The blood-brain barrier: clinical implications for drug delivery to the brain

ABSTRACT—The blood-brain barrier (BBB) determines whether or not a given drug can reach the central nervous system (CNS), either by passive diffusion or through carrier or receptor systems. Initial work focused on the structural and physico-chemical requirements favouring transport across the BBB as related to anatomical and physiological features. Such studies have had a significant effect on the design of CNS-active drugs with improved permeability across the BBB. Progress in pharmacology and neurosciences resulted in greater knowledge of CNS diseases and of potential therapies, but also created the need to develop new strategies to improve drug delivery to the brain. For a long time the BBB was considered to be a physical barrier, mainly represented by the cerebrovascular endothelium; however, transport of drugs to the brain may be limited by the metabolic activity of the BBB. The BBB should be regarded as a dynamic rather than a rigid barrier; it can be influenced by astrocytes and probably also by neuronal and hormonal stimuli, and its properties are also affected by diseases of the CNS. This may offer new strategies for targeting drugs to the brain.

Anatomy and physiology of the BBB

The concept of the blood-brain barrier (BBB) developed from the end of the nineteenth century onwards, starting with the German pharmacologist and physiologist Paul Ehrlich [1], and later Goldmann [2,3], Spatz and Walter [4,5]. The latter made a distinction between the BBB and the choroid plexus, whereas Krogh focused on active transport processes at the BBB [6]. At that time it was generally believed that the barrier function of the BBB was exerted by the glial sheets surrounding the entire surface of the neuronal capillaries [7]. However, in the mid-1960s Crone showed that Pappenheimer's pore theory for the filtration of substances by peripheral capillaries could also be extended to the BBB, implying an important functional role for the neuronal capillaries [8,9]. Subsequently Reese and Karnovsky, and Brightman and Reese, demonstrated that following intravenous injection of the electron-dense marker horseradish peroxidase, extravasation of this compound was indeed confined by the cerebrovascular endothelial cells, implying that these cells constitute the principal anatomical basis of the BBB [10,11].

As early as 1930 Spatz and Walter [4,5] suggested that the endothelial and epithelial lining of the choroid plexus also comprises a blood-cerebrospinal fluid (CSF) barrier, while the ependyma, which lines the brain tissue and bears a close morphological relationship to renal tubular epithelium, represents the brain-CSF barrier. An overview of all these barriers is given in Fig 1 [12].

Substances which do not pass the BBB may nevertheless reach the CNS by extravasation in the choroid plexus and the circumventricular organs such as the area postrema or median eminence because of their leakier endothelium, and subsequently by transependymal transport into the CSF [13,14].

The surface area available for exchange at the blood-CSF barrier is approximately 5,000 times less than that of the blood-brain barrier [15]. The BBB's large surface area makes it the most important barrier for drug delivery to the brain. Besides its main function of maintaining homeostasis in the CNS, the CSF is an important factor in the kinetics of substances in the CNS.

Peripheral and cerebrovascular endothelial cells

The endothelium of the microvessels in the brain shows various structural differences compared with other organs (Fig 2) [16]. Peripheral endothelial microvessels have fenestrations of approximately 50nm diameter between the endothelial cells, large enough to allow free exchange of water and solutes with the extracellular fluid. These fenestrations are not found in brain endothelial cells [17] and there are almost no pinocytotic vesicles in brain microvessel endothelium [18]. The transport of large proteins and colloidal lanthanum is effectively blocked by the presence of very tight junctions [10,11]. These tight junctions are a highly dynamic and heterogeneous system which regulates paracellular permeability [19]; in bovine brain microvessel endothelial cells they appear as 80 Ångstrom rectangular pores with a fractional pore area of 0.01% [20].

The endothelial cells representing the BBB contain many mitochondria, indicating that in addition to its physical barrier properties, the BBB may also function as a metabolic barrier. The upregulation of the P-glycoprotein efflux pump at the BBB in multi-drug resistance is further evidence of its barrier function [21].

