JPP Journal of Pharmacy and Pharmacology

JPP 2001, 53: 497–503

Received July 24, 2000
Accepted January 15, 2001
ISSN 0022-3573

Molecular and functional identification of large neutral amino acid transporters LAT1 and LAT2 and their pharmacological relevance at the blood–brain barrier

Yasuto Kido, Ikumi Tamai, Hiroshi Uchino, Fumio Suzuki, Yoshimichi Sai and Akira Tsuji

Abstract

We present here the evidence of molecular and functional expression of LAT1 and LAT2, subunits of the large neutral amino acid transporter system L, in cultured brain capillary endothelial cells of the rat. By means of the RT-PCR method, transcripts of LAT1, LAT2 and heavy chain of 4F2 antigen (4F2hc) were detected in rat primary cultured brain capillary endothelial cells (BCECs) and immortalized subline, RBEC1. The uptake properties of RBEC1, such as [^{3}H]leucine and L-[^{3}H]DOPA uptake, were similar to those of primary cultured BCECs. So, RBEC1 may retain almost native properties of the large neutral amino acid uptake activities. [^{3}H]Leucine uptake by RBEC1 showed two saturable components and the K_m values of the high- and low-affinity components were 8.92 ± 3.18 and 119 ± 45 μM, respectively. The K_m value of the high-affinity component agreed well with that of LAT1 and the amino acid transport selectivity of RBEC1 was similar to that of LAT1. Therefore, it is suggested that LAT1 is important at the blood–brain barrier of rats. Additionally, the K_m value of the low-affinity component was similar to that of LAT2. These observations indicate that LAT1 and LAT2 are involved as transporters for large neutral amino acids at the blood–brain barrier. Additionally, we concluded that RBEC1 is useful as an in-vitro model for evaluation of the pharmacological relevance of system L at the blood–brain barrier.

Faculty of Pharmaceutical Sciences, Kanazawa University, 13-1 Takara-machi, Kanazawa 920-0934, Japan

Yasuto Kido, Ikumi Tamai, Hiroshi Uchino, Yoshimichi Sai, Akira Tsuji

CREST, Japan Science and Technology Corporation, 4-1-8 Moto-machi, Kawaguchi 332-0012, Japan

Yasuto Kido, Ikumi Tamai, Hiroshi Uchino, Yoshimichi Sai, Akira Tsuji

Research Institute for Radiation Biology and Medicine, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-0037, Japan

Fumio Suzuki

Correspondence: A. Tsuji, Department of Pharmacobio-Dynamics, Faculty of Pharmaceutical Sciences, Kanazawa University, 13-1 Takara-machi, Kanazawa 920-0934, Japan. E-mail: tsuji@kenroku.kanazawa-u.ac.jp

Introduction

The blood–brain barrier is well known to have several transport systems that regulate the exchange of nutrients between circulating blood and the brain. These transport systems also play an important role in the permeation of xenobiotics across the blood–brain barrier (Tsuji & Tamai 1997, 1998). Amino acids are required for brain development and serve as a source of carbon skeletons for the tricarboxylic acid cycle, and as substrates for neurotransmitter synthesis and protein synthesis (Pardridge 1983). To regulate the amino acid levels in the brain extracellular fluid, there are several amino acid transporters in the blood–brain barrier and many studies have dealt with their functional analysis.

Large neutral amino acids in the plasma are taken up into the brain across the blood–brain barrier by system L, a large neutral amino acid transporter (LAT), which shows sodium-independent transport and has higher affinity than system L in peripheral tissue (Pardridge 1983; Sanchez del Pino et al 1995; Smith & Stoll 1998). System L at the blood–brain barrier is also important from the viewpoint of the delivery of amino acid-mimetic drugs, such as L-DOPA, to the central nervous

system. However, little is yet known about the molecular nature of system L at the blood–brain barrier.

Recently cDNA encoding components of system L, LAT1 and LAT2, were isolated from the rat, and it was shown that 4F2hc (CD98) was required for the functional expression of LAT1 and LAT2, in a *Xenopus laevis* oocyte gene expression system (Kanai et al 1998; Segawa et al 1999). These data supported the hypothesis that isoforms of system L and 4F2hc form heterodimers at the plasma membrane. Although these isoforms were detected in the whole brain, their distribution in the brain has not been clarified. Recently, LAT1 was cloned from bovine brain capillary and the selective expression of LAT1 mRNA at the bovine blood–brain barrier was demonstrated by Northern blotting (Boado et al 1999). These results suggest the functional importance of LAT1 at the blood–brain barrier, albeit there was little information about the LAT1 at the blood–brain barrier of other species and about other isoforms such as LAT2.

