BLOOD-BRAIN BARRIER PERMEABILITY TO DRUGS

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William H. Oldendorf

Research and Neurology Services, V. A. Wadsworth Hospital Center, Los Angeles, California and Department of Neurology, School of Medicine, University of California, Los Angeles, California

To affect central nervous system (CNS) cells directly, a drug must appear in the extracellular fluid (ECF) of the CNS. How much of an administered drug distributes to CNS ECF is determined by a number of interdependent factors. If taken orally, a molecule of drug must survive in the gut lumen, penetrate the intestinal wall, traverse the liver, resist degradation by enzymes in blood plasma and other organs, remain unionized in solution unattached to plasma proteins, and ultimately penetrate the blood-brain barrier (BBB). In this review it is assumed a drug is in the peripheral blood, and only those factors directly related to its entry into brain are discussed.

Many of the characteristics of BBB permeability to drugs referred to in this review have been long established and well described previously in this series (1) and in other reviews (2, 3). These well-established areas will be discussed briefly here but emphasis will be placed on what probably are the most interesting relevant developments during the past decade: the demonstration that the brain capillary cell wall almost certainly is the site of the BBB; the discovery that the brain has an appreciable extracellular space; the characterization of carrier-mediated BBB permeability; the development of reversibly lipophilic derivatives of drugs to promote BBB penetration; and the modification by capillary endothelial cell cytoplasm of some substances entering the brain.

RELATION OF CNS ECF TO GENERAL ECF

Since the last review of BBB permeability in this series (1), certain relationships of the CNS fluid compartments to each other and to fluid compartments outside the nervous system have been clarified. These compartments outside the CNS are here referred to as general compartments.

The blood plasma is a moving subcompartment of the general ECF that serves, through its bulk flow through the microcirculation, to produce short effective diffusion distances between all body cells. It is effective in this role because of the permeability of general capillary walls to all small molecules (4, 5). When injected intravenously all polar molecules with molecular weights less than 20,000–30,000 distribute to a fluid compartment (the general ECF) of about 4 times greater volume than the plasma. This distribution has an equilibration $T_{1/2}$ of considerably less than 1 min in small animals (6). Nonpolar molecules of any size distribute in an even shorter time to a space 10–15 times greater than the plasma volume (7). Polar molecules larger than about 30,000 mol wt remain for some time confined to the plasma compartment distributing to an anatomically undefined larger space with a $T_{1/2}$ of several hours (8).

The permeability of general capillaries to polar molecules is nonspecific other than being a function of molecular size. The cutoff appears to be at a molecular diameter of 70–90 Å. Larger molecules pass through general capillary walls inefficiently but nearly independently of molecular size once their diameter exceeds about 100 Å (9). In the CNS there is no such nonspecific capillary permeability, and most polar solutes in plasma exchange very slowly with brain ECF. This observed failure of many solutes to enter the brain is the basis of the concept of a BBB. Although many solutes fail to enter the brain, it is obvious that the BBB must be permeable to the brain's metabolic substrates and perhaps to some metabolic endproducts. In view of the instantaneous effects of some drugs, the BBB must also be reasonably permeable to them as well. Rather than being a completely impermeable barrier, the BBB is more like a selectively permeable barrier exercising criteria other than simple molecular size. It is through this selectively permeable barrier that exchanges take place between CNS ECF and the general ECF.

On the CNS side of the BBB, diffusional exchange readily takes place between various areas of brain and with the cerebrospinal fluid (CSF). The CSF is currently conceived as an aggregation of fluid in the ventricles and subarachnoid space which is an extension of the CNS interstitial ECF, because tracers introduced into CSF diffuse readily throughout the interstitial ECF of brain (10, 11). Similarly, plasma tracers that enter CNS ECF at the site of an induced lesion, where the BBB is not functioning, diffuse readily into the ECF of the surrounding healthy brain tissue (12).

In view of this concept of CSF as an extension of brain ECF, the term "blood-CSF barrier" is probably of limited usefulness because it refers to an empirical rate of exchange from blood to CSF. This exchange can occur at many sites such as the BBB, the ependymal layer of the choroid plexus, or at the arachnoidal membrane. These sites are of such divergent structure and function that it is impossible to establish an anatomical substrate for a blood-CSF barrier. For most substances it probably reflects predominantly the permeability of the BBB.

