Comparison of blood-brain barrier permeability in mice and rats using in situ brain perfusion technique

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MATERIALS AND METHODS

Reagents and animals. Iodo-[14C]antipyrine (67.5 mCi/mmol), [3H]methotrexate (15.0 Ci/mmol), [3H]quinidine (15.0 Ci/mmol), and [3H]quinine (14.5 Ci/mmol) were obtained from American Radiolabeled Chemicals (St. Louis, MO). [14C]tolbutamide (63.0 mCi/mmol), [14C]mannitol (19.7 mCi/mmol), [14C]phenylalanine (448.0 mCi/mmol), [14C]diazepam and [3H]methotrexate (15.0 Ci/mmol), [3H]quinidine (15.0 Ci/mmol), and [3H]quinine (14.5 Ci/mmol) were obtained from American Radiolabeled Chemicals (St. Louis, MO). [14C]tolbutamide (63.0 mCi/mmol), [14C]mannitol (19.7 mCi/mmol), [14C]phenylalanine (448.0 mCi/mmol), [14C]diazepam...
(55.0 mCi/mmol), [14C]warfarin (55.0 mCi/mmol), [3H]cimetidine (11.3 Ci/mmol), [3H]vincristine (6.1 Ci/mmol), [3H]cy-closporin A (9.9 Ci/mmol), [3H]vinblastine (14.5 Ci/mmol), [3H]alanine (52.0 Ci/mmol), and [3H]hypoxanthine (21.0 Ci/ mmol) were obtained from Amersham International (Buckinghamshire, UK). [3H]sucrose (12.3 Ci/mmol), [3H]glyburide (glucenclamide) (50.0 Ci/mmol), [3H]digoxin (15.0 Ci/mmol), and [3H]theophylline (18.5 Ci/mmol) were obtained from DuPont-New England Nuclear (Boston, MA). [14C]diazepam, [14C]diazepam, [14C]warfarin, and [14C]diazepam were obtained from Moravek Biochemicals (Brea, CA). Solvable was obtained from Packard Instruments (Downers Grove, IL). All other chemicals used in the experiments were of analytic grade.

The male ddY mice (20–25 g) and male Wistar rats (170–230 g) were obtained from Seac Yoshitomi (Fukuoka, Japan).

Surgical procedure. The right cerebral hemisphere of the mouse or rat was perfused by the method reported previously (28) with minor modifications. In brief, adult male mice (ddY, 20–25 g body wt) or adult male rats (Wistar, 170–230 g body wt) were anesthetized with pentobarbital sodium (50 mg/kg ip) (Dainippon Pharmaceutical, Osaka, Japan). In mice, the right common carotid artery was exposed with the aid of a microscope, the occipital and superior thyroid arteries were coagulated (20) and cut, and the right pterygopalatine artery was ligated. The right external carotid artery was then catheterized for retrograde infusion with 10 cm of polyethylene tubing (SP-10, Natsume, Tokyo, Japan) filled with heparinized saline; the opening of the catheterer was placed 2–3 mm above the bifurcation of the common carotid artery. The right common carotid artery was prepared for ligation by encircling the artery with surgical thread.

Perfusion fluid. Krebs-Henseleit buffer was prepared containing (in mM) 118.0 NaCl, 4.7 KCl, 1.2 MgSO4, 1.2 KH2PO4, 25 NaHCO3, and 2.5 CaCl2. Before an experiment, 10 mM D-glucose was added, and the perfusate was oxygenated for 10 min with 95% O2–5% CO2. The perfusate was adjusted to pH 7.4 with 1 M HCl. To determine the cerebral perfusion fluid flow, intravascular volume, or PS value, radiolabeled drugs were added to the perfusion fluid, and the mixture was equilibrated at 37°C with a water bath.

