Drug-Protein Binding and Blood-Brain Barrier Permeability

HIDEO TANAKA and KENJI MIZOJIRI
Developmental Research Laboratories, Shionogi & Co., Ltd., Toyonaka, Osaka, Japan

Accepted for publication July 30, 1998 This paper is available online at http://www.jpet.org

ABSTRACT
The permeability surface area (PS) product, an index of permeability of the blood-brain barrier (BBB), was measured by using the in situ perfusion method. In the cerebral circulation, the fraction of drug that permeates into the brain through the BBB is not only the unbound fraction but also the fraction dissociated from the protein in the perfusate. The sum of these two fractions, the apparent exchangeable fraction, was estimated by fitting the parameters of the BBB permeability under the condition of varying BSA concentrations in the perfusate. The unbound fraction of drugs in a buffer containing 0.5 mM BSA was measured by using the ultrafiltration method in vitro, and the apparent exchangeable fraction was measured in vivo by using the intracarotid artery injection method. The apparent exchange fraction was 100% for S-8510, 96.5% for diazepam, 90.9% for caffeine, 38.3% for S-312-d, 33.1% for propranolol, and 6.68% for (+)-S-145 Na, and each of these was higher than the corresponding unbound fraction in vitro in all drugs. The apparent exchangeable fractions, for example, were 8 times higher for diazepam and 38 times for S-312-d than the unbound fractions in vitro. The apparent exchangeable fraction of drugs was also estimated from the parameters obtained with the perfusion method. Because drugs can be infused for an arbitrary length of time in the perfusion method, substances with low permeability can be measured. The apparent exchangeable fractions obtained with this method were almost the same as those obtained with the intracarotid artery injection method.

In general, only the unbound fraction of drugs in media such as blood or protein-containing perfusate is thought to be transferred into body tissues. Thus, unbound fraction values measured with ultrafiltration or equilibrium dialysis in vitro are used not only for measurement of transfer rate into body tissues but also of blood-brain barrier (BBB) permeability. However, when we measured the BBB permeability of diazepam with the intracarotid artery (i.c.a.) injection method, we found that its cerebral concentration was much higher than that estimated based on the assumption that only the unbound fraction measured in vitro is able to penetrate the BBB. Because protein-bound drugs easily dissociate and permeate through the BBB in the cerebral circulation, the concentration of drug in the brain will be higher than that estimated from the unbound fraction in vitro. This suggested that use of the in vitro unbound fraction in determining BBB permeability might not be appropriate. Although this consideration has already been discussed (Pardridge and Landaw, 1984), the BBB permeabilities of most drugs are in fact calculated using in vitro unbound fractions with suitably acceptable results. However, methods based on the theory that drugs binding to protein dissociate rapidly and pass through the BBB may yield more accurate measurements of BBB permeability in vivo.

We therefore used the in situ perfusion method to measure the apparent exchange fractions of drugs with BBB permeability too low for measurement with the i.c.a. injection method and compare the results to observe the extent of their agreement.

The drugs used in the present study included diazepam, caffeine, and propranolol, as well as three drugs synthesized in our laboratories: (+)-S-145 Na, a thromboxane A2 receptor antagonist; S-312-d, a Ca2+ channel antagonist that improves cerebral blood flow and displays protection of central neurons; and S-8510, a benzodiazepine inverse agonist.

Experimental Procedures
Materials. [14C]Diazepam was obtained from Amersham International (Buckinghamshire, UK), [3H]Diazepam, [14C]caffeine, L-[3H]propranolol, [3H]glucose, [14C]sucrose, [3H]sucrose, [3H]water, and [14C]butanol were obtained from NEN Life Science (Boston, MA). (+)-[14C]S-145 Na ([+](1R-(1a,2az(2),3b,4a)]7-[3-[(U-14C)phenylsulfonfonyl]amino]bicyclo[2.2.1]hept-2-yl)-5-heptenoic acid sodium salt), [14C]S-312-d ([S]+)-methyl-3-isobutyl-6-methyl-4-(3-nitrophenyl)-4,7-dihydrothieno[2,3-b][14]-pyridine-5-carboxylate, and [14C]S-8510 [2-(isoxazol-3-yl)-3,6,7,9-tetrahydroimidazo[4,5-d]pyrazolo[4,3-b][14]-pyridine monophosphate monohydrate] were synthesized at Shionogi Research Laboratories (Shionogi & Co., Ltd., Osaka, Japan) and were confirmed to have radiochemical purities above 99% by HPLC. The labeled positions of the test drugs are shown in Fig. 1. All other reagents were of analytical grade.

