DRUG TARGETING

Breaking Down Barriers

The brain is guarded by a nearly impenetrable cellular barrier, but researchers are looking for ways to blast through this wall of defense—for the brain's own good

Any attempt to develop a new therapy for brain disease must deal with a vexing impediment. Neuroscience textbooks bury it in the appendix, Ph.D. programs give it a cursory treatment, and pharmaceutical companies have tried to ignore it. But the blood-brain barrier is a stubbornly real obstacle for potential drugs against many disorders of the central nervous system (CNS).

The barrier is built into the densely packed capillaries that feed the brain. The endothelial cells lining these tiny tubes are cemented together by junctions so tight that even ions have a hard time squeezing through. Transport proteins in the endothelial membrane act like bouncers, ushering sugars, amino acids, and other important players into the brain and making sure any undesirables that manage to slip in are promptly shown the way back out. Unlike the far leakier capillaries elsewhere in the body, brain capillaries form a superfine filter that protects the brain from any riffraff, such as toxins and viruses, circulating in the blood.

But this barrier also keeps out many would-be therapeutic agents. Molecules can cross the barrier only if they are escorted by the choosy transport proteins or if they are small enough and lipid-friendly enough to pass through one side of the endothelial cells and out the other. As a general rule, that means that only lipophilic molecules smaller than about 500 daltons can cross from blood to brain. But many drugs that show promise in animal studies for treating CNS disorders are much bigger—commonly weighing in at tens or hundreds of kilodaltons.

Now a small but growing community of predominantly academic researchers is studying the blood-brain barrier with an eye to improving drug delivery to the brain. Their research ranges from trying to better understand the basic biology to creating molecular Trojan horses that sneak anything from neural growth factors to therapeutic genes into the brain.

"It's an area that's been underresearched, and its significance hasn't been recognized," says David Begley, a neurophysiologist at King's College London. "There must be millions of good drugs sitting in pharmaceutical company stores simply because they can't be delivered," he adds. Drug companies have long been reluctant to tinker with the blood-brain barrier, but a recent surge in academic research on the subject might show them the way.

Traditionally, pharmaceutical companies have focused their efforts to develop CNS drugs on small molecules. It's worked for a few disorders, says William Pardridge, a blood-brain barrier researcher at the University of California, Los Angeles. He rattles them off: epilepsy, chronic pain, mood disorders, and schizophrenia. But a host of other disorders have proved far more recalcitrant, including Alzheimer's disease, Huntington's disease, stroke, and brain cancer.

Pardridge believes that the small-molecule approach to CNS drug development is fundamentally flawed. "It's based on two misconceptions: There's the misconception that you can come up with a small molecule for any disease and the misconception that all small molecules cross the blood-brain barrier."

In an editorial this year in *Drug Discovery Today*, Pardridge argued that the industry's fixation on small molecules is the reason there are relatively few CNS drugs on the market. The global market for CNS drugs, at $33 billion in 1998, was roughly half that of the global market for cardiovascular drugs, even though in the United States nearly twice as many people suffer from CNS disorders as from cardiovascular disease. The reason for this lopsidedness, he claims, is that more than 98% of all potential CNS drugs don't cross the blood-brain barrier.

Sugary solution

The first clue that a barrier separated the brain's blood supply from the body's came in the late 1800s. German bacteriologist Paul Ehrlich found that dyes injected into the body stained every organ except the brain. A student of his, Edwin Goldmann, later hit on the other side of the story: Dye injected into the cerebrospinal fluid stained the brain but not other organs. The anatomical basis of the barrier remained a mystery until the late 1960s, when electron microscope studies revealed the tight junctions between the endothelial cells lining the brain's capillaries.

If not for the blood-brain barrier, the capillaries—the branches of which meander more than 600 kilometers through the human brain—would provide a fantastic drug-delivery system, designed as they are to bring oxygen and nutrients virtually to the doorstep of every cell in the brain.

