Brain-to-blood active transport of β-alanine across the blood–brain barrier

Junko Komura, Ikumi Tamai, Mizuho Senmaru, Tetsuya Terasaki[1], Yoshimichi Sai, Akira Tsuji*

Division of Life Sciences, Graduate School of Natural Science and Technology, Kanazawa University, Takara-machi 13-1, Kanazawa 920, Japan

Received 29 July 1996; revised version received 12 November 1996

Abstract A high-affinity antiluminal uptake system for β-alanine was demonstrated in primary cultured bovine brain capillary endothelial cells (BCEC) for which K_t is 66.9 μM. β-alanine uptake was energy-, sodium- and chloride ion-dependent. β-amino acids strongly inhibited the uptake, while α- and γ-amino acids had a little or no inhibitory effect. In ATP-depleted cells, the uptake was stimulated by preloading β-alanine or taurine but not by L-leucine. These results suggest that β-alanine is actively transported across the antiluminal membrane of BCECs that is common to β-amino acids. The system may function for the efflux from the brain to blood.

Key words: Alanine, β-; Blood–brain barrier; Antiluminal uptake; Brain capillary endothelial cell; Active transport; Taurine

1. Introduction

Beta (β)-alanine is a neurotransmitter, the derivative of aspartate, and the structural analog of major inhibitory neurotransmitters γ-aminobutyric acid (GABA) and glycine, and neuroactive sulfur amino acid taurine. The physiological importance of high-affinity uptake systems for neurotransmitter amino acids is a subject of intensive study [1,2]. The transport of β-amino acids in Ehrlich cells has been studied principally by Christensen [3]. Some later studies showed that β-alanine and taurine share a common transport system, the so-called β-amino acid transport system, in various tissues [4,5]. The study of transport mechanism of β-alanine in brain was restricted in neurons, astrocytes or synaptosomal membrane vesicles [6,7]. It has recently been clarified whether such a β-amino acid transport system functions at the blood–brain barrier (BBB). We have studied the transport characteristics of taurine at the BBB in vitro and in vivo [8]. Taurine transport was dependent on Na^+ and Cl^- gradients and was inhibited by β-amino acids. We performed the in vitro study as a model of blood-to-brain transport (uptake from luminal membrane side) and of brain-to-blood transport (uptake from antiluminal membrane side). It is interesting that the antiluminal transport of taurine by using primary cultured bovine brain capillary endothelial cells (BCEC) also showed an active system. We have also found that the luminal transport of β-alanine by BCECs was mediated by an active transport mechanism which required Na^+ and Cl^- [9]. Therefore, it is attractive to investigate the transport of β-alanine across the brain to blood of BBB.

Although brain endothelial cells morphologically do not show obvious polarity, biochemical polarity is well established [10,11]. Some evidence suggests that polarity also exists concerning the transport of amino acids and glucose at the BBB [12–14]. It seems likely that the transport polarity of endothelial cells is important to the BBB function. However, transport polarity and its physiological significance are difficult to study by in vivo techniques. Primary cultured monolayers of bovine BCECs offer the advantage for studies of the transendothelial transport of various substances [15–18]. Furthermore, it is expected that the monolayers of BCECs remains polarized because several membrane functions have been reported to localize in the manner that is consistent with the brain capillary endothelial cells in vivo [8,12,19,20]. These features of the cultured cells enable us to study the polarized transport characteristics at the antiluminal membrane.

In this study, we have used an in vitro model to characterize the role of the endothelial component of the BBB in regulating β-alanine delivery across the brain. The purpose of the present study is to elucidate the characteristics of the antiluminal transcellular transport of β-alanine at the BBB by using the primary cultures of BCECs. Findings from this study will contribute to a better understanding of brain-to-blood transport of β-amino acids and related β-amino acid-mimetic drugs across the BBB.

