
4 Blood-Brain Barrier Modulations and Low-Level Exposure to Xenobiotics

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I. INTRODUCTION

Separation of the brain from the peripheral blood is crucial for protecting this most delicate and important organ from various insidious agents that circulate in the blood. Conversely, the separation must allow for the nutrition of the brain and the removal from it of waste products. The existence of a physical barrier that separates the brain tissue from the general circulation was first proposed 100 years ago, by Ehrlich, who discovered that injection of a series of dyes into laboratory animals resulted in uncolored brains, as opposed to highly stained visceral organs.¹ The blood-brain barrier (BBB) is formed during the late embryonic and early postnatal period. It is an endothelial barrier present in the capillaries throughout the brain, contact-influenced by neighboring astrocytes.² Electron microscopic studies reveal two major factors that distinguish brain endothelial cells from their peripheral relatives: first, they contain lower amounts of endocytic vesicles, and second, the space between adjacent cells is sealed by tight junctions; both factors restrict intercellular flux. These features enable the formation of a barrier that hinders the entry of most xenobiotics into the brain, and is actively involved in exporting such substances from the brain when they do enter it. Small lipophilic molecules enter the brain fairly freely, but hydrophilic molecules enter via active transport, and specific transporters exist for required nutrients such as glucose, L-DOPA, and certain amino acids.³

The physical and functional complexity of the BBB has hampered research efforts to delineate its components and fully understand its mode of action. Numerous experimental approaches were developed for evaluating BBB integrity; these include *in vitro* and *in vivo* systems as well as transgenic engineering approaches. The use of these methods has revealed several modulators of BBB functioning and has demonstrated intricate relationships between these modulators in their effects on BBB integrity. Impairments of any element of these chains of factors can disrupt BBB functioning, but the extent and duration of such disruptions apparently depend on the genetics, health, and wellbeing of the involved organism. In the following, we discuss these considerations as they relate to the issue of low-level exposure to xenobiotics.

II. THE PHYSICAL BASIS OF BLOOD-BRAIN BARRIER PROPERTIES

Low-level exposure to xenobiotics would first affect the circulation; to affect the brain, the xenobiotic must traverse the BBB. In certain cases, e.g., under exposure to anticholinesterases, these agents interact with and inhibit the catalytic activity of their target enzymes, cholinesterases, in peripheral and brain systems alike. The cellular

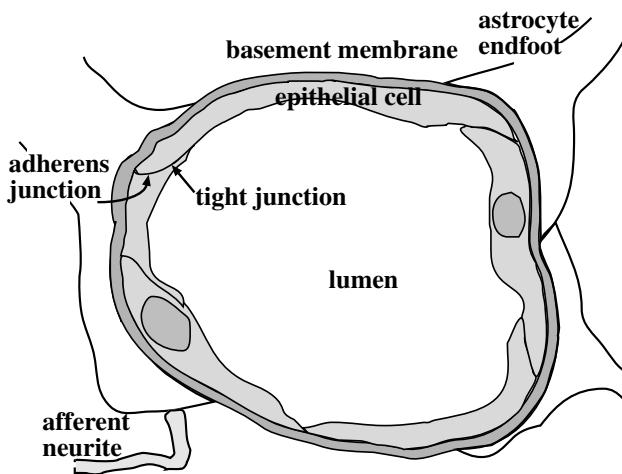


FIGURE 4.1 The physical components of the blood-brain barrier (BBB): Within the mammalian brain, blood vessels and microvessels transverse the brain tissue, bringing in essential compounds and removing metabolic end products. The three layers surrounding the microvessel lumen comprise the BBB, including endothelial cells lining the blood vessels, a basement membrane surrounding them, and astrocyte endfeet separating these structures from adjacent neurons, some of which interact with these astrocytes through contacting neurites. Two types of junctions connect endothelial cells to each other, tight and adherens junctions.

components of the BBB include the endothelial cells that line the inside of brain capillaries, the basement membrane surrounding them, and brain astrocytes, which constitute a third layer separating these blood vessels from the surrounding brain tissue. Intercellular BBB structures include adherens junctions, which attach endothelial cells to each other, as well as the tight junctions that seal them. Within these cells, surface membrane proteins transduce signals by activation of specific kinases, and the flow of information among the several cell types is affected by neuronal and astrocytic activities in the brain as well as by peripheral metabolic changes and external forces. Figure 4.1 presents a schematic view of these elements.

A. ENDOTHELIAL CELLS IN BRAIN VASCULATURE

Many have reported the special properties of endothelial cells in brain vasculature.⁴ Small hydrophobic molecules diffuse across the BBB; large and/or hydrophilic molecules may be transported only if a specific receptor or transporter exists. Thus, small hydrophobic molecules penetrate the brain by diffusion; nutrients such as glucose and certain amino acids are transported into the brain by specific transporters; and several large proteins like transferrin are transcytosed into the brain via specific receptors.

To enable these special properties, tight junctions connect brain endothelial cells, so that intercellular transport is extremely limited.⁵ The expression of P glycoprotein

(the multi-drug resistance *mdr* protein) on their surface membrane controls both penetration of small molecule drugs into the brain and their export from the brain. Genomic disruption of the *mdrla* (a.k.a. *mdr3*) gene causes extreme drug sensitivity, for example, to ivermectin.⁶ This finding highlights the importance of active transport mechanisms for the integrity of BBB functioning and may imply that cumulative exposure can modulate BBB properties.