In transport studies using animals, the rat is often employed because of the ease of handling. Therefore, for investigation of the transport function of the blood–brain barrier at the cellular level, it is convenient to use rat brain capillary endothelial cells (BCECs) as an in-vitro model, so that the results can be directly compared with in-vivo findings. Although a primary culture of rat BCECs has already been established, it has several practical disadvantages, such as a requirement for a large number of rats, because of the poor yield, and a long preparation time. Accordingly, for most of the assays in this study, we used the immortalized rat cell line, RBEC1, which retains several key features of BCECs as previously reported (Kido et al 2000).

The purpose of this study was to examine the expression of multiple LAT isoforms, LAT1 and LAT2, at the blood–brain barrier and their functional significance using an in-vitro model of the rat. Additionally we examined the suitability of RBEC1 as an in-vitro model for the estimation of drug transport into the brain via system L.

Materials and Methods

Materials

[^{3}H]Leucine (154 mCi mmol^{-1}) was purchased from Amersham (Buckinghamshire, UK), L-[^{3}H]DOPA was from American Radiolabeled Chemicals Inc. (St Louis, MO) and [^{14}C]inulin was from DuPont NEN (Boston, MA). Rat tail collagen (Type I) was from Collaborative Research Inc. (Grand Island, NY). Endothelial cell growth factor (ECGF) was from Sigma Chemical Co. (St Louis, MO). All other chemicals were commercial products of reagent grade.

Preparation and culture of rat brain capillary endothelial cells

Rat BCECs were isolated from cerebrum of male Wistar rats (SLC, Shizuoka, Japan), 4 weeks old, and cultured as described previously (Ichikawa et al 1996). When the cells reached confluence in approximately 10–12 days, they were used for the isolation of total RNA or for the uptake study.

RBEC1 cells, established by immortalizing the rat isolated BCECs by infection of recombinant plasmids containing origin-defective SV40 gene (Kido et al 2000), were used for the uptake study. The cells were cultivated as described previously (Kido et al 2000). For the uptake study, RBEC1 was seeded at 50000 cells cm^{-2} on collagen-coated culture dishes (16 mm diameter).

RT-PCR analyses

Total RNA was isolated from rat whole-brain, primary cultured BCECs and RBEC1 by means of the standard method using acid guanidinium–phenol–chloroform. Poly (A)$^+$ RNA was purified using Oligotex dT 30 (Takara, Ootsu, Japan). Reverse transcription (RT) was performed at 42°C for 1 h with 1 μg of Poly (A)$^+$ RNA, 0.05 μg of random primer, 0.125 μg of RNase Guard (GIBCO BRL, Gaithersburg, MD), 100 U of Super Script II (GIBCO BRL) in a 50-μL reaction volume. For subsequent PCR amplification, we used the primer sequences for rat LAT1, LAT2 and 4F2hc as follows: LAT1 primer set (sense: 5′-TCA AGC TCT GGA TCG AGC TGC TC-3′, and antisense: 5′-TCC TGT AGG GGT TGA TCA TCT CC-3′), LAT2 primer set (sense: 5′-GCC TGT GGT ATC ATT GTT GTA GG-3′, and antisense: 5′-AGT TGA CCC ATG TGA GCA GC-3′) and 4F2hc primer set (sense: 5′-CGA AGT GGA CAT GAA AGA TGT G-3′ and antisense: 5′-AAA CTA GGC CCT TCA CCT TCA G-3′). Amplification conditions for rat LAT1, LAT2 and 4F2hc were as follows: LAT1 cDNA (denaturation at 94°C for 10 s, annealing at 50°C for 30 s and extension at 72°C for 30 s through a total of 30 cycles), LAT2 cDNA (denaturation at 94°C for 10 s, annealing at 55°C for 30 s and extension at 72°C for 30 s through a total of 30 cycles) and 4F2hc cDNA (denaturation at 94°C for 10 s, annealing at 54°C for 30 s and extension at 72°C for 30 s through a total of 30 cycles). The PCR products were separated by electrophoresis in 1.5% agarose and visualized under UV light in the presence of ethidium bromide.