2. Materials and methods

2.1. Materials

[3-^{3}H(N)]β-Alanine (92.6 Ci/mmol) and [^{14}C(U)]sucrose (4.03 mCi/mmol) were purchased from New England Nuclear (Boston, MA). Horse serum was obtained from Gibco (Grand Island, NY). Rat tail collagen (type I) was from Collaborative Research Inc. (Bedford, MA), human fibronectin from Boehringer Mannheim GmbH (Mannheim, Germany), and bovine serum albumin (Fraction V) from Sigma Chemical Co. (St. Louis, MO). All other chemicals were commercial products of reagent grade.

2.2. Isolation and culture of BCECs

BCECs were isolated from cerebral gray matter of bovine brains by the method of Audus and Borchardt [21] with minor modifications. We have reported the details of the procedures for the preparation and the cell culture [22]. The isolated BCECs were stored at −100°C in culture medium containing 20% horse serum and 10% dimethylsulfoxide until use. Prior to seeding, the Transwell® polycarbonate membranes (12 mm diameter, 12 μm pore size, Costar, Cambridge, MA), were coated with rat tail collagen, sterilized in UV light, and coated with human fibronectin. Isolated BCECs were cultured at 37°C under 95% air and 5% CO_2. Transport experiments were performed when cells had reached confluence (in 10–12 days). These cultured cells were demonstrated to be endothelial cells by an immunostaining method

*Corresponding author. Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, Kanazawa University, Takara-machi 13-1, Kanazawa 920, Japan. Fax: (81) 762-34-4477

[1]*Present address:* Faculty of Pharmaceutical Sciences, Tohoku University, Aramaki-Aoba, Aoba-ku, Sendai, 980-77, Japan.

PII S0014-5793(96)01366-X

using von Willebrand Factor (Factor VIII related antigen, data not shown).

2.3. Uptake experiments

In order to measure antiluminal uptake, cultured BCECs were grown on Transwell® membranes. Cultured BCECs were washed 3 times with 1 ml of Na^+- and Cl^--free incubation solution (sucrose solution). Sucrose solution was consisted of 240 mM sucrose, 4 mM K gluconate, 2.8 mM $CaSO_4$, 1 mM $MgSO_4$, 10 mM D-glucose, 10 mM HEPES, and 0.1% bovine serum albumin, pH 7.4 at 37°C, 290 mOsm/kg. Uptake was initiated by adding 1.5 ml of incubation solution to the outside (antiluminal side) containing [^{3}H]β-alanine (10 nM) and [^{14}C]sucrose (50 μM), and adding 500 μl of sucrose solution to the inside (luminal side), minimizing the luminal-reuptake. Incubation solution was consisted of 141 mM NaCl, 4 mM KCl, 2.8 mM $CaCl_2$, 1 mM $MgSO_4$, 10 mM D-glucose, 10 mM HEPES and 0.1% bovine serum albumin, pH 7.4 at 37°C, 290 mOsm/kg. Moreover to minimize re-uptake from the luminal side, sucrose solution on the luminal side was replaced with fresh sucrose solution every 5 min. In order to terminate the transport reaction, cells were washed 3 times with 1 ml of the ice-cold incubation solution at the designated time. The washed cells were solubilized by incubating them with 300 μl of 1 M NaOH at room temperature for 60 min to quantitate the radioactivity associated with the cells. After neutralization with 60 μl of 5 M HCl, the resultant sample was put into a plastic vial containing 4 ml of Clear-sol I (Nacalai Tesque Inc., Kyoto, Japan). The radioactivity was then measured by a liquid scintillation counter, LSC-1000 (Aloka Co. Ltd., Tokyo, Japan). Protein content in cultured cells was measured by the Lowry method [23] using bovine serum albumin as a standard. Net uptake was expressed as the cell-to-medium concentration (C/M) ratio, obtained by dividing the uptake amount by the initial concentration (μl/mg protein), or as uptake rate (nmol/mg protein) after correction for extracellularly adsorbed β-alanine, estimated from the apparent uptake of [^{14}C]sucrose.

In order to estimate the kinetic parameters of [^{3}H]β-alanine uptake in cultured monolayers of BCECs, the uptake rate (J, nmol/mg protein/30 min) was fitted to the following equation, by using the non-linear least-squares regression analysis program, MULTI [24]:

$$J = J_{max}S/(K_t + S) \quad (1)$$

where J_{max} is the maximum uptake rate for the carrier-mediated process, S is a concentration of substrate, and K_t is a half-saturation concentration.

