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Relationship of octanol/buffer and octanol/water partition coefficients to transcellular diffusion across brain microvessel endothelial cell monolayers

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Summary

The importance of lipid solubility on the rate of passage of solutes across the bovine blood–brain barrier (BBB) was studied with an in vitro BBB model. The model consisted of primary cultured bovine brain microvessel endothelial cell monolayers mounted in a side-by-side diffusion cell. Nine compounds (sucrose, mannitol, urea, glycerol, butyrate, thiourea, caffeine, antipyrine, propanolol) with different octanol/water partition coefficients were selected in order to provide a wide range of lipid solubility. Time- and concentration-dependence, and the effect of bovine serum albumin on the rate of transport of these compounds across the monolayers was determined. Consistent with in vivo studies, a significant positive correlation between transcellular diffusion and octanol/buffer or octanol/water partition coefficients was observed. The passage of these compounds across the monolayers was also concentration-dependent, non-saturable, and reduced in the presence of bovine serum albumin. Results demonstrate that these compounds undergo passive diffusion across the monolayers in which the rate of transfer across the monolayers depends directly on their lipid solubility and indirectly upon molecular size. In addition, serum albumin is an apparent factor in determining the passive permeability of the monolayers.

Introduction

Brain microvessel endothelial cells forming the blood–brain barrier (BBB) are joined together by tight intercellular junctions, possess few pinocytotic vesicles and lack fenestrations. Because of these unique properties, the passage of most water-soluble solutes, including nutrients and drugs, across the BBB is restricted (Brightman, 1977; Bradbury, 1985). Water-soluble nutrients such as amino acids, hexoses, amines, purines, nucleosides, and monocarboxylic acids are transported across the BBB by specific carrier systems (Pardridge and Oldendorf, 1977). Additionally, some water-soluble drugs such as α-methyldopa, α-methyltyrosine, melphalan, and L-DOPA are transported across the BBB by the neutral amino acid transport system (Pardridge, 1985). However, many blood-borne solutes enter the brain compartment by passively diffusing through or absorbing into the lipid membranes of the BBB. Therefore, rates of passage of these molecules

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through the BBB depend directly on lipid solubility and inversely on their molecular size (Oldendorf, 1974a; Fenstermacher, 1981). Oldendorf (1974b), Levin (1980), and Cornford et al. (1982) have demonstrated that brain uptake of water-soluble solutes in vivo is related to both the octanol/water or octanol/buffer partition coefficient, and molecular weight.

Recently, we established and characterized monolayers of brain microvessel endothelial cells as an in vitro model for studying BBB permeability. The endothelial cells of the in vitro model retain transport, metabolic, and morphological properties associated with BBB endothelium in vivo (Audus and Borchardt, 1986a; Audus and Borchardt, 1986b; Baranczyk-Kuzma et al., 1986). This model offers several advantages over whole animal studies including elimination of tissues (neuronal and smooth muscle) that may not be involved in determining BBB permeability characteristics, the ability to readily manipulate the composition and temperature of the cellular environment, and avoids the high cost of animal purchase and maintenance. Compared with other in vitro BBB models such as isolated microvessels, this model facilitates study of transcellular passage of molecules as opposed to only cellular uptake.

In the present study we have characterized the relationship between the transcellular diffusion of nine compounds (sucrose, mannitol, urea, glycerol, butyrate, thiourea, caffeine, antipyrine, propanolol) across the monolayers of the model system and the octanol/water or octanol/buffer partition coefficients. In addition, we have investigated the effects of serum albumin on transcellular diffusion in the model system. This study provides further support for the use of this in vitro system as a model for studying cellular level permeability characteristics of the BBB to drugs.

Materials and Methods

Materials

^{14}C-labeled sucrose, mannitol, caffeine, antipyrine, sodium *n*-butyrate, thiourea, and urea were purchased from ICN Radiochemicals (Irvine, CA). ^{3}H-labeled sucrose and propanolol were purchased from New England Nuclear (Boston, MA) and Amersham-Searle (Arlington Heights, IL).