Because many drugs cannot penetrate the BBB, they have little effect on the CNS when administered systemically but since drugs in CSF diffuse readily into the brain interstitial ECF, the same drug that was without effect when administered systemically may evoke striking effects after ventricular injection (13).

The CSF acts as a "sink" for solutes in CNS ECF (14–16). The term "sink" refers to the net movement of solute from one region to another by diffusion down a concentration gradient for that solute existing between these regions. The concentration gradient removing solutes from the brain's ECF probably is maintained by constant dilution of the CSF by fresh choroidal secretion which is probably virtually free of all but those osmotically important solutes required to make it isotonic with the brain (17–19). Most (20, 21) but probably not all (22) of the CSF is formed in the choroid plexus. How much of the CSF originates from the choroid plexus and how much from the brain parenchyma is controversial and will probably continue to defy experimental clarification. Water moves so freely through living systems in response to slight osmotic and hydrostatic pressure gradients that results obtained under necessarily abnormal experimental conditions cannot accurately represent related processes taking place in the intact animal.

By virtue of this sink action, any solutes that are in higher concentration in the interstitial ECF than in the CSF move toward the ependymal or pial surfaces where they mix with CSF and are carried into blood by bulk flow through the arachnoidal villi. These and other aspects of CSF physiology are described in lucid depth in the 1967 monograph by Davson (23).

In addition to this sink removing solutes nonselectively from brain ECF into blood, certain anions such as iodide (24), thiocyanate (25), and pertechnetate (26), as well as diodrast (27), phenosulfonthalein (27), serotonin, and epinephrine (28), appear to be actively transported out of the ventricle into blood by the choroid plexus. Iodide (29) and potassium (30) probably are excreted from brain ECF into blood against a concentration gradient at the BBB.

The controversy about whether or not there is appreciable ECF in the CNS has been settled. Brain tissue that is allowed to expire in situ shows essentially no ECF by electron microscopy (31). That this observation is artifactual is supported by the demonstration of an abrupt increase in electrical impedance of brain several minutes after circulatory arrest (32), and by the relatively large amount of extracellular space observed by electron microscopy when the brain is frozen rapidly in life and lyophylized (33, 34). These observations and recent tracer distribution studies (15) indicate that the CNS has an extracellular space of 10–20% (35), of the same order as the mean extracellular space for the entire body (36). The virtual absence of ECF noted during routine electron microscopy is the result of postmortem movement of ECF into cells, probably in response to the high sodium gradient which can no longer be maintained when cellular membrane energy reserves are depleted.

ANATOMIC BASIS OF THE BBB

In the past decade considerable clarification of the structural basis of the BBB has been achieved by the electron microscopic definition of the distribution of horseradish peroxidase (mol wt $\sim$ 40,000) after intravenous (10, 37) or intraventricular injection (38, 39). Hydrolyzed horseradish peroxidase (mol wt 1900) retains sufficient peroxidase activity to allow its use as an electron microscopic marker (40). It has been used to demonstrate BBB tight junctions to this relatively small molecule.

The histologic site of the BBB has in the past been variously located at the capillary endothelial cell, its basement membrane, or the investing layer of astrocytic membrane. It is now generally believed that the barrier is based upon the continuous layer of tight-junctioned endothelial cells (10, 37, 41). These flattened cells are joined together about the complete periphery of each cell to the periphery of adjoining endothelial cells. They are bound together by what might be considered a "strip weld," whereas general capillaries are "spot welded" together. Implicit in this analogy is the absence of physically open narrow clefts between brain capillary endothelial cells. These clefts between general capillary cells are probably responsible for the passage of small polar molecules through general capillary walls such as found in skin and muscle. Such lipophobic molecules are incapable of penetrating lipid cell membranes, and their passage through the general capillary wall must be between cells rather than directly through the endothelial cell membranes and cytoplasm. The intercellular cleft is, logically, a likely site of passage of small molecules, and this is confirmed by electron microscopy of the general capillary using tracer substances (42).

The BBB is almost completely impermeable to macromolecules (43), and this may be related to the virtual absence of pinocytotic vesicles from brain capillary cytoplasm (37). These vesicles are a prominent feature of general capillary cell cytoplasm (44) and may account for the slight, but real, permeability of the general capillary to macromolecules (5).

