Recent Advances in Understanding Brain Capillary Function

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The endothelial cells in brain capillaries form a blood-brain barrier which limits and controls the movements of solutes between blood and brain. These cells contain continuous tight junctions and exhibit a low rate of pinocytosis, resulting in formation of a permeability barrier to macromolecules and many polar compounds. However, brain capillary endothelial cells also contain specialized transport systems that facilitate blood-to-brain transfer of some solutes and actively pump other solutes from brain to blood. Several investigators have developed methods to isolate microvessels from brain or to grow brain capillary endothelial cells in tissue culture. This review summarizes progress made with these model systems and discusses their usefulness in increasing our knowledge of brain capillary function.

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The endothelial cells in brain capillaries are sealed together by continuous, tight junctions and contain few pinocytotic vesicles. These properties limit the free exchange of polar molecules across the capillary wall and are responsible for formation of a blood-brain barrier [70]. Since alterations in this barrier can cause brain edema and hemorrhage, advances in understanding capillary function may lead to new therapies for acute brain disorders. Furthermore, the ability to selectively bypass the normal barrier should prove useful in treatment of certain infections, neoplasms, and enzyme deficiencies [56, 57]. During the past decade we and others have developed methods to isolate and study microvessels from brain and to grow the endothelial cells in tissue culture. These new techniques permit a more detailed investigation of the biochemical and cellular properties of brain capillaries. In this review we present selected advances in the field and attempt to correlate them to pathophysiology and therapeutics. A brain capillary model which incorporates these ideas is presented and will serve as the focus for this review. Two books provide a more comprehensive review of the blood-brain barrier [13, 69].

Properties of Brain Capillaries

Tight Junctions

The junctional contacts between endothelial cells in brain capillaries differ in two respects from those in the capillaries of other organs. First, the membrane fusion is continuous and leaves no gap between adjacent endothelial cells. A complete barrier of plasma membrane is thus created, separating the luminal contents of the blood from the interstitial fluid of the brain. Second, the structure of the intercellular fusion is extremely complex. Tight junction structure and permeability have been most thoroughly studied in epithelial tissues such as the intestinal mucosa, renal tubule, and bladder epithelium, and the tight junctions in brain capillaries appear similar. In each case, transmission electron microscopy reveals fusion of the external leaflets of adjacent plasma membranes producing a characteristic pentalaminar appearance. Using freeze-fracture techniques, it is possible to define the ultrastructure of the tight junction in more detail. With this method, the plasma membrane is split into ectoplasmic and protoplasmic surfaces. Replicas are then made and viewed by electron microscopy. Distinctive linear strands of membrane particles and complementary grooves are found at sites of tight junctional contact. Freeze-fracture replicas of tight junctions prepared from different tissues have different degrees of complexity [20]. The number, depth, and continuity of the strands appear to correlate with the permeability of the paracellular pathway. Epithelial tissues with the least effective barrier, as judged by a high rate of transcellular movement of tracers and a low electrical resistance, have either a small number of junctional strands or frequent discontinuities in the strands (for example, proximal renal tubule tissue). In contrast, tissues that produce a high electrical resistance and restrict the movement of small ions have multiple interconnecting and continuous strands (for example, urinary bladder

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tissue). By these morphological criteria, the tight junctions between endothelial cells in brain capillaries are continuous and very complex [25, 74]. That brain capillary junctions are indeed functionally very tight is supported by the restricted movement of ions from blood to brain [36], the inability of ionic lanthanum—an electron-dense tracer of low molecular weight—to cross the capillary wall [10], the limited ability of L-glucose, mannitol, and related polar organic molecules to cross the barrier [59], and the presence of very high electrical resistance across brain microvessels [21].

The biochemical composition of tight junctions is unknown and the molecular differences between junctions with varying degrees of tightness have not yet been investigated. Because alterations in tight junction integrity may underlie the enhanced permeability of the blood-brain barrier after various insults and manipulations [42], a more complete characterization of the chemical as well as the morphological properties of these contacts is an important goal for future research. Opening of tight junctions by osmotic agents is already carried out clinically to enhance the delivery of drugs to the brain [57].

Pinocytosis

Transendothelial vesicular transport is a potential route for the movement of plasma into brain. Under normal conditions, there are very few pinocytotic vesicles in the endothelial cells of brain capillaries [16, 70], whereas in muscle the cytoplasm of capillary endothelial cells is virtually filled with vesicles. These vesicles in muscle apparently take in (endocytosis) and release (exocytosis) material from outside the cell [75]. Since the vesicles appear freely mobile within the cytoplasm they are thought to ferry droplets of fluid across the vascular wall. It is also possible, however, that the vesicles transiently merge to form transcellular channels across the capillary wall [75].