Uptake measurements

When primary cultured rat BCECs and RBEC1 reached confluence after 11 and 3 days in culture, respectively, they were washed three times with 1 mL of uptake medium containing (mM): 122 NaCl, 3 KCl, 25 $NaHCO_3$, 1.2 $MgSO_4$, 0.4 K_2HPO_4, 1.4 $CaCl_2$, 10 D-glucose and 10 Hepes (pH 7.4) and pre-incubated at 37°C for 20 min. After the pre-incubation, uptake medium (0.25 mL) containing radiolabelled test compound was added to initiate uptake. In sodium-free uptake medium, Na^+ was replaced by choline or other cations as described in the Figure legends. The cells were incubated at 37°C for the designated time, then washed three times with 1 mL of ice-cold incubation solution to terminate the uptake. For the quantitation of the radioactivity taken up, the cells were solubilized with 1 N NaOH for 120 min, then neutralized with HCl, and the associated radioactivity was determined. Cellular protein content was measured by the Lowry method using BSA as a standard (Lowry et al 1951). Uptake was expressed as the cell-to-medium concentration ratio per mg of cellular protein (μL (mg protein)$^{-1}$) obtained by dividing apparent uptake amount (nmol min^{-1} mg^{-1}) by the substrate concentration (nmol min^{-1} μL^{-1}) in the uptake medium, after correction for nonspecific adsorption, which was estimated from the apparent adsorption or uptake of [^{14}C]inulin. The initial uptake rate was calculated by multiplying the cell-to-medium concentration ratio at 1 min by the substrate concentration. To estimate the kinetic parameters for the uptake of [^{3}H]leucine and L-[^{3}H]DOPA by RBEC1, the initial uptake rate was fitted to the equations 1 and 2 by means of nonlinear least-squares regression analysis using WinNonlin (Scientific Consulting Inc., Cary, NC):

$$v = V_{max} \times s/(K_m + s) \quad (1)$$

$$v = V_{max1} \times s/(K_{m1} + s) + V_{max2} \times s/(K_{m2} + s) \quad (2)$$

where v is the initial uptake rate of substrate (nmol min^{-1} (mg protein)$^{-1}$), s is the substrate concentration in the medium (μM), K_m is the Michaelis-Menten constant (μM), and V_{max} is the maximum uptake rate (nmol min^{-1} (mg protein)$^{-1}$) for the saturable process.

Results

Detection of rat LAT1, LAT2 and 4F2hc transcripts in rat BCECs

The expression of rat LAT1, LAT2 and 4F2hc in rat whole brain, rat primary cultured BCECs and RBEC1 was examined by the RT-PCR method using specific primers based on the respective nucleotide sequences of LAT1, LAT2 and 4F2hc (Figure 1). Whole brain was used as a control because LAT1, LAT2 and 4F2hc are all reported to be expressed in rat brain (Kanai et al 1998, Segawa et al 1999). RT-PCR products which correspond to LAT1, LAT2 and 4F2hc were observed from the poly (A)$^+$RNA of primary cultured BCECs and of RBEC1, as well as that of whole brain.

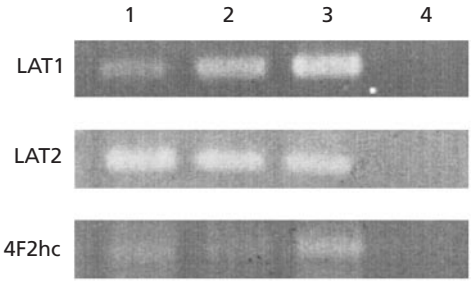

Figure 1 Detection of LAT1, LAT2 and 4F2hc in rat brain capillary endothelial cells by means of the RT-PCR method. The mRNA used for RT-PCR was isolated from whole brain (lane 1), rat primary cultured BCECs (lane 2) and RBEC1 (lane 3). As the negative control, water was used instead of mRNA for RT-PCR (lane 4). PCR products stained with ethidium bromide were visualized under UV light. The sizes of transcripts corresponding to LAT1, LAT2 and 4F2hc were 438 bp, 397 bp and 456 bp, respectively.

Uptake of leucine by RBEC1

Figure 2A shows the time course of uptake of [^{3}H]leucine (1 μM) by RBEC1 in the presence or absence of sodium ions. The profile in the absence of sodium, which was replaced with choline, was similar to that in the presence of sodium ions. To confirm the sodium-ion-independence of [^{3}H]leucine uptake by RBEC1, the effect of sodium-ion replacement with various cations was examined (Figure 2B). When sodium was replaced with choline, *N*-methylglucamine, lithium or potassium ions, the initial uptake of [^{3}H]leucine was comparable to that in the presence of sodium ions. Because the transport activities of LAT1 and LAT2 were reported to be independent of sodium ions (Kanai et al 1998; Segawa et 1999), all subsequent cellular studies were performed under the sodium-free condition to exclude any minor effect on leucine uptake such as sodium-dependent amino acid transporter system A or system ASC (Tayarani et al 1987).