B. ADHERENS AND TIGHT JUNCTIONS

A primary difference between endothelial cells of brain vasculature and the very similar cells that line peripheral blood cells relates to the composition and properties of the tight junctions between these cells.⁷⁻¹⁰

Adherens junctions are similar to the attachment structures of other cells in which their functions may be more easily studied on the molecular level. Yeast, for example, can express an analogue of the adherens junction, and its assembly was shown to depend upon the Ca^{++} -dependent protein kinase pathway.¹¹ Genetic studies in yeast are easy to perform, and since yeast has a well-described genome, the discovery of the genes that regulate junction formation is possible, and once the yeast gene is known, it is a relatively simple matter to discover its homologues in a mammalian genome.

Unlike adherens junctions, which form homophilic intercellular adhesion sites, tight junctions are complex structures recognized as being the molecular site of pericellular transport and its regulation.¹² In addition to adherens and tight junctions, brain capillary endothelial cells have transmembrane receptors for matrix proteins (e.g., integrins).⁹ Impairment of either cell-cell or cell-matrix interactions can disrupt the BBB, in processes that parallel those of the peripheral endothelium. However, such impairments occur much less frequently in the brain.

Several proteins, including cingulin and occludin, were shown to be essential for the function of tight junctions.^{13,14} More recently, junction-associated proteins such as Rho were reported to regulate tight junctions and perijunctional actin organization in polarized epithelia.¹⁵ Junction proteins are physically linked to cytoskeletal elements such as actin or linking proteins like β -catenin in a manner subject to modulation by phosphorylation or dephosphorylation of specific kinases and phosphatases. This suggests a potential opening of tight junctions by kinase regulation, however, no experimental evidence is yet available to demonstrate such opening *in vivo*. [Table 4.1](#) catalogs key proteins assumed to be associated with BBB junctions.

C. POTENTIAL INVOLVEMENT OF ACETYLCHOLINESTERASE

Like yeast, for nearly a century the fruit fly *Drosophila melanogaster* has served as a model for genetics studies. With the introduction of genetic engineering and genomic databases, this has also become a powerful tool for the discovery of genes and gene products that participate in physiological functions. The identification of a physiological defect in the insect can be quickly traced to a specific gene, and the homologous sequence in the mammalian genome can then be identified, where it then serves as a candidate gene for the similar function in the mammal. For instance, a defect in

TABLE 4.1**Protein Components and Candidate Components of the Blood-Brain Barrier**

Component	Intra/Extra Cellular ^a	Interaction Partners ^b	Reviewed in
7H6 antigen	Intra and extra	Tight junction	10
Acetylcholinesterase	Extra	Neurexin	16
Actin	Intra	Catenin	10
Band 4.1 protein	Intra	Cytoskeleton	16
Cadherin	Extra	Catenin, p120	10; 17
CASK ^c	Intra	Neurexin II β	16
β -catenin	Intra	Cadherin, actin	10
Cingulin	Intra	Tight junction	10
Gliotactin	Intra and extra	Neurexin IV	16
ICAM-1	Extra	ICAM	18
Neuroligin	Intra and extra	Neurexin II β	16
Neurexin	Intra and extra	Neuroigin 1, CASK	16
Nitzin	Intra	Cytoskeleton	16
Occludin	Extra	ZO-1/ZO-2/p130	10
p100	Intra	Cadherin	10; 19
p120	Intra	Cadherin/catenin	10; 17; 19
p130	Intra	Tight junction, ZO-1	10
PSD-95 ^d	Intra	Neuroigin 1, NMDA receptor	20
RPTP ^d	Intra	Cadherin/catenin	10
Selectin	Extra		21
src	Intra	Adherens junction	10
Tyrosine kinase	Intra	ZO-1, β -catenin	10
ZO-1	Intra	Tight junction, p130, tyrosine kinase	10
ZO-2	Intra	Tight junction	10

^aBBB components may function in extracellular locations (extra), convey signals within intracellular locations (intra), or do both.

^bIndependent factors to which attachment has been shown are separated by commas; aggregates of factors to which attachment has been shown are indicated by slashes.

^cPost-synaptic density.

^dCalmodulin-dependent protein kinase.

^dReceptor-type protein tyrosine phosphatase.

the hemolymph-neuron barrier, which serves a function analogous to the BBB in mammals, was shown to depend on the structural and functional integrity of the special septate junctions, which seal this barrier in insect larva.^{22,23} Disruption of these structures by genomic destruction of either of two different genes, neurexin IV and gliotactin, causes severe neuronal sensitivity to the high concentrations of K^+ in the hemolymph. This leads to paralysis and death of the developing insect larva. Such genomic disruption also impaired the subcellular targeting of coracle, a band 4.1 homologue that transduces signals from the cell membrane to the cytoskeleton.

Gliotactin is one of several structural homologues of the acetylcholine-hydrolyzing enzyme acetylcholinesterase (AChE) that were discovered in the past decade. Gliotactin, however, like the other AChE homologues, has no capacity for acetylcholine (ACh) hydrolysis. Intriguingly, AChE may compete with its structural homologues for their cell-cell interactions.^{16,24} This potential involvement of AChE has raised the question of which of the three variants, formed by alternative splicing of the human AChE pre-mRNA, may be involved in these interactions. These variants are: AChE-S, the synaptic form, AChE-E, the erythrocyte form, and AChE-R, a soluble monomeric form which, perhaps significantly for BBB physiology, has been shown to be over-expressed under stress.²⁵

