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Mouse Models of Neurological Disorders: A View From the Blood-brain Barrier

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## Abstract

The number of disease models that involve an aspect of blood-brain barrier (BBB) dysregulation have increased tremendously. The main factors contributing to this expansion have been an increased number of diseases in which the BBB is known to be involved, an increase in the known functions of the BBB, and an increase in the number of models and tools with which those diverse functions can be studied. In many cases, the BBB may be a target of disease; current thinking would include hypertensive encephalopathy and perhaps stroke in this category. Another category are those diseases in which special attributes of the BBB may predispose to disease; for example, the ability of a pathogen to cross the BBB often depends on the pathogen's ability to invoke transcytotic pathways in the brain endothelial or choroid plexus cell. Of special interest are those diseases in which the BBB may be the primary seat of disease or play a major role in the onset or progression of the disease. An increasing number of diseases are so categorized in which BBB dysfunction or dysregulation plays a major role; this review highlights such roles for the BBB including those proposed for Alzheimer's disease and obesity.

Key Words: Blood-brain Barrier, Models; Stroke; Obesity; Alzheimer's Disease; Multiple Sclerosis; Transcytosis; Paracellular; Neuroimmune; Choroid Plexus

## Introduction

Models of blood-brain barrier (BBB)-disease interactions have increased tremendously in number in the last two decades. Although there are many reasons for this, three main trends are largely responsible for the increase. First, there are more diseases which are thought to involve the BBB. At the same time, the explosion in animal models for any given disease have further enhanced this reason. Second, there are more better and models for studying the BBB. This allows functions to be investigated that previously could not be modeled. Third, the BBB has more functions than were previously appreciated. These functions can be studied and their dysfunction can participate in disease processes in ways which were not imagined even a few years ago. These three aspects are considered in more detail below.

The trends and future directions can be better appreciated by examining the state of BBB models 3-4 decades ago. Good sources for this are Rapoport's classic "Blood-Brain Barrier in Physiology and Medicine" published in 1976 [1] and Bradbury's "The Concept of a Blood-Brain Barrier" published in 1979 [2]. These books remain excellent sources for understanding the fundamentals of the BBB, but also provide snapshots of the status of BBB disease models some 30+ years ago. Many things have not changed: models of multiple sclerosis and stroke are still of major interest, debate continues about whether immune cells cross the BBB by penetrating between or through endothelial cells, and issues related to drug delivery to the CNS present major challenges. But many things have changed also: there are many more models of stroke as well as models of components of stroke (such as hypoxia/reoxygenation), immune cells are now widely believed to cross the BBB under normal physiologic conditions and not just during brain

infection, and drug delivery has become still more challenging with the discovery of, for example, efflux systems. Diseases most actively modeled in the 1970's were stroke, multiple sclerosis, trauma, cytotoxicity, invasion by cancer cells and leukemias, brain infections and encephalitides, convulsions and epilepsy, and hypertensive encephalopathy. BBB tissues were known to be enzymatically active and to be important in preventing monoamine transport across the BBB. Methods available allowed three generic types of studies to be done: the measure of uptake rates of substances which crossed the BBB very rapidly, such as hexoses, amino acids, or small lipid soluble molecules; the study of fluxes of substances which were metabolically stable such as electrolytes; studies of BBB disruption and edema, often using dye or fluid shift models. The conceptual view of the functions of the BBB these models helped to define and exploit were that of the nutritional and homeostatic regulatory interface, the lipid permeable cell membrane, and the barrier.

#### General Themes

The BBB models of diseases can be subdivided into 3 general categories as to their relation to the disease that they model. The first and traditionally the most common category is that of inducing some aspect of the disease by a mechanism that is clearly not the etiology of the disease. The purpose of these studies is to investigate the downstream consequences of the disease feature while recognizing that upstream events cannot be studied. An example of this model is induction of type I diabetes mellitus with streptozotocin. Although streptozotocin induces an insulinopenic hyperglycemic state, the hallmark of type I diabetes mellitus, streptozotocin is not the common etiology of diabetes mellitus in humans. As a result, the

downstream consequences of deficient insulin or hyperglycemia can be studied, but questions related to etiology, predisposition to disease, or genetics are not easily studied with this model.

