In Silico Modeling of Blood-Brain Barrier: Agent-Based Simulation of Cerebral Glucose Transport

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Abstract

We present a hierarchical agent-directed, agent-based model of the blood-brain barrier (BBB) and use it to simulate cerebral glucose transport. The BBB consists of brain capillary endothelial cells that are joined by tight junctions. The contiguous layer of endothelium protects the brain against harmful blood-borne substances, while simultaneously providing a pathway for the transport of metabolically important nutrients. Continuous, facilitated transport of glucose across the BBB is especially important because the brain has a very limited capacity for glucose storage. A detailed understanding of that transport and the BBB, however, remains elusive due to the inherent complexity of the system and its experimental inaccessibility. In recent years computer modeling and simulation techniques have been applied as experimental tools but are mostly limited to numerical simulations or data mining. The agent-oriented BBB model presented here is based on an established software framework for hierarchical, multi-agent simulation. The system model is capable of multi-scale simulations. We simulate BBB glucose transport and compare the results with experimental data from published literature.

1. INTRODUCTION

The blood-brain barrier (BBB) refers to a relatively impermeable interface between blood and brain. Structurally, the BBB consists of tightly joined endothelial cells lining the brain capillaries. They express elevated levels of specific enzymes that form an enzymatic barrier [1]. The main purpose of the BBB is to prevent the entry of extraneous or toxic substances into the brain and to simultaneously allow selective transport of metabolically essential nutrients [2,3]. The BBB also has active efflux mechanisms that can clear potentially toxic compounds from the brain into blood. The physiological integrity of the BBB is undoubtedly important to maintaining a homeostatic microenvironment for the cerebral tissue.

The protective role played by the BBB, however, has adverse implications for drug development and delivery strategies intended to treat brain disorders. First, the great majority of therapeutic compounds cannot passively penetrate the BBB. Small, uncharged, lipid-soluble molecules with high lipid solubility cross the BBB, but most of them fail to traverse the barrier in pharmacologically significant amounts. Consequently, only a few brain disorders respond consistently to such drugs [4]. Active efflux processes of the BBB contribute to drug exclusion [5]. Several drug development and delivery approaches are in use to overcome such obstacles, but results fall short of expectations and underscore the need for more useful insights into the biology of the BBB.

The multi-faceted complexity of the BBB poses challenges to understanding and manipulating the BBB in experimental or clinical settings. Compounding the difficulty are technological, statutory, or bioethical limitations in devising experiments to explore relevant aspects of the system *in vivo* [6]. Despite advancements in medical imaging and drug discovery research, experimental investigation of the BBB remains a difficult and costly effort. New techniques are being applied to build improved *in vitro* models, but they often lack key characteristics of the physiologic BBB [7].

To help overcome the predicament, researchers have increased their efforts to apply computational modeling techniques to capture aspects of BBB structure and dynamics [8-10]. The motivations are compelling: flexibility in design, reusability, economy of time and material, ease of manipulation and viewing, and overall versatility. To date, however, models have been mostly limited to numerical simulations based on sets of equations, or data mining in cases involving BBB partitioning and permeability prediction. A recent exception is an agent-based model of the BBB with promising results, but the model lacks the desired structural hierarchy to handle systems level simulations [11].

In this paper we introduce a newly developed agent-based model of the BBB and use it to simulate cerebral glucose transport. The model is based on a well-developed software framework with components designed and built for

modeling and simulation of mammalian organs [12]. The current model is capable of multi-scale simulations and of representing dynamic spatial heterogeneity.

2. BIOLOGY

2.1. Barrier Structure

The brain capillary endothelial cells that compose the BBB exhibit several distinguishing characteristics: tight junctions, lack of fenestrations, and minimal pinocytotic activity [2]. As a consequence of the tight junctions, the BBB exhibits high trans-endothelial electrical resistance [13]. The periendothelial structure of the BBB comprises pericytes, astrocytes, and neuronal contacts, as illustrated in Figure 1. Pericytes are connective tissue cells that are involved in multiple support functions. Astrocytic foot processes encapsulate the endothelium and play a vital role in the genesis, morphology, and function of the BBB. Neurons may also have an inductive effect on BBB formation. In addition, a basal lamina wraps around the abluminal surface of the endothelium, thereby reinforcing the structure and barrier function of the BBB.