The transport data were expressed as the mean ± SEM. The significance of differences was evaluated by using Student's t test or 1-way analysis of variance.

3. Results

3.1. Time course of β-alanine uptake

The time course of the antiluminal uptake of [^{3}H]β-alanine into cultured monolayers of BCECs is shown in Fig. 1. The value (expressed as C/M ratio) for [^{3}H]β-alanine was corrected for the extracellular space determined by using [^{14}C]sucrose.

Table 1
Effect of temperature and metabolic inhibitors on the antiluminal uptake of [^{3}H]β-alanine

Condition	Concentration (mM)	Uptake (% of control)[a]
Control		100
Low temperature (4°C)		0.1 ± 0.1*
Ouabain	0.5	43.2 ± 9.0*
DNP	1	29.9 ± 12.9*
NaN_3	10	38.3 ± 7.2*

BCECs were preincubated for 20 min at 37°C with each metabolic inhibitor in the absence of D-glucose except ouabain. The uptake of [^{3}H]β-alanine (10 nM) was measured at 37°C or 4°C for 30 min in the presence or absence of metabolic inhibitor and D-glucose.
[a]Each value represents the mean ± SEM of three to four experiments.
*$P < 0.05$ compared to control.

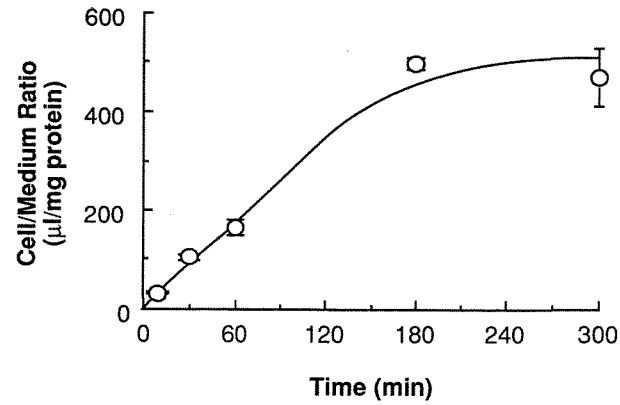

Fig. 1. Time courses of the antiluminal uptakes of [^{3}H]β-alanine. The uptake of [^{3}H]β-alanine (10 nM) was measured at 37°C. Each point represents the mean ± SEM of three to four experiments. When the SEM was small, it was included in the symbol.

The C/M ratio of [^{14}C]sucrose was 2 μl/mg protein (mean value of many experimental trials) and was almost constant within the experimental period. The data, corrected for the C/M ratio for sucrose, show that the uptake of [^{3}H]β-alanine was linear over a 60 min incubation period. Based on this result, all subsequent experiments were conducted for 30 min. The C/M ratio of [^{3}H]β-alanine uptake was determined to be 104.0 ± 6.8 μl/30 min/mg protein (mean ± SEM, $n = 4$), which was about 10-fold more than would be expected from the cellular volume, approximately 10 μl/mg protein [22]. Such an extensive accumulation of [^{3}H]β-alanine in BCECs was expected to be caused by a specific active transport across the membrane of BCECs.

3.2. Concentration dependence of β-alanine uptake

Fig. 2 shows the relationship between the initial antiluminal uptake rate and the concentration of β-alanine (1–100 μM). The antiluminal uptake was suggested to be composed of a single saturable processes when analyzed by means of an Eadie-Hofstee plot. A nonlinear least-squares regression analysis of the results based on Eqn. 1 yielded values of the kinetic parameters, K_t and J_{max}, of 66.9 ± 19.3 μM and 3.77 ± 0.66 nmol/30 min/mg protein, respectively.