The striking difference in vesicle density between capillary endothelial cells in muscle and those in brain may reflect a difference in intracellular regulators in the two vascular beds. Activation of pinocytosis in brain endothelial cells is reported after injury by ischemia [66], acute hypertension [78], and the increased cerebral blood flow associated with seizures [67]. In addition, osmotic opening of the blood-brain barrier may be mediated by pinocytosis as well as by separation of tight junctions [15]. Enhanced endocytosis was demonstrated in these disorders by ultrastructural studies using horseradish peroxidase, a protein with a molecular weight similar to that of plasma albumin and suitable for electron microscopic identification because of its electron-dense reaction product. Experimental data exist to implicate rises in cyclic adenosine monophosphate concentration in brain capillaries that exhibit an increased level of pinocytosis [42]. In addition, calcium appears to regulate exocytosis in systemic capillary endothelial cells [80]. Because increases in intracellular calcium activity frequently occur after cell injury [28], this mechanism could enhance capillary permeability in a number of disorders. However, one must be cautious about equating increases in vesicle density with increased transcellular transport, because in many cells endocytotic vesicles are destined for merging with lysosomes and internal digestion rather than for exocytosis. In fact, the small uptake of horseradish peroxidase found in endothelial cells after vascular perfusion of normal brain capillaries does not appear to be released on the antiluminal surface of the capillaries [16].

Basement Membrane

Surrounding the endothelial cells on their antiluminal surface is a basement membrane. The amorphous appearance of this thin layer is deceiving, as it is a complex structure composed of collagens and other proteins [18]. We believe that the basement membrane provides a tough external support for the endothelial cells (much like the cell wall of a bacteria or plant cell) and may limit distortion of the microvessel during hydrostatic or osmotic stress. If so, release of proteases during ischemic injury could lead to degradation of structural proteins and weaken the resistance of the microvessel wall to luminal forces. This weakening could cause small vessel hemorrhage, especially as the blood volume of the capillary bed increases after loss of autoregulation or in acute severe hypertension. The basement membrane is thinner in immature as compared with mature brain [26]. This fact may explain the high incidence of microvessel brain hemorrhages in infants [77]. Breaks in basement membrane continuity may also lead to endothelial cell migration. In this way the basement membrane could influence capillary budding and growth. This type of relationship between cell growth and differentiation with extracellular matrix is under active investigation in other tissues [71].

The basement membrane does not appear to hinder the diffusion of large molecules but may be capable of binding selected compounds based on electrical charge [44]. Whether this binding influences barrier function is unknown.

Glial Foot Processes

Foot processes from protoplasmic astrocytes abut the brain side of the microvascular basement membrane. Lateral contacts between these processes are incomplete and they do not prevent the movement of material from the interstitial fluid into the basement membrane. Although the function of the glial feet is unknown, they probably contribute to the synthesis of the basement membrane [1]. They may also selectively secrete and absorb solutes such as potassium [13, 43]. The fact that carbonic anhydrase [72] and glutamine

synthetase [58] are predominantly found in glial cells implicates them in NH_3 and H^+ inactivation. It also seems possible that glial cells could influence the behavior of endothelial cells by secreting factors into the basement membrane that control endothelial cell growth or function. In fact, the expression of brain-specific features by the endothelial cells may be determined by signals released by the glial cells into the basement membrane [23].

Carrier-Mediated Transport

Despite the barrier created by the plasma membrane and tight junctions of the endothelial cells, certain polar molecules rapidly cross the capillary wall. D-glucose and the essential large neutral L-amino acids are the most completely investigated compounds of this type. Movement of D-glucose into brain occurs by a mechanism that is stereospecific and saturable at high concentrations of the sugar [49]. From in vitro experiments with isolated brain capillaries [3] it seems likely that glucose carriers are present on both the luminal and antiluminal surfaces of the endothelial cells. As shown in Figure 1, glucose crosses the capillary wall by entering the endothelial cell from the blood side, equilibrating in the cytosol, and leaving through the antiluminal plasma membrane in response to a concentration gradient created by the oxidation of this substrate by neurons and glia. Only a small fraction of the glucose is consumed by the endothelial cells; the remainder is available for release into the interstitial fluid. In fact, when studied in isolation, brain capillaries use fatty acids and ketone bodies as a source for energy [7]. In this way glucose is conserved as an energy substrate for the neurons and glia.

