recently, the existence of the BBB was demonstrated by intravenous administration of radioactive histamine into a mouse, followed 30 minutes later by sacrifice of the mouse and whole-body imaging. The histamine was found to label all tissues of the body except the CNS (the brain and spinal cord).[3] Researchers believe the BBB's function is to protect the CNS from toxic molecules (including xenobiotic toxins that an organism may ingest in food as well as endogenously-formed toxic molecules).

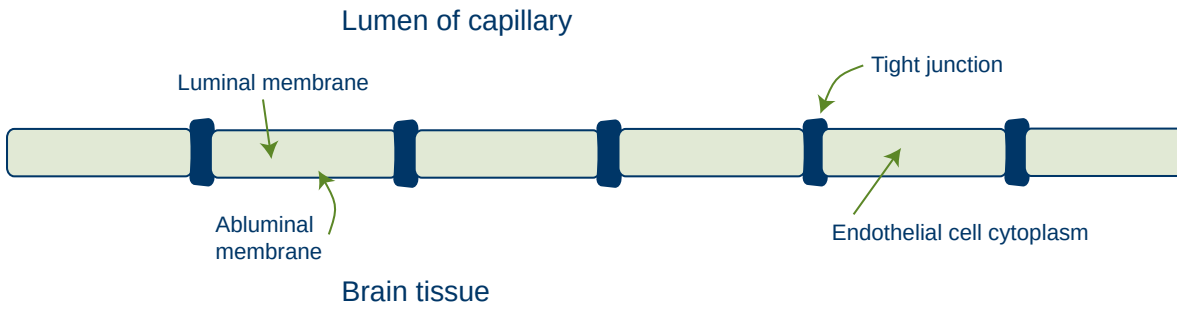
1.2. Dearth of Drugs for CNS Diseases with High Unmet Need

Most small-molecule drugs (98%) behave like histamine and fail to cross the BBB. No large-molecule drugs cross the BBB, with the exception of a few natural peptides and proteins such as insulin (which, as is discussed later, is transported across the BBB via specific receptors), and those specifically designed to do so.

Most small-molecule drugs (98%) behave like histamine and fail to cross the BBB.

As will be discussed later, currently successful small-molecule CNS drugs possess a set of molecular characteristics, including relatively high lipid solubility and a molecular weight under 400–500 daltons (Da). These molecular parameters enable the drugs to cross the BBB via passive diffusion. There is only a small subset of CNS diseases that respond to such current drugs: psychiatric diseases (depression, bipolar disorder, schizophrenia, and attention-deficit hyperactivity disorder [ADHD]), chronic pain, migraine, and epilepsy. Other CNS conditions are generally not treatable with current (mainly small-molecule) CNS drugs and usually have no or few treatment options. (Current small-molecule drugs for Alzheimer's disease [AD] do exist. However, they provide at best modest symptomatic relief for some patients and in no way affect the course of the disease.) These CNS conditions of high unmet medical need are listed in Table 1.1.

Figure 2.1. Structure of Brain Endothelium



Source: Haberman Associates

Like all capillaries, brain capillaries are surrounded by a type of extracellular matrix called a basement membrane. Ninety percent of the brain side of the capillaries is covered by astrocyte foot processes. Neither the basement membranes nor the astrocyte foot processes constitute diffusion barriers to molecules crossing the BBB. However, the endothelial cells themselves constitute a formidable barrier to diffusion, with the tight junctions being mainly responsible for the barrier nature of this endothelium. Moreover, the astrocytes appear to be involved in inducing and maintaining the barrier characteristics of brain endothelium.

The brain has a very high vascular density. The human brain contains over 100 billion capillaries, and the distance between capillaries is approximately 50 micrometers. Because this distance is so small, nearly every neuron in the human brain is perfused by its own capillary.[3] Therefore, if a drug is capable of readily crossing the BBB, it has ready access to its target neurons.

For a drug to cross the BBB, it must do so via passive diffusion or active transport. As discussed earlier, passive diffusion requires a drug molecule to possess certain molecular characteristics, especially small molecular size and lipid solubility. In active transport, small- or large-molecule

TT genotype in the same locus. Since this polymorphism fell within a large block of linkage disequilibrium (i.e., a genetic region with multiple polymorphisms that tend to be inherited together), this polymorphism may not be involved in causation of multidrug resistance but may be linked to the causative polymorphism.

One way to avoid multidrug resistance in epilepsy, as suggested by this study, is to develop drugs that are not P-gp substrates. For example, UCB's marketed drug Keppra (levetiracetam) is not a substrate for P-gp and shows relatively low levels of resistance in clinical practice.[15] UCB has a structurally related compound, brivaracetam (Rikelta), in Phase III clinical trials.