A second category is that in which the disease being studied arises from essentially the same etiology in the model as in the human and follows essentially the same course. Pure examples of these are rare and the most useful models. An example in this category is that of maturity onset obesity in a mouse strain which is not otherwise prone to obesity. In a version of this model, randomly selected mice are fed regular chow for an extended period. A subset will tend towards a higher level of obesity than the average, just as in the human population. In a variation of this version, a high fat diet and sedentary conditions will accentuate the differences in body weight, just as occurs in humans.

A third category of model is that in which the relation between the etiology of the disease and the inducer of the model are unclear. This leads to ambiguity as to what extent the model recapitulates the disease. An example of this is the use of experimental allergic encephalomyelitis (EAE) to model multiple sclerosis. There are several EAE models that primarily vary in the method used to induce disease, but most activate the immune system against components of the nervous system. As the etiology of multiple sclerosis is unknown, it is unclear to what degree, if any, the various methods used to induce EAE resemble the etiology of multiple sclerosis.

#### Diseases Affecting or Involving the BBB

The number of diseases in which the BBB is thought to be affected has increased dramatically. Included are some of the most important diseases of Western society such as

Alzheimer's disease, obesity, epilepsy, and diabetes mellitus [3-8]. The role of the BBB in many diseases has also shifted from being a passive target to an active participant, even a prime participant, in disease. Two examples of diseases in which the BBB may play the role of prime participant are obesity and Alzheimer's disease; these are considered in detail below.

Other models suggesting a primary role for the BBB in disease states are those relating to immune cell and pathogen penetration of the BBB. Diseases involving immune infiltration are often mistakenly thought to occur because a disruption of the BBB allows the immune cells to leak into the brain. It is now clearly understood that immune cells and brain endothelial cells participate in an elaborate cross talk termed diapedesis [9]. Passage of immune cells across the BBB involves activation of both the immune cell and BBB cells [10;11]. Blockade of key receptors on either brain endothelial cells or the immune cells can halt or retard diapedesis. This is most dramatically shown by treatment with natalizumab, an antibody that binds to immune cells to block alpha(4) integrin binding and is effective against multiple sclerosis (3206).

Multiple sclerosis is a disease in which the BBB plays a major role. EAE is used as a model of multiple sclerosis [12]. There are several versions of induction of EAE that produce either relapsing and non-relapsing disease [13;14]. EAE models clearly support a role of the BBB in mediating onset and progression of disease [15;16]. The EAE models have also been important tools in the investigation of how immune cells cross the BBB [17]. Work with these models also shows that disruption of the BBB is secondary to immune cell invasion, not the other way round [18-20]. Other aspects of BBB function are also likely affected by induction of EAE and, presumably, in multiple sclerosis such as cytokine transport [21]. The EAE model has also served as an example of how neuroimmune activation can facilitate immune cell invasion

for other conditions and diseases such as spinal cord injury [22].

HIV-1 invasion of the CNS occurs when immune cells infected with the virus cross the BBB [23]. Immune cells containing HIV-1 are activated and more likely to adhere to brain endothelial cells than non-infected cells [24]. Models of this disease also show that immune cell invasion produces BBB leakage and disruption rather than the BBB disruption allowing immune cells to leak into the brain [25]. HIV-1 can also cross the BBB as free virus and does so by interacting in a complex manner with the cells which constitute the BBB [26;27]. This complexity occurs not just with initial binding, but with subsequent interaction with cellular machinery that controls transcytosis [28-31]. Indeed, the neuroAIDS field has produced an important example of how pathogen and BBB cells interact to activate one another and to forward the progression of disease [24;32]. This interaction has consequences in addition to the facilitation of passage of pathogen across the BBB [23;33]. For example, the immune activation induced by the neuroAIDS process [34] activates other components of the neurovascular unit, secretions from BBB cells, and alters expression of p-glycoprotein at the BBB [35-37]. This latter effect, in turn, affects the delivery of antivirals to the CNS [38;39].

These principles apply to most other neuropathogens which enter the CNS by crossing the vascular BBB or choroid plexus [40]. That is, the virus, bacteria, or parasite induces alterations in the BBB function which facilitate the passage of the agent across the barrier as opposed to leaking across an already disrupted BBB [41-44]. The agent often induces cooperation of the BBB, taking over some aspect of endogenous machinery.