Insulin does not appear to modify the activity of glucose transport into the brain of animals during acute experiments [49]. Most work to date suggests that the concentration of glucose in the blood and its rate of metabolism by the brain cells are the primary factors that determine the rate of glucose movement across the capillary wall [49]. The number of glucose carriers appears more than adequate to provide for normal brain energy metabolism. When the concentration of glucose in the blood falls to low levels, however, the capillary carriers may become a rate-limiting step in the availability of glucose for brain metabolism [5]. Limited glucose availability paradoxically may be accentuated in diabetic patients, in whom chronic elevations of the blood glucose appear to reduce the number of glucose transport carriers in the brain capillary wall [31, 51]. This would make the diabetic patient especially vulnerable to a sudden lowering of blood glucose, as inadequate transport of glucose into the brain could occur at blood glucose concentrations that are tolerated well by nondiabetic patients.

Transport carriers for large neutral amino acids are present in capillary endothelial cells and allow for the entry of essential amino acids into the brain [64]. Because the same carrier facilitates the movement of several amino acids, the potential exists for competition among different amino acids for transport into brain. Thus, patients with phenylketonuria have a high concentration of phenylalanine in the blood, which may limit the movement of large neutral amino acids such as leucine and valine into the brain [60]. Deficient transport of these essential amino acids into brain may alter protein synthesis, especially early in brain development, when demand for these amino acids is high. Furthermore, because synthesis of certain neurotransmitters appears to be rate-limited by the availability of amino acid precursors, alterations in the concentration of neurotransmitters could also result from the same type of competition at the capillary wall [35]. Thus, dietary intake may influence neurotransmitter levels in the brain. Similarly, in liver failure one theory proposes

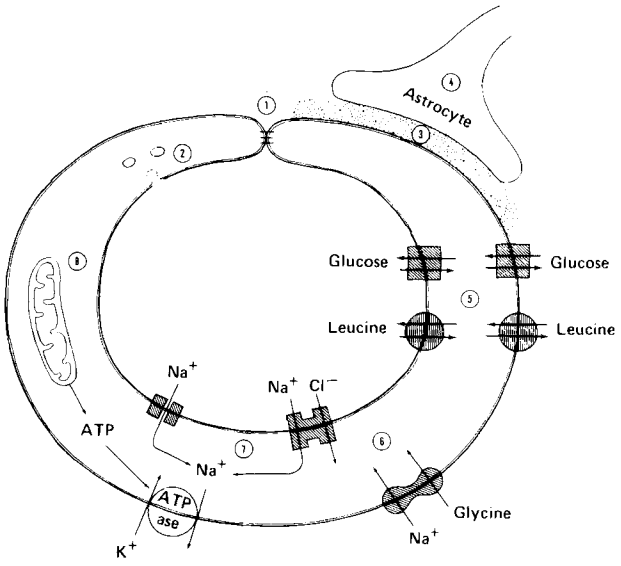

Fig 1. Model of brain capillary. The tight junctions (1) *that join endothelial cells in brain capillaries are continuous and complex and they limit the diffusion of large and small solutes. Very few pinocytotic vesicles* (2) *are found in the cytoplasm; this potential route for transendothelial transport is inoperative in normal brain capillaries. The basement membrane* (3) *provides structural support for the capillary and may influence endothelial cell function. Foot processes of astrocytes* (4) *encircle the capillary but do not create a permeability barrier. Transport carriers* (5) *for glucose and essential amino acids facilitate the movement of these solutes into brain. Active transport systems* (6) *appear to cause efflux of certain small amino acids from brain to blood. Na^+ pores and NaCl carriers on the luminal surface of the endothelial cell and Na^+, K^+-ATPase on the antiluminal surface* (7) *account for movement of ions across the brain capillary. Mitochondria* (8) *produce the adenosine triphosphatase needed for energy dependent transport processes. Not shown are receptor sites for agents that may regulate the permeability of this barrier.*

that changes in the amino acid concentration of the blood may lead to abnormal neurotransmitter levels in the brain and contribute to the encephalopathy [41]. Therapeutic maneuvers designed to increase the concentration of selected neurotransmitters in the brain must consider competition and rate-limiting transport sites at the capillary wall.