Small, electrically neutral, lipid-soluble molecules cross the BBB by passive diffusion, whereas polar and lipid-insoluble compounds do not. The endothelial tight junctions block the paracellular route, while the lack of fenestrations and the absence of pinocytotic activity effectively hinder transcellular influx of the molecules. Metabolically essential nutrients such as glucose and amino acids are instead transported across the BBB by facilitated diffusion mediated by transmembrane proteins [2].

2.2. Glucose Transport

Because glucose is the main cerebral energy source and the brain has a limited capacity to store glycogen (polymeric storage form of glucose), continual transport of glucose is critical for proper cerebral metabolism. The human brain normally consumes glucose at a relatively constant rate of about 23 μmol/100 g/min [3], and the BBB provides a robust transport mechanism to meet that demand [14,15].

BBB glucose transport is mediated almost exclusively by the facilitative glucose transporter protein type 1 (GLUT1) [16]. Consequently, influx of glucose into the brain is dependent on functional properties and substrate specificity of GLUT1 proteins. The level of transporter expression in the BBB is also important; a change in GLUT1 expression is associated with a number of brain abnormalities or disorders. In addition, a differential distribution of GLUT1 exists between the luminal and abluminal membranes, and the difference may play a key role in the regulation of glucose transport [6].

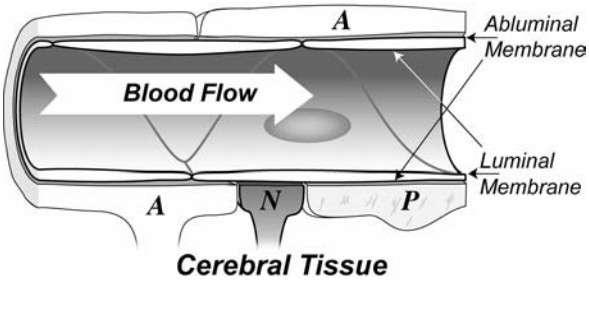

Figure 1. A representative longitudinal section of a cerebral capillary of the blood-brain barrier. Shown are pericytes (P), neuronal contacts (N), and astrocytic foot processes (A) that surround the microvascular endothelium of the BBB. The luminal membrane of the endothelium borders the capillary lumen, and the abluminal membrane adjoins the periendothelial structure.

3. MODEL

3.1. Conceptual Model

At an abstract level, brain capillaries and adjacent structures form a complex network that can be viewed as consisting of three compartments: the capillary lumen, the endothelium and the cerebral tissue. The capillary lumen refers to the inner space of the cerebral microvasculature. The endothelium represents the barrier elements of the BBB. The luminal and abluminal membranes of the endothelium separate the compartments and prevent inter-compartmental bulk flow of solutes. The cerebral tissue represents the perivascular region excluding the barrier elements.

Pertaining to BBB glucose transport are GLUT1 transporters that are distributed throughout the luminal and abluminal membranes at densities that depend on local conditions. GLUT1 proteins can transport glucose molecules in either direction. Only a minuscule amount of glucose influx occurs by simple diffusion [6].

3.2. Agent-Based Model

To represent the networked architecture of the BBB, we use directed graphs in which the inter-nodal movement of solutes is modeled as message passing. Directed graphs provide a tractable means to represent the topographical structure of the system at whatever level of detail or complexity as needed. A cylindrical barrier segment agent that represents a normalized length of BBB occupies each graph node in the model.

As shown in Figure 2, a barrier segment agent is represented as three separate 3D grid spaces that correspond to the abstract compartments. A fine-grained space (Grid A) represents the capillary lumen. A second fine-grained space (Grid B) wraps around Grid A to represent the endothelium. Another space (Grid C) surrounds Grid B to represent the cerebral tissue. The outermost grid locations of Grid B juxtaposed to Grid A and Grid C constitute the luminal and abluminal membranes, respectively.

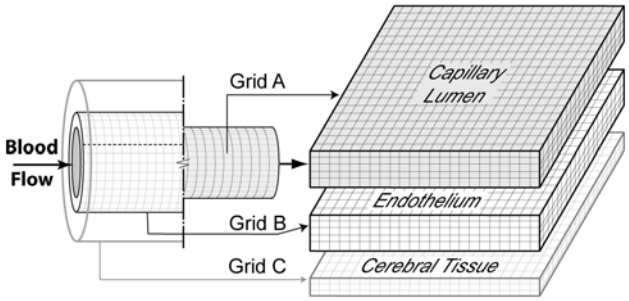

Figure 2. Schematic of a barrier segment agent. One barrier segment is placed at each node of the directed graph. 3D grid spaces represent the compartments of the abstract BBB system. Sublevel objects or agents representing cellular entities such as macromolecules are distributed within the latticework. Individual grid points can have properties and rules that govern their interactions with mobile objects and their neighbors.