Gliotactin, like several other AChE homologues, is equipped with an extracellular domain, a transmembrane peptide and C-terminal peptide that protrudes into the cytoplasm and can transduce signals into cells. In particular, it interacts with proteins, which modulate the cytoskeleton. Therefore, these discoveries present the entire series of AChE homologues and their yet unidentified binding partners as promising candidates to participate in control of the integrity of the BBB and transduction of signals that regulate its functioning. The impressive conservation of these inter- and intracellular factors, and the chain of interactions by which they may affect cytoskeletal properties, suggest at a mechanism by which AChE levels, and/or the specific chemical properties of its variants, affect the integrity of the BBB. Kaufer et al.²⁶ have recently discovered a feedback process that leads to AChE-R accumulation under exposure to anticholinesterases. This points to the AChE protein as a modulator that may be intimately involved in BBB disruption under exposure to such agents. That no embryonic impairment in BBB functioning is known in mammals most likely attests to the essential role played by the BBB in mammalian embryonic development, as early lethality of such a mutant would preclude its discovery. [Figure 4.2](#) summarizes the evolutionary conservation of the structural properties of AChE as these may be involved in BBB integrity.

D. SIGNAL-TRANSDUCING ELEMENTS

Appropriate functioning of the BBB and its capacity to respond to environmental insults evidently depend on fast, accurate, and sensitive transduction of appropriate signals from the periphery into the brain and vice versa. Over the past few years, several molecular components were discovered which ascertain such a flow of information and ensure its reliability. The role of guanine nucleotides in regulating BBB properties is of special interest. Endothelial capillary cells are polarized, being long and flat structures linked by tight junctions. GTP-Binding Rho proteins are responsible, in these cells, for the particular organization of the filamentous actin fibers that ensure their polarization.¹⁵ Further, transduction of intracellular signals is based, in most polarized epithelial cells, primarily on PDZ domain proteins. Named for three members of this family, PDZ proteins include the post-synaptic density protein, PSD-95, the *Drosophila* tumor-suppressor protein, discs-large (DlgA), and the tight-junction protein, ZO-1; they are often found at the plasma membrane and transduce signals into the cell, affecting cytoskeletal organization.^{27,28} That this is also the case

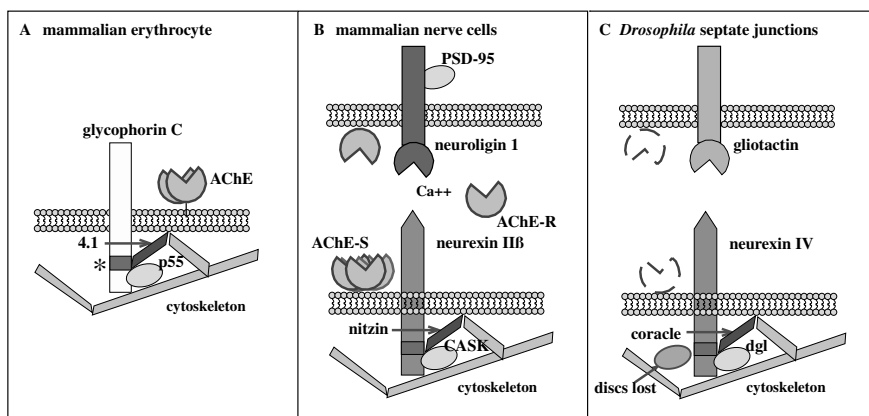


FIGURE 4.2 AChE and its structural homologues are potentially involved in BBB functioning. Shown are schematic drawings of membrane signaling and cytoskeletal components which involve AChE and its structural homologues. (A) In the mammalian erythrocyte, glycophorin C is located on the surface membrane, with one domain protruding into the cytoplasm. A conserved element within this domain (starred) interacts with the band 4.1 protein that serves as an anchor to the cytoskeleton. Another region in the cytoplasmic domain of glycophorin C binds p55, a PDZ protein. It is not yet known whether the AChE-E dimers, which are anchored by a glycophosphoinositol moiety to the outer surface of the erythrocyte membrane, are involved in glycophorin's role of modulating the erythrocyte structure. (B) In mammalian nerve cells, the major AChE isoform is AChE-S tetramers. The AChE-homologous neuronal membrane proteins, neuroigins, are expressed in the developing brain and in excitatory synapses. At least one of the neuroigins, neuroigin 1, interacts with at least one of the neurexins, neurexin II β , in a Ca^{++} -dependent manner. Both neuroigin and neurexin protrude into the cytoplasm, and both interact with PDZ proteins such as PSD-95 and CASK. Neurexins further associate with neuronal band 4.1 homologues, like nitizin,¹⁶ creating a link with the cytoskeleton. Modulation of AChE properties under low-level exposure to an anticholinesterase (e.g., accumulation of AChE-R monomers) may therefore alter neuroigin-neurexin interactions, transducing signals to the neuronal cytoskeleton. (C) In *Drosophila* septate junctions, the AChE-homologous protein, gliotactin, protrudes into the cytoplasm. Another transmembrane protein, neurexin IV, shares with other neurexins an extracellular domain that may interact with the core domain module that is common to AChE and neuroigins. Neurexin IV also includes cytoplasmic glycophorin C elements; these regions interact with the insect band 4.1 homologue, coracle, as well as with the PDZ protein dgl and the multi-PDZ domain protein, discs lost. Therefore, neurexin IV interactions with either gliotactin or the cytoskeleton are essential for maintaining septate junction integrity. It is not yet known whether AChE itself (broken-line structure) is expressed in these junctions.

for BBB components has recently been demonstrated in *Drosophila* embryos by Bellen and co-workers, who found a third protein that is essential for the integrity of septate junctions formation.²⁹ This protein, discs-lost, uses multiple PDZ domains to interact with intracellular components in a manner dependent on septate junction interactions. In general, PDZ domains interact with the carboxy terminal end of their

target proteins.²⁷ Therefore a multi-PDZ protein can aggregate a series of target proteins within the cell, simultaneously transducing multiple signals.²⁹ This enables an extremely sensitive biosensor activity, as is expected from a system designed to protect the brain from low-level exposures.