The active metabolism of certain neurotransmitter precursors by the endothelial cells (i.e., by L-dopa-decarboxylase present in high concentrations in the endothelial cells of brain capillaries) provides an additional barrier to the successful manipulation of neurotransmitter levels in the brain. This topic has been reviewed in the *Annals* [37].

Many polar molecules cannot readily enter the brain because the capillary contains no appropriate carriers. This principle has clinical application. Mannitol is similar in molecular weight to glucose but is excluded from the brain because the capillaries contain no appropriate carriers. Its entry into brain is, therefore, limited to a very slow diffusion process. When the concentration of this sugar alcohol is raised in the blood the resulting inequality of osmolarity between the blood and brain is offset by water movement out of the brain. By this mechanism, mannitol and other osmotic diuretics dehydrate brain tissue with a normal capillary barrier.

Active Transport

Energy dependent secretory activity has long been attributed to the choroid plexus in its role of producing and regulating the composition of the cerebrospinal fluid. Brain interstitial fluid is also closely regulated, however, and active transport pumps on the endothelial cells of brain capillaries may play an important role in maintaining both the volume and the composition of this extracellular fluid [13]. The high density of mitochondria in the endothelial cells of brain capillaries supports the role of these cells in energy dependent processes [61]. The active transport pumps tend to work in a one-way or vectorial direction. For example, the concentration of potassium in the extracellular fluid of brain is 2.8 mM, which is significantly lower than its 3 to 5 mM concentration in blood. Furthermore, changes in the blood concentration of potassium do not result in changes in the interstitial fluid concentration of potassium [22]. Two cellular mechanisms at the capillary appear important in this homeostasis. The luminal (blood surface) plasma membrane and tight junctions of the capillary wall have a low permeability to potassium [36], while, as shown in Figure 1, the antiluminal (brain surface) plasma membrane contains sodium, potassium-adenosine triphosphatase (Na^+, K^+-ATPase), which is capable of pumping potassium from the interstitial fluid into the endothelial cells in the brain-to-blood direction [4]. The affinity of capillary Na^+, K^+-ATPase is half-saturated at a potassium concentration of 2.8 mM; therefore, it should be responsive to physiological deviations in the concentration of potassium in brain interstitial fluid [32]. This capillary mechanism would supplement the potential role of glial cells in maintaining a stable concentration of potassium in sites around neuronal activity [43], and would allow for the long-term maintenance of a concentration gradient for potassium between blood and brain.

The antiluminal location of Na^+, K^+-ATPase in brain capillary endothelial cells is similar to the cellular polarity of many fluid-transporting epithelial cells. In such tissues, transepithelial movement of water is coupled to the transcellular transport of Na^+ and Cl^-. The major energy-requiring step is the active extrusion of Na^+ across the antiluminal membrane mediated by Na^+, K^+-ATPase. When this movement is coupled to a passive entry mechanism for Na^+ on the luminal membrane, the net result is active transport of Na^+ across the cell from lumen to interstitial space. Simultaneous transfer of Cl^- maintains electroneutrality, and water follows to offset osmolar gradients. Secretion of cerebrospinal fluid by the choroid plexus involves this general scheme [81]. Since it has been proposed that brain capillaries also contribute to cerebrospinal fluid production [52], it is not surprising to find that they have a similar transport polarity for ions. Recent studies show at least two separate Na^+ transport systems on the luminal membrane [2]. One is inhibited by low concentrations of the diuretic amiloride and is therefore similar to the Na^+ pore found in many tight epithelia. The other luminal Na^+ transport system is inhibited by furosemide and is probably a Na^+-Cl^- cotransport system. Thus transport of Na^+ and water from blood to brain across the capillary involves entry of Na^+ into the endothelial cell across the luminal membrane via either of two transport systems, followed by active pumping of Na^+ from endothelial cell to brain interstitial fluid across the antiluminal membrane by Na^+, K^+-ATPase.

Transport carriers for small neutral amino acids may show a similar polar distribution between the luminal and antiluminal surfaces of the capillary wall. These amino acids for the most part are not "essential" and can be synthesized in brain. This group includes glycine, which acts as an inhibitory neurotransmitter. The small neutral amino acids do not readily enter the brain from the blood, but in some studies they appear to be actively transported from brain to blood across the capillary wall [46, 48, 54]. From such in vivo studies, however, it is difficult to prove that active efflux of amino acids occurs across the capillaries [9], and further confirmation will require more direct measurement of transendothelial amino acid fluxes. Nevertheless, this vectorial transport would be best explained by an asymmetrical distribution of transport carriers

between the two membranes of the brain capillary. We have proposed that the antiluminal surface of the capillary contains a sodium-dependent cotransport system which moves sodium down a concentration gradient and the amino acid up a concentration gradient [6]. The sodium gradient necessary for this active transport is maintained by Na^+, K^+-ATPase in the endothelial cell. Organic acids may be actively transported out of the brain by a similar mechanism or by an exchange process for metabolites either produced by the endothelial cells or present in the blood [8].