Models of the BBB

The multiplication of models can be illustrated by the increased number of models for stroke or diabetes mellitus. Stroke models or models relating to some aspect of stroke have expanded greatly in the last few decades and demonstrated important changes in BBB tight junctions in the post-ischemic state [45]. For example, there are currently five major categories of ischemic models: embolic middle cerebral artery occlusion, endovascular filament middle cerebral artery occlusion, permanent transcranial middle cerebral artery occlusion, transient transcranial middle cerebral artery occlusion, and cerebrocortical photothrombosis. These models are not simply variants on how to produce the same end point, but have subtle differences including how the BBB is affected or responds. There are also “component models” that involve replication of a major aspect of stroke [46]. For example, hypoxia/reoxygenation models lower the ambient oxygen to a level and for a time that affects BBB and CNS function to study the effects of hypoxia. Oxygen levels are then returned to normal to study effects of reoxygenation, a period when damage from oxidative stress can occur. In this model, unlike the focal ischemia models, blood flow does not cease, no physical damage is done to endothelial cells by a plug or clot, and hypoxia/reoxygenation is global, not localized. As stroke involves all of these, hypoxia/reoxygenation is not a stroke model, although it models a major component of stroke. Results may be applicable to other diseases involving hypoxia or hypoxia with reoxygenation, such as sleep apnea, asthma, chronic obstructive pulmonary disease, cardiac arrest, and cardiopulmonary bypass.

Stroke models have also been used to study how drug delivery mechanisms may differ in the post-stroke BBB or how potential therapeutics affect BBB disruption in stroke [47-49]. Alterations in relevant BBB transporter systems, such as those for peptides and cytokines, or

expression of other BBB proteins have also been studied in stroke or stroke-prone models [50-52]. Modeling can lead to therapeutic strategies such as increasing brain retention of potential therapeutics by inhibiting brain-to-blood efflux systems [53].

Stroke is usually considered a disease associated with aging, but one form, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), occurs in persons under 40 years of age [54]. CADASIL results from a mutation in the Notch3 gene. Although the mutation is throughout the vascular bed, it seems that only the central nervous system is affected. Mouse and pig models recapitulate various aspects of the disease, developing a CADASIL-like picture. Loss of pericytes is prerequisite to the development of altered BBB permeability and hypoperfusion (R Cecchelli, personal communication). Interestingly, pericyte drop out is also key to the develop of disruption of the blood-retinal barrier in diabetes mellitus [55;56].

Most models of diabetes mellitus use streptozotocin which destroys the beta cells of the pancreas. This produces an insulinopenic hyperglycemia and so models type I diabetes mellitus. However, as discussed below, aspects of the BBB are also affected in obesity. Most models of obesity are likely also producing animals with insulin resistance or hyperinsulinemic hyperglycemia. The latter would model diabetes mellitus type II. These models provide new opportunities to study the effects of type II diabetes mellitus on the BBB.

The BBB can be involved indirectly in models of diseases not typically thought to have a BBB component. For example, the lysosomal storage diseases result from defects in enzyme production, function, or translocation, allowing the accumulation of various storage products [57]. Dysfunction of both peripheral tissues and the CNS occurs with the accumulation of these

storage products. In many of these diseases, normal function can be returned to the peripheral tissues by treatment with enzyme. However, these enzymes are large and do not cross the BBB of adults. As such, lysosomal storage diseases represent a major challenge for CNS drug delivery [58;59]. They also represent an excellent model for the development of drug delivery systems for large proteins. Animal models with the enzyme of interest knocked out will give a clear signal of clearing of storage product when therapeutic amounts of the enzyme are delivered to the brain. Most drug development for this disease has concentrated on delivering enzyme as a cargo attached to some other substance that will cross the BBB rapidly, the oft-called Trojan horse approach. More recent work has noted that the perinatal BBB expresses function mannose-6 phosphate receptor which transports into the brain two enzymes, GUSB whose deficiency causes Sly syndrome and sulfamidase whose deficiency causes Sanfilippo syndrome [60;61]. Thus, the brains of neonates in which these enzymes are knocked out can be successfully treated by giving peripheral enzyme [62-64]. However, the transporter function is lost with maturity and is virtually absent by adulthood. Recent work has shown that epinephrine can re-induce transporter function in adults [65]. Thus, lysosomal storage diseases may provide an excellent model for the study of transcytotic events and their induction at the BBB. Recent work has also suggested that the BBB is not itself resistant to the pathologies of lysosomal storage disease (DJ Begley, personal communication). Knockouts for MPS IIIA have a decreased brain vascular space, altered penetration of diazepam, and increased penetration of glycine. This opens the possibility that among the targets of these diseases is the BBB itself.