Perfusion system. Cerebral perfusion fluid flow (using [14C]diazepam) or PS value of various drugs were calculated, based on the parenchymal brain uptake from the perfusion fluid. The perfusion fluid containing radiolabeled drug was infused into the right external carotid artery at a constant rate by an infusion pump (model 55-1111, Harvard Apparatus, Harvard, MA), and the right common carotid artery was ligated immediately. The perfusion rate was 1.0 ml/min in mice, and perfusion was continued for 15, 30, or 60 s. The perfusion was terminated by decapitation. The right cerebral hemisphere was immediately excised, weighed, and placed in a scintillation vial. Samples were digested at 80°C in 1.5 ml of Solvable under airtight conditions. After cooling, samples were treated with 200 µl of 30% H2O2, neutralized by addition of 100 µl of 6 M HCl, and prepared for scintillation counting by addition of 15 ml of scintillation cocktail (Clearsol I, Nacalai Tesque, Kyoto, Japan). Immediately after the decapitation, a 20-µl sample of perfusate was placed in a scintillation vial and prepared for scintillation counting by addition of 15 ml of scintillation cocktail. The radioactivity of the brain or perfusate samples was measured with a liquid scintillation counter (model LS6500, Beckman Instruments, Fullerton, CA).

Determination of perfusion rate, carotid perfusion pressure, and perfusion delay time. Carotid perfusion pressure was measured in anesthetized mice by connecting the catheter inserted into the right external carotid artery to a strain gauge transducer (model TP-400T, Nihon Kohden, Tokyo, Japan) and to the cylinder containing perfusate via a T connector. Carotid perfusion pressure was determined as the pressure during perfusion minus the open catheter pressure (catheter resistance). Before measurement of carotid perfusion pressure, systemic arterial blood pressure was measured from the right external carotid artery. We chose the perfusion rate on the basis of the relationship between perfusion rate and carotid perfusion pressure. Using this rate, we measured the time required for the perfusion fluid to reach the cortical blood vessels after starting the infusion pump. Anesthetized mice were prepared for perfusion, and a hole was made in the skull above the right hemisphere. Cortical arteries were observed before and during infusion into the right external carotid artery. The time when blood was replaced with perfusate after starting the pump was recorded. Using this rate, we also measured the contribution of systemic blood to the total flow. Mice were prepared and perfused with perfusate containing no radiotracer. Fifteen seconds after the start of perfusion, 0.5 µCi of iodine-125]antipyrine and 2.0 µCi of [3H]sucrose were injected as a bolus into the tail vein, blood was sampled from the femoral artery at 5-s intervals, and the mouse was decapitated 30 s after the injection. The right cerebral hemisphere and blood samples were prepared for scintillation counting as described in Perfusin system.

Calculations. Cerebral perfusion fluid flow (Fpf, ml·g⁻¹·s⁻¹) and the contribution of systemic blood to total flow [f = F[pf]/F[total], where F[ab] is systemic blood flow (ml·g⁻¹·s⁻¹)] and F[total] is the sum of F[pf] and F[ab] are given as

\[ F_{pf} = C_{brain}(T)/[T']/C_{pf} \tag{1} \]

\[ f = C_{brain}(T)/[Ak/(hN - B)] \times \left( e^{-RT} - e^{-BRT} \right) \tag{2} \]

where T is time of decapitation (s), Cbrain is the parenchymal brain concentration of tracer (pmol/g), Cpf is the concentration of tracer in the perfusion fluid (pmol/ml), V is cerebral distribution volume (ml/g), and k is a transfer coefficient (ml·g⁻¹·s⁻¹) (28). To determine the constants A, B, D, and E, the femoral arterial concentration of C[pm,art] (pmol/ml) of iodine-125]antipyrine was fitted to Eq. 3 by means of a nonlinear least-squares procedure (MULTI) (30)

\[ C_{pm,art} = Ae^{-RT} + De^{-RT} \tag{3} \]

Brain uptake of a Drug during the perfusion is given by Eq. 4 (back diffusion was ignored) or Eq. 5 (back diffusion was not ignored), where Cbr,art is the brain arterial concentration (pmol/ml), K1 is influx clearance (ml·g⁻¹·s⁻¹), and k2 is efflux rate constant (s⁻¹). During perfusion, Cbr,art equals Cpf and is fixed, and therefore we may divide Eq. 5 by Cpf to obtain Eq. 6, where K0 (ml/g) is the ratio of Cbrain to Cpf

\[ dC_{brain}/dt = K_1 \times C_{br,art} - k_2 \times C_{brain} \tag{4} \]

\[ dC_{brain}/dt = K_1 \times C_{br,art} - k_2 \times C_{brain} \tag{5} \]

\[ dK_p/dt = K_2 - K_2 \times C_{brain} \tag{6} \]