The two structural features of CNS capillaries that seem to explain their impermeability to most hydrophilic molecules are the tight endothelial cell junctions and the absence of pinocytosis. The physiological basis for these unique characteristics of CNS endothelial cells is unknown but it has been speculated to be a function of the production of a humoral agent by the astrocytic membranes which affects the apposed capillary cell membrane causing it to form tight junctions and stop pinocytosis (45).

Neither the brain capillary basement nor surrounding astrocytic membrane contribute to the BBB according to electron microscopic observations that the electron-dense protein ferritin (39) and horseradish peroxidase (10) introduced into the ventricle diffuse readily through the ECF into the pericapillary space between astrocytic membrane and capillary cell. These tracers then freely pass through the capillary basement membrane, enter the intercellular cleft, and stop at the tight junctions (10).

Thus, if a molecule is to pass through the CNS capillary wall, it must pass directly through the lumenal endothelial cell membrane, the thin layer of cytoplasm, and the outer cell membrane (41). It presumably is the permeability of these two membranes that determines the permeability of the BBB. In 1946 August Krogh stated that the BBB had the permeability characteristics of a biological membrane (46). The membrane of the brain capillary endothelial cell apparently fulfills this prediction. In this review, the term "BBB" refers to this structural complex of two endothelial cell membranes and interposed cytoplasm. The monograph by Crone & Lassen (47) is an excellent compilation of recent thinking on capillary permeability.

MEASUREMENT OF BBB PERMEABILITY

BBB permeability to a solute is usually defined as an all or none phenomenon. All statements regarding BBB permeability to a substance should be quantitated because the BBB is measurably, though sometimes only slightly, permeable to all small molecules.

BBB permeability for many substances can be easily determined by the simultaneous carotid injection of a labeled test substance and a highly diffusible internal reference substance (48, 49). By measuring the amounts of the injected substances remaining in the brain 15 sec after carotid injection, the amount of test substance lost to brain in a single passage through the microcirculation can be measured. A surprisingly large percentage of many drugs and metabolites are taken up by brain in a single passage (48, 49). For instance nicotine, ethanol, imipramine, caffeine, heroin, procaine, and antipyrine are all nearly completely deposited in brain (49). This route of administration and method of measurement is useful, however, only for substances which penetrate BBB rapidly. For many solutes no measurable uptake occurs in a single brain passage after carotid injection.

Because drugs are seldom administered into the carotid system, a clinically more relevant estimate can be made by measuring brain concentrations after systemic administration. The usual routes of administration are intravenous, subcutaneous, intramuscular, or intraperitoneal. In most instances only the intravenous route should be used, as the rates of absorption into blood from other routes are often much slower than the rate of BBB penetration, and brain concentrations accordingly reflect rate of absorption rather than BBB permeability. The literature concerning BBB permeability to drugs describing measurements of brain concentration after systemic administration is so voluminous and involves so many diverse substances and techniques that individual review here is impossible.

In addition to BBB permeability, brain concentrations of a drug after systemic administration will be affected by retention of the drug at the injection site, plasma protein binding, accumulation of drug by liver, kidney or other tissues, the rate of systemic degradation of the drug, and the degree of brain tissue binding. These factors must be considered before a firm relationship can be established between a systemically administered dose and the intrinsic BBB permeability to the drug.

Molecular Criteria for BBB Permeability

To penetrate the endothelial cell membranes, a molecule must escape the polar environment of the blood plasma and enter the nonpolar environment of the lipid of the plasma membrane. It must subsequently escape this plasma membrane, enter the cytoplasmic water and repeat these transitions at the outer plasma cell membrane.

The transition from blood plasma to inner plasma membrane is largely predictable on the basis of the molecule's relative affinities for plasma proteins, water, and membrane lipid. If strongly bound to plasma protein, this macromolecular complex cannot escape into the lumenal membrane because it is unlikely it will ever achieve the energy level required to make a water-lipid phase transition.