To obtain the kinetic parameters of the sodium-independent uptake by RBEC1, the concentration dependence of leucine uptake was examined (Figure 3A). When the result was analysed in the form of an Eadie-

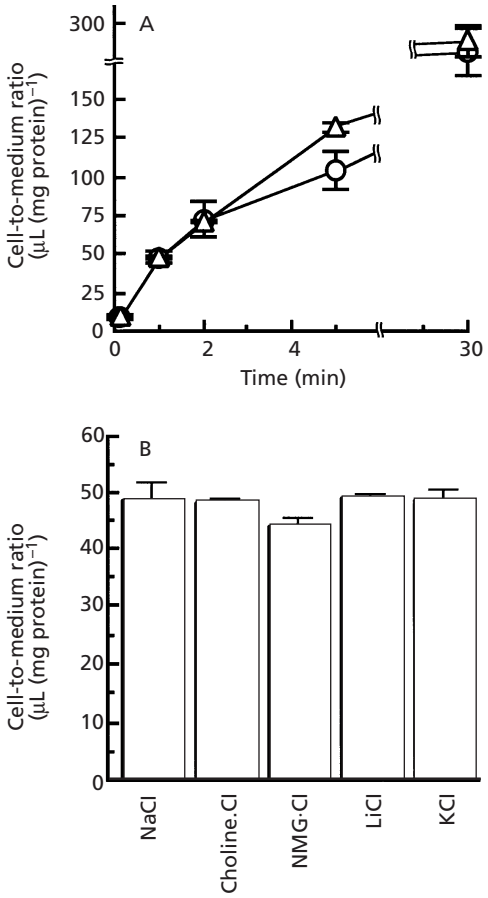

Figure 2 Sodium-ion dependence of [^{3}H]leucine uptake by RBEC1. A. Time course of [^{3}H]leucine (1 μM) uptake by RBEC1. Uptake of [^{3}H]leucine was measured in sodium-containing (○) or sodium-free (△) medium. B. Cation dependence of [^{3}H]leucine (1 μM) uptake by RBEC1. When sodium ions were replaced with other cations, chloride was used as the counter anion. The [^{3}H]leucine uptake was measured at pH 7.4 and 37°C for 1 min. Each point or column represents the mean ± s.e.m. of 3 or 4 experiments. NMG, N-methyl-glucamine.

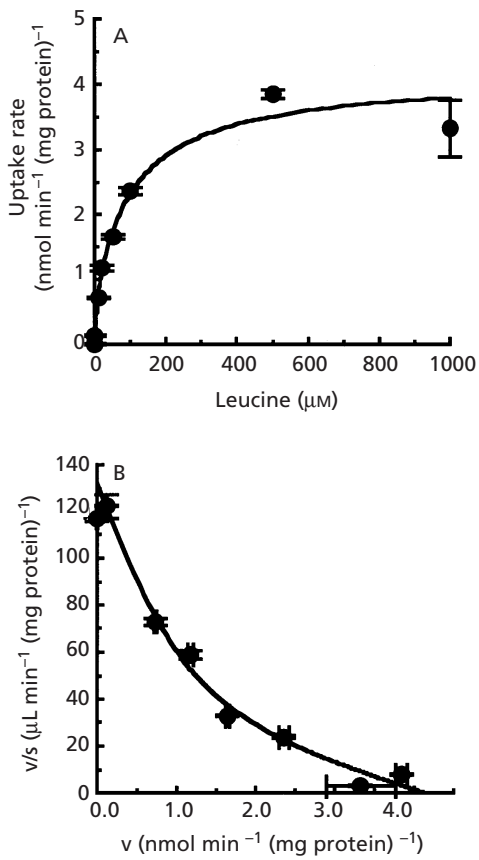

Figure 3 Concentration dependence of leucine uptake by RBEC1. A. The uptake of leucine at various concentrations was measured at pH 7.4 and 37°C for 1 min in sodium-free medium (replaced with choline$^+$). The solid line represents total uptake rate based on equation 2, using nonlinear least-squares regression analysis. B. Eadie-Hofstee transformation of the data, from which kinetic parameters were determined. Each point represents the mean ± s.e.m. of 3 or 4 experiments.