To determine K1 and k2, the K0 of the tracer was fitted to Eqs. 4 and 6 by means of a nonlinear least-squares procedure (MULTI) (30), and we judged the suitability of the equation by whichever obtained a smaller Akaike information crite-
ion (AIC) (30). The PS values were calculated from Eq. 7 (28)

\[ PS = -F_{lw} \times \ln \left(1 - \frac{K_pF_{lw}}{F_{lw}}\right) \]  

Measurement of the octanol-water partition coefficient. The octanol-water partition coefficients were measured by the method described below. n-Octanol and 0.1 mM phosphate buffer (pH 7.4) were used as the organic and the aqueous phases, respectively. The initial concentrations of \(^3\)H-labeled and \(^1\)C-labeled compounds in the aqueous phases were 333 and 16.7 nCi/ml, respectively. An aliquot (3 ml) of drug-containing aqueous phase was mixed with an equivalent volume of octanol and shaken vigorously for 1 h at 37°C. The organic and the aqueous phases were then separated by centrifugation at 738 \(g\) for 5 min. The organic and the aqueous phases (1 ml each) were placed in a scintillation vial and prepared for scintillation counting by addition of 15 ml of scintillation cocktail. The radioactivity was measured with a liquid scintillation counter.

Data analysis. The PS values were plotted against the values of the octanol-water partition coefficients divided by the square root of the molecular weight. In this plot, the data for eight compounds reported by Takasato et al. (28), i.e., sucrose, mannitol, urea, glycerol, ethylene glycol, trimethyl-ene glycol, thiourea, and anti-pyrene, and the regression line for these compounds were additionally superimposed. Moreover, the PS values were compared between mice and rats. A slope, an intercept, a coefficient of correlation \((r^2)\), and a \(P\) value were determined by multiple regression analysis.

RESULTS

Perfusion pressure and rate. In anesthetized mice after ligation of the pterygopalatine artery, the systolic pressure was 126 ± 8 (means ± SE) mmHg, and the diastolic pressure was 113 ± 7 (means ± SE) mmHg at the carotid artery. As shown in Fig. 1, the carotid pressure increased in proportion to the perfusion rate from 0.6 to 1.2 ml/min. On the basis this relationship, a perfusion rate of 1.0 ml/min was selected for the in situ brain perfusion in this study. At this rate, the time required to replace the blood in cortical arteries with perfusate after starting the pump was 2 s \((n = 5)\). Therefore, 2 s was subtracted from the total perfusion time, and the corrected perfusion times are used for calculation.

![Fig. 1. Relationship between carotid perfusion pressure and perfusion rate. Data are means ± SE \((n = 5)\). The straight line represents the correlation line for the data. The shaded area represents the arterial blood pressure range of the anesthetized mouse.](Image)

Intravascular volume and cerebral perfusion fluid flow. A shown in Fig. 2, the distribution volume of \(^3\)H-inulin was almost constant \((11.4 ± 0.4 \mu l/g; \text{means ± SE, } n = 9)\) after perfusion for 15–60 s. This distribution volume of \(^3\)H-inulin was used to correct the intravascular volume. Cerebral perfusion fluid flow calculated from brain uptake data of \(^1\)C-diazepam for 5 s was 71.3 ± 6.7 \(\mu l \cdot g^{-1} \cdot s^{-1}\) (means ± SE, \(n = 3\)).

![Fig. 2. \(^3\)H-inulin distribution volume in the mouse brain determined using the in situ brain perfusion technique. Data are means ± SE \((n = 3)\).](Image)

PS values of mice and rats. Figure 3 shows the brain uptake (apparent distribution volume)-time profiles of 21 drugs in mice and rats. For several drugs (e.g., phenylalanine, quinine, and thiourea), the distribution volumes increased linearly with time up to 60 s. For others (e.g., iodoantipyrine), the uptake rate decreased with time. Table 1 summarizes the values of molecular weight, octanol-water partition coefficient (pH 7.4), \(K_1\), \(k_2\), and PS values (Eqs. 4–7) in mice and rats. In addition, as shown in Fig. 4, we plotted the PS values in mice and rats against the values of the octanol-water partition coefficients divided by the square root of the molecular weight. The line shows the relationship for eight drugs that were transported only by passive diffusion across the BBB in rats \((\log(PS) = -1.17 + 0.848 \times \log(\text{partition coefficient/square root (molecular weight)}); r^2 = 0.882, P < 0.001)\) in a previous study (28). In this study, drugs that were transported only by passive diffusion (e.g., sucrose, mannitol, thiourea, and iodoantipyrine) lie on the line, whereas drugs that were transported into the brain by facilitated uptake.
systems (e.g., alanine, phenylalanine, and glucose) lie above the line, and drugs that were subject to active efflux (e.g., vincristine and quinidine) lie below the line.