Methods

Bovine brain microvessel endothelial cells were isolated from the gray matter of the cerebral cortex as described by Audus and Borchardt (1986a). Briefly, the gray matter was minced, enzymatically dispersed, centrifuged over dextran and then a 50% Percoll (Sigma Chemical Co., St. Louis, MO) gradient to separate endothelial cells from myelin, fat, and cell debris. Isolated endothelial cells were frozen for late use at −70°C in culture medium supplemented with 10% DMSO and 20% horse serum.

Regenerated cellulose membranes (nominal molecular weight cut-off 160,000 Da; Sartorius Filters, Hayward, CA) were cut into 15 mm diameter discs and placed in 100 mm plastic culture dishes (Corning Glass Works, Corning, NY). The discs were coated with rat-tail collagen and human fibronectin as described in Audus and Borchardt (1986b) and sterilized under the UV light for 1 h. After frozen endothelial cells were thawed and washed with culture medium, approximately 3 million cells were seeded into the culture dish containing the cellulose discs. Endothelial cells were incubated at 37°C with 5% CO_2 and 95% humidity. Cellulose discs containing complete monolayers (10–14 days after seeding) were removed aseptically from the culture dishes and used for transendothelial transport studies.

Horizontal side-by-side diffusion cells (Crown Glass Co., Somerville, NJ) were used for transendothelial transport studies. Regenerated cellulose discs with and without endothelial cell monolayers were placed in the diffusion cells and monolayers oriented so that the monolayer was always on the donor chamber side of the disc. The diameter of diffusion area was 9 mm and the total cell protein in that area approximately 0.08 mg (Lowery et al., 1951). The thermal jacket surrounding the sample chambers was thermostated at 37°C with a circulating water bath. The volume of the diffusion cell sample chambers was maintained at 3.0 ml during experiment and continually stirred with teflon stirring bar magnets driven at a constant

speed (600 rpm) by an external drive console (Crown Glass Co., Somerville, NJ). One sample chamber was designated donor chamber and the other the receptor chamber. The donor chamber was pulsed with a radiolabeled compound (about 0.5 μCi for ^{14}C-labeled compounds and 10 μCi for [^{3}H]propanolol) and [^{14}C]- or [^{3}H]sucrose (3 μCi for ^{3}H, 0.1 μCi for ^{14}C) to start experiments. A 0.1 ml aliquot of assay buffer was removed from the receptor cell at various times after pulsing, 10 ml of scintillation cocktail (3a70; Research Products Int., Mt. Prospect, IL) was added, and samples assayed for ^{3}H and ^{14}C with a Beckman 6800 scintillation counter (Beckman Instruments, Fullerton, CA). In order to maintain constant volume at both chambers, an aliquot (0.1 ml) of assay buffer was added back to the receptor chamber after each sample withdrawal. Composition of the assay buffer containing bovine serum albumin (BSA) was as follows: 150 mM NaCl, 4 mM KCl, 3.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, 15 mM HEPES, pH 7.4, 5 mM glucose, 1% BSA. The other assay buffer contained 122 mM NaCl, 25 mM $NaHCO_3$, pH 7.4, 10 mM glucose, 3 mM KCl, 1.2 mM $MgSO_4$, 0.4 mM K_2HPO_4, and 1.4 mM $CaCl_2$. Unless specified, the latter buffer was used for all experiments.

Viability of the endothelial cells of the monolayer was checked periodically by trypan blue exclusion and remains at 90–100% throughout the time period of the experiments.

The amount of compound transported was calculated by the following equation:

$$^{3}\text{H or } ^{14}\text{C compound transported} = \frac{M \cdot V \cdot S \cdot (1 \times 10^{12})}{P \cdot S \cdot 0.083 \text{ mg}} \text{ (pmol/mg/min)}$$

where M = concentration of labeled sample compound; V = volume of the chamber (0.003 liter); P = pulse in dpm; S = sample in dpm; T = time of sample taken after pulsing (min).