E. ASTROCYTE CONTRIBUTIONS TO BLOOD-BRAIN BARRIER PROPERTIES

Janzer and Raff recognized the key function of astrocytes that surround brain capillaries in the dynamic properties of the BBB.³⁰ Specific interactions between astrocyte endfeet, which surround brain capillaries, are essential to ensure BBB integrity. The discovery of astrocytic responses to altered ion (e.g., Ca^{++}) concentrations in their environment sheds new light on the specificity of astrocyte interactions and their importance for ensuring BBB integrity.³¹ In a tissue co-culture model, astrocytes were shown to affect the integrity of the tight junctions between adjacent endothelial cells.³² More recently, astrocytes were demonstrated to enhance the defense of capillary endothelial cells against reactive oxygen species.³³ Thus, astrocytes both signal higher centers of the brain that the BBB has been disrupted and themselves receive signals from higher centers that cause them to modulate the BBB.

III. FUNCTIONAL CHARACTERISTICS OF THE BLOOD-BRAIN BARRIER

Designed to protect the brain from penetrance and accumulation of unwanted molecules and cells, the BBB has distinct properties at central and peripheral structures. To properly control the inward and outward flow of constituents to and from the brain, it must sense the needs of both the brain and the peripheral system. Therefore, both the electrophysiological activity in the brain and peripheral properties such as blood pressure must affect BBB properties. Similarly, changes in BBB integrity inevitably affect brain functioning; for example, BBB disruption will allow the passage into the brain of serum constituents, which are known to affect neuronal electric activity (e.g., amino acids). The relative contribution of such agents to brain function under BBB disruption awaits further investigation.

A. INWARD AND OUTWARD MOVEMENT ACROSS THE BLOOD-BRAIN BARRIER: PHYSIOLOGICAL CONSIDERATIONS

Blood-brain barrier properties largely depend on the surrounding brain tissue. This is evident from a recent study that demonstrated the development of an intact BBB in brain tissue transplants in a manner dependent on the site of transplantation.³⁴ The integrity of BBB functioning is affected by cellular glutathione and is sensitive to oxidative stress.³⁵ Neuronal activity is another important factor in BBB functioning, perhaps through the activation of afferent axons innervating brain microvasculature. Indeed, both psychotropic drugs and nicotine impair dopamine transport across the BBB,³⁶ suggesting that BBB functioning is affected by the state of cholinergic or

dopaminergic neuronal activity, and that BBB integrity is imperative for preventing neurotoxicity under exposure to dopamine analogs (e.g., MPTP).³⁷ Thus, human cerebromicrovascular endothelium was shown to possess dopaminergic receptors linked to adenylyl cyclase, suggesting signal transduction activities. Adrenergic influences on BBB control were reported by Sarmiento et al.,³⁸ who also demonstrated the influence of electrical stimulation of locus coeruleus on the rat BBB permeability to sodium fluorescein. Similar effects in a cell culture model were shown by Borges.³⁹

B. CHOLINERGIC INVOLVEMENT IN BLOOD-BRAIN BARRIER FUNCTIONING

The importance of ACh innervation to cortical capillaries has been suggested on the basis of a body of biochemical and morphological data, and indicates the underlying mechanism. Purified capillaries are capable of releasing ACh in a Ca^{++} -dependent mechanism, in response to K^{+} depolarization or electrical stimulation.⁴⁰ Moreover, specific cholinergic machinery was identified in isolated microvessels from goat cerebral cortex, as demonstrated by measuring AChE and choline acetyl transferase (ChAT) activities.⁴¹ ChAT activity in bovine cerebral cortex capillaries does not originate from the endothelial cells, nor do they release ACh in response to electrical stimulation. Rather, cerebrovascular ACh apparently has a neuronal origin.⁴⁰ The origin of the perivascular cholinergic terminals was examined in rat brains in which the nucleus basalis of Mynert, accounting for 70% of cortical ChAT activity, was lesioned. No change was observed in the microvessel-associated ChAT activity in the lesioned animals, ruling out the basal forebrain as the origin of this pathway.⁴⁰ The existence of released ACh hints at the presence of receptors that respond to the signal, and, indeed, muscarinic ACh receptors were identified in rat brain cortical capillaries.^{42,43} Taken together, all this points to the involvement of cholinergic innervation of cerebral microvessels in cerebral blood flow and BBB permeability, which is an essential requirement under various physiological and pathological insults. The cholinergic involvement in BBB functioning is particularly important under exposure to cholinesterase inhibitors such as organophosphates or carbamates, since these induce a feedback response of AChE accumulation, which would lead to cholinergic hypo-functioning.^{26,44} Therefore, AChE may affect BBB disruption through two inter-related mechanisms, which involve its catalytic capacity for ACh hydrolysis or its structural resemblance to gliotactin, neurologins, or related proteins.^{20,29}

C. PERICELLULAR CELL PASSAGE ACROSS BLOOD-BRAIN BARRIER STRUCTURES

One of the roles of the BBB likely involves protection of the brain from invasive bacteria, viruses, and fungi. However, when under BBB disruption any of these parasites invades the brain, the immune system must respond. This implies that under certain conditions, lymphocytes cross the BBB and reach those sites in the brain where their protective functions are needed. The existence of tight junctions between endothelial

cells in brain vasculature complicates this process and requires specific signaling to ensure the specificity of the pericellular transport. Also, the endothelial monolayer needs to be re-sealed once this transport has been completed. A recent study demonstrates that lymphocyte migration through brain endothelial cell monolayers involves signaling through endothelial I-CAM-1 via a Rho-dependent pathway, thus expanding the list of BBB-involved molecules.¹⁸