As shown in Fig. 5, a good correlation (1:1) was obtained between the \( PS \) values in mice and in rats \([\log(PS_{\text{mouse}}) = (-0.499 \pm 0.0215) + (0.884 \pm 0.068) \times \log(PS_{\text{rat}}); r^2 = 0.898, P < 0.0001, \text{means} \pm \text{SE}]\). There were no significant differences in the \( PS \) values between mice and rats.

**DISCUSSION**

In this study, we used the in situ brain perfusion technique to compare the \( PS \) values of various drugs at the BBB between mice and rats. Several techniques have been established to measure \( PS \) values at the BBB quantitatively (1, 18, 28). To examine transport saturation, competition, and inhibition by altering the concentration of solute or inhibitor in the perfusate and to investigate the effects of pH, osmolality, and ionic content (21), the in situ brain perfusion technique is particularly suitable, because the \( PS \) value at the BBB can be determined accurately over a 10-fold range from a minimum of \( 10^{-5} \text{ ml} \cdot \text{g}^{-1} \cdot \text{s}^{-1} \), and the drug concentration in the fluid of brain arteries is equal to that in the perfusate. This technique has several other advantages, as described below. Because the contribution of systemic blood flow to the right
cerebral hemisphere flow was only 3.2%, serum protein binding can be neglected. The effects of metabolism in other tissues, such as the liver, can be ignored because the perfusate is introduced directly into the cerebral hemisphere. For instance, the PS values of trimethylene glycol and glycerol at the BBB measured by using the intravenous injection technique are larger than those using the in situ brain perfusion technique, because these compounds are immediately converted in the liver to glucose and CO₂, which readily permeates into the brain (4, 8, 10). Therefore, we considered that the in situ brain perfusion technique was the most suitable technique to determine the PS values at the BBB.

Table 1. Comparison of the PS values across blood-brain barrier between mouse and rat

