N_2 supplement, GIBCO) in 35 mm culture dishes.

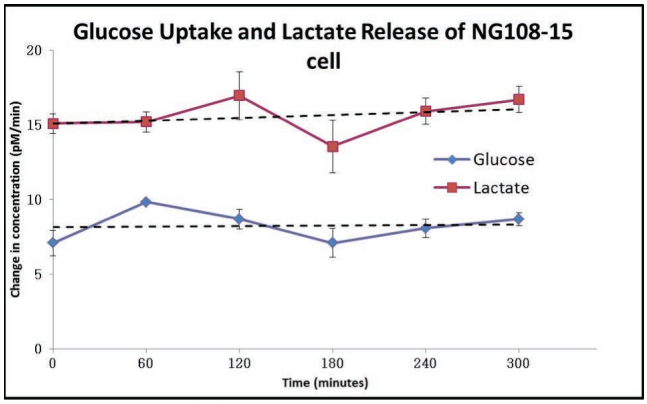

Figure: 2 Rate of Glucose uptake and Lactate release by NG108-15 cells.

2.2 Metabolic Measurements of NG108-15 cells

Glucose, Lactate, ATP, ADP and Pyruvate measurements were performed on the differentiated NG108-15 cells by using commercially available assay kits (BioVision, Inc). After obtaining the baseline, 10mM of 2-Deoxy-Glucose, 10mM Sodium Cyanide and 10mM Sodium Malonate were added to measure their inhibitory effect on the biochemical pathways in the cell.

2.3 Development of the Neuronal Metabolism Model

Two independent models of Glycolysis[2] and Mitochondrial[3] metabolism were combined. These two models were coupled by incorporating reactions for pyruvate transport into the mitochondria and conversion to Acetyl CoA by pyruvate dehydrogenase. The Malate-Aspartate shuttle was also included to maintain NAD^+/NADH concentrations. The equilibrium constants were modified to match the experimental data[4]. This was implemented in MATLAB Simbiology (Fig.1). This metabolic model was then coupled with a modified Hodgkin-Huxley mathematical model [5] to predict cellular responses.

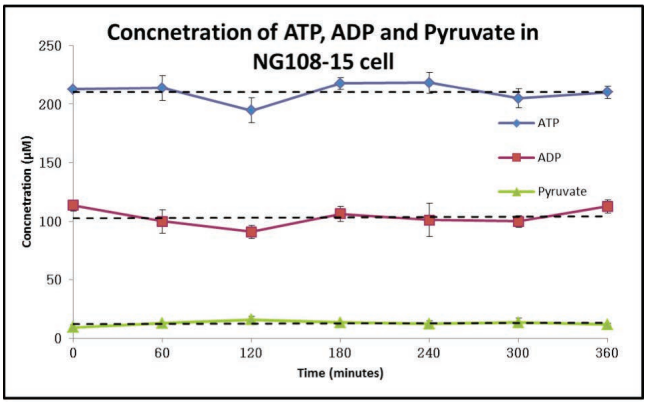

Figure: 3 Intracellular levels of ATP, ADP and Pyruvate in NG108-15 cells.

3. RESULTS and DISCUSSION

The reconstructed metabolic model of NG108-15 cells has been calibrated by performing real time metabolic measurements such as glucose uptake, lactate release, rate of ATP/ADP turnover and intracellular pyruvate levels. The measured glucose uptake and lactate release were 8.25±1.06 and 15.47+1.24 pM per min per cell respectively (Fig.2). The intracellular ATP, ADP and pyruvate levels were found to be 210.1±7.2, 103.5±6.3 and 12.8±2.1 µM respectively (Fig. 3). NG108-15 cells possess cancer cell like properties such as increased glycolysis and lower ATP levels. The effect of different metabolic inhibitors such as 2-Deoxy-Glucose, Cyanide and Malonate was also tested on metabolism and on action potential (AP) generation (data not shown). AP peak shape was measured in NG108-15 cells by performing patch clamp electrophysiology. Controls and drug-modified action potentials were simulated using the whole-cell model and were fitted to the experimental data (Fig.4). The calibrated model was able to predict NG108-15 cellular metabolism based on the membrane electrical properties.

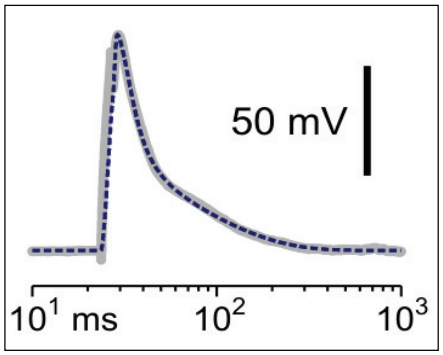

Figure 4: Fitted simulation (dotted line) to control action potential recording (solid line) of NG108-15 cell.

4. CONCLUSION

The developed metabolic model was validated by a series of experiments and is able to predict the changes in cellular metabolism based on an electrophysiological readout. A fully developed model could be a powerful tool for High Information Content Screening. In conclusion, we have developed and validated a complex whole cell model for detection of cellular responses to external stimuli.

5. ACKNOWLEDGMENTS

We thank Dr.Nirenberg (NIH) for supplying the NG108-15 cell line. This study was supported by NIH R01 EB005459 Grant.

6. REFERENCES

[1] Hoffman, A. F. and Garippa, R. J. A pharmaceutical company user's perspective on the potential of high content screening in drug discovery. *Methods Mol Biol*, 3562007), 19-31.

[2] Lambeth, M. J. and Kushmerick, M. J. A computational model for glycogenolysis in skeletal muscle. *Ann Biomed Eng*, 30, 6 (Jun 2002), 808-827.

[3] Cortassa, S., Aon, M. A., Marban, E., Winslow, R. L. and O'Rourke, B. An integrated model of cardiac mitochondrial energy metabolism and calcium dynamics. *Biophys J*, 84, 4 (Apr 2003), 2734-2755.

[4] Hussain, F., Dutta, R. G., Jha, S. K., Langmead, C. J. and Jha, S. Parameter discovery for stochastic biological models against temporal behavioral specifications using an SPRT based Metric for simulated annealing. *Intl. Conf. on Comp. Adv. in Bio and Medical Sciences*, Feb. 2012.

[5] Mohan, D. K., Molnar, P. and Hickman, J. J. Toxin detection based on action potential shape analysis using a realistic mathematical model of differentiated NG108-15 cells. *Biosens Bioelectron*, 21, 9 (Mar 15 2006), 1804-1811.