Hofstee plot, two saturable components were obtained (Figure 3B). Nonlinear regression analysis yielded K_m values of 8.92 ± 3.18 and 119 ± 45 μM, and V_{max} values of 0.94 ± 0.38 and 3.20 ± 0.33 nmol min^{-1} (mg protein)$^{-1}$, for the high- and low-affinity components, respectively.

The inhibitory effects of several amino acids and amino acid analogues on the [^{3}H]leucine uptake are summarized in Table 1. The initial uptake of [^{3}H]leucine was strongly inhibited by 1 mM cysteine, histidine, isoleucine, leucine, phenylalanine, tyrosine and a system-L-specific inhibitor, 2-aminobicyclo[2,2,1]heptane-2-carboxylic acid (BCH). Acidic amino acids, aspartate and glutamate, a basic amino acid, arginine, and system-A-specific inhibitor, *N*-methylaminoisobutyric acid (MeAIB) showed no inhibitory effect. Amino acid-mimetic xenobiotics such as baclofen, L-DOPA and melphalan significantly reduced the [^{3}H]leucine uptake.

Uptake of L-DOPA by RBEC1

We examined whether RBEC1 is a suitable in-vitro model for evaluation of drug transport via system L at the blood–brain barrier. Figure 4A shows the time course of L-[^{3}H]DOPA (1 μM) uptake in the presence and absence of sodium ions, and in the presence of BCH without sodium ions in the uptake medium. Replacement of sodium ions with choline did not affect the L-[^{3}H]DOPA uptake by RBEC1, but L-[^{3}H]DOPA uptake was strongly inhibited by BCH. The initial uptake of

Table 1 Inhibitory effects of various amino acids and amino acid analogues on [^{3}H]leucine uptake by RBEC1.

	Inhibitor	Relative uptake (% of control)
Amino acids	Cysteine	35.3 ± 1.10*
	Histidine	26.7 ± 1.64*
	Isoleucine	25.2 ± 0.90*
	Leucine	9.2 ± 0.20*
	Phenylalanine	10.8 ± 0.33*
	Tyrosine	32.4 ± 1.62*
	Alanine	96.4 ± 2.21
	Glycine	102.6 ± 1.94
	Proline	99.5 ± 1.80
	Serine	96.3 ± 2.49
	Aspartate	93.4 ± 2.64
	Glutamate	101.6 ± 4.00
	Arginine	102.4 ± 2.76
Amino acid analogues	Baclofen	86.0 ± 1.70*
	L-DOPA	21.5 ± 1.23*
	Melphalan in 1% DMSO	53.9 ± 2.00*
	(1% DMSO)[a]	107.7 ± 4.48
	BCH	21.3 ± 0.75*
	MeAIB	94.9 ± 2.79

Uptake of [^{3}H]leucine (1 μM) was measured at pH 7.4 and 37°C for 1 min in the sodium-free medium containing each inhibitor. The concentration of melphalan was 200 μM and those of other inhibitors were 1 mM. Melphalan was dissolved in the medium containing 1% DMSO. Each value represents the mean ± s.e.m. of 3 or 4 experiments. *$P < 0.05$, compared with the control value. [a]Control study for melphalan effect in the presence of 1% DMSO.

L-DOPA in the absence of sodium ions was saturable with a high affinity, exhibiting a K_m value of 28.2 ± 7.03 μM and a V_{max} value of 0.56 ± 0.04 nmol min^{-1} (mg protein)$^{-1}$ (Figure 4B).

Uptake of leucine and L-DOPA by primary cultured rat BCECs

Because RBEC1 is an immortalized cell line, we examined whether RBEC1 cells retain the uptake properties of primary cultured rat BCECs. The uptake of [^{3}H]leucine (1 μM) and L-[^{3}H]DOPA (1 μM) by the primary cultured rat BCECs was measured at 1 min in the absence of sodium ions (Table 2). The uptake of both substrates was reduced in the presence of the respective unlabelled compound in a concentration-dependent manner, and the uptakes were strongly inhibited in the presence of 1 mM BCH. These results are comparable with those obtained in RBEC1 cells.