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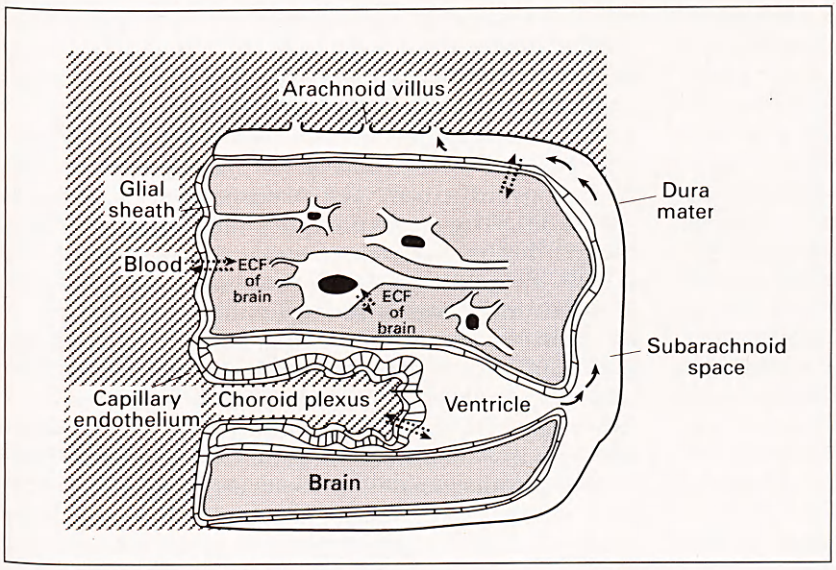

Fig 1. *Schematic representation of the anatomical barriers for drug transport to the brain and relationship between blood-brain barrier and blood-CSF barrier.* Arrows indicate possibilities for solute exchange and transport (redrawn from Bradbury) [12].

Other cell types present in and surrounding the BBB may influence its permeability and functionality. Astrocytes [22,23,24] influence the tightness of the tight junction when co-cultured with cerebrovascular cells [25], while neurons also seem to have an effect on BBB function [26].

The BBB is not a homogeneous system. Due to fenestrations, the endothelium of the circumventricular organs, such as the area postrema or the median eminence [14], is leakier than in other parts of the brain and is therefore not representative of the whole brain. Similarly, the bloodflow to the various brain regions may differ considerably, which suggests that there may be quantitative differences in drug transport across the BBB [14].

Anatomically the BBB is clearly not a static, homogeneous, impermeable barrier. Its permeability is dynamically regulated with special features (physical and metabolic) arising from the absence of fenestrations, the relative lack of pinocytotic vesicles, the presence of tight junctions and mitochondria, and from the influence of astrocytes, pericytes and neurons, and of blood constituents such as hormones. These regulatory systems may have some influence on permeability and so affect drug delivery to the CNS.

Implications for drug delivery to the brain

Drugs that have their site of action in the brain should in general enter the brain across the BBB. Drug delivery to the brain may be enhanced by:

- increasing the lipophilicity of the drug;
- using prodrugs that are dissociated into the active substance and the prodrug moiety after passing the BBB;
- using carrier/receptor systems present at the BBB (passive drug targeting);