Perfusion Method. Male Sprague-Dawley rats (Clea Japan, Inc., Tokyo) age 9 weeks were used. The rats were anesthetized with...
pentobarbital; the occipital artery, superior thyroid artery, and pterygopalatine artery were clotted or ligated; and the external carotid artery was cannulated in a retrograde manner with a polyethylene tube (PE50, Intramedic, Sparta, MD) (Takasato et al., 1984). Krebs-Henseleit buffer (118 mM NaCl, 14.7 mM KCl, 2.5 mM CaCl2, 1.2 mM MgSO4, 1.2 mM KH2PO4, 25 mM NaHCO3, 10 mM d-glucose, pH 7.4) was used as a perfusate. This buffer was bubbled with a mixture of 95% O2 and 5% CO2, filtered through Millex HV filter (Millipore, Bedford, MA), incubated at 36°C, and infused with an infusion pump (Harvard Apparatus, S. Natick, MA). In our laboratories and used in this study include (+)-S-145 Na, a thromboxane A2 receptor antagonist; S-312-d, a Ca2+ channel antagonist that improves cerebral blood flow and offers protection of central neurons; and S-8510, a benzodiazepine inverse agonist.

Local Cerebral Perfusion Flow in Rat Forebrain. A mixture of 7.4 kBq (1.03 μg/ml [14C]diazepam and 37 kBq (0.017 μg/ml [3H]sucrose was perfused for 10 s, and the animal was decapitated. Local cerebral perfusate flow (LCPF: represented Q in equation) was obtained using the equation:

\[ Q = \frac{C_{in}(T)}{C_{in} \cdot T} \]  

(1)

where \( C_{in}(T) \), the parenchymal brain concentration (dpm/g) of [14C]diazepam at time \( T \) was calculated as the measured brain concentration minus intravascular tracer concentration, with the latter equal to the product of regional vascular volume measured by [3H]sucrose multiplied by perfusion fluid concentration of tracer. \( C_{in} \) is the concentration (dpm/g) of unbound [14C]diazepam in the perfusate, and \( T \) is perfusion time (s).

Apparent Exchangeable Fraction in Brain Microcirculation Determined with Perfusion Method. Apparent exchangeable fractions for 3.7 kBq (0.36 μg/ml [14C]caffeine, 3.7 kBq (1.3 μg/ml [14C]-S-312-d, and 37 kBq (18 μg/ml [14C](-)-S-145 Na were measured with the perfusion method. Each [14C]-labeled tracer and [3H]sucrose were added to perfusate. The BSA concentration in perfusate was varied between 0 and 1 mM. Perfusion with [14C]caffeine and [14C]-S-312-d was performed for 10 s, and perfusion with [14C](-)-S-145 Na was performed for 30 s; the value of \( C_{in}(T)/C_{in\cdot tot} \cdot T \cdot Q \) was obtained for each concentration of BSA. \( C_{in}(T) \) of each tracer was the parenchymal brain concentration. \( K_{d,app} \) was calculated by fitting to eq. 2 (refer to Appendix for details):

\[ \frac{C_{in}(T)}{C_{in\cdot tot} \cdot T \cdot Q} = \frac{1 - \exp(-k_{dt})}{1 + [P]/K_{d,app}} \]  

(2)

The apparent exchangeable fraction \( f_{app} \) was obtained from eq. 14 (Partridge and Fierer, 1990) given in the Appendix.

