Folate Transport at the Blood-Brain Barrier
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**Purpose**
Folates (vitamin B9) play a critical role in DNA synthesis, gene regulation and the production of the universal methylation agent, S-adenosylmethionine. In mammals, three major transport systems have been identified to mediate gastrointestinal absorption and distribution of folates to major organs (Fig. 1). The proton-coupled folate transporter (PCFT) functions optimally at low pH ($K_m=1\mu M$) and primarily facilitates the uptake of folates into enterocytes. Expressed ubiquitously in mammalian tissues is the reduced folate carrier (RFC), which serves as a low affinity organic anion antiporter ($K_m=2-7\mu M$). In the central nervous system, particularly at the choroid plexus, folate receptor alpha (FRα) constitutes a major transcytosis pathway ($K_m=1nM$) for folates into the cerebrospinal fluid. Mutations and/or presence of antibodies against FRα can cause severe folate deficiency resulting in childhood neurodegeneration characterized by ataxia, dyskinesia, epilepsy and abnormal brain myelination. The aim of this project was to investigate folate transport at the blood-brain barrier (BBB), an area of the brain largely unstudied in relation to folate transport.

**Methods**
Applying qPCR and immunoblotting, the expression of PCFT, RFC and FRα was examined in: i) human (hCMEC/D3) and rat (RBE4) brain microvessel endothelial cell lines representative of the BBB, ii) primary cultures of human brain microvessel endothelial cells (hBMVEC) and iii) isolated rat and mouse brain capillaries. To investigate folate transport at the BBB, uptake of $^3$H-folic acid (FA; specific PCFT and FRα substrate) was evaluated in hCMEC/D3 monolayers grown on 24-well plates. Transport assays were performed at different extracellular pHs (i.e., 5.5 and 7.4) as well as in the presence of standard PCFT inhibitors (i.e., 4,4’-diisothiocyanostilbene-2,2’-disulfonic acid or DIDS, bromosulfophthalein or BSP and sulfasalazine). $^3$H-FA cellular accumulation was measured by liquid scintillation counting.

**Results**
The folate transporters demonstrated robust expression (gene and protein) in all BBB model systems with western blots revealing multiple protein bands for PCFT (50-60kDa) and RFC (58-75kDa), indicative of differential glycosylation. Furthermore, $^3$H-FA uptake by hCMEC/D3 cells was stimulated by an acidic extracellular pH, with time-dependent uptake being higher at acidic pH 5.5 compared to pH 7.4. At both pH conditions, $^3$H-FA uptake was linear for over 2 min and reached a plateau after 15 min. The uptake was also susceptible to inhibition by DIDS (51%), BSP (45%) and sulfasalazine (32%).

**Conclusion**
The detection of PCFT and RFC in various BBB model systems suggests a potential role for these transporters in folate permeability at the BBB, especially when FRα mutations impair the predominant mode of brain folate uptake at the choroid plexus. Transport assays with FA reveal a potential PCFT-mediated folate transport in hCMEC/D3 cells due to the observed sensitivity to extracellular pH and susceptibility to established PCFT inhibitors. Future work will characterize the kinetic properties and direct involvement of H+ as a driving force of this pH-sensitive folate transport at the level of the BBB. Modulating folate transport at the BBB could potentially constitute a novel strategy for the treatment of neurometabolic disorders caused by folate deficiency. (Supported by Natural Sciences and Engineering Research Council of Canada operating grant and Ontario Graduate Scholarship)

![Figure 1: Transport of folate (F) across the small intestine (A) and choroid plexus (B).](image_url)