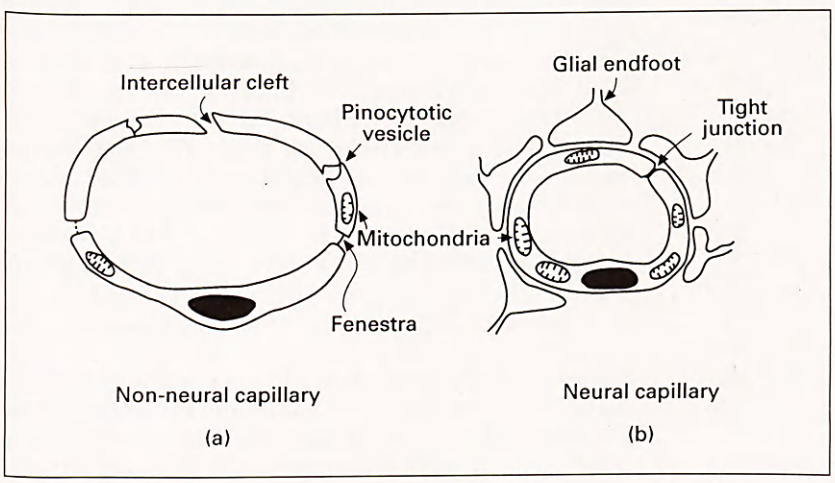

Fig 2. *Schematic representation of some of the relevant anatomical characteristics of neural and non-neural microvessels.* The neural microvessel differs from the non-neural one by the presence of very tight junctions, the absence of pinocytotic vesicles and fenestrations, and the relatively high number of mitochondria (redrawn from Reed *et al*) [18].

- identifying and using upregulated transport systems at the BBB in disease states (active drug targeting).

Lipophilic drug delivery

Lipophilic drugs enter the brain relatively easily by passive transcellular diffusion. This mechanism applies to most psychopharmacological drugs used clinically; an example is the induction anaesthetic thiopentone [27]. However, the uptake of these drugs is not restricted to the brain alone but occurs also in most peripheral tissues. Therefore peripheral side-effects may occur in addition to effects on the central nervous system (CNS).

Prodrug delivery

A prodrug of a CNS-active compound consists of the attachment of a chemical moiety to the parent drug, usually to render the combined molecule more lipophilic. These prodrugs (eg esters) may be hydrolysed in the target tissues (but also in blood) by various esterases. The delivery of prodrugs is affected by variations in protein binding (only the unbound fraction is available for transport to the target tissue), in ionisation (ionised drugs pass through membranes poorly), and in the pharmacokinetics of the prodrug. These factors may greatly change the amount of the parent compound that is retrieved from the prodrug and consequently affect its concentration in the target organ. Nevertheless, an interesting approach has been the development of prodrugs coupled to sterically hindered esters with good stability in plasma and with a degree of lipophilicity that did not reduce their transport to the brain by restrictive protein binding [28,29]; they also entered the brain readily and high concentrations were maintained in the brain while plasma concentrations decreased rapidly.

Other approaches to prodrug delivery are the so-called double ester prodrugs [30], and the dihydropyridine/pyridinium salt redox-type reactions [31,32,33]. The former implies the application of a special double ester that avoids the problems that occur with the sterical hindrance esters when the prodrug has to be hydrolysed again. This procedure can be applied to create lipophilic prodrugs that can enter the brain and are relatively quickly hydrolysed. The latter approach employs the dihydropyridine form of the drug which is lipophilic enough to enter the brain and which will subsequently be oxidised by NADH-linked dehydrogenases to the charged and therefore hydrophilic pyridinium compound. This 'traps' the compound in the brain, because its diffusion out of the brain will be slow.

Carrier/receptor mediated drug delivery

The CNS requires an adequate supply of nutrients and other factors to function properly. These are often hydrophilic substances that need to be transported across the BBB and the blood-CSF barrier by specialised carrier/receptor systems, each responsible for blood-to-brain transport of a group of closely related substrates. An overview of the values of the main kinetic parameters of several of these transport systems with their substrates is given in Table 1 [34,35,36, 37,38].

Probably the most successful use of a carrier for drug delivery to the brain is the use of L-dopa in Parkinson's disease. L-dopa is transported into the CNS by the large neutral amino-acid carrier and is then decarboxylated to yield dopamine, the active moiety. Since the enzyme decarboxylase, responsible for conversion to the active drug, is not solely located in the CNS, peripherally acting decarboxylase inhibitors which do not penetrate the BBB have to be administered concurrently. Bodor *et al* have proposed the use of a general dihydropyridine promoiety which uses the pyridinium carrier for transport, after which it is oxidatively cleft in the CNS and the drug released [39]. This so-called chemical delivery system (CDS) has been applied with variable results. Examples include the transport of gamma aminobutyric acid (GABA) [40] and estradiol [41] across the BBB. It seems that the use of the dihydropyridine-CDS depends on the structure of the drug to which it is linked and that linkage to larger drugs could hinder the enzymatic cleavage of the prodrug [42].