<table>
<thead>
<tr>
<th>Drug</th>
<th>Molecular Weight</th>
<th>Octanol-Water Partition Coefficient</th>
<th>( K_1, \text{ml} g^{-1} \cdot \text{s}^{-1} \times 10^5 )</th>
<th>( k_2, \text{s}^{-1} \times 10^5 )</th>
<th>PS, \text{ml} g^{-1} \cdot \text{s}^{-1} \times 10^5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mouse</td>
<td>Rat</td>
<td>Mouse</td>
</tr>
<tr>
<td>Sugar</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Sucrose</td>
<td>342.30</td>
<td>0.000246</td>
<td>3.94 ± 1.71</td>
<td>1.67 ± 0.107</td>
<td>3.95</td>
</tr>
<tr>
<td>2 Mannitol</td>
<td>182.20</td>
<td>0.00167</td>
<td>3.35 ± 0.649</td>
<td>5.39 ± 0.530</td>
<td>3.35</td>
</tr>
<tr>
<td>3 Glucose</td>
<td>180.20</td>
<td>0.00107</td>
<td>460 ± 19.7</td>
<td>664 ± 64.44</td>
<td>15.9</td>
</tr>
<tr>
<td>Amino Acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 Alanine</td>
<td>89.00</td>
<td>0.00130</td>
<td>31.2 ± 3.71</td>
<td>35.9 ± 5.76</td>
<td>31.2</td>
</tr>
<tr>
<td>5 Phenylalanine</td>
<td>165.00</td>
<td>0.0318</td>
<td>2,060 ± 161</td>
<td>3,920 ± 201</td>
<td>2,440</td>
</tr>
<tr>
<td>Acidic Drug</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 Glibenclamide</td>
<td>494.00</td>
<td>85.8</td>
<td>53.2 ± 6.70</td>
<td>168 ± 23.2</td>
<td>27.8</td>
</tr>
<tr>
<td>7 Tolbutamide</td>
<td>270.00</td>
<td>2.64</td>
<td>246 ± 45.7</td>
<td>142 ± 11.0</td>
<td>23.7</td>
</tr>
<tr>
<td>8 Valproic acid</td>
<td>144.21</td>
<td>1.39</td>
<td>210 ± 17.5</td>
<td>720 ± 107</td>
<td>21.6</td>
</tr>
<tr>
<td>9 Warfarin</td>
<td>308.32</td>
<td>16.7</td>
<td>758 ± 146</td>
<td>947 ± 71.8</td>
<td>28.0</td>
</tr>
<tr>
<td>10 Methotrexate</td>
<td>454.46</td>
<td>0.0344</td>
<td>6.82 ± 0.890</td>
<td>5.89 ± 1.19</td>
<td>6.82</td>
</tr>
<tr>
<td>Basic Drug</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11 Vincristine</td>
<td>923.00</td>
<td>70.1</td>
<td>7.50 ± 1.99</td>
<td>79.8 ± 303</td>
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<tr>
<td>12 Vinblastine</td>
<td>909.00</td>
<td>79.6</td>
<td>48.0 ± 8.75</td>
<td>323 ± 3.94</td>
<td>48.1</td>
</tr>
<tr>
<td>13 Quinidine</td>
<td>324.40</td>
<td>113</td>
<td>213 ± 30.9</td>
<td>181 ± 11.3</td>
<td>30.3</td>
</tr>
<tr>
<td>14 Quinine</td>
<td>324.40</td>
<td>24.4</td>
<td>253 ± 14.6</td>
<td>230 ± 17.3</td>
<td>257</td>
</tr>
<tr>
<td>15 Cimetidine</td>
<td>252.00</td>
<td>2.16</td>
<td>8.90 ± 0.629</td>
<td>8.92 ± 1.40</td>
<td>8.01</td>
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<tr>
<td>16 Theophylline</td>
<td>180.20</td>
<td>0.912</td>
<td>54.8 ± 2.65</td>
<td>153 ± 17.9</td>
<td>26.4</td>
</tr>
<tr>
<td>17 Hypoxanthine</td>
<td>136.00</td>
<td>0.0909</td>
<td>39.3 ± 7.05</td>
<td>35.8 ± 6.90</td>
<td>15.0</td>
</tr>
<tr>
<td>18 Idoantypyrine</td>
<td>314.10</td>
<td>15.2</td>
<td>3,250 ± 567</td>
<td>4,980 ± 448</td>
<td>32.7</td>
</tr>
<tr>
<td>Neutral Drug</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19 Thiourea</td>
<td>60.10</td>
<td>0.100</td>
<td>50.8 ± 3.75</td>
<td>35.2 ± 2.11</td>
<td>50.9</td>
</tr>
<tr>
<td>20 Cyclosporin A</td>
<td>1,023.00</td>
<td>105</td>
<td>647 ± 37.6</td>
<td>1,070 ± 224</td>
<td>35.5</td>
</tr>
<tr>
<td>21 Digoxin</td>
<td>780.90</td>
<td>53.2</td>
<td>3.29 ± 0.225</td>
<td>7.21 ± 1.77</td>
<td>3.29</td>
</tr>
</tbody>
</table>

Values are means ± SE. Octanol-water partition coefficients were determined in this laboratory. Influx clearance (\( K_1 \)) and efflux rate constant (\( k_2 \)) were calculated by means of a nonlinear least-square procedure (MULTI). Permeability coefficient-surface area product (PS) values were calculated using Eq. 7.
It is important to select an appropriate perfusion rate for mice. If the perfusion rate is too slow, the contribution of systemic blood flow from vertebral arteries to total flow in the right hemisphere increases, despite ligation of the right common carotid artery. In this case, the advantages of this technique, i.e., no protein binding and the fluid in brain arteries being identical with the perfusate, are attenuated. However, if the perfusion rate is too fast, the perfusion pressure in microvessels is too high and integrity of the BBB is compromised. We considered 1.0 ml/min to be an appropriate perfusion rate, because the carotid perfusion pressure generated by this rate is higher than the systolic pressure and lower than the pressure required to destroy the BBB in rats (160–190 mmHg) (11). This rate in mice (assuming that the weight of the right cerebral hemisphere is 0.13 g) is comparable with the value predicted (1.1 ml/min) from the relationship between brain weight (0.6 g) and perfusion rate (4.98 ml/min) in rats. The perfusion pressure caused by this perfusion rate was considered not to injure the BBB in mice, because the distribution volume of [3H]inulin showed no significant change during perfusion for 15–60 s (9–13 µl/g) and was close to the brain capillary volume (8 µl/g from Ref. 20 and 6–9 µl/g from Ref. 28) previously reported. The cerebral perfusion fluid flow calculated from the brain uptake of [14C]diazepam in 5 s was 7.13 × 10−2 ml · g−1 · s−1 (the cerebral perfusion fluid flow measured in rats is 6–13 × 10−2 ml · g−1 · s−1, see Ref. 28) and is sufficiently higher than cerebral blood flow (17). Furthermore, the contribution of systemic blood flow to total flow was only 3% in mice during the perfusion into the external carotid artery, similar to the values (3–5%) reported in rats (28). We conclude that the right cerebral hemisphere of mice was adequately perfused at this rate.