Discovery and Design of Drugs That Use Nutrient Transporters to Cross the BBB

Solute Carrier Transporters in Active Efflux from the BBB

Academic and drug discovery and development researchers have been focusing mainly on the role of P-gp and to a lesser extent other ABC transporters in active efflux from the brain across the BBB. However, drugs and other substances that enter the brain and are subject to efflux transport must cross both the abluminal and luminal membranes (and the intervening cytoplasm) of BBB endothelial cells to move from the brain to the blood. P-gp and other ABC transporters are present exclusively in the luminal membrane.

The transporters that are involved in transporting drugs or other small-molecule compounds across the abluminal membrane for transport by P-gp and/or other ABC efflux pumps are members of the solute carrier (SLC) transporter family.[3] These proteins, which include, for example, the organic anion transporter (OAT) and the OAT polypeptide subfamilies, are energy independent exchangers of numerous endogenous substances (e.g., amino acids, sugars, metal ions, peptides, steroids). (In contrast, ABC transporters are ATP energy dependent.) SLC transporters can also serve to transport drugs from the brain back into BBB endothelial cells. Researchers hypothesize that they act in concert with P-gp and/or other ABC family members that are present in the luminal membranes of endothelial cells to transport compounds out of the brain and back into the blood. This is illustrated in Figure 3.1.

cell by a process known as receptor-mediated endocytosis and may then be transported across the abluminal membrane of the endothelial cell. This whole process is called receptor-mediated transcytosis.[3,35,36]

Table 4.1 lists examples of receptors that are currently exploited for development of large-molecule drugs that cross the BBB. This list was taken from the claims section of a recent patent application submitted by William Pardridge and his colleagues.[37] It would be conceivable to use other RMT systems. But the systems listed in Table 4.1 are well studied, which probably accounts for their selection. Of the natural biomolecules that are transported by these receptors, insulin, insulin-like growth factor (IGF), and leptin are protein hormones or growth factors, transferrin functions to transport iron into the brain, and LDL functions to transport lipids such as cholesterol into the brain.

Table 4.1. Selected Receptor-Mediated Transport Systems Used for Development of Large-Molecule Drugs That Can Cross the BBB

Insulin receptor
Transferrin receptor
Leptin receptor
Insulin-like growth factor receptor
Low-density lipoprotein (LDL) receptor

Source: Haberman Associates

Molecular Trojan Horses

In designing large-molecule drugs that exploit RMT systems to enter the brain, researchers use what are called "molecular Trojan horses" (MTHs). These are biomolecules that are either peptide or protein ligands for the target BBB receptors (e.g., receptor-binding sequences from insulin), or they are MAbs that are specific for the target receptors. In designing protein drugs that can transverse the BBB, researchers typically construct fusion proteins between the desired therapeutic protein and an MTH.

The Pardridge laboratory and ArmaGen have published several recent scientific reports on experimental versions of potential protein and nucleic-acid drugs based on use of MTHs to cross the BBB. One such drug is a fusion protein between a chimeric MAb to the human insulin receptor (HIR) and the neurotrophin (i.e., a member of a class of growth factors that promotes the survival of neurons) brain-derived

The CORVUS system can be used in animal models to validate gene targets of siRNAs and other drug candidates in the CNS and to validate potential siRNA therapeutics themselves. The development of siRNA-CORVUS complexes for human therapies will require additional work (for example, on chemically modified siRNAs) and collaboration with a biotechnology company (such as one of the leading specialty companies now developing siRNA therapeutics).

4.4. Need for Basic Research to Find Additional Receptors That Can Be Exploited to Get Large-Molecule Drugs across the BBB

Many BBB researchers would like to have better receptors to exploit to get large molecules across the BBB via RMT. Receptors that have been commonly studied as targets for RMT, such as the insulin and transferrin receptors, are not brain specific but are also expressed in other tissues. Thus large-molecule drugs that incorporate MTHs that target these receptors will be delivered not only to the brain but to other tissues as well. This can result in the need for increased doses of these drugs, with added expense and the potential for adverse effects. Moreover, the commonly studied receptors are involved with transport of essential nutrients or signaling molecules into the brain. Drugs carrying MTHs that target these receptors would be expected to compete with transport of these endogenous substances. As discussed earlier, drugs that incorporate MTHs that target the DTR would not compete with endogenous substances. However, the DTR is not brain specific but is found in other tissues as well.

