Genome-Scale Reconstruction of the Human Astrocyte Metabolic Network

N Sowmya Manojna | BE17B007

Introduction

Astrocytes are non-neuronal, electrically inexcitable glial cells present in the brain. These cells take part in a variety of tasks such as neuro-modulation, vaso-modulation, containment of neural inflammations and maintenance of neural homeostasis.

Analysis of astrocyte metabolic networks is pivotal in understanding several neurological disorders, as dysfunctional astrocytes are ubiquitous in several neuro-pathological conditions. This paper presents the first detailed astrocyte metabolic network reconstruction at the genomic scale, comprising 5,659 reactions and 5,007 metabolites.

The model uses a Flux Balance Analysis approach to predict astrocytes' normal physiological and pathological states, specifically Ischemia (condition where the brain receives reduced blood flow and hence, decreased glucose and oxygen).

Modelling approach

Data Collection:

The genomic, transcriptomic, biochemical and physiological data used in this model have been assembled as proposed by Baart and Martens (2012). A statistical analysis of the transcriptomics data (obtained from GEO (GSE53404)) was performed to model the astrocyte metabolic phenotype. Mapping of the genes to their respective enzymatic product and analysis of gene expression arrays were done using Bioconductor. All enzyme-associated chemical reactions were obtained from the HMA.

Manual Refinements:

Manual refinements of the data such as correction of the stoichiometric matrix, reversibility of reactions and spatial location of metabolites were performed. Presence of all enzymes in the mature astrocyte was ensured and the network was mass-energy balanced. Gaps in the network, blocked reactions and dead-end metabolites were identified, and reactions, where these dead-end metabolites were produced or consumed, were added. The connection of metabolites across different components was done by adding suitable transport and exchange reactions. The presence of all added enzymes was validated against the HPD.

Mathematical modelling:

A stoichiometric modelling approach was used to integrate the refined metabolic reconstruction obtained. The equations used in the model are as follows:

- $S_{m \times n} \times v = 0$
- $v_{min}(i) \leq v(i) \leq v_{max}(i)$

S is the stoichiometric matrix where m - # of metabolites, n - # of reactions and v - vector of reaction fluxes. Default maximum flux and minimum flux limits were set to +1000 units and -1000 units, respectively. Experimental data of excitatory astrocyte metabolism was used to restrict the flux space. Simulation of Ischemic state in the astrocytes was done by gradual drop of 20% in the glucose and oxygen supply to the brain.

Model Validation:

Flux Balance Analysis (FBA) was used to validate the model. Flux restriction alterations that enabled finer predictions were incorporated. Objective functions that best replicated the astrocyte metabolic reaction fluxes such as maximisation of ATP production and glutamate uptake coupled with glutamine release were chosen. The physiological implications of the objective function in case of maximisation of ATP was to steer the metabolite fluxes to contribute towards the cellular energy demand, while glutamate intake and glutamine release is maximised during neural excitation, to reduce glutamate toxicity in the extracellular space.

Model evaluation in Ischemic condition was done by random sampling of the flux space. Statistical significance of the results was calculated using Z-scores, and compared against OMIN, HMDB and NCBI literatures across different biological states.