Plasma Protein Binding

Plasma protein binding has been adequately discussed in other reviews (50, 51). The review by Mayer & Guttman contains an exhaustive review of the literature relating to specific studies of drug binding. For our purposes the important result of protein binding is the establishment of the concentration of free drug in plasma, because it is this unbound fraction that may possibly traverse the BBB. It is assumed the protein molecule, by virtue of its size and hydrophilic character, will not penetrate the BBB. Thyroxin (T_4) is an example of penetration of only the unbound plasma fraction. Unbound plasma T_4 represents much less than 1% of the total plasma T_4. Although CSF T_4 is very much lower than total plasma T_4, its absolute concentration is approximately equal to the unbound plasma concentration (52). This suggests that unbound T_4 readily equilibrates between plasma and brain ECF.

Competition for protein binding sites may result in displacement of one substance by another, raising its unbound plasma concentration and consequently its CNS concentration. Christensen (53) has suggested that displacement of T_4 from its protein binding sites by another drug could explain some of the observed drug effects on metabolic rate. Such a displacement, with a rise in free plasma T_4, could be expected to result in a rapid rise in CNS T_4.

Bilirubin is neurotoxic but there is little CNS effect even when jaundice is severe because of protein binding and removal of bilirubin from free solution. The CSF concentration, our only clinical index of CNS bilirubin, is much lower than in plasma (54), and brain staining at necropsy is minimal. Administration of sulfadiazine may displace bilirubin and, in the newborn, result in significant neurotoxicity (55).

Lipid Versus Water Affinity

Although protein binding is the dominant factor affecting BBB permeability to many solutes, the most important relationship for most solutes is their relative affinities for water and lipid because these establish the ease with which a solute molecule can escape plasma or cytoplasmic water and enter membrane lipid. This relative affinity can be established in vitro by measuring the lipid/water partition coefficient. Many nonpolar substitutes for plasma membrane lipid have been used with similar results, but none can precisely substitute for the living membrane lipid and for its carrier transport systems that confer upon the living membrane highly specific affinities.

Ionization greatly influences BBB penetration by a drug making it more hydrophilic and lipophobic thus favoring its retention by the water phase. The charge-dipole interaction between an ion and its surrounding water molecules is much stronger than the dipole-dipole interaction between a molecule hydrogen-bonded to water. An ion is, by virtue of this interaction, surrounded by a shell of several water molecules each of which, by hydrogen bonding, is anchored in the water phase (56). The state of ionization of a drug may also alter its affinity for plasma protein binding sites. Accordingly, the degree of dissociation at blood pH is a major determinant of permeability to a drug. If a drug is largely unionized at pH 7.4, its entry into brain

is favored (57, 58). It is generally believed that only the unionized fraction penetrates the BBB (58). Because the blood pH is quite stable and can shift in life only about ± 0.5, changes in blood pH are not nearly as important for most drugs in altering penetration of BBB as are gut pH changes because a very great range of pH is encountered in the gut. Drugs such as thiopental having a pK near 7.4 could, however, undergo a considerable change in ionization with the slight shifts in blood pH encountered in respiratory and metabolic acid-base imbalances.

Whereas the hydrophilic character of an ionized molecule is dominated by its electrical charge, the relative lipid versus water affinity of the unionized molecule is largely determined by its hydrogen bonding capability. For most large organic molecules, water affinity is dominated by their hydrogen bonding capability.

The lipid/water partition coefficient of a substance can be estimated by examining its structure and adding up the total number and relative hydrogen-bonding capabilities of polar groups. Stein (56) has classified the apparent strength of hydrogen bonding by various common organic groups and related them to Collander's earlier observations (59) of membrane permeability. To define the apparent strength of hydrogen bonding by organic groups, Stein assigned a weighted numerical value (N) proportional to apparent hydrogen bonding strength. $N = 2$ was attributed to the –OH group of alcohols, sugars, glycols, carboxylic acids and to the $-NH_2$ group of primary amines. $N = 1$ was attributed to the –N(R)H of secondary amines and to the –CO group of carboxylic acids, amides, and aldehydes, $N = 1/2$ was attributed to the –CO– of esters, and $N = 0$ to the –O– of ethers. Knowledge of the bonding strengths of these and other groups is vital for predicting membrane permeability. Stein estimated that (56) each additional full-strength hydrogen bond to water decreased the likelihood of a water-membrane transition by a factor of 6–10.