Another interesting example is the transport into the brain of alkylating drugs like melphalan [43] and d,l-NAM [44] (both are nitrogen mustard amino-acid derivatives) by the large neutral amino-acid carrier. Other drugs like baclofen, a centrally acting muscle-

Table 1. Blood-brain barrier transport systems*

Transport system	Substrate	Kd (ml/min g)	Km (mM)	Vmax (nmol/min g)
Hexose	Glucose	0.023	9	1600
Monocarboxylic acid	Lactate	0.028	1.9	120
Large neutral amino-acid	Phenyl-alanine	0.018	0.12	30
Basic amino-acid	Lysine	0.007	0.10	6
Acidic amino-acid	Glutamate	0.002	–	–
Amine	Choline	0.003	0.22	6
Purine	Adenine	0.006	0.027	1
Nucleoside	Adenosine	0.001	0.018	0.7
Thiamine	Thiamine	0.029	0.004	0.03
Thyroid	T3	–	0.001	0.1

*Data from references 34, 35, 36, 37, 38.

relaxing agent, are stereoselectively transported to the brain [45] presumably by the large neutral amino-acid carrier, while AZT is passively transported across the BBB and actively into and out of the CSF by the choroid plexus [46,47]. In addition, various peptides are transported to the brain by passive or active (peptide) transport systems [48,49].

The so-called chimaeric peptide approach has also gained considerable attention. It comprises the covalent binding of a peptide to another peptide that is transported across the BBB by a receptor system, eg transferrin or insulin [50]. Although it increased the transport of these chimaeric peptides into the brain, the absolute amount of drug uptake was quite small. Similarly, the transport of liposomes coupled to the Fab fragment of the OX-26 monoclonal antitransferrin receptor antibody seems to exhibit an increased binding or uptake by the brain and/or endothelium [51]. However, the amounts taken up in the brain were again quite small, while the presence of a relatively high density of transferrin receptors in the spleen and red blood cells resulted in a significant uptake of the liposomes in these tissues.

Upregulated transport systems at the BBB in disease state (active drug targeting)

The BBB functions as an interface between blood and the brain. Its function can be influenced by astrocytes, pericytes, neurons, and many substances in the blood such as hormones. Little is known about the changes in BBB function in disease state, in particular in diseases of the CNS. Several processes may be altered in such circumstances, eg protein binding capacity and blood flow [52,53]. In addition the BBB may be 'opened' in inflammation or by tumours. Preliminary results in rats indicated that in inflammation the transport of hydrophilic compounds across the BBB is increased, possibly because of their changed plasma kinetics [54]. Less is known about the up- or down-regulation of transport systems at the BBB in disease states. There are indications that this may occur, eg the expression of ICAM-1 and ICAM-2 was down-regulated in a mammary adenocarcinoma in rats [55], while in inflammation these molecules were up-regulated [56]. Following stimulation with lipopolysaccharide and interleukin-1 and 6, lymphocyte binding was increased three-fold and could be blocked by monoclonal anti-VLA4 and anti-CD11a/CD18 (LFA-1) antibodies [57]. Thus disease states may yield important pointers towards achieving selectivity in BBB transport by identifying up-regulated transport systems at the BBB.

General remarks

When an endogenous carrier or receptor is being used, interactions between endogenous ligands and the drug may be prominent. The endogenous ligands often have a higher affinity for the carrier. Drug delivery is therefore dependent on and complementary to ligand kinetics. Predictable and reproducible transport into the CNS can be difficult in such a situation. Clinically relevant interactions between dietary amino-acids and L-dopa therapy, resulting in the 'on-off' syndrome in Parkinson's disease, have been reported [58]. On the other hand, drug interaction with the carrier may interfere with the transport of endogenous ligands, especially by drugs with a high affinity for the carrier system, and may lead to unphysiological situations.