Mast cells represent another cell type that might penetrate the brain through BBB structures. This is of considerable importance because of the key role of mast cells in autoimmune demyelinating diseases.^{45–47} When interacting with myelin basic protein, mast cells degranulate to induce by exocytosis immediate demyelination.^{48,49} Under normal circumstances mast cells are located in leptomeninges and are concentrated along blood vessels, especially in dorsal thalamic nuclei.⁵⁰ Exposure to steroid hormones induces in mast cells massive secretion of, for example, histamine.⁵¹ Several of the other neuromodulators, neurotransmitters, and growth factors secreted by mast cells can alter BBB properties.⁵² Using confocal microscopy and vital dyes, Silverman et al.⁵³ very recently demonstrated rapid penetrance of mast cells through BBB structures into nests of glial processes. This transport may account for the rapid increases in mast cell populations after physiological manipulations.

D. CELL CULTURE, ORGAN SYSTEMS, AND IMAGING APPROACHES IN BLOOD-BRAIN BARRIER RESEARCH

The complexity and plasticity of BBB properties called for experimental dissection of the disruption process in both *in vitro* and *in vivo* conditions. Multiple cell and organ cultures, animal models, and measurement techniques have been developed, each of which addresses some of the issues involved. The development of research into BBB characteristics was initially approached in avian embryos, where transplanted endothelial quail cells invaded a developing chick chimera.⁵⁴ A simpler cell culture model of the BBB was developed by Rubin and co-workers.⁵⁵ More recently, an immortalized cell line created from vascular endothelial cells was used to develop another model of the BBB in co-cultures with glioma cells and was used to demonstrate nitric oxide-induced perturbations of these cells.⁵⁶ In another cell culture model, hypoxia was shown to increase the susceptibility to oxidative stress and intercellular permeability.⁵⁷

Measurements of Evans blue penetrance proved useful in analyzing BBB properties in animal models.⁵⁸ Recent technological breakthroughs in brain imaging now offer a previously impossible view into the integrity of human BBB under various conditions. Imaging the human brain is widely used in the clinical and research settings by two major methods: (1) computerized tomography (CT) and (2) magnetic resonance imaging (MRI). In both methods, standard techniques use contrast agents to enhance signals and unmask brain pathologies. Both approaches are therefore aimed at delineation of the site, duration, and extent of potential BBB disruption in CNS pathologies.

Iodine is the only heavy atom that possesses the chemical properties suitable for intravascular use in CT analyses. The currently available iodinated contrast agents are

nonionic, are highly hydrophilic, and have low osmolarity and minimal toxicity. The paramagnetic atom gadolinium (Gd), with seven unpaired electrons, forms a stable complex with diethylenetriamine pentaacetic acid (DTPA) and is the contrast material used in MRI. The DTPA complex is well tolerated and even minimal concentrations lead to marked shortening of its observed relaxation times and increase in signal intensity.

Both contrast agents normally do not cross the BBB. When injected intravenously, neither Gd nor DTPA affects BBB integrity. In contrast, intra-arterial injection of iodinated contrast agents was shown to disrupt normal BBB functioning due to both osmotic and chemotoxic effects (reviewed by Sage et al.).⁵⁹ This study, performed to determine the safety of currently used contrast agents, is of considerable significance for predicting the risks involved in low-level exposure to xenobiotics, as it indicates that even minor arterial elevation of the concentration of potentially harmful agents may by itself disrupt BBB functions.

In healthy individuals with normally functioning BBB, CT and MRI contrast agents cannot accumulate in the extracellular fluid of the brain parenchyma. Therefore, brain structures are not enhanced and remain relatively transparent in the imaging scans. In cases when the permeability of the BBB is increased because of a pathological process, the passage of iodinated agents (in CT) or paramagnetic DTPA complex (in MRI) leads to enhancement of signals. This occurs through X-ray attenuation, creating enhanced brain images in CT scans, or shortening of relaxation times, which results in sharper images in MRI. Such alterations in BBB penetrance may be local or massive, reflecting brain tumors, infectious disease, or cerebrovascular impairments. [Figure 4.3](#) demonstrates examples for such imaging analyses of human BBB disruption.

E. TRANSGENIC ENGINEERING MODELS FOR BLOOD-BRAIN BARRIER STUDIES

Genetic manipulations of the molecular mechanisms controlling BBB functioning yield new insights into the corresponding physiological or pathological circumstances and the dissection of their effects on BBB integrity. Several transgenic and knockout models have unraveled key elements involved in BBB functioning. These included several intentional as well as serendipitous studies. Mice with a genetic disruption in the *mdr1a* gene (multiple drug resistance), encoding the drug-transporting P-glycoprotein, which resides in the BBB, display up to 10-fold increases in their dexamethasone uptake into the brain.⁶⁰ The effect of cytokine overproduction on BBB functioning was checked in transgenic mice that overexpress interleukin 3 (IL3) or interleukin 6 (IL6). The IL3 transgenics develop progressive demyelination and infiltrated CNS lesions associated with BBB defects.⁶¹ The effects observed in IL6 transgenics were even more dramatic: extensive breakdown of the BBB was evident in the cerebellum of IL6-overproducing mice, followed by subsequent inflammation, reactive gliosis, axonal degeneration, and macrophage accumulation.⁶² CuZn-superoxide dismutase (SOD) was discovered to have protective effects against trauma-induced BBB disruption, in a model of mice that overexpress human SOD.⁶³ These