Apparent Exchangeable Fraction in Brain Microcirculation Determined with i.c.a. Injection Method. Each rat was anesthetized with pentobarbital, and 0.2 ml of a mixture of test compound and a reference compound was rapidly injected from the carotid artery. The animal was decapitated at 5 s after the injection, and the brain uptake index was calculated from the ratio of test to reference values of the administered solution and that in the brain (Oldendorf, 1970). The test compounds used were 3.7 kBq (0.04 μg/ml [3H]diazepam, 37 kBq (3.6 μg/ml [14C]caffeine, 74 kBq (0.034 μg/ml [3H]propranolol, 18.5 kBq (6.4 μg/ml [14C](-)-S-145 Na, 200 kBq (100 μg/ml [14C](-)-S-145 Na, 37 kBq (15 μg/ml [14C]-S-8510, [3H]Water or [14C]butanol was used as the reference compound. For comparison of the values obtained with different reference compounds, brain uptake index was converted to the extraction \( E \) with N-isopropyl-p-iodoamphetamine (Partridge et al., 1985). The BSA concentration in the administered solution was varied between 0 to 1 mM. \( E \) was measured for each concentration, and the apparent dissociation constant \( K_{d,app} \) was calculated by fitting to eq. 3, derived from the model in Fig. 2 (Partridge and Landaw, 1984):

\[ E = 1 - \exp \left( -\frac{k_{dt}}{1 + [P]/K_{d,app}} \right) \]  

(3)

where \( k_{dt} \) is the rate constant of drug transport from blood to brain (s\(^{-1}\)); \( t \) is the mean brain capillary transit time (s); and \([P]\) is the concentration of BSA (M). The apparent exchangeable fraction \( f_{app} \) was obtained from eq. 14 (Partridge and Fierer, 1990) given in the Appendix. The fitting of eqs. 2 and 3 to brain permeability-protein concentration in the perfusate curve was performed with least-squares regression analysis (Yamaoka et al., 1981). The \( K_{d,app} \) ± S.D. predicted on the basis of fitting the experimental data to eqs. 2 and 3 is shown in Table 2. The data were analyzed using the Damping-Gauss-Newton method, except for S-8510 (in the i.c.a. injection method) and caffeine (in the perfusion method), which analyzed with the Simplex method.

**PS Products Determined with Perfusion Method.** PS products for 3.7 kBq (0.04 μg/ml [3H]diazepam, 37 kBq (3.6 μg/ml [14C]caffeine, 74 kBq (0.034 μg/ml [3H]propranolol, 18.5 kBq (6.4 μg/ml [14C]-S-312-d, and 200 kBq (100 μg/ml [14C](-)-S-145 Na, 37 kBq (15 μg/ml [14C]-S-8510, [3H]Water or [14C]butanol was used as the reference compound. For comparison of the values obtained with different reference compounds, brain uptake index was converted to the extraction \( E \) with N-isopropyl-p-iodoamphetamine (Partridge et al., 1985). The BSA concentration in the administered solution was varied between 0 to 1 mM. \( E \) was measured for each concentration, and the apparent dissociation constant \( K_{d,app} \) was calculated by fitting to eq. 3, derived from the model in Fig. 2 (Partridge and Landaw, 1984):

\[ E = 1 - \exp \left( -\frac{k_{dt}}{1 + [P]/K_{d,app}} \right) \]  

(3)

where \( k_{dt} \) is the rate constant of drug transport from blood to brain (s\(^{-1}\)); \( t \) is the mean brain capillary transit time (s); and \([P]\) is the concentration of BSA (M). The apparent exchangeable fraction \( f_{app} \) was obtained from eq. 14 (Partridge and Fierer, 1990) given in the Appendix. The fitting of eqs. 2 and 3 to brain permeability-protein concentration in the perfusate curve was performed with least-squares regression analysis (Yamaoka et al., 1981). The \( K_{d,app} \) ± S.D. predicted on the basis of fitting the experimental data to eqs. 2 and 3 is shown in Table 2. The data were analyzed using the Damping-Gauss-Newton method, except for S-8510 (in the i.c.a. injection method) and caffeine (in the perfusion method), which analyzed with the Simplex method.