For all three spaces, the properties of grid points can be homogeneous or heterogeneous depending on the specific requirements of the problem being solved. Specifically, different types of sublevel agents such as cells and transporters can be assigned to one or more grid points. In addition, the movement of solute objects is subject to one or more lists of rules defined by their intrinsic properties or by agents occupying the source and target locations. Each barrier segment agent handles the inter-compartmental movement of solutes, while the aggregate blood flow in the capillaries is simulated collectively as message passing between interconnected barrier segment agents; the solute objects are treated as messages.

Cellular entities and macromolecules for glucose transport and metabolism are also represented as agents. These include BBB endothelial cells, glucose transporters, and neuronal cells of various types. Functionally less active components such as solutes and non-specific proteins are represented as objects. An agent or object in the model may map to more than one entity or molecule in the biological system, or vice versa, and it can occupy one or more grid points depending on the scale and concentration parameter values.

Glucose transporters bind and transport glucose objects across the membranes. The process is initiated when a glucose object makes a random contact with the transporter, triggering a binding event. Then, after a specified time delay, the transporter delivers its bound object to the target space. Random walk diffusion determines the movement of individual glucose objects, and the resulting net movement of glucose tends toward regions with fewer glucose objects (lower glucose concentrations), facilitated by the glucose transporters. The probabilistic properties of binding and transport are governed by adjustable parameters.

Simulation of the BBB model is directed by an autonomous experiment agent. It controls the simulation environment and encapsulates the model from the underlying software platform. It handles simulation related tasks including resources management, runtime experimental setup, activity scheduling, and data observation within the agent-based platform Swarm, using standard Swarm protocols. Swarm, as a platform for agent-based modeling, is a collection of libraries (written in Objective-C) that provides standardized support for object-oriented, hierarchical modeling of multi-agent systems [17].

4. EXPERIMENTS AND RESULTS

We conducted a series of simulations covering a range of simulated plasma glucose levels to observe the effects on the simulated steady-state cerebral glucose concentration. The results were then compared to experimental data and a Michaelis-Menten model obtained from a previously published *in vivo* study of glucose transport in the human brain [18].

Parameter values governing BBB properties were assigned to complete a crossmodel validity assessment and to reflect physiological conditions observed *in vivo*. Table 1 lists parameters used to modulate relevant properties of glucose transport. At each run, specified densities of glucose transporters were distributed randomly in the spaces representing luminal and abluminal membranes. A luminal to abluminal GLUT density ratio of 2 was imposed to match the normal phenotype observed *in vivo* [6]. Parameters pertaining to the properties of transport mechanics include the probability of a forward or reverse transport action and time delays for binding, transport, and recovery. Thus, in silico trans-membrane glucose transport occurs in an assumptive bi-directional, temporal manner. Parameter values were randomly drawn from specified ranges, each covered by a uniform distribution, and resulted in essentially symmetric transport properties between the luminal and abluminal glucose transporters.

For each simulation, a bolus of glucose objects was placed ("injected") into Grid A (the capillary lumen) at the initial time step. Thereafter a constant luminal level of glucose was maintained. The cerebral glucose concentration level was recorded when the system reached steady state. Simulated plasma glucose levels ranged between 3 and 30 mM (increments of 3.3 mM; 5 independent runs per level) to cover the experimentally investigated concentrations.

Table 1. Parameters used in the model for glucose transport

Parameter	Value(s)
Glucose passage by simple diffusion	0.5%
Luminal GLUT density	0.6
Abluminal GLUT density	0.3
Luminal FW[§] transport probability	0.25 - 0.75
Abluminal FW transport probability	0.25 - 0.75
Glucose binding and transport time	5 - 10 time steps
GLUT post-transport recovery time	1 time step

[§] FW = forward

Figure 3 includes the simulated results. The in silico cerebral glucose levels were plotted as a function of corresponding simulated plasma glucose levels. These values were compared with the experimental data from a nuclear magnetic resonance (NMR) study [18], which investigated a broad range of glucose concentrations *in vivo*. A crossmodel validity study was undertaken to compare the simulation results with values obtained from a standard, parameterized, irreversible Michaelis-Menten model that had been fitted to the *in vivo* data.