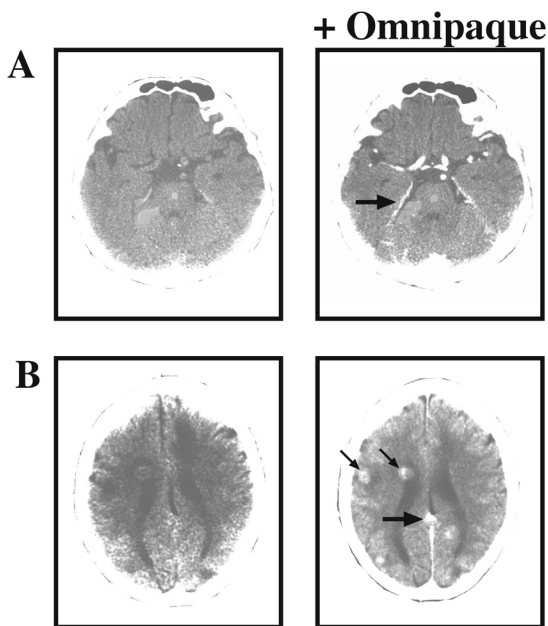


FIGURE 4.3 Blood-brain barrier disruption as revealed by computerized tomography: A. Normal CT scan, before and after injection of the enhancement material, Omnipaque™. Note the enhancement (hyperdense white) in brain arteries and vein sinuses (wide arrows) without penetration into the brain parenchyma. B. A case of multiple brain metastases in a 41-year-old female with a history of breast cancer. Following injection of Omnipaque™, several round white regions appear, reflecting focal BBB disruption in the regions harboring tumors (thin arrows).

mice were further found to display improved neurological recovery following traumatic brain injury,⁶⁴ which emphasizes the importance of oxidative stress in BBB disruption.

IV. MODULATORS OF BLOOD-BRAIN BARRIER FUNCTIONS AND THEIR INTERRELATIONSHIPS

Several considerations point to specific natural compounds and therapeutic agents as potential modulators of BBB functions. The rapid kinetics of BBB transport implicates post-translational control mechanisms in this process, simply because there is insufficient time to allow the slow transcription and translation processes to take place. The intimate relationship with vasculature properties points to vasoactive agents as potential modulators, and the necessity for penetrance of cells from the

immune system suggests the involvement of immunomodulators. These, and drug transporters, should all communicate with the complex array of molecules and cellular structures that together compose the BBB.

Kinase cascades, universal pathways for rapid signal transduction in numerous biological processes, were naturally investigated for their potential relevance to BBB functioning. To regulate BBB properties, such kinase cascades should be induced in the various cell types that comprise the BBB. This prediction is verified by the finding that a pituitary adenylyl cyclase-activating polypeptide is successfully transported across the BBB, preventing the ischemia-induced death of hippocampal neurons.⁶⁵ The next logical step in this kinase cascade is tyrosine phosphorylation, which may increase tight junction permeability.⁶⁶ That such phosphorylation is actively involved in regulating BBB transport processes is evident from findings of phosphorylation of endothelial Na-K-Cl co-transport protein under changes in tonicity and hormones.⁶⁷

A. NITRIC OXIDE AND VASOACTIVE AGENTS INVOLVEMENT

A primary mediator that was demonstrated to participate in meningitis-induced BBB disruption is nitric oxide (NO). NO is produced in response to exposure to bacterial endotoxins by the host endothelial cells. In an animal model of lipopolysaccharide-induced meningitis, BBB disruption and NO production sites in the brain co-localized,⁶⁸ and NO-synthase inhibitors reduced the meningeal-associated alterations in BBB permeability.⁶⁹ NO is likely produced by astrocytes, and it decreases endothelin-1 secretion by brain microvessel endothelial cells.^{70,71}

Agents that regulate vasoactive processes, such as bradykinin and angiotensin, were shown to effect biochemical opening of the BBB.⁷² In tissue culture experiments, such agents were further demonstrated to modulate tight junction structures in BBB endothelial cells co-cultured with astrocytes.³² In cultured A431 cells, the signaling cascade induced by these agents was shown to involve tyrosine phosphorylation and reorganization of the tight junction protein ZO-1, processes that are also mediated by epidermal growth factor-1 (EGF1).⁷³

B. IMMUNOMODULATORS AND MULTI-DRUG TRANSPORTERS

The passage of immunomodulators across the BBB has been the subject of much research activity, especially because of the known impairment in BBB functioning in autoimmune diseases such as multiple sclerosis (MS).⁷⁴ It is generally considered that basic mechanisms of brain inflammation involve massive, yet transient, disruption of BBB functioning that plays an important role in the acute episodes of several autoimmune diseases.⁷⁵ This may indicate that individuals with an inherited susceptibility to autoimmune responses are a high-risk group for low-level exposure to xenobiotics.

The mdra1 genomic disruption studies noted above have pointed to this multi-drug transporter protein as a rate-limiting factor in the bidirectional transport of drugs across the BBB. This finding explained the drug-induced neurotoxicity under chemotherapy and opens interesting options for developing BBB-regulating drugs.

Interestingly, the mouse *mdr1a* gene is also the earliest known endothelial cell differentiation marker during BBB development.⁷⁶

V. CONDITIONS INDUCING BLOOD-BRAIN BARRIER DISRUPTION

The pathophysiological origin of BBB impairments is of major clinical interest for several reasons. Impairment is dangerous, as it may cause extreme susceptibility to adverse drug responses, which would necessitate individualized drug dosage; however, breaching the barrier is sometimes useful to enable delivery of needed drugs to the brain (for example, under bacterial, fungal, or viral brain infection, or in cases of malignant brain tumors).