Modifications of drug transport across the BBB can be satisfactorily accomplished only by designing methodologies which specifically modify the transport profile of the compound in question. Since general modification of permeability is not possible, a specific approach is necessary for each drug [59,60]. This implies that in designing new drugs for optimal CNS delivery with minimal peripheral side-effects, careful attention should be given to the 'delivery system' in combination with a particular BBB transport system.

References

1 Ehrlich P. Das sauerstoffbedürfnis des Organismus; eine farbenanalytische Studie. Hirschwald, Berlin, 1885.
2 Goldmann EE. Die äussere und innere Sekretion des gesunden and kranken Organismus im Lichte der vitalen Färbung. *Beitr Klin Chirurg* 1909;**64:**192–265.
3 Goldmann EE. Vitalfärbung am Zentralnervensystem. *Abh Preuss Akad Wiss Phys Math* KL 1913;**I:**1–60,
4 Spatz H. Die Bedeutung der vitalen Färbung für die lehre vom Stoffaustausch zwischen dem Zentralnervensystem und dem übrigen Körper. *Arch Psychiat Nervenkr* 1933;**101:**267–358.
5 Walter FK. Die allgemeinen Grundlagen des Stoffaustausches zwischen dem Zentralnervensystem und dem übrigen Körper. *Arch Psychiat Nervenkr* 1930;**101:**195–230.
6 Krogh A. The active and passive changes of inorganic ions through the surfaces of living cells and through living membranes generally. *Proc Roy Soc B* 1946;**133:**140–200.
7 Dempsey EW, Wislocki GB. An electron microscopic study of the blood-brain barrier in the rat, employing silver nitrate as a vital stain. *J Biophys Biochem Cytol* 1955;**1:**245–56.
8 Pappenheimer JR, Renkin EM, Borrero LM. Filtration, diffusion and molecular sieving through peripheral capillary membranes. *Am J Physiol* 1951;**183:**221–34.
9 Crone C. The permeability of brain capillaries to non-electrolytes. *Act Physiol Scand* 1965;**64:**407–17.
10 Reese TS, Karnovsky MJ. Fine structural localisation of a blood-brain barrier to exogenous peroxidase. *J Cell Biol* 1967;**34:**207–17.
11 Brightman MW, Reese TS. Junctions between intimately apposed cell membranes in the vertebrate brain. *J Cell Biol* 1969;**40:**648–77.
12 Bradbury M. *The concepts of the blood-brain barrier.* John Wiley & Sons, London 1979, p.32.
13 Bates IP. Permeability of the blood-brain barrier. *Trends in Pharmacological Sciences* 1985;**6:**447–50.
14 Gross PM, Sposito NM, Pettersen SE, Fenstermacher JD. Differences in function and structure of the capillary endothelium in grey matter, white matter and a circumventricular organ of rat brain. *Blood Vessels* 1986;**23:**261–70.
15 Pardridge WM. Blood-brain barrier transport of nutrients. *Fed Proc* 1986;**45:**2047–9.
16 Reed DJ. Drug transport into the central nervous system. In: *Antiepileptic drugs: mechanisms of action.* Glaser GH, Pentry JK, Woodbury DM (eds). Raven Press, New York, 1980, pp 199–205.

17 Lee JC. Evolution in the concept of the blood-brain barrier phenomenon. In: *Progress in Neuropathology*, vol I. Zimmermann HM (ed). Grune and Stratton, New York 1971, pp 84–145.

18 Cervos-Navarro J. Electronmikroskopischer Befund an den Capillaren der Hirnrinde. *Arch Psychiat Nervenkr* 1963;**204:**484–504.

19 Madara JL. Tight junction dynamics: is paracellular transport regulated? *Cell* 1988;**53:**497–8.

20 Van Bree JBMM, De Boer AG, Danhof M, Ginsel LA, *et al.* Characterisation of *in vitro* blood-brain barrier: effects of molecular size and lipophilicity on cerebrovascular endothelial transport rates of drugs. *J Pharmacol Exp Ther* 1988;**247:**1233–9.