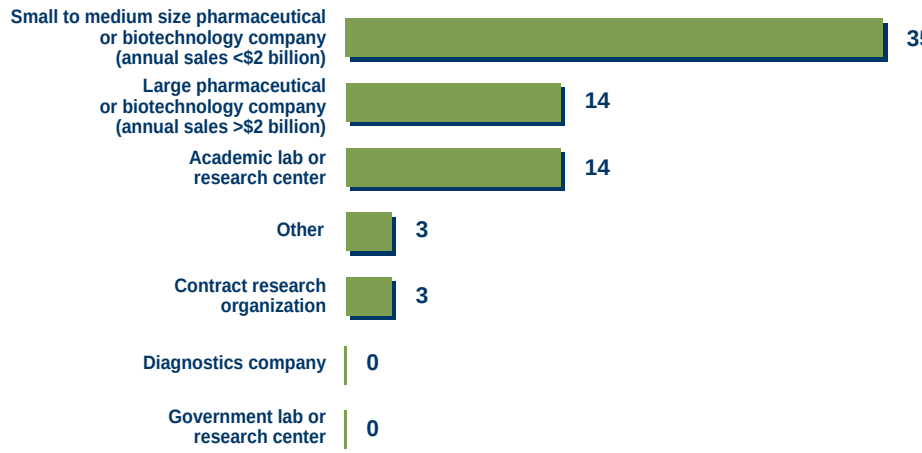
Some BBB researchers are therefore applying such technologies as genomics and proteomics to the discovery of new transporters. This research is in an early stage. ArmaGen's BBB genomics program, which is aimed at discovering novel transporters, was discussed earlier. This program is an extension of the Pardridge group's transporter discovery programs. Other academic researchers have also been conducting genomics and proteomics research aimed at discovery of novel BBB transporters.

5.1. Blood-Brain Barrier Survey Results

Cambridge Healthtech Institute, in conjunction with this report, ran a Blood-Brain Barrier Survey, the results of which are included in this report as an Appendix. The 69 survey respondents represent a range of companies involved in CNS research and drug discovery/development. Half of the respondents are in small to medium-sized pharmaceutical or biotechnology companies, 20% are in large pharmaceutical or biotechnology companies, and 20% are academic researchers (Figure 5.1).

Figure 5.1. Respondents by Organization Type

Please classify your organization.



n = 69

Source: Insight Pharma Reports Blood-Brain Barrier Survey—January 2008

According to the survey, the respondents have a significant involvement in the areas of CNS drug discovery and development that represent the greatest unmet medical needs and are in most cases not treatable with current drugs. (A few of the respondents [8%] are involved in devices or nutritional products, which are beyond the scope of this report.) Of respondents who answered the question of what areas they are involved in (Figure 5.2), 55% cite involvement in neurodegenerative disease and 25% in brain cancers. Thirty-four percent cite involvement in pain and 21% in psychiatric diseases, which have very significant areas of unmet need even though there are some marketed drugs that treat these conditions.

PG: The combination of having a good validated target and a drug that you know really works on it. CNS diseases are always difficult to develop drugs for—delivery is still a big issue. The pharmacokinetics, especially, as well as the route of administration of existing delivery technologies, are a big hurdle for large markets, because most CNS treatments are based on large complexes that cannot be administered orally. I think that has more to do with a marketing problem, or a patient-uptake problem, than with the technology problem of the BBB itself.

CHI: Are you using imaging methods to evaluate brain penetration and target occupancy of drugs?

PG: We use a lot of fluorescence microscopy for tissue slides. We do some whole-body imaging, but not much. I prefer to be able to see, inside the tissue, how the targeting complexes end up with the determined pharmacokinetic profile, followed by disease efficacy studies. The resolution of whole-body imaging is not yet strong enough to provide a really good mechanistic view of what your delivery is doing other than a gross estimation of tissue retention.

William M. Pardridge, MD

Chairman & Chief Scientific Officer, ArmaGen Technologies, Santa Monica, CA

Cambridge Healthtech Institute: Please describe ArmaGen's CNS drug discovery and development program.

WP: We are focused on biopharmaceuticals, recombinant proteins, and monoclonal antibodies as new therapeutics for the brain. These large molecules do not cross the blood-brain barrier. Hence, they cannot be developed as new drugs for the brain without first solving the blood-brain barrier problem.

Our approach to solving the blood-brain barrier problem is to reengineer a protein- or monoclonal antibody-based pharmaceutical as a bi-functional fusion protein. We fuse the protein therapeutic to our molecular Trojan horse (e.g., a genetically engineered chimeric or humanized monoclonal antibody), which crosses the blood-brain barrier on the endogenous insulin receptor. We have developed a number of products with this technology and have published numerous articles describing various fusion constructs.