Functions of the BBB

Traditional studies have focused on BBB disruption. Classically, diseases such as multiple sclerosis, stroke/ischemia, trauma, Alzheimer's disease, epilepsy, CNS cancers, hypertensive encephalopathy, and sepsis have been modeled [21;66-69]. More recently, neuroAIDs, diabetes mellitus, and chronic pain syndromes have been shown to have disruptions in the BBB [70-72].

A wide variety of approaches have been used to study BBB disruption and these have given different aspects to the overall picture. Injection of dyes was the original method used by Erlich in the 1880's that led eventually to the elucidation of barrier function and was used for decades [73]. This method has enjoyed a resurgence in use in the last decade or so, possibly because little special equipment or regulatory oversight is needed. The method, however, requires skill and practice to give true results. Main sources of error are inadequate washout of the vascular space, disruption of the BBB from washout of the vascular space, or administration of too high a dose of dye. The latter occurs when the amount of dye given exceeds the binding capacity of serum albumin. These common errors all act to overestimate BBB disruption. Ultrastructural studies ultimately showed the anatomical basis for the BBB [74] and subsequently showed that most disruptions of the BBB [75] result from increased vesicular pathways (transcytosis) with or without an accompanying opening of tight junctions (paracellular pathways). Nevertheless, many current studies, especially those devoted to use of in vitro methods, focus on tight junctions. Radioactive studies using a series of molecular weight markers can often distinguish between disruption from transcytosis, in which the measure of disruption is similar regardless of the molecular size of the marker, vs paracellular mechanisms, in which smaller molecular weight markers often give larger measures of disruption [76;77].

In comparison to disruption, other aspects of BBB function during disease are relatively understudied. This is unfortunate, as it is likely that these other functions change much more early in the course of disease and likely are much more relevant to all but the terminal aspects of disease progression than is disruption. Changes in BBB lipid composition, receptor binding kinetics and function, transporter function, immune cell trafficking, enzymatic activity, and secretory capacity have been shown to change in models of aging, diabetes mellitus, sepsis, neuroimmune activation, multiple sclerosis, neuroAIDS, and Alzheimer's disease [6;9;21;23;78-86]. Changes in transporter function as central to progression of obesity and Alzheimer's disease is discussed below, but other important examples exist. For example, whereas disruption of the BBB begins after several weeks of streptozotocin-induced diabetes mellitus, the transport of insulin across the BBB is altered within 72 h [72;87]. Whereas the BBB can become disrupted in neuroAIDS or with seizures, these conditions cause alterations in p-glycoprotein expression [88;89]. This, in turn, alters the ability of anti-virals and anti-epileptic medications to accumulate within the CNS.

Modeling of many diseases shows that the BBB is involved or altered on many levels [90]. For example, in neuroAIDS: the BBB is minimally disrupted [25;42;91]; activation of BBB cells and immune cells leads to enhanced adhesion of immune cells to the BBB and enhanced viral entry [25;33;34;92;93]; infected immune cells, free virus, and viral proteins including the neurotoxic gp120 and TAT cross the BBB [23;26;33;94;95]; BBB surface glycoprotein composition likely determines which HIV-1 strains are taken up or transported by the BBB [29]; BBB efflux systems impair the accumulation by the brain of many antivirals [38;39;96]; BBB secretions and cell surface expression of immune-active cytokines and

chemokines is increased by virus or viral proteins [32;97;98]. These changes affect many aspects of AIDS and neuroAIDS including disease initiation and progression, neuroimmune activation, and drug delivery. Such multi-level changes in the BBB also occur in models for diabetes mellitus and sepsis/neuroimmune activation.

Another example of the expanded understanding of the sorts of roles the BBB may play in diseases is provided by epilepsy. Classically, the BBB has been noted to be disrupted in seizures with such disruption affected by parameters such as aging and gender [99;100]. More recently, drug delivery has become of great interest with the realization that an efflux system located at the BBB termed p-glycoprotein retards the accumulation by brain of most anti-seizure medications [101;102]. Indeed, the some 30% of epileptic patients who are resistant to anti-seizure medications may be so because of overexpression of p-glycoprotein [103]. Even more recently, it has been shown that seizure activity increases expression of p-glycoprotein at the BBB, thus complicating control of epilepsy [88;89]. Finally, immune cell-BBB interactions may be key to turning experimental seizure into epilepsy. Seizure activity induces selectin expression at the BBB which increases immune cell adhesion and perhaps CNS trafficking [8]. Blockade of immune cell-BBB adhesion inhibits both induction of acute seizures and chronic recurrent seizure activity.