A. PATHOPHYSIOLOGICAL INDUCTION OF BLOOD-BRAIN BARRIER PENETRANCE

Several diseases are known which are associated with BBB disruption. These include brain tumor metastases; epilepsy and the more severe condition of status epilepticus; cerebrovascular disorders; autoimmune diseases such as multiple sclerosis; acute cerebral infarcts; meningeal carcinomatosis; and ischemic white matter lesions.^{77–84} Several genetic polymorphisms are known which increase the susceptibility to BBB disruption. These include polymorphisms in glutathione transferase, important for protection against oxidative stress,⁸⁵ and malfunctioning variants of serum BChE (e.g., “atypical” *BCHE*).⁸⁶ In particular, such mutations increase the risk of BBB disruption that is involved with exposure to anticholinesterases or to lead sulfate batteries, with subsequent increased risk for Parkinson’s disease.^{87,88}

Disruption of the BBB was reported in MS patients examined by contrast-enhanced MRI.⁸⁹ Blood-brain barrier disruption in MS patients was suggested to be the initial event in the development of the brain lesions that are characteristic of the advanced stages of this disease.⁹⁰ It correlates with the severity of symptoms, and an earlier age of the disease onset.⁹¹ Another pathological condition in which BBB breakdown was demonstrated is epilepsy. Disruption was demonstrated by computerized tomography (CT) in a patient following generalized seizure and by Evans blue penetration in a rat model of pentylenetetrazol-induced seizures.^{92,93}

Cerebrovascular pathologies are abundant in Alzheimer’s disease (AD) and are demonstrated by changes in the endothelium, amyloid depositions in the cerebral blood vessels, and disruption of the BBB.⁹⁴ A possible mechanism that underlies this phenomenon may be drawn from *in vitro* studies using a BBB model of a monolayer of vascular endothelial cells. Amyloid β -peptide, which deposits in plaques of AD patients, induced in these cells permeability to albumin and apoptotic cell death.⁹⁵ The potential clinical relevance of this finding was emphasized by intracarotid infusion of amyloid β -peptide, which resulted in BBB damage.⁹⁶

BBB disruption has also been reported for CNS infections, primarily in meningitis, where it is used as a differential diagnostic tool. In an acute cytokine-induced mouse model of meningitis, endothelial selectins (glycoproteins involved in cell adhesion) were demonstrated to contribute toward the disruption of the BBB.²¹

HIV-1 infection of the CNS was also suggested to involve a component of chronic brain tissue inflammation and BBB disruption, resulting in neuronal injury and death, which lead to cognitive, motor, and behavioral impairments.⁹⁷

B. BLOOD-BRAIN BARRIER DISRUPTION FOLLOWING ACUTE INSULTS

Blood-brain barrier disruption following ischemia is well documented. Carotid artery occlusion, followed by reperfusion resulted in transendothelial leakage of a marker horseradish peroxidase in the hippocampus.⁹⁸ Unilateral BBB permeabilization in the cortex and striatum subregions was demonstrated in a rabbit model of ischemic hemisphere using contrast-enhanced MRI.⁹⁹ Extreme temperature changes appear to be an additional factor influencing BBB integrity, as both cold and heat stress impair it. Cold injury in mice induced the penetrance of Evans blue, immediately following the injury, with reversal to the normal situation of intact BBB only 24 h post-injury.¹⁰⁰ In a milder model, infusion of hypothermic saline into the left carotid artery of rats resulted in disruption of the BBB in the left hemisphere, which did not occur with a normothermic solution.⁹³ The effects of hyperthermia, on the other hand, were checked in a model of local heating of a rat's head. BBB opening was observed from 6 h to 3 days post-injury.¹⁰¹ Similar results were noted in rats that were exposed to general heat stress (38°C) for 4 hours.¹⁰² The involvement of the NO pathway in this phenomenon¹⁰³ was indicated by the up-regulation of neuronal NO synthase activity, which coincided with BBB breakdown in distinct brain regions.¹⁰⁴

Traumatic brain injury, simulated by a model of closed head injury to mice, had also been shown to result in disruption of the BBB.¹⁰⁵ The temporal resolution of this disruption was monitored by MRI in rats subjected to closed head injury. Blood-brain barrier disruption appeared immediately after the impact, and declined gradually, until full reversal to control levels 30 min post-injury.¹⁰⁶ Opening of the BBB was similarly demonstrated in response to acute anticholinesterase exposure, however, low-level exposure has not yet been tested. BBB disruption under anticholinesterase exposure was proven to be seizure-dependent, as it could be blocked by the use of anticonvulsant agents.¹⁰⁷ The anticholinesterase effect on BBB ultrastructure did not impair endothelial tight junctions. Yet, an increased number of endothelial vesicles were observed, suggesting increased transcytosis as the mechanism involved.¹⁰⁸