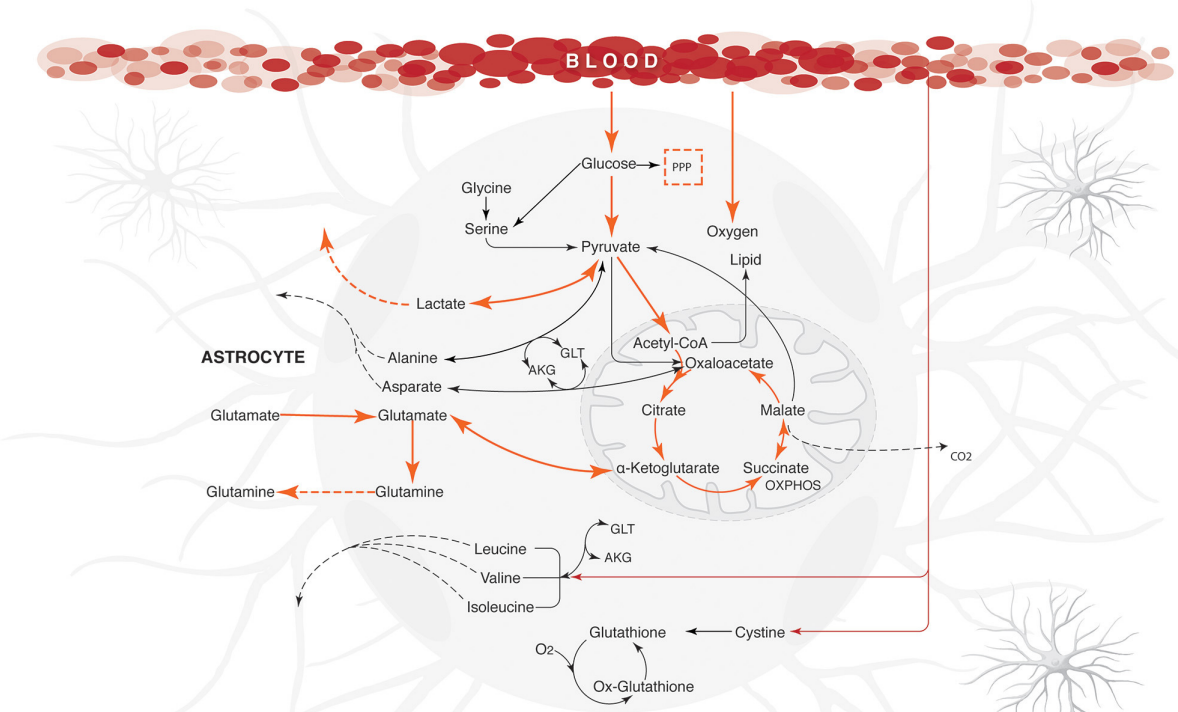
Results and Discussions

Analysis of the flux distributions by the model provides a systems-level perspective of the astrocyte metabolism.

Normal Physiological Conditions:

Under normal physiological conditions, the following was observed:

- 35% of total flux passes through transferase enzymes such as glutathione peroxidase, catalase and glutathione S-transferase, which play a crucial role in protection from oxidative damage and detoxification respectively.
- 39% of the reactions are part of central metabolic reactions such as the TCA cycle, glycolysis, PPP and oxidative pathways.
- 58% of the reactions are found to have a direct association with different genes, and 59% of all reactions were present in subcellular locations, cytosol and mitochondria.



Astrocytes under normal physiological conditions

Orange arrows indicate active reactions under normal physiological conditions

Objective function - ATP Maximization

Maximisation of ATP production was shown to have a positive correlation with the glucose and oxygen uptake, fluxes through glycolysis, TCA cycle and oxidative phosphorylation. The fluxes through key enzymes involved in glycolysis, glucose intake and pyruvate release were calculated and found to correlate with experimentally available data. The model also provided computational evidence to support the hypothesis that oxidative phosphorylation in Astrocytes generates small amounts of reactive oxygen species.

Objective function - Glutamate uptake and Glutamine release(GUGR) Maximisation

The flux distribution was analysed at different glutamate uptake rates while maximising GUGR, and the following results were obtained with an increase in glutamate uptake:

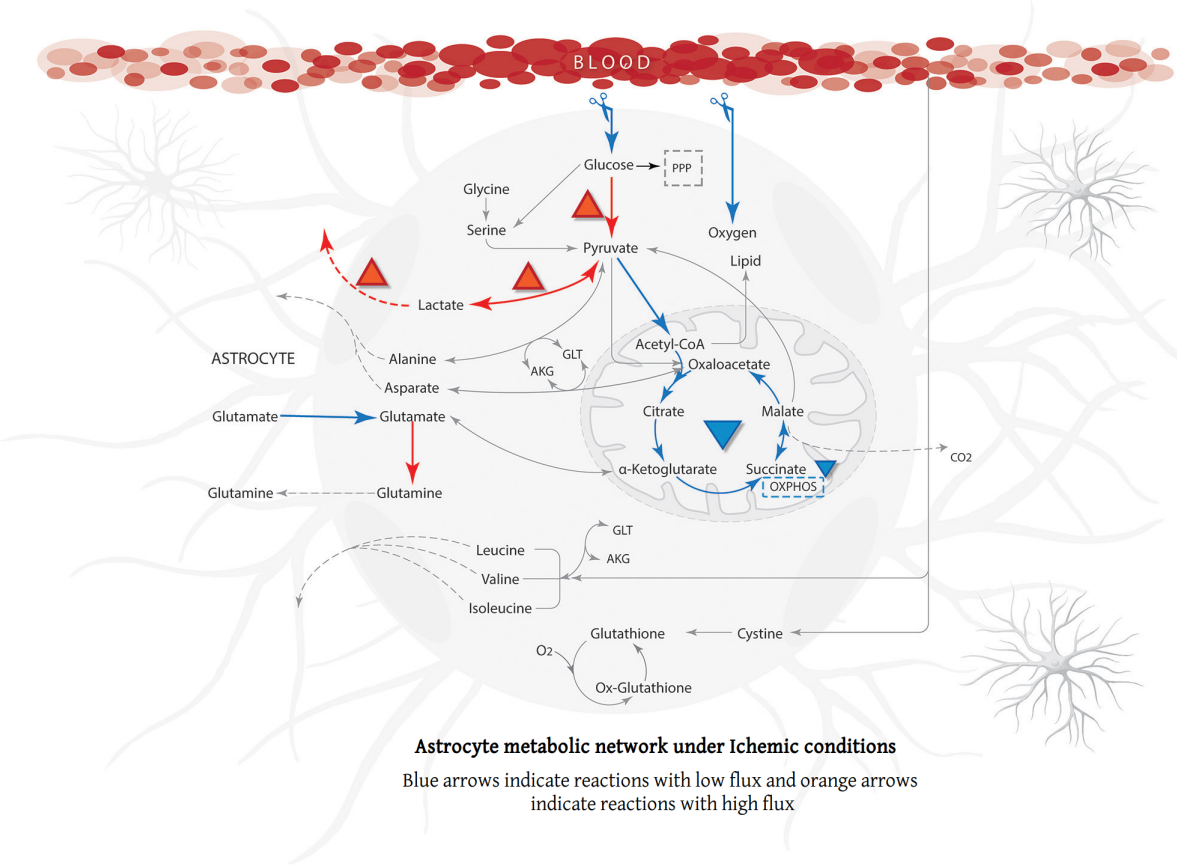
- Glucose, oxygen uptake rates and ATP production are positively correlated
- α-ketoglutarate and lactate fluxes also increase
- Glutamine release was also found to increase

Increased ATP production can be explained physiologically by the working of the Sodium-Potassium-ATP pumps in the astrocytes, which mediate the transmembrane sodium gradient and drive the glutamate-sodium pumps in the astrocytes. The functioning of the Na-K-ATPase pumps require ATP and hence, maximisation of GUGR, also results in a maximisation of ATP production.

Ischemic Conditions:

Simulations of Ischemic condition was performed by gradually decreasing the glucose and oxygen uptake rates by 20%. The following flux distributions were obtained with a decrease in the glucose and oxygen fluxes:

- Fluxes through several metabolic pathways such as fatty acid pathways, TCA cycle, oxidative phosphorylation decreased
- Fluxes through anaerobic glycolysis pathways increase
- Lactate production increases
- Glutamate uptake and ATP generation also decreases



Astrocyte metabolic network under Ichemic conditions

Blue arrows indicate reactions with low flux and orange arrows indicate reactions with high flux

The increase in fluxes associated with anaerobic glycolysis can be accounted as an attempt to increase ATP production and increase GUGR (dependent on ATP availability). The increase in lactate flux can be physiologically explained by the replenishment of NAD+ and ATP production through glycolysis under anaerobic conditions. However, it was found that the ATP production diminished and this was hypothesised to be a result of cell energy balance compromise.

Summarising the results under Ischemic condition, reduction in glucose and oxygen fluxes hampered the glutamate uptake and glutamine release in astrocytes. The hypoxic conditions also resulted in increased lactate flux, decreased ATP production and rapid intracellular acidification. The results were validated with experimental data.

Benefits of Computational modelling and FBA

The systems-level approach of this Astrocytes model has enabled us to understand and analyse cascading reactions and their effects under normal physiological conditions and pathological conditions. Besides, it enables us to identify key metabolic routes in different pathological conditions, better biomarkers, drug targets and drug evaluations. The model also allows us to study these reactions at various levels such as enzymatic classification, gene association, subcellular spatial locations and metabolic pathways.

FBA based approach was advantageous to model the system without detailed kinetic parameters of the reactions and to study the effects of perturbations in the network. This particular model is highly annotated, extensively manually curated, intricate, freely-accessible and integrable. The model is available on Biomodels (MODEL1608180000). Given the complexity of the metabolic space of astrocytes, experimental determination and quantification of all metabolic fluxes under different pathological conditions would be very difficult and time-consuming.

Drawbacks of the model

Dynamics of the systems cannot be captured due to the steady-state approach used by FBA. The physiological basis underlying the objective function could potentially vary under different pathological conditions.