#### Diseases Caused by the BBB: The Case for Alzheimer's and Obesity

Diseases in which the BBB plays a central or primary role could be those in which either the BBB is the original site of pathophysiology or in which altered BBB function is key to disease progression. Multiple sclerosis and stroke including CADASIL have been considered

above. To this list can be added De Vivo's disease, which includes familial seizures and mental retardation resulting from underexpression of the GLUT-1 transporter at the BBB [104].

Encephalopathy occurs when the BBB is disrupted during hypertensive emergencies; although formerly thought to be entirely caused by physical disruption from high pressure, more recent investigations suggest that activity of the renin-angiotensin-aldosterone system in general and of angiotensin II in particular may be important. In a broader sense, epilepsy resistance to anti-seizure medications, which occurs in about 30% of cases, is caused by overexpression of p-glycoprotein at the BBB, and CNS infections and infestations arise because of their ability to interact with the BBB in a way to negotiate their permeation into the CNS. Arguments which place the BBB in a causal role in Alzheimer's disease and obesity are illustrative of the general concepts of this review.

#### Alzheimer's Disease

Several hypotheses have been advanced that would involve the BBB/cerebrovasculature (table I) in AD. These include BBB disruption, defective glucose transport, and release of neurotoxins from the BBB. One of the most recent hypotheses is the neurovascular hypothesis [3]. This states that deficient efflux of amyloid  $\beta$  peptide ( $A\beta$ ) from the brain contributes to its accumulation and toxicity within the CNS. That  $A\beta$  is removed from the CNS by a rapid saturable efflux system has been well documented by several laboratories [105-108]. In addition,  $A\beta$  efflux is decreased in both transgenic mice and the SAMP8, a mouse strain with a natural mutation that results in an age-dependent increase in  $A\beta$  and cognitive defects [105;109;110].

Efflux of A $\beta$  from brain has been suggested to be mediated by low density lipoprotein receptor-related protein-1 (LRP-1) and by p-glycoprotein [107;111-113]. A $\beta$  is a ligand for liver LRP-1 which likely plays an important role in its clearance from blood. Immunohistochemistry studies show that LRP-1 levels decrease in brains of AD patients [4]. However, other studies suggest that the relations among LRP-1, A $\beta$ , and p-glycoprotein are complex and may involve other ligands or transporters [106;114]. A model in which LRP-1 at the BBB was knocked down with antisense given by intracerebroventricular infusion showed that brain A $\beta$  levels increased about 40% and mice developed cognitive impairments [115]. These results are consistent with a major role in AD for the BBB efflux of A $\beta$  by an LRP-1 dependent system.

Other work has suggested that blood-to-brain transport of blood-borne A $\beta$  could contribute to brain levels of A $\beta$  with the receptor for advanced glycation end products (RAGE) acting as the primary transporter [116;117]. The contribution that circulating A $\beta$  makes to CNS levels of A $\beta$  is not established and so the role of blood-to-brain transport is less clear. Findings suggest, however, that just as A $\beta$  efflux is decreased in AD, blood-to-brain transport is increased.

Given the evidence that the BBB transporters are altered to favor increased influx and decreased efflux of A $\beta$ , the question arises as to what dictates these changes. Isoforms of A $\beta$  are differentially transported by LRP-1 and with aging in the SAMP8, efflux of A $\beta$ 1-42 is more significantly lost than efflux of A $\beta$ 1-40 [105;118]. This would favor retention of the more toxic form of A $\beta$ 1-42 with aging. Dimerization and complexing with other LRP-1 ligands also retards A $\beta$  efflux [119]. Models of inflammation induced by lipopolysaccharide also recapitulate the proposed findings in AD, increasing influx and decreasing efflux of A $\beta$ . Interestingly, administration of the nonsteroidal antiinflammatory drug indomethacin prevents the

lipopolysacchride-induced impairment of A $\beta$  efflux [120]. This is consistent with the proposed beneficial role of indomethacin in AD [121;122].

## Obesity

Obesity is characterized by resistance to leptin, a 17 kDa protein secreted by fat tissue [123]. Leptin crosses the BBB by way of a saturable transport system where it interacts with its receptors located throughout the brain [124]. Most studied of the CNS sites is the arcuate nucleus. Here, leptin acts through its receptor to inhibit orexigenic peptides such as neuropeptide Y and orexin and to stimulate anorexigenic peptides such as melanocortin [125]. Leptin inhibits feeding through these mechanisms and also increases caloric expenditure. These two factors together result in a decrease in fat mass. As fat mass decreases, so does leptin levels in blood and the CNS; this, in turn, decreases anorexia and caloric expenditure. Thus, a negative feedback loop is formed between adipose tissue and feeding/caloric expenditure. Resistance to these actions of leptin or an absence of leptin results in obesity.

