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Transport of prostaglandin E_1 across the blood–brain barrier in rats

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Abstract

The transport of prostaglandin E_1 (PGE_1) across the blood–brain barrier (BBB) was characterized using an in-situ rat brain perfusion technique. The uptake of [^{3}H]PGE_1 was not affected by short-chain monocarboxylic acids (butyric acid and valeric acid). On the other hand, uptake of [^{3}H]PGE_1 was significantly inhibited by medium-chain monocarboxylic acids such as hexanoic acid, enanthic acid and octanoic acid. These medium-chain monocarboxylic acids showed a more potent inhibitory effect on [^{3}H]PGE_1 uptake with increasing number of carbon atoms. In contrast, there was no decrease in [^{3}H]PGE_1 transport by any dicarboxylic acids with 5–8 carbon atoms. Valproic acid decreased [^{3}H]PGE_1 uptake, whereas *p*-aminohippuric acid, a substrate for the organic anion transporter family, did not inhibit [^{3}H]PGE_1 transport. Bromocresol green, an inhibitor of prostaglandin transporter (PGT), strongly decreased [^{3}H]PGE_1 transport across the BBB. In addition, digoxin and taurocholate, substrates for organic anion transporting polypeptide subtype 2 (Oatp2), significantly inhibited [^{3}H]PGE_1 uptake. RT-PCR analysis revealed that PGT mRNA and Oatp2 mRNA are expressed in a capillary-rich fraction from rat brain. Thus, it is suggested that PGE_1 transport across the BBB is mediated by some specific transport systems, possibly by the members of the Oatp family.

Introduction

Prostaglandins (PGs) are 20-carbon unsaturated carboxylic acids with a cyclopentane ring. PGs are produced by a variety of cell types following physiological and pathological stimuli and exert multiple effects, normally as local hormones or autacoids. PGs are organic anions with pKa values of approximately 5, thus they mainly exist as charged molecules at physiological pH. The in-vitro partition coefficients (log P) for PGs including PGE_1 are around 0.7 and cell membranes, such as the plasma membrane of erythrocytes, are impermeable to PGs (Bito & Baroody 1975). In addition, PGE_2 was transported at the same rate as inulin across the canine cortical collecting tubule (CCCT) cell monolayer on Millipore filters, suggesting that the only pathway for PGE_2 across the CCCT cell monolayer is the paracellular pathway (Garcia-Perez & Smith 1984). Systemic administration of the PGs, such as PGD_2, PGEs (PGE_1, PGE_2) and $PGF_{2\alpha}$, induce neurophysiological actions such as sleep induction, change in body temperature and regulation of food intake (Horton 1964; Holmes & Horton 1968; Suzuki et al 1986; Nishino et al 1989). In addition, it has been reported that intravenous administration of PGE_2 to urethane-anesthetized rats induced dose-related hyperthermia (Eguchi et al 1988). Furthermore, the hyperthermic response was blocked by administration of SC-19220, an antagonist of fever centrally induced by PGE, into the cerebral ventricles (Eguchi et al 1988). These observations indicate that PGs penetrate to the brain parenchyma from the blood circulation across the blood–brain barrier (BBB). Since PGs diffuse poorly across the plasma membrane as described above, some transport system would be necessary for the transfer of PGs from the bloodstream to the brain across the BBB. However, the transport system involved in prostaglandin influx through the BBB is so far not well understood.

Recently, it has been suggested that PGE_1 protects neurons from apoptosis. Kawamura et al (1999) reported that PGE_1 decreased the incidence of apoptotic cell death in rat pheochromocytoma PC12 cells. The preventive effect of PGE_1 on apoptotic

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cell death was blocked by bromocresol green (BCG), an inhibitor of prostaglandin transporter (PGT), as well as a PGT antisense oligonucleotide, suggesting the involvement of PGT in the blockade of apoptosis by PGE_1. Also, apoptosis in neuronal cells in the spinal cord was inhibited by the systemic administration of lipo-PGE_1 (PGE_1 incorporated in lipid microspheres) (Kawamura et al 1997). Thus, PGE_1 may be effective for the treatment of neurodegenerative disorders, especially Alzheimer's disease and Parkinson's disease. In order to effectively exert the neuroprotective effects by administration of PGE_1, it is important to understand the molecular mechanisms underlying PGE_1 influx into the brain across the BBB.

In this study, PGE_1 transport across the BBB was analysed using an in-situ brain perfusion technique that allows an accurate assessment of the BBB permeability, based on the cerebrovascular permeability–surface area (PS) product. In addition, mRNA expressions of PGT and organic anion transporting polypeptide (Oatp) subtype 2 in brain capillaries were examined.

Materials and Methods

Materials

[5,6(n)-^{3}H]PGE_1 (1.81 TBq mmol^{-1}), [2-^{14}C]diazepam (2.07 GBq mmol^{-1}) and [U-^{14}C]sucrose (18.0 GBq mmol^{-1}) were purchased from Amersham Pharmacia Biotech (Buckinghamshire, UK). All other chemicals used were of the highest purity available.