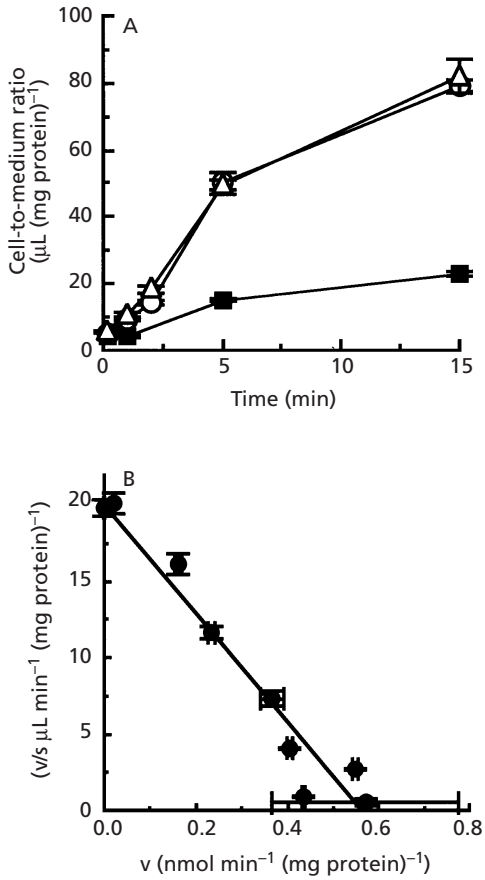

Figure 4 Sodium-ion and concentration dependence of L-DOPA uptake by RBEC1. A. Time course of L-[^{3}H]DOPA (1 μM) uptake by RBEC1. Uptake of L-[^{3}H]DOPA was measured in sodium-containing (○), sodium-free (replaced with choline$^+$) (△) and 1 mM BCH-containing (■) medium at pH 7.4 and 37°C. B. Eadie-Hofstee plot of L-DOPA uptake. Kinetic parameters were determined using equation 1, using nonlinear least-squares regression analysis. L-DOPA uptake at various concentrations was measured at pH 7.4 and 37°C for 1 min in the sodium-free medium. Each point represents the mean ± s.e.m. of 3 or 4 experiments.

Discussion

In this study, we demonstrated, by using primary cultured rat BCECs and a cell line, that LAT1 is expressed and functions for leucine transport at the blood–brain barrier of the rat. The expressions of LAT1 and 4F2hc in rat primary cultured BCECs and RBEC1 were detected by the RT-PCR method. By Northern blot analysis, LAT1 mRNA of 3.5 kb was also detected in RBEC1 (data not shown). LAT2 was also detected by RT-PCR analysis in primary cultured rat BCECs and RBEC1. These results indicated that both LAT1 and LAT2 are expressed in the blood–brain barrier.

Table 2 Uptake of [^{3}H]leucine and L-[^{3}H]DOPA by primary cultured rat BCECs.

Substrate	Inhibitor	Cell-to-medium ratio (μL (mg protein)$^{-1}$)
[^{3}H]leucine	Control	69.6 ± 0.42
	Leucine (20 μM)	45.4 ± 0.78*
	Leucine (1 mM)	5.20 ± 0.41*
	BCH (1 mM)	11.6 ± 1.46*
L-[^{3}H]DOPA	Control	12.5 ± 0.30
	L-DOPA (50 μM)	7.65 ± 0.71*
	L-DOPA (1 mM)	0.59 ± 0.22*
	BCH (1 mM)	1.68 ± 0.09*

Uptake of [^{3}H]leucine (1 μM) and L-[^{3}H]DOPA (1 μM) was measured at pH 7.4 and 37°C for 1 min in sodium-free medium in the absence or presence of the indicated concentrations of inhibitors. Each value represents the mean ± s.e.m. of 3 or 4 experiments. *$P < 0.05$, compared with the control value.

We functionally analysed the leucine transport activity by using RBEC1, which is an immortalized cell line of rat BCECs (Kido et al 2000). Replacement of sodium ions with several cations did not affect the uptake of [^{3}H]leucine by RBEC1 (Figures 2A and 2B). The magnitude of the sodium-independent component of leucine uptake observed in RBEC1 was comparable with that of the leucine influx into the brain observed by using the in-vivo brain uptake index (BUI) method (Sershen & Lajtha 1976). Sodium-independent uptake of leucine by RBEC1 was saturable and an Eadie-Hofstee plot exhibited two saturable components (Figure 3B). Smith et al (1985) reported that leucine influx into the brain, evaluated with a brain perfusion technique, showed a better fit to a model with two saturable components than to that with a one-saturable-component model plus nonsaturable diffusion. In particular, the low K_m obtained in RBEC1 was close to that of LAT1 ($K_m = 18.1$ μM) and also close to the values obtained by the brain perfusion technique ($K_m = 14 \sim 29$ μM) (Kanai et al 1998; Smith et al 1985, 1987). Further, the K_m of the low-affinity component in RBEC1 ($K_m = 119$ μM) was similar to that of LAT2 ($K_m = 120$ μM) (Segawa et al 1999). These results of kinetic analysis support the view that LAT1 and another component, presumably LAT2, are functionally expressed at the blood–brain barrier of rats.