**Fig. 1.** Compounds synthesized in our laboratories and used in this study include (+)-S-145 Na, a thromboxane A2 receptor antagonist; S-312-d, a Ca2+ channel antagonist that improves cerebral blood flow and offers protection of central neurons; and S-8510, a benzodiazepine inverse agonist.

**Fig. 2.** A compartmental model of transport of protein-bound drugs into the brain in vivo during passage through the brain microcirculation. [PD], protein/drug complex; [P], free protein; [D], free drug in the plasma compartment; [Db], free drug in the brain compartment. \( k_{1} \) is the rate constant of drug dissociation (s\(^{-1}\)); \( k_{2} \) is the rate constant of drug association (mol\(^{-1}\) s\(^{-1}\)), \( k_{3} \) is the rate constant of drug dissociation (s\(^{-1}\)), and \( k_{4} \) is the rate constant of drug from plasma transport through the BBB (s\(^{-1}\)). Because plasma proteins do not cross the BBB, the transport of protein-bound drugs into the brain occurs via a free intermediate mechanism that involves obligatory dissociation of protein-bound drugs into the free intermediate state before BBB transport.
Table 1

<table>
<thead>
<tr>
<th>BSA Concentration, mM</th>
<th>0</th>
<th>0.02</th>
<th>0.05</th>
<th>0.1</th>
<th>0.2</th>
<th>0.5</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>ml/min/ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>1.98 (0.412)</td>
<td>1.97 (0.147)</td>
<td>1.90 (0.242)</td>
<td>1.96 (0.314)</td>
<td>1.70 (0.078)</td>
<td>1.35 (0.120)</td>
<td>0.90 (0.058)</td>
</tr>
<tr>
<td>3.5</td>
<td>2.43 (0.472)</td>
<td>2.38 (0.348)</td>
<td>2.30 (0.288)</td>
<td>2.33 (0.275)</td>
<td>2.05 (0.144)</td>
<td>1.46 (0.151)</td>
<td>1.21 (0.111)</td>
</tr>
<tr>
<td>5</td>
<td>2.81 (0.212)</td>
<td>2.89 (0.463)</td>
<td>2.75 (0.620)</td>
<td>2.63 (0.350)</td>
<td>2.69 (0.135)</td>
<td>2.05 (0.149)</td>
<td>1.61 (0.138)</td>
</tr>
</tbody>
</table>

*Mean (S.D.) (n = 4–6).*

---

**Results**

**LCPF in Rat Forebrain.** The flow rate of perfusate from pump was set at three levels (2.5, 3.5, and 5 ml/min), and LCPF was measured in BSA concentrations in perfusate from 0 to 1 mM (Table 1). At each pump flow rate, LCPF in the hemisphere of the forehead was smaller than the pump flow rate but gradually decreased at higher concentrations of BSA. LCPF was calculated using the in vivo apparent exchangeable fraction of \[^{14}\text{C}\]diazepam measured by i.c.a. injection method.

**Protein Binding In Vitro.** Each drug was dissolved in the Krebs-Henseleit buffer containing 0.5 mM BSA (Fraction V; Sigma, St. Louis, MO), and protein binding was measured with the ultrafiltration method using an MPS-3 filter (Amicon, Beverly, MA). In this experiment, the adsorption of each drug to the PMS-3 filter was measured and used to correct the protein binding.