In Vitro Studies of Brain Capillary Function

Isolated Brain Capillaries

Methods for isolating and purifying brain capillaries were first published less than ten years ago [14, 33, 53, 62, 79]. The several different available techniques yield small, branching segments of intact microvessels consisting of endothelial cells joined by complex tight junctions and surrounded by a basement membrane. These purified microvessels are metabolically active and readily oxidize glucose, fatty acids, β-hydroxybutyrate, and pyruvate to carbon dioxide [7, 14, 32]. The transport of glucose [3, 45], amino acids [6, 17, 34, 40, 73], and ions [19, 27, 32] into isolated brain capillaries has been studied extensively. These investigations are particularly useful in identifying transport processes on the antiluminal membrane of the endothelial cell, and their findings have contributed to the model in Figure 1.

The availability of isolated brain capillaries also provides the opportunity to study structural and enzymatic properties of the blood-brain barrier at a biochemical level. For example, capillary basement membrane can be isolated and characterized [18, 29], receptors for hormones and neurotransmitters can be catalogued [38, 39, 55, 65], the presence of specific enzymes such as those involved in neurotransmitter metabolism can be determined [37], and the synthesis of prostaglandins studied [30, 50]. There are, however, several limitations to using freshly isolated brain microvessels. Studies are restricted to short incubations, thus it is not possible to study the long-term response of microvessels to injury. In addition, investigations of transport by isolated capillaries are limited to studies of solute movement into the cells rather than across a layer of the cells. Finally, while growth of new brain capillaries is a prominent reaction in several diseases, this proliferative response cannot be studied with isolated microvessels. These problems can be circumvented by using purified capillary endothelial cells grown in tissue culture.

Cultured Brain Capillary Endothelial Cells

During the past few years, several laboratories have developed methods for culturing endothelial cells from brain microvessels [11, 24, 63, 68, 76]. In this issue of the *Annals*, Bowman and colleagues [12] describe some of the features of cultured endothelial cells prepared from bovine brain. These cells form tight junctions, contain few pinocytotic vesicles, and produce a permeability barrier when grown on collagen-coated nylon mesh. Since these features are responsible for formation of the blood-brain barrier, the cultured cells should provide a useful model for studying brain capillary function.

Extension of these studies to carrier-mediated transport across the in vitro barrier is an exciting prospect for the future. Furthermore, the ability to study transcellular transport in either the "blood-to-brain" or "brain-to-blood" directions with the same preparation should lead to a better understanding of the mechanisms involved in active transport across the blood-brain barrier. Since receptors for neurotransmitters are present in brain microvessels [38, 39, 55, 65], the regulation of transport processes can be studied.

Cells in culture secrete a basement membrane capable of influencing cell growth and differentiation [71]. In brain capillaries, both endothelial cells and glial foot processes contribute to the synthesis of basement membrane [1], and signals for endothelial cell function are probably present in this extracellular matrix. Since the cells involved in basement membrane synthesis can be grown in culture, it is now possible to examine the interrelationships between basement membrane and endothelial cell function. Other approaches to the investigation of cell interaction in the capillary-glial cell complex include exposure of each cell type to media collected from cultures of the other cell, and coculture of endothelial cells with glial cells. In the latter type of experiment, culture of brain endothelial cells in the presence of glial cells has resulted in the reappearance of an enzyme normally present in brain microvessels in vivo [23].

Another potential application for cultured endothelial cells is the investigation of factors that activate capillary proliferation in brain lesions. The ability to control capillary proliferation is important because in neoplasms these new capillaries sustain tumor growth [47]. On the other hand, after ischemic brain damage the growth of new capillaries may be important for recovery of function. In both cases, the newly formed blood vessels do not exhibit normal barrier properties and may be a site for the formation of brain edema. Very little is known about the control of neovascularization in these neurological diseases, and the culture of endothelial cells should provide a useful assay system to search for activators and inhibitors of brain capillary angiogenesis. If we can learn to manipulate brain capillary growth, new forms of therapy would become feasible for diseases in which arrest or stimulation of neovascularization would have a clinical benefit.

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