As shown in Fig. 3, the apparent distribution volume-time profiles of various drugs, obtained by in situ brain perfusion studies in mice and rats, were classified into two groups. In one group, the rate of increase of the apparent brain distribution volume did not alter with time (linear type), and in the other group, it decreased with time (saturation type), indicating that the efflux rate of the drug from the brain to the blood is rapid in the latter group. The PS values at the BBB obtained in mice were plotted against the values of the octanol-water partition coefficients divided by the square root of the molecular weight (Fig. 4). As for drugs that do not have a specific transport system, it was reported that the permeability coefficients at the BBB and the octanol-water partition coefficients have a good correlation (6, 14, 27), and a correlation line was derived from Takasato’s report (Fig. 4, solid line) (28). Data for drugs that are thought to be transported only by passive diffusion, such as sucrose, mannitol, thio-urea, and iodoantipyrine (28), lie on the line. On the other hand, values for drugs that are transported into the brain by facilitated diffusion, such as alanine (2), phenylalanine, and glucose (9, 19), lie above the line, whereas values of drugs that are actively transported out of the brain by P-glycoprotein, such as vincristine (25), vinblastine (7), digoxin (26), and quinidine (13), lie below the line. Similar results were obtained in rats, in accordance with previous reports (3, 18). Other drugs giving values below the line (e.g., tolbutamide and glibenclamide) might also be actively transported out of the brain. We have previously reported that tolbutamide was transported by a carrier-mediated system that differs from P-glycoprotein (28). Moreover, our preliminary studies indicate that glibenclamide may be a substrate of P-glycoprotein (unpublished data).

As shown in Fig. 5, a good correlation between the PS values of drugs in mice and rats was obtained, indicating that there is no significant species difference in the permeation of drugs across the BBB between mice and rats. This observation is consistent with a prior comparison of rats and neonatal rabbits (6). However, species differences in the PS values of some drugs (e.g., vincristine and warfarin) were noted in the present study. One possibility is that species differences in the transport systems of rats and mice may differ. A second possibility is that for drugs with small K_p values, individual differences of vascular volume may influence the estimated K_p measurement.

Recently, P-glycoprotein gene knockout mice were developed and used to investigate the transport mechanisms of drugs or toxic compounds (15, 16). In the future, knockout mice lacking other transporter genes may be available to investigate the transport mechanisms of endogenous compounds, drugs, and toxic compounds. However, the in situ brain perfusion technique in rats remains useful because of the amount of reported data available for comparison. Our findings in mice should further extend this utility.

In conclusion, a good relationship was obtained between the perfusion rate and the carotid perfusion pressure in the in situ mouse brain. In our studies, we find that a perfusion rate of the 1.0 ml/min is optimal, providing a pressure higher than the systolic blood pressure without impairing the integrity of the BBB. At this rate, the intravascular volumes and cerebral perfusion fluid flows in mice were similar to those in rats. In addition, the contribution of systemic blood to total flow in the hemisphere was very small. The PS values at the BBB determined by using the in situ brain perfusion technique in mice and rats were compared with the predicted values based on the relationship between lipophilicity and molecular weight. The PS values for drugs transported by passive diffusion were almost equal to the predicted values, whereas the PS values of drugs transported into the brain by facilitated diffusion were greater than the predicted values, and the PS values of drugs actively transported out of the brain were smaller than the predicted values. Finally, there was a very good correlation (1:1) in the PS values between mice and rats, indicating that the PS values of various drugs are very similar in these two species despite the likely involvement of different transport mechanisms.
REFERENCES