3.3. Effect of temperature and metabolic inhibitors on the uptake of β-alanine

The effects of temperature and metabolic inhibitors on the antiluminal uptake of [^{3}H]β-alanine are summarized in Table 1. The uptake of [^{3}H]β-alanine at 4°C was less than 1% of the control value (37°C), showing a marked temperature dependence. Addition of 1 mM DNP, an uncoupler of oxidative phosphorylation, or 10 mM sodium azide, a respiratory chain inhibitor, significantly reduced the uptake of [^{3}H]β-alanine at 37°C by the antiluminal membrane. Moreover 0.5 mM ouabain, an inhibitor of Na^+,K^+-ATPase, reduced the antiluminal uptake of [^{3}H]β-alanine.

3.4. Effect of ion replacement in the incubation solution on the uptake of β-alanine

The effect of cations or anions on the antiluminal uptake of [^{3}H]β-alanine are summarized in Table 2. The substitution of Na^+ with choline or *N*-methyl-D-glucamine in the presence of Cl^- completely abolished antiluminal uptake of [^{3}H]β-alanine.

Table 2
Effect of sodium and chloride ions on the antiluminal uptake of [^{3}H]β-alanine

A: Na^+ replacement with cations in the presence of Cl^-	
Cation	Uptake (% of control)[a]
Control	100
Choline	0.0*
N-Methyl-D-glucamine$^+$	0.0*
Sucrose	0.0*
B. Cl^- replacement with anions in the presence of Na^+	
Anion	Uptake (% of control)[a]
Control	100 ± 3.5
Br^-	32.4 ± 11.4*
SCN^-	10.8 ± 0.8*
Gluconate$^-$	2.1 ± 0.6*

The uptake of [^{3}H]β-alanine (10 nM) was measured at 37°C for 30 min.
A: Cation indicates that Na^+ in the incubation solution was replaced by other cations or sucrose.
B: Anions indicate that Cl^- in the incubation solution was replaced by other anions.
[a] Each value represents the mean ± SEM of three to four experiments.
*$P < 0.05$ compared to control.

In the presence of Na^+, [^{3}H]β-alanine uptake showed a marked anion-dependence. Replacing Cl^- with Br^- or SCN^- significantly reduced antiluminal uptake. Gluconate almost completely abolished the uptake of [^{3}H]β-alanine. Among the anions tested, the ability to stimulate the Na^+-dependent β-alanine uptake was in the following order: $Cl^- > Br^- > SCN^- \gg$ gluconate$^-$. Moreover, the replacement of NaCl with sucrose completely reduced the uptake of [^{3}H]β-alanine. These results suggest that antiluminal uptake of β-alanine is dependent on Na^+ and Cl^-.

3.5. *Effect of structural analogs and various amino acids on the uptake of* β-*alanine*

The effects of various compounds on the antiluminal uptake of [^{3}H]β-alanine are summarized in Table 3. The uptake of [^{3}H]β-alanine was strongly inhibited by β-amino acids (i.e., taurine and hypotaurine). Nipecotic acid, a β-amino acid, possessing a ring structure, significantly inhibited the uptake of [^{3}H]β-alanine. An α-amino acid (L-alanine) and γ-amino acid (GABA) also reduced the uptakes of [^{3}H]β-alanine. Furthermore, L-phenylalanine inhibited the antiluminal uptake. In contrast, neither L-leucine nor L-glutamic acid inhibited the uptake of [^{3}H]β-alanine.

3.6. *Countertransport effect on the uptake of* β-*alanine*

Countertransport by cultured cells that were ATP-depleted with 10 mM NaN_3 is summarized in Table 4. The uptake of [^{3}H]β-alanine was significantly stimulated by the preloading of 150 μM of unlabeled β-alanine, or 60 μM or 90 μM taurine for 20 min. In contrast, preloading of 90 μM of L-leucine did not stimulate the antiluminal uptake of [^{3}H]β-alanine.