C. PSYCHOLOGICAL AND PHYSICAL STRESSORS IMPAIR BLOOD-BRAIN BARRIER FUNCTIONING

Friedman et al.⁵⁸ have demonstrated enhanced brain penetrance under psychological stress of relatively small molecules such as anticholinesterases, as well as larger dye-protein complexes and DNA plasmids. This stress-induced process putatively explains some of the nervous system-associated sequelae reported by Gulf War veterans, who were exposed to unknown doses and combinations of potentially harmful xenobiotics, particularly anticholinesterases. The anticipated chemical warfare agents would have irreversibly blocked AChE. For prophylactic protection from these agents, Gulf War soldiers were administered pyridostigmine, a reversible carbamate

cholinesterase inhibitor which has a quaternary ammonium group that under normal circumstances prevents its transport across the BBB. Pyridostigmine is routinely used to treat peripheral neuromuscular junction deficiencies in myasthenia gravis patients,¹⁰⁹ and was shown to cause mild, primarily peripheral side effects during peacetime clinical tests in healthy volunteers.¹¹⁰ However, pyridostigmine use during the Gulf War caused a significant increase in reported CNS symptoms. Similarly, in animal experiments the dose of pyridostigmine required to block 50% of brain AChE in stressed mice was found to be 100-fold lower than that required in non-stressed mice, indicating a breakdown of the BBB.⁵⁸ More recently, heat stress, even extreme, reportedly failed to induce penetration of pyridostigmine into the brain of guinea pigs.¹¹¹ That BBB disruption depends on the status of neuronal activity in a brain-region specific manner was demonstrated in a study that compared stress-induced increase in BBB permeability in control and monosodium glutamate-treated rats, which reported increased BBB disruption in the hypothalamus and decreased in the brain stem, as compared with control animals.¹¹² Other reports demonstrate AChE overproduction in response to anticholinesterase exposure and to increases in interleukin 1.^{26,113,114} Therefore, a long-term outcome of low-level exposure to an anticholinesterase may be a hypocholinergic state, due to entry of the agent into the brain, and the induction of AChE expression and excessive AChE-R accumulation.

D. BLOOD-BRAIN BARRIER AS A COMPLEX TRAIT WITH GENETIC AND PHYSIOLOGICAL COMPONENTS: PROSPECTS

Blood-brain barrier properties are probably a complex genetic trait, in which the correlation of genotype to phenotype is difficult to dissect. Such a trait is termed complex or, if the phenotype is measured through a continuous variable, a quantitative trait. In certain cases, complex traits induce a susceptibility to a disease that depends upon environmental conditions. One example is the extreme adverse response to pyridostigmine treatment during the Gulf War that was found in a homozygous carrier of the “atypical” *BCHE* variant. The *BCHE*, with minor expression in the brain, gene encodes the butyrylcholinesterase (BChE, a.k.a. serum cholinesterase), that sequesters anticholinesterases such as pyridostigmine and prevents their reaction with AChE.⁸⁶ However, “atypical” BChE is incapable of binding pyridostigmine. Hence, homozygous carriers of this variant are at risk for extremely adverse responses, especially under the stress associated with war, to pyridostigmine doses in the circulation that would not affect individuals with normal *BCHE*. Therefore, the indirectly related *BCHE* gene becomes an important consideration for BBB disruption under the combination of stress and anticholinesterase exposure.

Loci in the genome that affect traits that may be quantified are called quantitative trait loci (QTL). Since many complex traits can be measured through a continuous variable (e.g., anxiety through cortisol measurements, Alzheimer’s disease through cognition tests), QTL may serve as a general term for complex traits. Although the identification of QTL in humans and in model organisms is in its infancy, the QTL paradigm fits BBB properties from many points of view. Thanks to the human genome project and the development of related technologies, the detection of BBB genes will soon be aided by high-density single nucleotide polymorphism

(SNP) marker maps, which will allow population-based studies of greater significance than the current family-based studies. The human genome project, soon to be completed, will further provide important information on the sequence of all of the relevant genes and the homologies of their protein products. However, even with all the genome sequenced, significant additional work to assess functionality will be required. Expression analysis of endothelial cell genes through high-density microchip arrays will provide an independent dimension to increase the efficiency and efficacy of the QTL aspects of BBB research. Comparative genetics will also bring essential insights for functional determination. Finally bioinformatics and theoretical developments emerging from it will allow integration of all the various aspects of this multidisciplinary field to achieve the appropriate results. All of these efforts together will be needed to shed more light on the issue of BBB properties.

VI. SUMMARY

A comprehensive survey of the recent literature reveals an increasingly complex collection of BBB constituents and functions. For example, under low-level exposure to anticholinesterases, BBB integrity may be compromised because of four interrelated processes:

1. Anticholinesterase blockade of the ACh hydrolytic capacity of AChE induces a short-term hypercholinergic activation in the brain, leading by a rapid yet long-term feedback process to accumulation of an excess of AChE-R and modification of the cholinergic status in a manner affecting BBB properties at a later phase.
2. Anticholinesterase-AChE interactions may modify the flexible 3-dimensional structure of the AChE protein and change its capacity to compete in protein-protein interactions with its non-neuronal signal transducing homologues (e.g., gliotactin), or its neuronal homologues, like neurexins. This could alter astrocyte or neuron properties that control BBB functioning.¹¹⁵
3. Anticholinesterase-induced AChE-R may differ from the normally present AChE-S in its ability to affect BBB integrity. Therefore, the combination of AChE's catalytic and structural properties with the anticholinesterase-induced feedback response would have a more dramatic effect on BBB properties than would any of these processes alone.
4. In individuals prone to adverse responses to stress stimuli, all of the above processes may be exacerbated in a complex manner, combining genetic and physiological mechanisms.

Blood-brain barrier disruption would affect brain functioning because of penetration of the brain by peripheral compounds that may modulate the properties of glia and neurons. Therefore, the consequences of breaching the BBB, even for a short duration and in a limited area, may persist for long periods and involve larger brain areas. In an era when breakthroughs in molecular genetics that allow a previously unimagined dissection of biological processes, and with technological developments

that provide a dynamic real-time view of brain functions, the BBB represents a medical and scientific frontier awaiting exploration.

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