As shown in Table 1, the initial uptake of leucine was inhibited by neutral amino acids and an amino acid analogue, BCH. It has been shown by functional analysis using *Xenopus laevis* oocytes that the amino acid selectivity of LAT1 is restricted to large neutral amino acids, in contrast to LAT2, which prefers small neutral amino acids (i.e., alanine and glycine) (Kanai et al 1998; Segawa et al 1999). The finding that leucine (1 μM) uptake was not inhibited by alanine or glycine in RBEC1 is consistent with the involvement of LAT1 rather than LAT2. In an in-vivo BUI study, the influx into the brain of [^{14}C]phenylalanine, which is a well known substrate of system L, was not inhibited by glycine or alanine (Oldendorf 1971). Furthermore, self-inhibition by the unlabelled amino acid of the uptake of [^{14}C]alanine or [^{14}C]glycine was not observed, and these two amino acids did not penetrate the blood–brain barrier via a selective carrier system (Oldendorf & Szabo 1976). In addition, the brain perfusion technique showed that glycine had no inhibitory effect on the permeation of [^{14}C]phenylalanine (Smith et al 1987). These previous selectivity studies on amino acid uptake in-vivo and the present functional analysis by RBEC1 indicate that contribution of LAT1 is more significant than that of LAT2 in the influx of large neutral amino acids from plasma to brain, although the contribution of each of them is not clear in the circulating blood.

Because the blood–brain barrier is formed by BCECs linked to each other by tight junctions and is impermeable to hydrophilic compounds, system L could be useful for the delivery into the brain of hydrophilic amino acid-mimetic drugs. Several clinically used drugs, such as L-DOPA, melphalan and baclofen, have been suggested to be transported by system L across the blood–brain barrier (Pardridge 1983; van Bree et al 1988; Takada et al 1991). In the current study using RBEC1, L-DOPA and melphalan showed strong inhibition of [^{3}H]leucine uptake, whereas baclofen showed weak inhibition. The L-[^{3}H]DOPA uptake by RBEC1 was sodium independent and strongly inhibited by BCH (Figure 4A). These results indicate that L-DOPA is mainly taken up by RBEC1 via system L. The kinetic analysis of L-DOPA transport showed a high-affinity transport with a K_m value of 28.2 μM (Figure 4B), which is lower than that obtained by the in-vivo BUI method ($K_m = 430$ μM) (Pardridge 1983). The reason for the difference in kinetic parameters is not clear, but may be partially ascribed to the dilution of the infusate by the circulating blood or inhibition by amino acids in plasma in-vivo. Consequently, the sensitivities of LAT for amino acid-mimetic drugs in RBEC1 seem to be comparable with those in animal studies.

In transport analysis using cell lines, there is a potential problem of up- or down-regulation of transporters during cultivation and passaging. As shown in Table 2, the [^{3}H]leucine and L-[^{3}H]DOPA uptake activities at 1 min of primary cultured rat BCECs were

quantitatively similar to those of RBEC1 (Figures 2A and 4A). As the uptake properties of primary cultured rat BCECs, including concentration dependence and inhibition by BCH (Table 2), were also similar to those of RBEC1 RBEC1 appears to retain the native properties of the large neutral amino acid uptake activity.

In conclusion, we have demonstrated the existence of LAT1 and 4F2hc in primary cultured rat BCECs and RBEC1 by RT-PCR and have evaluated the uptake properties, such as K_m value and amino acid specificity, in RBEC1. LAT2 was also detected in rat BCECs and the low-affinity K_m for leucine uptake by RBEC1 showed a good agreement with that of LAT2. These results indicate that plural isoforms of system L are expressed in the blood–brain barrier. In addition, the L-DOPA transport features of RBEC1 were similar to those of primary cultured rat BCECs. Accordingly, RBEC1 is suggested to be an appropriate in-vitro model for evaluation of drug transport via system L at the blood–brain barrier. Further studies on the polarity of LAT1 and LAT2 in the cells, and the expression of other system L isoforms, are required to establish the physiological roles and pharmacological relevance of system L at the blood–brain barrier.