Octanol/buffer partition coefficients were determined for each compound as follows. About 0.5 μCi of labeled test compound in transendothelial assay buffer was added to 150 μl buffer. An equal volume of *n*-octanol (Aldrich Chemical Co, WI) was added, and the tube was vortexed for 1 min at high speed and then centrifuged for 1 min in a microcentrifuge (Model 5414, Brinkmann Instruments, NY) to separate oil and buffer phase. A sample of 100 μl was taken from both oil and buffer phase and prepared for scintillation counting. The partition coefficient was then calculated as follows.

$$\text{Partition coefficient} = \frac{^{14}\text{C or } ^{3}\text{H cpm}_{\text{octanol}}}{^{14}\text{C or } ^{3}\text{H cpm}_{\text{buffer}}}$$

Octanol/water partition coefficients were determined in the same manner as above except that buffer was replaced by water.

Data points in figures represent the mean of diffusion rates for at least 3 different monolayers. The standard error of the mean (S.E.M.) was generally 10–15% of the mean. Correlation coefficients were calculated and a one-sample *t*-test for correlation coefficients applied as described by Rosner (1982).

Results and Discussion

Brain microvessel endothelial cells form a complete monolayer on the cellulose discs after approximately 10 days in culture. Generally, after about 14–15 days in culture the endothelial cell monolayer is more permeable perhaps due to cell death or other morphological changes in the monolayer. Fig. 1 illustrates the differences in permeability to labeled sucrose observed for 10–18-day-old monolayers. Sucrose diffuses across endothelial cell monolayers intercellularly and does not cross biological membranes to an appreciable extent. Thus, significant sucrose leakage would indicate that the monolayer incompletely covers the cellulose disc either because of physical damage or because incomplete growth. Since a decreased permeability of the monolayers to sucrose was consistently observed with 10–14-day-old monolayers, only 10–14-day-old monolayers were used for experiments in this study. Endothelial cells forming 10–14-day-old monolayers also retain specific markers for BBB endothelium (Audus and Borchardt, 1986a; Baranczyk-Kuzma et al., 1986)

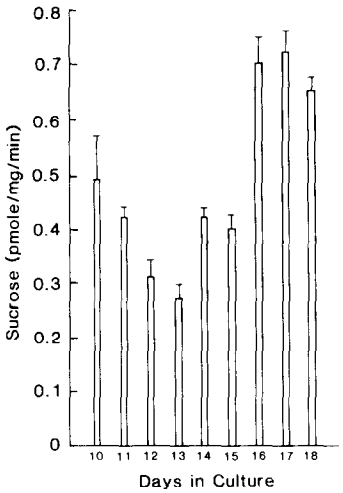

Fig. 1. Brain microvessel endothelial cell monolayer permeability to [^{14}C]sucrose 10–18 days after seeding onto growth surface.

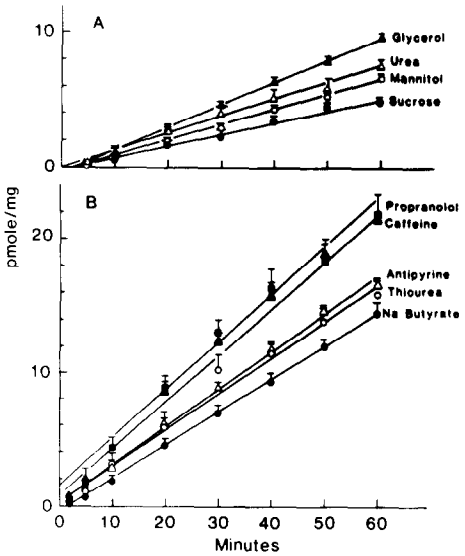

Fig. 2. Time-dependence of the passage of labeled solutes across brain microvessel endothelial cells grown on collagen-coated regenerated cellulose membranes. A: amount of sucrose, mannitol, urea, and glycerol transported at 37°C. B: amount of sodium butyrate, thiourea, antipyrine, caffeine, and propranolol transported at 37°C.

while older monolayers may or may not. Sucrose leakage across each monolayer used in these experiments was monitored and transcellular diffusion data collected only from those monolayers exhibiting low sucrose leakage (0.4 pmol/mg/min).

