The dam breaks: disruption of the blood-brain barrier in diabetes mellitus

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At the end of the century before the last, a young graduate student performed an experiment that would forever change the way we look at the microvasculature of the brain. He injected basic dyes into the blood and noted that nearly every tissue of the body was colored by the dye except for the central nervous system (CNS). This experiment and the observation that bile salts could cause seizures when injected into the brain but not intravenously were the seminal 19th century observations giving rise to the concept of a blood-brain barrier (BBB).

The young graduate student, Paul Ehrlich, would get many things right in his career, winning a Nobel prize in 1908 for his work on antibiotics. But the BBB he got wrong. Ehrlich thought the brain did not stain because the dye was not taken up by brain tissue, not because a barrier prevented the dye from reaching the brain. Indeed, that a BBB really existed and that it resided at the level of the capillary bed and the choroid plexus were not finally established until the late 1960s. Elegant physiological experiments by Davson and Segal (10) and electron microscopy studies by Reese and Karnovsky (24) demonstrated the basis of the BBB: the capillary bed of adult mammals is modified to exclude the production of a plasma ultrafiltrate. The major modifications include a greatly decreased rate of pinocytosis, a lack of intracellular pores and fenestrae, and obliteration of the intercellular space between brain endothelial cells by tight junctions that essentially “cement” apposing endothelial cells together.

The lack of production of an ultrafiltrate means that circulating proteins such as albumin do not exude from blood into brain. This is the basis for Ehrlich’s 120-year-old observation: basic dyes bind to albumin so tightly that they are a visible proxy for plasma protein passage across the BBB. On the one hand, this is good news for the brain: circulating rifriff is kept out of a pristine CNS by the BBB; on the other hand, how is the brain to be nourished without the production of a plasma ultrafiltrate? The realization that the BBB also addresses this latter need of the CNS is the basis for the modern view of the role of the BBB in CNS function and disease. In addition to the role of barrier, the BBB also decides what shall be imported into and what shall be exported from the CNS. Hence, in addition to being a barrier, the BBB has nutritional, communication, and homeostatic roles (1, 4, 5, 9, 11, 18, 25, 26).

Old concept meets ancient disease in the article of Huber et al. (13) in this issue of American Journal of Physiology-Heart and Circulatory Physiology. Diabetes mellitus was known to the ancients, but modern medicine still struggles with effectively treating it. Microvascular and macrovascular disease affecting peripheral tissues, including the peripheral nervous system, macrovascular disease affecting the CNS, and microvascular disease at the retinal-blood barrier are well-known hallmarks of advanced diabetes, regardless of whether the diabetes is caused by insulinopenia or insulin resistance. Given all this, it could be erroneously assumed that study of the brain microvasculature (also known as the BBB) in diabetes is a huge field. In fact, only a handful of studies have been done. A search engine pairing of “blood-brain barrier” and “diabetes mellitus” finds 34 articles; pairing “blood-brain barrier” with “multiple sclerosis” or “HIV-1” finds 394 and 100 articles, respectively. This lack of work, in part, has been because it was thought that brain microvasculature and brain function were both preserved in diabetes. Slowly, both of these perceptions have changed.

It is now clear that diabetics have alterations in cognitive function (16). There have also been hints that BBB function is changed, at least in insulinopenic animal models of diabetes. Morphological alterations of the capillary bed and alterations of transporter function have been noted (12, 19). The latter includes an alteration in how insulin itself is transported across the BBB as well as the ability of insulin to control leptin transport (3, 17). However, the work to date simply illustrates that the area of BBB function in diabetes is ripe for study and that even fundamental questions are unanswered.

Huber et al.’s (13) paper begins to fill this void. It has an elegant, simple approach to studying one of the most fundamental aspects of the BBB: its barrier function. The work clearly shows that barrier function fails with increasing duration of diabetes. Huber et al. (13) take advantage of a classic but seldom-used approach to the study of barrier function, that of using various sized molecular weight markers to measure leakiness (20, 28). As the ultrastructural work of Reese and Karnovsky (24) showed, BBB impermeability is imposed by tight junctions that block leakage between endothelial cells (the paracellular pathway) and by other modifications (a decreased number of intracellular pores and fenestrae and decreased rate of pinocytosis) that limit leakage across a cell (the transcytotic pathway). Traditionally, the transcellular pathway is considered to be molecular weight independent (because the 50- to 100-nm-diameter vesicles are so much larger than even the largest marker, albumin, with a 7-nm diameter), whereas the paracellular pathway can display molecular weight dependence as disrupted tight junctions begin to close. Traditional studies show that most BBB disruption is predominantly transcytotic, not paracellular (20). However, recent work has suggested that tight junctions are physiologically regulated, so that the paracellular pathway may have some flux, partially opening under unknown, physiological influences.

Huber et al.’s (13) work clearly shows a time-dependent, molecular weight-dependent failure of barrier function. At first, it may seem that the molecular weights of sucrose, inulin, and albumin at 342, 5,000, and 65,000 Da are biased toward the low end. But leakiness relates to volume of the molecules, and this is better approximated by the square root of the molecular weight than the molecular weight itself. The square root values of 18.4, 70, and 255 approximate a geometric progression based on 4 and so are ideally suited to assessment of molecular dependent leakage. The molecular weight dependent-pattern suggests that paracellular pathways predominate in failure of the diabetic BBB.
This is consistent with work showing altered tight junction proteins in diabetic animals (8). This, in turn, raises a fundamental question: is such failure a nonspecific response in a very sick animal or does it give a clue to tight junction regulation? Insulin, glucose, and triglycerides each have direct effects on brain endothelial cell function (2, 6, 7, 21); could one of them be important in tight junction regulation also?

One of the most important observations of the paper by Huber et al. (13) is that not all regions of the BBB are equally susceptible to disruption. Midbrain was the first to show an enhanced leakage to sucrose after 28 days of diabetes, followed by hippocampus, cortex, and basal ganglia. Other regions showed no disruption even after 90 days of diabetes. Such regional variation is consistent with work showing complex temporal and regional alterations in BBB transporters after insults to the CNS (23, 27). Such variation illustrates that the BBB is not a simple monolitic barrier but a complex regulatory interface that is itself regulated by global and regional factors. Future work will need to relate regional BBB changes to altered functions of the CNS.

The work by Huber et al. (13) gives us a firm basis for future work on the diabetic BBB. As such, the work opens up the field to a series of tantalizing questions. For example, healthy aging does not lead to BBB disruption, but the aged BBB is more susceptible to disruption (22). Does diabetes mellitus also increase susceptibility of the brain microvasculature to injury from hypertension and stroke? If so, how much does BBB susceptibility contribute to the worse outcomes diabetics have with hypertension or after stroke? Is the BBB altered in insulin-resistant diabetes? Well over 90% of humans with diabetes are insulin resistant, not insulin deficient, but almost all basic research is done in insulinopenic models. The explosion of work in feeding has produced many rodent models of obesity, most of which have insulin resistance. It is already established that insulin transport itself is impaired in these models (14, 15). Finally, given that the BBB is more than just a barrier, are its other functions altered as well? What effect does diabetes have on the nutritional, homeostatic, communicative, secretory, and enzymatic functions of the BBB? Is immune cell trafficking into the CNS, an event that is highly regulated by an interplay between the immune and brain endothelial cells, altered? Some of the early work cited above would suggest that these “higher” functions of the BBB are indeed altered. The third century of BBB research will have to revisit tight junction regulation also?

REFERENCES