4. Discussion

For the first time, the characteristics of brain-to-blood (antiluminal) transport of β-alanine have been investigated by primary cultured bovine BCECs. Antiluminal uptake of β-alanine is temperature- and metabolic energy-dependent (Table 1). The sensitivity to metabolic inhibitors suggested that the antiluminal passage of β-alanine across BCECs was dependent to some degree on the metabolic status of the cells. Na^+-dependent β-alanine uptake requires anion; especially, Cl^- is the most preferred among the tested anions (Table 2). Further, the antiluminal uptake of β-alanine consisted of a single saturable process (Fig. 2). In our previous study, the metabolite of taurine was not observed during 30 min incubation by BCECs (37°C) [8]. Similarly, we assumed that the metabolism of β-alanine was negligible in the present study. Accordingly, the antiluminal uptake of β-alanine was ascribed to the transport. It was demonstrated that [^{3}H]β-alanine is actively transported by a carrier-mediated mechanism from antiluminal side, consuming energy supplied by Na^+ and Cl^- gradients, as similarly observed for the luminal transport of β-alanine [9] and antiluminal transport of taurine by BCECs [8]. At this point of view, the antiluminal transport of β-alanine by BCECs has similar characteristics compared with the luminal transport [9].

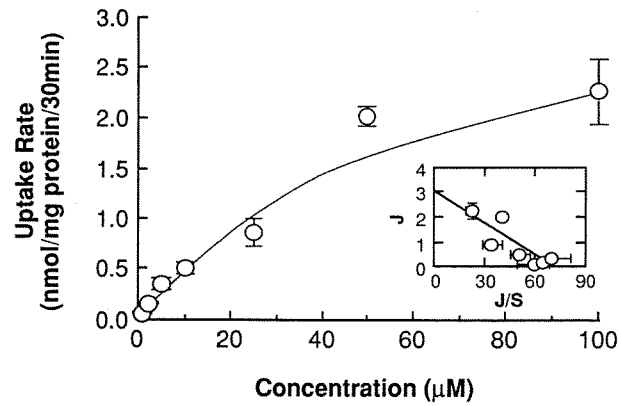

Fig. 2. Concentration dependence of the antiluminal uptake of [^{3}H]β-alanine. Initial uptake rates at various concentrations of β-alanine (1–100 μM) were measured at 37°C for 30 min. Each point represents the mean ± SEM of three to four experiments. When the SEM was small, it was included in the symbol. The solid line represents saturable uptake rate generated from Eqn. 1, using the MULTI fitted parameters. Inset: Eadie-Hofstee plots of [^{3}H]β-alanine uptake.

Table 3
Effect of amino acids and structural analogs on the antiluminal uptake of [^{3}H]β-alanine

Inhibitor	Concentration (mM)	Uptake (% of control)[a]
Control		100 ± 3.5
Taurine	0.5	6.1 ± 0.3*
Hypotaurine	0.5	5.5 ± 0.3*
L-α-Alanine	0.5	66.7 ± 5.9*
GABA	0.5	58.3 ± 4.2*
Glycine	0.5	82.9 ± 6.0*
Nipecotic acid	5.0	32.4 ± 2.9*
Cysteine	5.0	26.4 ± 2.2*
Methionine	5.0	41.0 ± 4.6*
L-Phenylalanine	5.0	66.5 ± 9.3*
L-Leucine	5.0	90.9 ± 5.6
L-Glutamate	5.0	100.9 ± 11.3

The uptake of [^{3}H]β-alanine (10 nM) was measured at 37°C for 30 min in the presence of each compound.
[a]Each value represents the mean ± SEM of three to four experiments.
*$P < 0.05$ compared to control.

Recent studies have investigated whether taurine and β-alanine share a common carrier. Most of these studies concluded that taurine and β-alanine are transported via the common transporter specific to β-amino acids [4,5,25]. In neurons and astrocytes [6], and cultured glioma cells [26], β-alanine competitively interacts with the transport system for taurine. In contrast, Breckenbridge et al. [27] claimed that taurine was an ineffective inhibitor of β-alanine transport by brain slices. Furthermore, the ileal β-alanine transport system is not identical with that for taurine, since L-leucine was a high-affinity inhibitor of β-alanine transport, but had no effect on taurine transport [28]. We assessed the inhibitory effect of β-amino acid analogs and other amino acids on the transport of β-alanine by BCECs to address these contradictory findings. As seen in Table 3, [^{3}H]β-alanine uptake by BCECs was not inhibited by 5 mM L-leucine, being consistent with the fact that taurine uptake by antiluminal membrane of BCECs was not inhibited by 5 mM L-leucine [8]. This result indicates the β-alanine transport system is probably different between ileum and BCECs. β-amino acids such as taurine and hypotaurine, and structurally analogous compounds, significantly inhibited the uptake of β-alanine (Table 3). This indicates that β-alanine transport is highly selective for β-amino acids. Furthermore, countertransport of [^{3}H]β-alanine was observed by preloading unlabeled β-alanine or taurine (Table 4). These observations suggest that the affinity for the β-amino acid transporter depends on the number of carbon atoms between the positive and negative charges, as suggested previously for taurine transport across the BBB [8].