One of the earliest techniques to circumvent the barrier for therapeutic purposes—and the first to be used in humans, more than 20 years ago—was developed by neuroscientist Stanley Rapaport and neurosurgeon Edward Neuwelt, then at the National Institutes of Health in Bethesda, Maryland. The idea behind the approach is to break the barrier down—temporarily—by injecting a sugar solution into arteries in the neck. The resulting high sugar concentration in brain capillaries sucks water out of the

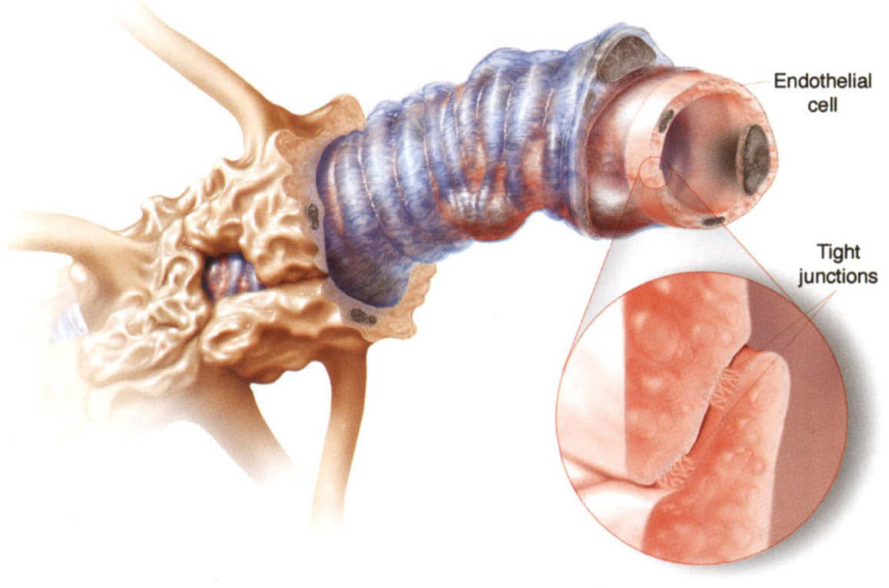
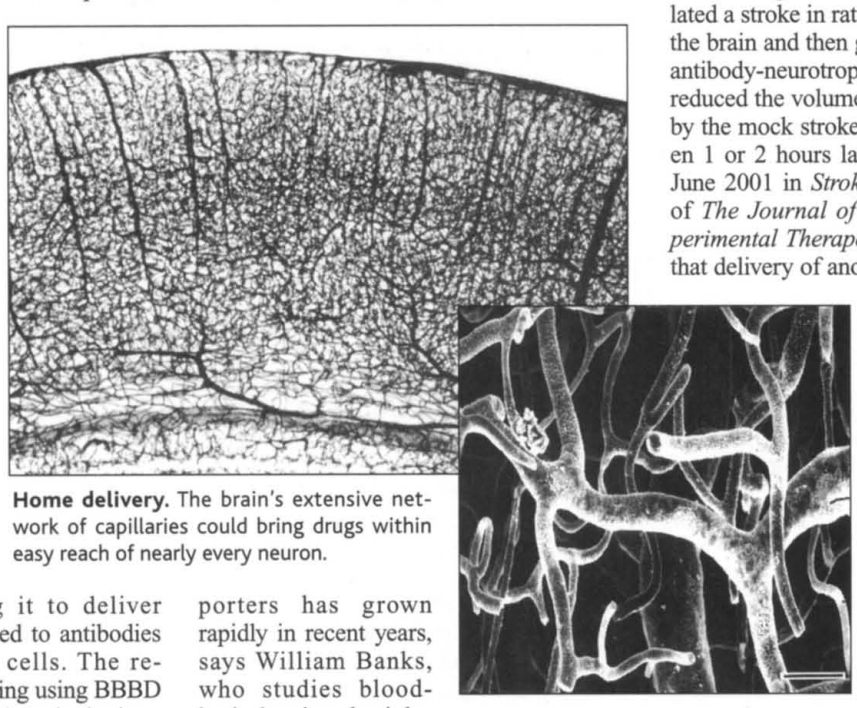


ILLUSTRATION: C. SLAYDEN

Tight security. Brain capillaries are surrounded by different types of cells, but the real barriers are the tight junctions between endothelial cells in the capillary lining.

endothelial cells, shrinking them and opening gaps between cells. In current practice, the effect lasts 20 to 30 minutes, during which time drugs that wouldn't normally cross the barrier have a free pass.