21 Cordon-Cardo C, O'Brien JP, Casals D, Rittman-Grauer L, *et al.* Multidrug-resistance gene (P-glycoprotein) is expressed by endothelial cells at the blood-brain barrier sites. *Proc Nat Acad Sci USA* 1989;**86:**695–8.

22 Janzer RC, Raff MC. Astrocytes induce blood-brain barrier properties in endothelial cells. *Nature* 1987;**325:**253–7.

23 Arthur FE, Shivers RR, Bowman PD. Astrocyte-mediated induction of tight junctions in brain capillary endothelium: an efficient *in vitro* model. *Dev Brain Res* 1987;**36:**155–9.

24 Tao Cheng JH, Brightman MW. Development of membrane interactions between brain endothelial cells and astrocytes *in vitro*. *Int J Dev Neurosci* 1988;**6:**25–37.

25 Minakawa T, Bready J, Berliner J, Fisher M, *et al. In vitro* interaction of astrocytes and pericytes with capillary-like structures of brain microvessel endothelium. *Lab Invest* 1991;**65:**32–40.

26 Pardridge WM. *Peptide drug delivery to the brain.* Raven Press, New York, 1991.

27 Ghoneim MM, Kortilla K. Pharmacokinetics of intravenous anaesthetics: implications for clinical use. *Clin Pharmacokin* 1977;**2:**344–72.

28 Greig NH, Daly EM, Sweeney DJ, Rapoport SI. Pharmacokinetics of chlorambucil-tertiary butyl ester, a lipophilic chlorambucil derivative that achieves and maintains high concentrations in brain. *Cancer Chemother Pharmacol* 1990;**25:**311–9.

29 Greig NH, Stahle PL, Shetty HU, Genka S, *et al.* High performance liquid chromatography analysis of chlorambucil-tertiary butyl ester and its active metabolites, chlorambucil and phenylacetic mustard, in plasma and tissue samples. *J Chromatogr* 1990;**534:**279–86.

30 Krogsgaard-Larsen P, Christensen A. GABA agonists. Synthesis and structure-activity studies on analogues of isoguvacine and THIP. *Eur J Med Chem* 1979;**14:**157–64.

31 Bodor N, Brewster M. Problems of drug delivery to the brain. *Pharmacol Ther* 1982;**19:**337–86.

32 Bodor N, Kaminski JJ. Prodrugs and site-specific chemical delivery systems. *Ann Rep Med Chem* 1987;**22:**303–13.

33 Bodor N. Redox delivery system for targeting drugs to brain. *Ann NY Acad Sci* 1987;**507:**289–306.

34 Pardridge WM. Transport of nutrients and hormones through blood-brain barrier. *Fed Proc* 1984;**43:**201–4.

35 Crone C. Facilitated transfer of glucose to brain tissue. *J Physiol (Lond)* 1965;**181:**103–13.

36 Pardridge WM. Carrier mediated transport of thyroid hormones through the blood-brain barrier. Primary role of albumin-bound hormone. *Endocrinology* 1979;**105:**605–12.

37 Dembri A. Caracteristiques de transport des analogues structuraux des hormones thyroidiennes à travers la barrière hémato-encephalique. *C R Soc Biol* 1981;**181:**249–57.

38 Spector R. Myo-inositol transport through the blood-brain barrier. *Neurochem Res* 1988;**13:**785–7.

39 Bodor N, Shek E, Higuchi T. Delivery of a quaternary pyridinium salt across the blood-brain barrier as its dihydropyridine derivative. *Science* 1975;**190:**155–6.

40 Anderson WR, Simpkins JW, Woodard PA, Winwood D, *et al.* Anxiolytic activity of a brain delivery system for GABA. *Psychopharmacology (Berlin)* 1987;**92:**157–63.