---

**Appendant Exchangeable Fraction in Brain Microcirculation Determined with i.c.a. Injection Method.** The extraction (E) of each drug was determined with the i.c.a. injection method. The experimentally observed values are indicated by the symbols, and the E predicted by fitting the experimental data to eq. 3 is indicated by a solid line (Fig. 4). Although E decreased with increase in BSA concentration in the i.c.a. injection method, the degree of decrease varied depending on the drug. Extraction of S-8510 was not affected by BSA at all, whereas E of diazepam and caffeine were slightly affected and that of (+)-S-145 Na decreased sharply with increasing BSA concentration. The \(K_{d, \text{app}}\) of S-8510 was 223,121 mM, whereas that of (+)-S-145 Na was 0.0358 mM, indicating the slow dissociation of latter compound from BSA.
The ratio of drug concentration in the brain at time $T$ to the total amount of drug perfused in the brain until time $T$, whereas $E$ compares the ratio of the reference compound to the test compound in the perfusate with the corresponding ratio in the brain. Although different methods were used, similar changes were noted for these indices of BBB permeability by BSA. These findings showed that both methods yield an accurate determination of BBB permeability.

The apparent dissociation constants ($K_{d,app}$) and apparent exchangeable fraction ($f_{app}$) obtained with the i.c.a. injection method and the perfusion method in the BSA concentration (0.5 mM) are shown in Table 2. Unbound fractions measured with the ultrafiltration method in vitro are also shown for comparison.

The apparent exchangeable fraction was higher than the in vitro unbound fraction for every compound tested. These observations indicate that compounds bound to BSA under static conditions will dissociate under dynamic conditions in the cerebral circulation. The apparent exchangeable fraction of S-312-d was 38% and much higher than the in vitro findings of 1% in the unbound fraction, indicating that this compound has a high protein-binding ratio but readily dissociates in the cerebral circulation. The apparent exchangeable fraction of diazepam was about 8 times the in vitro unbound fraction, whereas those of other compounds were less than twice as high as the in vitro unbound fraction. For caffeine, there was little difference between the two. It has been reported that the in vitro unbound fraction of propranolol was 0.3 in buffer containing 4% BSA (Gariepy et al., 1990) and 0.29 in buffer containing 3% BSA (Jones et al., 1984). These
values are almost the same as the apparent exchangeable fraction ($f_{app}$) in the cerebral circulation observed in the present study.

The $K_{d,app}$ obtained with the perfusion method was slightly lower than that obtained with the i.c.a. injection method for S-312-d and (+)-S-145Na. The $K_{d,app}$ of caffeine was 4.99 mM with the i.c.a. injection method but very high with the perfusion method. Although the $K_{d,app}$ obtained with the two methods appeared to differ, the apparent exchangeable fraction ($f_{app}$) at 0.5 mM BSA calculated from the $K_{d,app}$ was 90.9% for the i.c.a. injection method and not significantly different from the value of 100% for the perfusion method. Indeed, the $K_{d,app}$ can vary markedly based on the fitting estimation for drugs whose permeability is little affected by BSA, but the apparent exchangeable fraction, which is estimated from $K_{d,app}$ is approximately equal to the almost unbound fraction.

### Comparison of Unbound Fraction In Vitro and Apparent Exchangeable Fraction In Vivo of Diazepam

The unbound fraction of diazepam was extremely decreased by 0.017 mM BSA in the buffer solution in vitro and decreased further as the BSA concentration increased (Table 3). On the other hand, the apparent exchangeable fraction of diazepam was decreased only a little by an increase in the concentration of BSA. In 0.17 mM BSA, the ratio of the apparent exchangeable fraction to the unbound fraction was 3.6, and in 0.5 mM BSA, it was 8; this meant that an 8-fold higher concentration of unbound fraction of diazepam existed in the cerebral circulation and more drug contributed to protein binding in the cerebral circulation. Protein-bound drugs have been reported to be transported into the cerebral circulation.

### Discussion

In general, drugs penetrating into tissues are thought to be those in the unbound fraction in blood, and in vitro values are usually used to estimate drug transport. Detailed analytical methods are available for measuring protein binding through the use of equilibrium dialysis and ultrafiltration. Two protein-binding sites for diazepam and (+)-S-145 Na were indicated with Scatchard plot analysis and Michaelis-Menten plot analysis, and the corresponding dissociation constants, $K_{d1}$ and $K_{d2}$, of each compound were obtained (Table 2). Caffeine and S-8510 yielded straight lines on Scatchard plots and thus appeared to each have one binding site. In contrast, only the apparent dissociation constant $K_{d,app}$ can be obtained by fitting data obtained with the i.c.a. injection and perfusion methods. Although the values of $K_{d,app}$ were almost the same for caffeine and (+)-S-145 Na, they differed greatly for diazepam and S-8510. The significance of these differences is unclear at present. $K_{d,app}$ was measured for dissociation from albumin in the cerebral circulatory system assuming a single binding site. Although there may be sites with greater or lesser dissociability for compounds from albumin, the $K_{d,app}$ was measured for overall dissociation from albumin in the cerebral circulation.