Betz and Goldstein [10] concluded that small neutral amino acid transport system (A-system) is located on the antiluminal side of the isolated brain capillaries. However, substrates may enter endothelial cells through both membranes simultaneously in the isolated brain capillary. Asymmetrical transport of β-alanine by cultured cells is not known. In the present study, L-phenylalanine significantly inhibited the antiluminal β-alanine transport (Table 3). On the contrary, luminal transport of β-alanine was not inhibited by L-phenylalanine [9]. L-Phenylalanine is exclusively transported via the Na^+-independent L-system in various tissues [29]. However, it is unlikely that β-alanine is partly transported by the L-system because the antiluminal transport of β-alanine was completely diminished in Na^+-free condition (Table 2A). Since the excess concentration of phenylalanine (5 mM) was used to inhibit uptake of β-alanine, it may not be the catabolic effect of phenylalanine. The important point with respect to the inhibitory effect by phenylalanine occurred only at the antiluminal membrane. The kinetic parameters of luminal side which we had obtained previously were $K_t = 25.3 \pm 2.5$ (μM) and $J_{max} = 6.90 \pm 0.48$ (nmol/30 min/mg of protein) [9]. The antiluminal kinetic parameters were estimated to be $K_t = 66.9 \pm 19.3$ (μM) and $J_{max} = 3.77 \pm 0.66$ (nmol/30 min/mg of protein) in the present study. Therefore, the transport efficiencies estimated by the values of J_{max}/K_t (ml/30 min/mg protein) indicate that β-alanine is transported more efficiently in the luminal (0.27) [9] than the antiluminal (0.06) membranes. Based on our studies, the features of β-alanine transport across BCECs differs between the luminal and antiluminal sides, seemingly due to one or more factors. For instance, one factor was the differing energy requirements of individual carriers. Another factor was significant differences in the bidirectional β-alanine transport where an apparent existence of unequal numbers of carriers on luminal and antiluminal surfaces of BCECs could have resulted in different efficiency of the systems. These findings suggest the functional polarity of cultured bovine BCECs.

In summary, we have demonstrated the polar, saturable, Na^+- and Cl^--dependent and active transport system for β-alanine at the antiluminal membrane of primary cultured BCECs. Furthermore, it was also shown that β-alanine is transported by taurine-sensitive β-alanine carrier system at the BBB.

Table 4
Countertransport effect on the antiluminal uptake of [^{3}H]β-alanine by ATP-depleted cultured monolayers of BCECs

Preloaded amino acid	Preloaded amino acid (μM)	Uptake (% of control)[a]
Control	–	100.0 ± 6.1
β-Alanine	150	207.3 ± 34.8*
Taurine	60	168.8 ± 9.3*
	90	175.2 ± 11.9*
L-Leucine	90	124.5 ± 10.8

BCECs were preincubated for 20 min with 10 mM NaN_3 in the absence of D-glucose and with each compound. The uptake of [^{3}H]β-alanine (10 nM) was measured at 37°C for 30 min with 10 mM NaN_3 in the absence of D-glucose. The designated concentration in the table was non-radioactive β-amino acids.
Each value represents the mean ± SEM of four experiments.
*$P < 0.05$ compared to control.

Acknowledgements: The authors thank Mr. Khoichiro Kirihara, Meat Inspection Center of Kanazawa City (Kanazawa, Japan) for providing us with fresh bovine brains. This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan, grants from the Japan Health Sciences Foundation, Drug Innovation Project, and from Taisho Pharmaceutical Co., Ltd.