The passage of the nine radiolabeled compounds across brain microvessel endothelial cell monolayers increases linearly with time as shown in Fig. 2. The movement of these molecules across the monolayers is also concentration-dependent but not saturable (data not shown).

Except for propranolol, results in Table 1 demonstrate that the rate of solute diffusion across endothelial cell monolayers grown on the regenerated cellulose discs is less than the diffusion of solutes across collagen-coated cellulose discs alone. Additionally, there is no correlation ($r = -0.15$) between rates of solute passage across the cellulose disc and octanol/water or octanol/buffer partition coefficients (data not shown). Therefore, rates of solute diffusion, other than perhaps propranolol, across endothelial cell monolayers grown on collagen-coated regenerated cellulose membranes is not limited by the permeability properties of the cellulose disc. The cellulose membrane may be a limitation to the study of the permeability of the monolayers to propanolol in this model system.

The relationship between solute diffusion across bovine microvessel endothelial cell monolayers and octanol/water or octanol/buffer partition coefficients adjusted for molecular weight is shown in

TABLE 1

COMPARISON OF SOLUTE DIFFUSION ACROSS COLLAGEN-COATED REGENERATED CELLULOSE MEMBRANE WITH AND WITHOUT BRAIN MICROVESSEL ENDOTHELIAL CELL MONOLAYERS PRESENT

Compound	Monolayer (pmol/mg/min)	No monolayer (pmol/mg/min)
Sucrose	0.87	4.86
Mannitol	0.98	4.85
Urea	1.26	9.31
Glycerol	1.40	6.70
Sodium butyrate	2.33	6.86
Thiourea	3.21	10.74
Caffeine	4.88	6.21
Antipyrine	3.52	6.08
Propranolol	4.70	5.14

Fig. 3. A significant positive correlation exists between rates of transcellular diffusion and the octanol/water partition coefficient with ($r = 0.82$, $P < 0.005$) and without propanolol ($r = 0.97$, $P < 0.0005$) included in the analysis. Similarly, a significant positive correlation exists between transcellular diffusion and the octanol/buffer partition coefficient ($r = 0.84$, $P < 0.005$) with or without propanolol included in the analysis.

The rate of transcellular diffusion of solutes across the monolayers of this in vitro BBB model is reduced by addition of BSA to the transendothelial cell assay buffer as shown in Table 2. However, as shown in Fig. 4, a significant positive correlation between the rate of solute transcellular diffusion and octanol/buffer coefficient ($r = 0.85$, $P < 0.005$) still exists. As above, a significant positive correlation between transcellular diffusion and octanol/water partition coefficient is also observed without propanolol ($r = 0.89$, $P < 0.005$) included in the analysis (data not shown). There is not a correlation between transcellular diffusion and octanol/water partition coefficients with propanolol ($r = 0.55$, $P > 0.05$) included in the analysis. The large difference in correlations in the latter case may be reflective of the significant protein binding of propanolol (Pardridge et al., 1983).