Pardridge and coworkers have reported the permeation of protein-bound compounds through the BBB (Cornford et al., 1983; Pardridge et al., 1983; Terasaki et al., 1986). In addition, albumin-bound drugs have been reported to be transported into the liver (Weisiger et al., 1981; Forker and Luxon, 1983). In the present study, we demonstrated the participation of protein-bound drugs in BBB permeation for diazepam, caffeine, propranolol, S-312-d, (+)-S-145Na, and S-8510 with...
obtained with the i.c.a. injection method (Table 2). However, the perfusion method was very large and differed greatly from that from K tration (2) the effect of BSA on 

leagues were equivalent to those obtained with our perfusion method. Although the mechanism by which protein-bound drugs permeate tissues is not known, the report of Horie et al. (1988) that conformational change of albumin occurs in contact with isolated rat hepatocytes is of interest because this would suggest that dissociation of compounds from protein may occur due to the conformational change of albumin when it comes into contact with endothelial cells in brain capillaries. If this does occur, the strength of the hydrogen bonding, which plays an important role in the binding of drugs to albumin, may be related to the apparent exchangeable fraction.

We developed a new method to measure the apparent exchangeable fraction from data obtained with the perfusion method and compared the values with those obtained with the i.c.a. injection method that had already been reported by Pardridge and Landaw (1984). The \( K_{d,app} \) values for (+)-S-145 Na and S-312-d obtained with the perfusion method were nearly the same as those obtained with the i.c.a. injection method. On the other hand, \( K_{d,app} \) for caffeine obtained with the perfusion method was very large and differed greatly from that obtained with the i.c.a. injection method (Table 2). However, the apparent exchangeable fractions \( f_{app} \) of caffeine calculated from \( K_{d,app} \) were 100% for the perfusion method and 90.9% for the i.c.a. injection method and thus differed only a little. In the case of caffeine, which undergoes less dissociation from BSA and has a large apparent exchangeable fraction, the different \( K_{d,app} \) may have been predictable on the basis of fitting estimation from different experimental methods.

The \( K_{d,app} \) obtained under anesthesia with ether was smaller than that obtained with pentobarbital because blood flow is more rapid under ether anesthesia (Pardridge and Fierer, 1990). If this is true, it should not be possible to apply the apparent exchange fraction obtained with the i.c.a. injection method to the perfusion method when LCPF is larger than the cerebral blood flow of the i.c.a. injection method. Therefore, the \( K_{d,app} \) for (+)-S-145 Na was measured by changing the perfusion rate (Fig. 6). The effects of BSA on the \( C_{br}(T)/(C_{in,tot} \cdot T \cdot Q) \) for (+)-S-145 Na were unchanged when the perfusion velocity was increased from 2.5 to 5 ml/min, and no difference in \( K_{d,app} \) was found. These observations confirmed that \( K_{d,app} \) was not affected by a change in the blood flow rate.

In conclusion, the following findings validated the use of the perfusion method for determination of the apparent exchangeable fraction: 1) the apparent exchangeable fractions obtained with the i.c.a. injection method reported by Pardridge and colleagues were equivalent to those obtained with our perfusion method, 2) the effect of BSA on \( C_{br}(T)/(C_{in,tot} \cdot T \cdot Q) \) measured with the perfusion method was similar to that on \( E \) measured with the i.c.a. injection method, and 3) equal and stable values of \( K_{d,app} \) were obtained with different perfusion velocities.