References

Boado, R. J., Li, J. Y., Nagaya, M., Zhang, C., Pardridge, W. M. (1999) Selective expression of the large neutral amino acid transporter at the blood–brain barrier. *Proc. Natl Acad. Sci. USA* **96**: 12079–12084

Ichikawa, N., Naora, K., Hirano, H., Hashimoto, M., Masumura, S., Iwamoto, K. (1996) Isolation and primary culture of rat cerebral microvascular endothelial cells for studying drug transport in vitro. *J. Pharmacol. Toxicol. Methods* **36**: 45–52

Kanai, Y., Segawa, H., Miyamoto, K., Uchino, H., Takeda, E., Endou, H. (1998) Expression cloning and characterization of a transporter for large neutral amino acids activated by the heavy chain of 4F2 antigen (CD98). *J. Biol. Chem.* **273**: 23629–23632

Kido, Y., Tamai, I., Okamoto, M., Suzuki, F., Tsuji, A. (2000) Functional clarification of MCT1-mediated transport of monocarboxylic acids at the blood–brain barrier using in vitro cultured cells and in vivo BUI studies. *Pharm. Res.* **17**: 55–62

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265–275

Oldendorf, W. H. (1971) Brain uptake of radiolabeled amino acids, amines, and hexoses after arterial injection. *Am. J. Physiol.* **221**: 1629–1639

Oldendorf, W. H., Szabo, J. (1976) Amino acid assignment to one of three blood–brain barrier amino acid carriers. *Am. J. Physiol.* **230**: 94–98

Pardridge, W. M. (1983) Brain metabolism: a perspective from the blood–brain barrier. *Physiol. Rev.* **63**: 1481–1535

Sanchez del Pino, M. M., Peterson, D. R., Hawkins, R. A. (1995) Neutral amino acid transport characterization of isolated luminal and abluminal membranes of the blood–brain barrier. *J. Biol. Chem.* **270**: 14913–14918

Segawa, H., Fukasawa, Y., Miyamoto, K., Takeda, E., Endou, H., Kanai, Y. (1999) Identification and characterization of a Na(+)-independent neutral amino acid transporter that associates with the 4F2 heavy chain and exhibits substrate selectivity for small neutral D- and L-amino acids. *J. Biol. Chem.* **274**: 19745–19751

Sershen, H., Lajtha, A. (1976) Capillary transport of amino acids in the developing brain. *Exp. Neurol.* **53**: 465–474

Smith, Q. R., Stoll, J. (1998) Blood–brain barrier amino acid transport. In: Pardridge, W. M. (ed.) *Introduction to the Blood–Brain Barrier*. Cambridge University Press, Cambridge, UK, pp 188–197

Smith, Q. R., Takasato, Y., Sweeney, D. J., Rapoport, S. I. (1985) Regional cerebrovascular transport of leucine as measured by the in situ brain perfusion technique. *J. Cereb. Blood Flow Metab.* **5**: 300–311

Smith, Q. R., Momma, S., Aoyagi, M., Rapoport, S. I. (1987) Kinetics of neutral amino acid transport across the blood–brain barrier. *J. Neurochem.* **49**: 1651–1658

Takada, Y., Greig, N. H., Vistica, D. T., Rapoport, S. I., Smith, Q. R. (1991) Affinity of antineoplastic amino acid drugs for the large neutral amino acid transporter of the blood–brain barrier. *Cancer Chemother. Pharmacol.* **29**: 89–94

Tayarani, I., Lefauconnier, J. M., Roux, F., Bourre, J. M. (1987) Evidence for an alanine, serine, and cysteine system of transport in isolated brain capillaries. *J. Cereb. Blood Flow Metab.* **7**: 585–591

Tsuji, A., Tamai, I. (1997) Blood–brain barrier function of P-glycoprotein. *Adv. Drug Delivery Rev.* **25**: 287–298

Tsuji, A., Tamai, I. (1998) Blood–brain barrier transport of drugs. In: Pardridge, W. M. (ed.) *Introduction to the Blood–Brain Barrier*. Cambridge University Press, Cambridge, UK, pp 238–247

van Bree, J. B., Audus, K. L., Borchardt, R. T. (1988) Carrier-mediated transport of baclofen across monolayers of bovine brain endothelial cells in primary culture. *Pharm. Res.* **5**: 369–371