Neuwelt, now at Oregon Health & Science University in Portland, still uses the technique, called blood-brain barrier disruption (BBBD), primarily to deliver chemotherapy drugs to brain cancer patients. Animal studies suggest that the method increases drug delivery to the brain 10- to 100-fold over injection into the neck arteries without the sugar solution. BBBD has been used to treat hundreds of patients and is currently in phase I and II clinical trials at nine U.S. institutions, as well as one in Canada and one in Israel. The National Cancer Institute has twice turned down proposals for a controlled phase III trial of the technique, however. Designing a phase III BBBD trial is exceptionally tough, Neuwelt says, because appropriate brain cancer patients are relatively rare and because only a few institutes are qualified in the technique.



Home delivery. The brain's extensive network of capillaries could bring drugs within easy reach of nearly every neuron.

In the laboratory, Neuwelt's team is working on other applications for BBBD, including using it to deliver chemotherapy drugs attached to antibodies that home in on cancer cells. The researchers are also investigating using BBBD to shuttle therapeutic genes into the brain—either to make tumor cells more susceptible to chemotherapy or to replace defective genes in neurodegenerative disorders.

But others see limitations to the BBBD approach. For one, it is invasive and requires considerable expertise, says Raymond Bartus, senior vice president of life sciences research and development at Alkermes, a biotech company in Cambridge, Massachusetts. Bartus and colleagues pioneered an alternative method for opening the barrier using intravenous injections of a compound called RMP7, which binds to receptors on the surface of endothelial cells and kicks off a biochemical cascade that loosens the tight junctions.

Although the technique showed promise in early clinical trials, Bartus says Alkermes has more or less given up on the project. The method would be useful only for a small number of patients, such as those suffering from brain cancer, and thus wasn't worth continued investment, he says. BBBD has a similarly limited scope, he points out.

Both procedures are unacceptably risky, according to Pardridge. Disrupting the barrier—even for brief periods—leaves the brain vulnerable to infection and damage from toxins, he explains. Even some substances that circulate harmlessly through the peripheral bloodstream, such as the protein albumin, can have deleterious effects if they get into the brain, he says.

Escort service

Pardridge and others are investigating another option. They hope to take advantage of the transporter proteins in the endothelial membrane. The number of known transporters has grown rapidly in recent years, says William Banks, who studies blood-brain barrier physiology at Saint Louis University in Missouri. And that has changed the way people view the barrier: "It's not only a wall but a gate."

Pardridge and his team are looking for ways to fool the gatekeepers. Many of their initial studies have focused on a receptor that binds transferrin, a protein critical for transporting iron. When an iron-laden transferrin molecule on the blood side of a capillary binds to the receptor, a dimple forms in the endothelial cell membrane and the transferrin gets sucked into the cell inside a tiny bubble of membrane. This bubble makes its way to the other side of the pancake-shaped cell (a mere 300 nanometers away), where it fuses with the membrane and spits the transferrin out into the brain to deliver its iron.

In studies with rats, Pardridge's team has managed to trick transferrin receptors into ferrying an unusual cargo across the barrier: molecules called neurotrophins that protect neurons and encourage them to grow.

One day, says Pardridge, such a strategy could protect the brain from damage caused by strokes. Many of the few-dozen known neurotrophins have been shown to reduce stroke damage in animals, but the molecules are too big to pass the barrier on their own. The only way to deliver them into lab animals' brains has been to drill a hole in the skull and inject the compounds—a procedure widely judged too invasive and impractical for humans.