TABLE 2

INHIBITION OF SOLUTE DIFFUSION ACROSS BRAIN MICROVESSEL ENDOTHELIAL CELL MONOLAYERS BY BOVINE SERUM ALBUMIN (BSA)

Compound	BSA (pmol/mg/min)	No BSA (pmol/mg/min)	% Inhibition
Sucrose	0.42	0.87	52
Mannitol	0.61	0.98	38
Urea	1.44	1.26	0
Glycerol	0.38	1.40	73
Sodium butyrate	1.14	2.33	51
Thiourea	2.62	3.21	18
Caffeine	4.04	4.88	17
Antipyrine	3.46	3.52	2
Propranolol	1.76	4.70	63

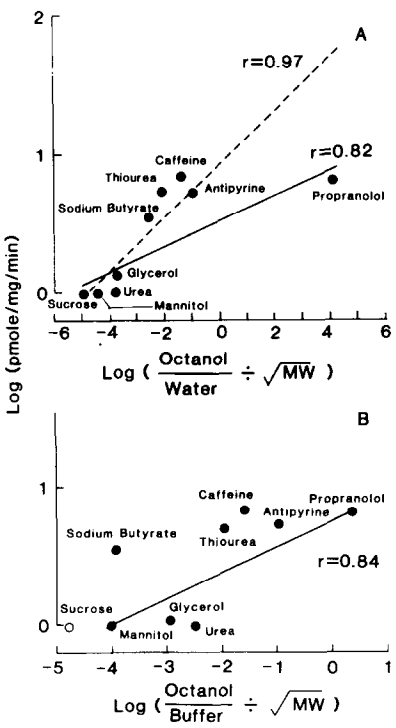

Fig. 3. Relationship between rate of solute passage across brain microvessel endothelial cell monolayers and increasing solute lipophilicity. A: rate of transcellular diffusion vs octanol/water partition coefficient. Solid line represents linear regression through all data points and the dashed line, linear regression through all data points excluding propranolol. B: rate of transcellular diffusion versus octanol/buffer partition coefficient.

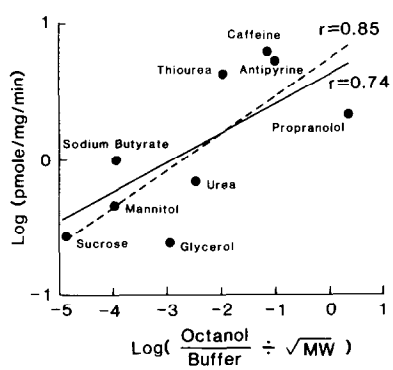

Fig. 4. Relationship between rate of solute passage across brain microvessel endothelial cell monolayers and octanol/buffer partition coefficient in the presence of bovine serum albumin. Solid line represents linear regression through all data points and the dashed line, linear regression through all data points excluding propranolol.

While significant binding to BSA may explain reduced transcellular diffusion of propanolol (Pardridge et al., 1983), the inhibition (see Table 2) of the transcellular diffusion of glycerol, sucrose, butyrate, and mannitol across the monolayers may not. Recent reports do suggest that the networks of fibrous surface glycoproteins contribute to the overall molecular sieving properties of the endothelial cell. Thus, binding of proteins to this fibrous matrix may alter the permeability of the endothelial cell (Mason et al., 1977; Curry and Michel, 1980; Turner et al., 1983). Pardridge et al. (1985) have shown that a significant fraction of BSA binds to isolated brain microvessel endothelium in a non-specific manner. Such protein–cell interactions may in part explain the effects of BSA on the permeability of the monolayers of this model system. Further study of the interactions of BSA and other macromolecules with endothelial cell monolayer, and resultant monolayer permeability changes is continuing in this laboratory.

In summary, the relationship between the rates of solute passage across brain microvessel endothelial cell monolayers and partition coefficients demonstrated in this in vitro model are consistent with similar in vivo brain uptake studies by Oldendorf (1974b), Levin (1980), and Cornford et al. (1982). In addition, we have provided some evidence that serum albumin may play a role in determining the permeability of the endothelial cell monolayer. Finally, the results of this study, together with a previous ones by Audus and Borchardt (1986a and b), would indicate that this in vitro system may be an appropriate model for cellular level investigations of the permeability characteristics of the BBB in vivo to drugs.

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