Appendix

The permeability clearance \( (K_{pa}) \) defined for drug concentration \( (C_{in}) \) in the perfusate flowing into brain tissues is experimentally obtained using the perfusion method.

\[
C_{br}(T) = K_{in} \cdot C_{in} \cdot T
\]  

(5)

\( C_{br}(T) \) is the parenchymal brain concentration of drug, which is calculated as measured brain concentration minus intravascular drug concentration, at perfusion time \( (T) \). First, when no protein is contained in the perfusate and the drug is 100% unbound \( (C_{in,tot}) \), the relationship between \( K_{in} \) and PS product can be described using the capillary model shown in Fig. 7.

\[
PS = -Q \cdot \ln \left( 1 - \frac{K_{in}}{Q} \right)
\]  

(6)

\[
\text{Perfusion rate} \quad \triangle : 2.5 \text{ ml/min} \\
\text{O : 3.5 ml/min} \\
\text{D : 5.0 ml/min}
\]
The velocity of drug transfer \((v)\) from perfusate into the brain is expressed in eq. 7 based on the definition of \(K_{in}\)

\[
v = K_{in} \cdot C_{in,tot}
\]

(7)

Given the mass-balance characteristics of drugs in a microscopic region with distance \(x\) from the entrance at a steady state, eq. 8 can be derived from the definition of PS using \(Q\) as the perfusate flow rate:

\[
v = Q \cdot C_{in,tot} \left[1 - \exp\left(\frac{-PS}{Q}\right)\right]
\]

(8)

Moreover, PS can be expressed in eq. 9 using the model in Fig. 2

\[
k_{o}f = \frac{\text{PS}}{Q}
\]

(9)

By substitution of eq. 9 into eq. 8, we obtained eq. 10 from eqs. 7 and 8

\[
C_{in,tot} = \frac{Q \cdot C_{in,tot} \left[1 - \exp\left(-k_{o}f \cdot t\right)\right]}{K_{in}}
\]

(10)

The intracerebral concentration \(C_{br}(T)\) of eq. 5 is expressed in eq. 11

\[
C_{br}(T) = K_{in} \cdot C_{in,tot} \cdot T
\]

(11)

eq. 11 is substituted into eq. 10 to yield eq. 12

\[
C_{in,tot} = \frac{C_{br}(T)}{T \cdot Q \left[1 - \exp\left(-k_{o}f \cdot t\right)\right]}
\]

(12)

When the perfusate contains protein and drug binds to the protein, if the apparent exchangeable fraction \(f_{app}\) is considered regarding \(C_{in,tot}\), eq. 13 is obtained, and \(f_{app}\) can be expressed by eq. 14:

\[
f_{app} \cdot C_{in,tot} = \frac{C_{br}(T)}{T \cdot Q \left[1 - \exp\left(-k_{o}f \cdot t\right)\right]}
\]

(13)

\[
f_{app} = \frac{K_{d,app}}{K_{d,app} + [P]}
\]

(14)

Eq. 14 is substituted into eq. 13, and when the denominator and numerator on the left are each divided by \(K_{d,app}\), eq. 2 of the text is obtained:

\[
\frac{C_{br}(T)}{C_{in,tot} \cdot T \cdot Q} = \frac{1 - \exp\left(-k_{o}f \cdot t\right)}{1 + [P]/K_{d,app}}
\]

\(K_{d,app}\) and \(k_{o}f\) are obtained by calculation using the least-squares method based on the changes in \(C_{br}(T)/(C_{in,tot} \cdot T \cdot Q)\) when the brain is perfused with various BSA concentrations present in the perfusate.

Acknowledgments

We thank Tohru Nagasaki and Yoshihiko Katsuyama for synthesis of labeled compounds. The assistance of Yuka Iwamoto is also gratefully acknowledged.

References


Send reprint requests to: Dr. Hideo Tanaka Developmental Research Laboratory, Shionogi & Co., Ltd., 12-4, 5-chome, Sagisu, Fukushima-ku, Osaka 553-0002, Japan. E-mail: hideo.tanaka@shionogi.co.jp