References

[1] Curtis, D.R. and Johnston, G.R. (1974) Ergb. Physiol. 69, 97–188.
[2] Schousboe, A. (1981) Int. Rev. Neurobiol. 22, 1–45.
[3] Christensen, H.N. (1964) J. Biol. Chem. 239, 3584–3589.
[4] Miyamoto, Y., Balkovetz, D.F., Leibach, F.H., Mahesh, V.B., and Ganapathy, V. (1988) FEBS Lett. 231, 263–267.
[5] Jessen, H. (1994) Biochim. Biophys. Acta 1194, 44–52.
[6] Larsson, O.M., Griffiths, R., Allen, I.C., and Schousboe, A. (1986) J. Neurochem. 47, 426–432.
[7] Kanner, B.I. and Bendahan, A. (1990) Proc. Natl. Acad. Sci. USA 87, 2550–2554.
[8] Tamai, I., Senmaru, M., Terasaki, T. and Tsuji, A. (1995) Biochem. Pharmacol. 50, 1783–1793.
[9] Komura, J., Tamai, I., Senmaru, M., Terasaki, T., Sai, Y. and Tsuji, A. (1996) J. Neurochem. 67, 330–335.
[10] Betz, A.L. and Goldstein, G.W. (1978) Science 202, 225–227.
[11] Vorbrodt, A.W. (1993) in: The Blood-Brain Barrier (Pardridge, W.M., ed.), pp. 137–164, Raven Press, New York.
[12] Betz, A.L., Firth, J.A. and Goldstein, G.W. (1980) Brain Res. 192, 17–28.
[13] Tovar, A., Tews, J.K., Torres, N. and Harper, A.E. (1988) J. Neurochem. 51, 1285–1293.
[14] Knudsen, G.M., Pettigrew, K.D., Patlak, C.S., Hertz, M.M. and Paulson, O.B. (1990) J. Cereb. Blood Flow Metab. 10, 698–706.
[15] Smith, K. and Borchardt, R.T. (1989) Pharm. Res. 6, 466–473.
[16] Takakura, Y., Kuentzel, S.L., Raub, T.J., Davies, A., Baldwin, S.A. and Borchardt, R.T. (1991) Biochim. Biophys. Acta 1070, 1–10.
[17] Tsuji, A., Tamai, I., Sakata, A., Tenda, Y. and Terasaki, T. (1993) Biochem. Pharmacol. 46, 1096–1099.

[18] Saheki, A., Terasaki, T., Tamai, I. and Tsuji, A. (1994) Pharm. Res. 11, 305–311.
[19] Audus, K.L., Bartel, R.L., Hidalgo, I.J. and Borchardt, R.T. (1990) Pharm. Res. 7, 435–451.
[20] Tsuji, A., Terasaki, T., Takabatake, Y., Tenda, Y., Tamai, I., Yamashima, T., Moritani, S., Tsuruo, T. and Yamashita, J. (1992) Life Sci. 51, 1427–1437.
[21] Audus, K.L. and Borchardt, R.T. (1986) Pharm. Res. 3, 81–87.
[22] Terasaki, T., Takakuwa, S., Moritani, S., and Tsuji, A. (1991) J. Pharmacol. Exp. Ther. 258, 932–937.
[23] Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[24] Yamaoka, K., Tanigawara, Y., Nakagawa, T., and Uno, T. (1981) J. Pharm. Dyn. 4, 879–885.
[25] Yuasa, S. and Hamprecht, B. (1987) J. Neurochem. 49, 528–535.
[26] Martin, D.L. and Shain, W. (1979) J. Biol. Chem. 254, 7076–7084.
[27] Breckenbridge, R.J., Nicholson, S.H., Nicol, A.J., Suckling, C.J., Leigh, B. and Iversen, L. (1981) Biochem. Pharmacol. 30, 3045–3049.
[28] Munck, L.K. and Munck, B.G. (1992) Am. J. Physiol. 262, G609–G615.
[29] Christensen, H.N. (1984) Biochim. Biophys. Acta 779, 255–269.