41 Brewster ME, Estes KS, Percahlski R, Bodor N. A dihydropyridine conjugate which generates high and sustained levels of the corresponding pyridinium salt in the brain does not exhibit neurotoxicity in cynomolgus monkeys. *Neurosci Lett* 1988;**87:**277–82.

42 Boddy AV, Zhang K, Lepage F, Tombret F, *et al. In vitro* and *in vivo* investigations of dihydropyridine-based chemical delivery systems for anticonvulsants. *Pharm Res* 1991;**8:**690–7.

43 Greig NH. Drug delivery to the brain by brain-blood barrier circumvention and drug modification. In: *Implications of the blood-brain barrier and its modification*, Vol 1. *Basic science studies*, Neuwelt EA (ed). Plenum, New York, 1989, pp 311–67.

44 Takada T, Greig NH, Vistica DT, Rapoport SI, *et al.* Affinity of antineoplastic amino-acid drugs for the large neutral amino-acid transporter of the blood-brain barrier. *Cancer Chemother Pharmacol* 1991;**29:**89–94.

45 Van Bree JBMM, Heijligers-Feijen CD, de Boer AG, Danhof M, *et al.* Stereoselective transport of Baclofen across the blood-brain barrier in rats as determined by the unit impulse response methodology. *Pharm Res* 1991;**8:**259–62.

46 Masereeuw R, Jaehde U, Langemeijer MWE, de Boer AG, *et al. In vitro* and *in vivo* transport of Zidovudine (AZT) across the blood-brain barrier and the effect of transport inhibitors. *Pharm Res* 1994;**11:**324–30.

47 Spector R. Nucleoside and vitamin homeostasis in the mammalian central nervous system. *Ann NY Acad Sci* 1986; **481:**221–30.

48 Banks WA, Kastin AJ. Peptide transport systems for opiates across the blood-brain barrier. *Am J Physiol* 1990;**259:**E1-E10.

49 Segal MD, Zlokovic BV. *The blood-brain barrier amino acids and peptides.* Kluwer, Lancaster, 1990.

50 Pardridge WM. Recent advances in blood-brain barrier transport. *Ann Neurol Pharmacol Toxicol* 1988;**28:**25–39.

51 Micklus MJ, Greig NH, Tung J, Rapoport SI. Targeting of liposomes to the blood-brain barrier in rats. *Drug Delivery* 1993;**1:**21–6.

52 Juhler M. Simultaneous determination of regional cerebral blood-flow, glucose metabolism, and pH in acute experimental allergic encephalomyelitis. *J Cerebral Blood Flow Metab* 1987;**7:**578–84.

53 Benet LZ, Massoud N, Gambertoglio JG (eds). *Pharmacokinetic basis for drug treatment.* Raven Press, New York, 1984.

54 de Vries HE, Prins ME, de Boer AG, *et al.* (unpublished results).

55 Wu NZ, Klitzman B, Dodge R, Dewhirst MW. Diminished leukocyte-endothelium interaction in tumor microvessels. *Cancer Res* 1992;**52:**4265–8.

56 Osborn L. Leukocyte adhesion to endothelium in inflammation. *Cell* 1990;**62:**3–6.

57 de Vries HE, Moor ACE, Blom-Rosemalen MCM, de Boer AG, *et al.* Lymphocyte adhesion to brain capillary endothelial cells *in vitro*. *J Neuroimmunol* 1994;**52:**1–8.

58 Nutt JG, Woodward WR, Hammerstad JP. The on-off phenomenon in Parkinson's disease: relation to levodopa and transport. *N Engl J Med* 1984;**310:**483–8.

59 Hunt CA, MacGregor RD, Siegel RA. Engineering targeted *in vivo* drug delivery. 1. The physiological and physicochemical principles governing opportunities and limitations. *Pharm Res* 1986;**3:**333–44.

60 Boddy AV, Aarons L, Petrak K. Efficiency of drug targeting: steady-state considerations using a three-compartmental model. *Pharm Res* 1989;**6:**367–72.

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