Visual assessment of the results in Fig. 3 showed that the simulation results and the experimental data were similar. The simulated cerebral and plasma glucose levels had a correlation coefficient of 0.976, strongly suggesting a linear relationship. Further analysis of both sets of data produced a bivariate regression equation with the slope of 0.33 for the simulated data versus 0.31 obtained for the experimental data. The dashed line in Fig. 3 represents the regression equation for the experimental data.

We also compared our simulation results with values obtained from the standard, symmetric, irreversible Michaelis-Menten model, the solid curve in Fig. 3. Expected cerebral glucose values generated by the irreversible Michaelis-Menten model are nonlinear, but nevertheless correspond reasonably well with the simulation results. In contrast, reversible Michaelis-Menten models predict a linear relationship between cerebral and plasma glucose, as does the in silico model. Simulation results from both the reversible Michaelis-Menten model [18] and the agent-based model presented here exhibit linearity in the studied range, consistent with the experimental data.

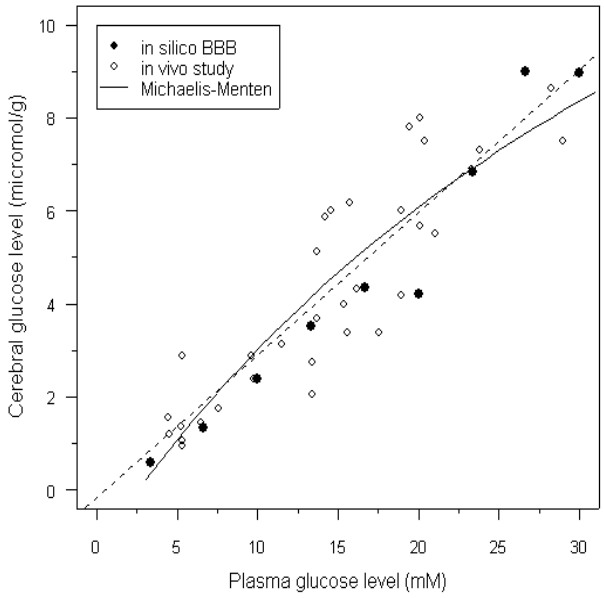

Figure 3. A scatter plot of steady-state cerebral glucose levels as a function of plasma glucose concentration. Simulation results are compared with experimental data obtained from a previously published NMR study. Each solid circle represents the mean value of five independent simulations. The dashed line corresponds to the regression line of the *in vivo* cerebral and plasma glucose concentrations. The solid curve represents the irreversible Michaelis-Menten model. Our simulated results agree well with the experimental data; both show a clear linear relationship between cerebral and plasma glucose levels. Predicted glucose values from the irreversible Michaelis-Menten model are nonlinear, but correspond reasonably well with our simulated results and the experimental data.

5. DISCUSSION

In order to gain deeper insights into how a complex system such as the mammalian BBB functions *in vivo* under normal and diseased conditions, with and without therapeutic interventions, we will need to coordinate exploratory modeling in silico with biological experimentation [19]. We will need simulation tools that are easily assembled from modular, validated components that map logically, in both form and function, to their biological counterparts. The models and simulations will need to mimic not only the biology and its behavior, but also the actual experimental conditions, and so the models and their components will need to be designed for experimentation. We will need cross-validated components of the same functional subsystem, but modeled at different scales and levels of resolution, that can be exchanged as needed. We are currently far from that vision. This paper, however, represents an important step in that direction.

We present an agent-directed, agent-based model of the BBB and the associated glucose transport mechanism. The model was parameterized based on physiologically comparable values. The simulation results were compared against previously published experimental data. The standard, symmetric Michaelis-Menten model was included in the comparative analysis.

The simulated results agree well with the experimental data. Both sets of data show a strong linear relationship between plasma and brain glucose concentrations. For the concentration range studied, the extent of nonlinearity in the predicted values from the irreversible Michaelis-Menten model is not striking, and so it too is reasonably consistent with the data. Possible explanations for the observed differences between results simulated using the agent-based and continuous models include (1) differences in how the steady states are approximated; (2) systemic experimental or modeling errors; and (3) normal variations between experiments.

Cerebral glucose transport in mammals is a complex process that spans multiple components, levels, and scales. The components include blood flow and glucose concentration, biochemical and molecular characteristics of GLUT, and cerebral glucose metabolism to name a few. Traditional continuous state models are ineffective in capturing the complexity, and they do not facilitate

exploratory modeling. Agent-based simulation models are both more tractable and intuitive. Nevertheless, they are still simple representations of the BBB and glucose transport across the barrier. The model herein moves one step closer to a more realistic and systemic representation of the BBB. Guided by additional data the model can easily accommodate new or more detailed system characteristics. In the most general sense, the agent-based model offers a way to explore ideas and test hypotheses that otherwise may be impossible to test experimentally.