Leptin interactions with the BBB have been studied in several animal models, especially those of obesity. The earliest studies were those of van Heek and [126;127] in two models of obesity prone mice. These two studies showed that as obesity increased, these mice passed through a phase of resistance to peripherally administered leptin while remaining sensitive to centrally administered leptin. This is consistent with a block in the blood-to-brain transfer of leptin and suggested that failure of transport at the BBB preceded that of resistance at the arcuate nucleus. Pharmacokinetic studies in an obesity of maturity model, the Koletsy rat, and several other obesity models showed transport of leptin across the BBB to be decreased in proportion to

the degree of obesity [128-131]. Studies with the brain perfusion model showed that leptin resistance at the BBB is complex, induced by both circulating factors, such as leptin inducing its own self-inhibition, and longer term factors inducing down regulation [128;132].

Investigation of transport of leptin across the BBB in various models has revealed several interesting characteristics. The transport rate is not static but influenced by obesity, ovarian factors, starvation, triglycerides, insulin, glucose, and epinephrine [133-136]. Effects of lipopolysaccharide, cholecystokinin, and alcohol administration have also been studied [137-139]. The nature of the BBB transporter protein for leptin is of great interest. Most work has indicated that at the vascular BBB, the short form of the leptin receptor likely acts as the leptin transporter. However, the Koletsky rat does not express functional receptors, yet is still able to transport leptin across the BBB by a saturable mechanism[131;140;141]. Therefore, there is likely a leptin transporter at the BBB other than the short form. Megalin has been proposed as the leptin transporter at the choroid plexus [142].

The role of the BBB in modifying leptin actions in the CNS likely affects many of leptin's other effects. Leptin has effects on immune function, bone density, cognition, breathing, reproduction, and other functions, most mediated through CNS sites [143-150]. Leptin has receptors throughout the brain as well, including on non-neuronal cells such as astrocytes [151], so many of these actions may be mediated through different CNS locales and cell types. The rate of transport of leptin across the BBB varies among brain regions [152]. As such, the regional differences obesity has on transport likely means that obesity has differential effects on the leptin actions mediated through the CNS. Leptin also has functioning receptors within the subfornical organ, a circumventricular organ [153]. Similarly, there is evidence for some arcuate nucleus

neurons projecting into the median eminence [154]. This suggests a dynamic interaction of CNS leptin receptor sites that are inside and outside the BBB as has been proposed for other peptides and regulatory proteins such as amylin and interleukin.

BBB interactions with other peptides and proteins related to feeding and metabolism have been studied in various models of disease. Starvation, immune activation with lipopolysacchride, obesity, and hypertriglyceridemic models have been used to study ghrlein or insulin transport [87;155-160]. Urocortin transport across the BBB is induced by leptin and tumor necrosis factor-alpha administration[161;162].

## Conclusions

Our understanding of the BBB continues to evolve. As it does, the BBB is discovered to be involved ever more intimately in an ever wider array of diseases. This understanding has been greatly fostered and defined by models of the BBB and models of disease states. As these models continue to conceptualize the BBB not only as a physical barrier but also as a regulatory interface between the CNS and blood, more roles in disease states will likely be discovered. Most exciting are a series of diseases in which the BBB and its dysfunctions play key roles to disease onset and development.

Table I. Theories Relating Alzheimer's Disease to the Blood-brain Barrier and the Cerebrovasculature

Leaky Blood-brain Barrier	[163]
Tortuous Capillary Bed	[164]
Defective Glucose Transporters	[83]
Microvascularopathy	[165]
Cerebrovascular Degeneration	[166]
Brain Endothelium and the Release of Neurotoxins	[82]
Permeation of Aluminum-glutamate Complexes	[167]
Inhibition of Brain Endothelial Cell Proliferation	[168]
Decreased Vitamin B12 Transport	[169]
Vascular Cognitive Impairment	[170]
Altered Insulin Transport into Brain	[171]
Decreased Cerebral Blood Flow	[172]
A $\beta$ Formation of Ionophores	[173]
Neurovascular Hypothesis: Decreased A $\beta$ Efflux from Brain	[4]

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