Latentiation

The BBB is responsible for a very restricted distribution of many lipid-insoluble drugs to the brain. The penetration of BBB can be greatly increased by shielding hydrogen bond-forming sites by substituting relatively lipophilic groups. If the brain has the enzymatic capability of removing these added lipophilic groups, the original compound is regenerated and has in effect penetrated the BBB. This process of adding lipophilic groups that can be removed in the body with regeneration of the original compound has been termed "latentiation" (60) and will probably be widely applied to cause drugs to enter the brain. This is one form of a more general pharmacological strategy in which the body is caused to generate a drug in a given location by providing a suitable precursor. The first, although inadvertent, application of a lipid-soluble, reversible drug derivative to promote brain effects was the synthesis of heroin by acetylating the two hydroxyl groups of morphine (61). This greatly increases the amount of the drug entering the brain (49) and, once in the brain, it is deacetylated through 6-monoacetyl morphine to morphine, in which form it probably is most pharmacologically active (62).

The BBB is quite impermeable to all known CNS transmitter substances (48), and this has prevented raising CNS dopamine levels in parkinsonism by the systemic administration of dopamine. Its precursor, L-dopa, despite its great total hydrogen

bonding capability, freely penetrates the BBB by virtue of its affinity for the large neutral amino acid carrier system (48). Cells of the brain's dopaminergic system can then decarboxylate it to dopamine. Lipophilic derivatives of dopamine should freely penetrate the BBB and, if brain cells can remove these lipophilic groups, dopamine should be regenerated. Various 3,4-O-derivatives of dopamine have been synthesized (63, 64), and some have been shown to activate dopamine receptors (65). Whether or not this approach will prove therapeutically useful remains to be established. Such a ploy for raising brain dopamine levels is unphysiological in that lipid-mediated BBB penetration would result in a distribution to the entire brain. Because deacetylases are ubiquitous, dopamine levels probably would be raised throughout the brain. This is quite different from forcing an increased output from deficient dopaminergic cells by introducing an excess of precursor because such dopamine presumably would be distributed specifically to its usual receptor areas (largely in basal ganglia), creating a quite different distribution from that likely to be found after administration of lipophilic derivatives. Conversely, latentiation of dopamine may be effective in some cases in which the dopaminergic cells are so deficient that they have essentially completely lost their decarboxylating function. In such cases raised brain tissue dopamine levels might still be created by reversible lipophilic derivates.

Similar attempts have been made to develop reversible lipophilic derivatives of norepinephrine (66) and various other amines (67). Using this general approach, many possibilities of raising brain concentrations of central transmitter substances, neurohumoral agents, antibiotics, and other drugs remain to be explored.

Biotransformation Within the Endothelial Cell

In addition to penetration of the inner and outer endothelial cell membranes, a drug must traverse the intervening thin layer of cytoplasm. That biotransformation can occur within this cytoplasm is indicated by the presence of monoamine oxidase (68) and the demonstration of dopamine fluorescence (69) in this cytoplasm. This cytoplasmic action may serve to enhance the effectiveness of the BBB in blocking the entry into the brain of systemic amines that might otherwise exert a CNS effect when blood concentrations change. This capability of brain capillary endothelial cytoplasm may bring about biotransformation of certain drugs before they enter brain ECF where they can affect neuronal activity.

Carrier Mediated BBB Transport

Although the BBB is impermeable to many polar small molecules, it is permeable to some metabolic subtrates such as glucose (70, 71), despite their extremely lipophobic character. Several specific BBB carrier systems have been demonstrated, and these exhibit sufficient affinity that they can, for their transported substances, compete successfully against even strong hydrogen bonding to water. Although many such carrier systems probably exist in the BBB, four independent carriers have been clearly defined. There is one carrier for D-glucose and related hexoses (48), one for large neutral amino acids, one for basic amino acids (48, 72), and one for short-chain monocarboxylic acids (73). The presence of these carrier sites, presumably located

in the endothelial cell membranes, creates membrane affinities not predictable from simple lipid/water partition coefficients. Such carrier sites, being of restricted number, are saturable. They also exhibit stereospecificity (74). Although little attention has been directed to this area, the BBB penetration of some drugs may be accelerated by these carriers beyond that which would have been predicted by examination of the molecular structure for hydrophilic sites. The BBB penetration of amphetamine, for example, is partially saturable (75).

In general, BBB selective permeability for a large number of substrates resembles that of the red cell plasma membrane (48).

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