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REFERENCES

[1] Allt, G. and J.G. Lawrenson. 2000. "The Blood-Nerve Barrier: Enzymes, Transporters, Receptors—A Comparison with the Blood-Brain Barrier." *Brain Research Bulletin 52*(1):1-12.

[2] Davson, H.; B. Zlokovic; L. Rakic; M.B. Segal. 1993. *An Introduction to the Blood-Brain Barrier*. CRC Press, Florida, USA.

[3] Paulson, O.B. 2002. "Blood-Brain Barrier, Brain Metabolism and Cerebral Blood Flow." *European Neuropsychopharmacology 12*:495-501.

[4] Pardridge, W.M. 2003. "Blood-Brain Barrier Drug Targeting: The Future of Brain Drug Development." *Molecular Interventions 3*(2):90-105.

[5] Gottesman, M.M. and I. Pastan. 1993. "Biochemistry of Multidrug Resistance Mediated by the Multidrug Transporter." *Annual Review of Biochemistry 62*:385-427.

[6] Pardridge, W.M. 1998. *Introduction to the Blood-Brain Barrier: Methodology, Biology, and Pathology*. Cambridge University Press, Cambridge, U.K.

[7] Lundquist, S. and M. Renftel. 2002. "The Use of *In Vitro* Cell Culture Models for Mechanistic Studies and as Permeability Screens for the Blood-Brain Barrier in the Pharmaceutical Industry—Background and Current Status in the Drug Discovery Process." *Vascular Pharmacology 38*:355-364.

[8] Clark, D.E. 2003. "In silico prediction of blood-brain barrier permeation." *Drug Discovery Today 8*(20):927-933.

[9] Feher, M.; E. Sourial; J.M. Schmidt. 2000. "A simple model for the prediction of blood-brain partitioning." *International Journal of Pharmaceutics 201*:239-247.

[10] Oyler, G.A.; R.B. Duckrow; R.A. Hawkins. 1992. "Computer simulation of the blood-brain barrier: a model including two membranes, blood flow, facilitated and non-facilitated diffusion." *Journal of Neuroscience Methods 44*:179-196.

[11] Qutub, A.A. and C.A. Hunt. 2002. "Glucose Transport Across the Blood-Brain Barrier." In *Proceedings of the Second Joint EMBS/BMES Conference* (Houston, Texas, USA, October 23-26) *3*:2224-2225.

[12] Hunt, C.A.; G.E.P. Ropella; M.S. Roberts; L. Yan. 2004. "Biomimetic In Silico Devices." *Computational Methods in Systems Biology, Second International Workshop, CMSB 2004* (Paris, France, May 26-28).

[13] Pardridge, W.M. 1999. "Blood-Brain Barrier Biology and Methodology." *Journal of NeuroVirology 5*(6):556-569.

[14] Knudsen, G.M.; E. Rostrup; S.G. Hasselbalch. 2004. "Quantitative PET for Assessment of Cerebral Blood Flow and Glucose Consumption under Varying Physiological Conditions." *International Congress Series 1265*:189-200.

[15] Christensen, T.G.; N.H. Diemer; H. Laursen; A. Gjedde. 1981. "Starvation Accelerates Blood-Brain Glucose Transfer." *Acta Physiologica Scandinavica 112*: 221-223.

[16] Mueckler, M. 1994. "Facilitative Glucose Transporters." *European Journal of Biochemistry 219*:713-725.

[17] Minar, N.; R. Burkhart; C. Langton; M. Askenazi. 1996. "The Swarm Simulation System: A Toolkit for Building Multi-Agent Simulations." Santa Fe Institute, Santa Fe, NM, *www.swarm.org*.

[18] Gruetter, R.; K. Ugurbil; E.R. Seaquist. 1998. "Steady-State Cerebral Glucose Concentrations and Transport in the Human Brain." *Journal of Neurochemistry 70*:397-408.

[19] Davis, P.K. 2000. "Dealing with Complexity: Exploratory Analysis Enabled by Multiresolultion, Multiperspective Modeling." In *Proceedings of the 32nd Winter Simulation Conference* (Orlando, Florida, USA, December 10-13), 293-302.

BIOGRAPHIES

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