Pardridge and colleagues tested a new hybrid transport method: They attached a neurotrophin called brain-derived neurotrophic factor to antibodies that attach to the transferrin receptor and prompt endothelial cells to engulf them. The researchers simulated a stroke in rats by blocking an artery to the brain and then gave the rats a shot of the antibody-neurotrophin combo. The injection reduced the volume of cerebral cortex killed by the mock stroke by up to 70% when given 1 or 2 hours later, the team reported in June 2001 in *Stroke*. And in the May issue of *The Journal of Pharmacology and Experimental Therapeutics*, the team reported that delivery of another neurotrophin via the same antibody has an even more powerful protective effect.

The team hopes to use this method to ferry other cargo across the blood-brain barrier, including material for gene therapy. Earlier efforts, using viruses to deliver genes into the human brain, spurred potentially deadly immune responses. But the researchers can sneak genes across, sans virus, by enclosing genetic material in membrane bubbles coated with antibodies tuned to the transferrin receptor. The approach works with a single IV injection, and in the July issue of *Molecular Therapy*, Pardridge's team reported doubling the lifespan of mice with brain tumors by using the technique. In that case, the team delivered so-called antisense messenger RNA to block expression of the gene for epidermal growth factor, which is often hyperactive in especially nasty brain tumors.

The transferrin receptor isn't the only baggage handler pulling molecules into the brain. Pardridge has also developed a drug-delivery system based on an insulin receptor, and tests in monkeys suggest it is 10 times more effective than the transferrin receptor system, he says. And he has begun a blood-brain barrier genomics project to identify more transporters. His team is isolating the capillary endothelium from rat and human brain tissue, identifying genes specific to the blood-brain barrier, and fish-

CREDITS: (TOP TO BOTTOM) W. PARDRIDGE/UCLA; H. DUVERNOY *ET AL.*, *BRAIN RESEARCH BULLETIN* 11, 419 (1983)

ing through them for transporters. So far, about 15 transporters are known; Pardridge estimates there are at least 50, all told.

The advantage of the transporter approach, many researchers say, is its flexibility. "You could get almost anything across in principle," says Tom Davis, a pharmacologist at the University of Arizona in Tucson. But while Davis agrees the approach is promising, he cautions that therapies based on it would probably be expensive, given the "exotic chemistry" involved.

Another stumbling block, argues Jörg Kreuter, a pharmaceutical scientist at Johann Wolfgang Goethe University in Frankfurt, Germany, is that only limited amounts of drug can be delivered this way. The traffic is limited by the number and carrying capacity of the transporters, as well as by the number of drug molecules that can attach to each antibody, he says. "You have a very big engine and very few passengers," he says. That might be fine for neurotrophins, which are effective in low doses, says Begley, but it might not work as well in cases where large quantities of the drug are needed.

Nanotricks

Looking for another entryway into the brain, Kreuter, Begley, and others have found that polymer nanoparticles can be used to sneak drugs across the barrier. The tangled balls of polymer have grooves and pockets that can be stuffed with drugs. Nanoparticles are typically hundreds of nanometers across—big enough to transport almost any molecule, even strips of DNA, Kreuter says. He has used the particles to deliver the chemotherapy drug doxorubicin, for instance, to rats with brain tumors. Nanoparticle drug injections cured up to 40% of the rats, which normally die in 10 to 20 days. Six months later, the tumors were gone—only a bit of swelling and scar tissue remained.

How nanoparticles cross the barrier is a matter of some debate. Pardridge argues that the technique is something like BBBD in disguise. Detergents added to keep the particles from clumping together have been shown to disrupt the barrier, he says, either by loosening the tight junctions between the endothelial cells or by dissolving their membranes. But Kreuter and others argue that they have good evidence that the particles are sucked up by the cells without disrupting the barrier, although they acknowledge the process isn't fully understood. "We don't know everything about the mechanism, but we can measure the pharmacological effects," Kreuter says.

Worth the investment?

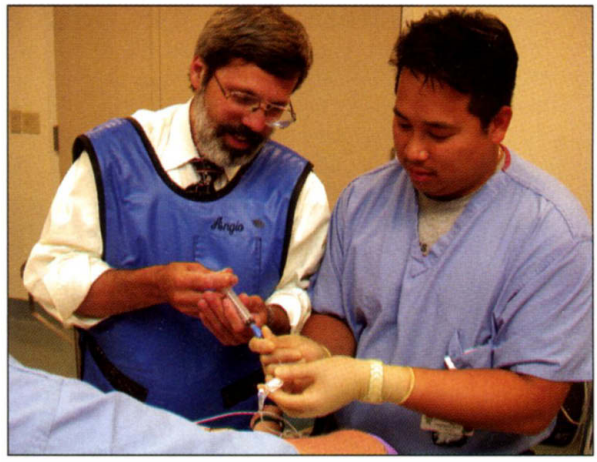
Many researchers say blood-brain barrier research is just gathering momentum. A recent Gordon conference in Tilton, New Hampshire, attracted almost everyone working in the field—150 researchers from 11 countries. It wasn't a huge crowd, as far as biomedical meetings go, but was larger than similar gatherings in past years. "That's been the problem in blood-brain barrier research—the community is small but the problem is big," says Tom Jacobs, scientific leader of the Neural Environment Cluster, an advisory group at the National Institute of Neurological Disorders and Stroke (NINDS) in Bethesda, Maryland.

Although enthusiasm runs high among those in the field, many researchers are frustrated that the pharmaceutical industry hasn't expressed much interest in their work—and isn't doing much of its own. "I'm aware of no company that has a focused R&D group for developing technology for getting drugs across the blood-brain barrier," says Davis. Others, including some who work in industry, agree. "It's not only surprising, it's not cost effective," Davis says. "You spend tremendous amounts of money developing drugs. You should also spend money on technology to deliver the drugs to the organ of choice."

This neglect in part reflects a historic bias toward developing small-molecule therapeutics. "Industry has more expertise with small molecules," says Andreas Reichel, a pharmacologist at Schering in Berlin. "People are more comfortable working with what they know."

But Reichel says awareness of the blood-brain barrier is growing in industry. Companies now screen compounds earlier in the drug development process to see if they will cross the barrier. In vitro and in silico models of the barrier have helped avert many dead ends, he says.

Still, for the most part, industry has taken a wait-and-see attitude toward delivery systems for large molecules. "If there were a clear high-value target that was really valuable for patients, we'd make the investment," says Jim Baxter, executive director of pharmacokinetics dynamics and metabolism at Pfizer's Groton, Connecticut, research facility. The cost of drug development is so high, he says, that putting additional money into sophisticated delivery systems for large molecules is too great a risk. "Until we saw that it's the only way, we'd rather try the more precedented [small-molecule] methods."



Strong medicine. Edward Neuwelt (left) injects a sugar solution that briefly opens the blood-brain barrier.

Pardridge scoffs at this logic. "It's completely ludicrous," he says: A neuroprotective drug for the treatment of stroke would be a "high-value drug." For every disorder that hasn't responded to small-molecule drugs, he says, "they'd have a blockbuster drug."

But even he acknowledges that more research is needed to get large-molecule drug-delivery systems into the clinic. For now, everyone agrees most of that work will have to come from the academic side. Although some researchers complain that getting funding for blood-brain barrier research has been tough, the situation may be improving, at least in the United States. An initiative to be announced this fall by NINDS will include money for blood-brain barrier research, says Jacobs. He says researchers in this field have made a compelling case that understanding the barrier is critically important for understanding and treating CNS disease.

–GREG MILLER

Blue genes. Bubbles coated with antibodies for the transferrin receptor deliver genes to the entire brain (*left*), unlike ones coated with nonspecific antibodies (*right*).

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