In-situ brain perfusion

Experiments with animals were performed in accordance with the Guide for Animal Experimentation, Hiroshima University, and the Committee of Research Facilities for Laboratory Animal Sciences, Graduate School of Biomedical Sciences, Hiroshima University. PGE_1 transport at the BBB was investigated using the in-situ rat brain perfusion technique developed by Takasato et al (1984). The perfusion fluid was a bicarbonate-buffered physiological saline including [^{3}H]PGE_1 (6.6 nM) and [^{14}C]diazepam (0.89 μM). The pH of the perfusion fluid was adjusted to pH 7.4 and the osmolality was corrected by changing the concentration of sodium chloride in the buffer (293.4 ± 0.9 mOsm kg^{-1}). In brief, male Wistar rats (250–350 g) were anesthetized by intraperitoneal injection of pentobarbital (30 mg kg^{-1}) and the external carotid artery was cannulated with polyethylene tubing for retrograde perfusion. The right pterygopalatine, occipital and superior arteries were tied off. Immediately before perfusion, the heart was cut and the common carotid artery was ligated to infuse the oxygenated perfusion fluid at 37°C into the brain capillaries through the internal carotid artery. The rat brain was perfused for 15 s at a flow rate of 5 mL min^{-1}. At the end of the perfusion, the rat was decapitated and the brain was quickly removed from the cranium. The half (right) brain hemisphere was weighed and digested at 50°C for 2 h with NCSII (0.5 mL per 0.15 g wet tissue weight) (Amersham Pharmacia Biotech, Buckinghamshire, UK). Subsequently, 10 mL of ACSII (Amersham Pharmacia Biotech) per 0.5 mL NCSII was added and the radioactivity was measured by dual-channel liquid scintillation counting. The K_{in} of [^{3}H]PGE_1 uptake (normalized by gram tissue weight) was calculated as follows:

$$K_{in,\,PG} = [^3\text{H}]PGE_1 \text{ brain concentration}/(\text{perfusion time} \cdot [^3\text{H}]PGE_1 \text{ perfusate concentration})$$

The tissue concentration of [^{3}H]PGE_1 was corrected for perfusate [^{3}H]PGE_1 trapped in the brain intravascular space determined by the radioactivity of [^{14}C]sucrose in the brain and in the perfusate. The brain intravascular volume, experimentally determined by using [^{14}C]sucrose, was $7.85 \pm 0.68\ \mu$L g^{-1} tissue (mean ± s.e.m., n = 3). The cerebral perfusate flow rate (Q_{pf}) was estimated by using the K_{in} of [^{14}C]diazepam as follows:

$$Q_{pf} = [^{14}\text{C}]\text{diazepam brain concentration}/(\text{perfusion time} \cdot [^{14}\text{C}]\text{diazepam perfusate concentration})$$

It is postulated here that [^{14}C]diazepam is completely extracted through the brain capillaries and that there is no efflux of [^{14}C]diazepam from the brain into the capillaries during the passage of the perfusate through the brain. Finally, the PS product of PGE_1 across the BBB was estimated by the following equation:

$$\text{PS product} = -Q_{pf} \ln(1 - K_{in,\,PG}/Q_{pf})$$

Preparation of brain capillary-rich fraction

Brain capillary-rich fraction was prepared according to the reported method (Ohtsuki et al 2002). In brief, the brain was quickly isolated from the cranium after decapitation and the meningeal membranes and the cerebellum were removed from the excised brain. The tissue was then weighed and homogenized with a glass/Teflon Potter homogenizer (13 strokes at 1000 rpm) in a five-fold volume of 122 mM NaCl, 25 mM $NaHCO_3$, 3 mM KCl, 0.4 mM K_2HPO_4, 1.4 mM $CaCl_2$, 1.2 mM $MgSO_4$, 10 mM D-glucose and 10 mM HEPES (pH 7.4). Dextran solution was added to the homogenate (final concentration of dextran was 16%) and the mixture was centrifuged at 4500 *g* for 20 min at 4°C in an Avanti 30 Compact Centrifuge with rotor F0630 (Beckman Instruments Inc., CA). The pellet was used as the brain capillary-rich fraction for RT-PCR analysis.

RT-PCR analysis

Total RNA was extracted from the brain capillary-rich fraction and the whole brain using MagExtractor RNA kit (TOYOBO, Osaka, Japan) (Sasaki et al 2001). Primers were synthesized chemically on the basis of the sequence of rat PGT and Oatp2. The sequences of sense strand and antisense strand for rat PGT were 5′-GAGCAG TCTCACCACAATCG-3′ and 5′-GGCTCGGCAAAG TCATCCAC-3′, respectively. Those for rat Oatp2 were 5′-GCCTAAGTATCTGGAACAGCAA-3′ and 5′-CAG CGAGTATATGAAACAGCCA-3′, respectively. RT-PCR

was performed using a ReverTra Dash RT-PCR kit (TOYOBO, Osaka, Japan) according to the following schedule: denaturation at 95°C for 1 min, annealing at 69°C (for PGT) or 62°C (for Oatp2) for 1 min, extension at 72°C for 30 s and 40 cycles (for PGT) or 28 cycles (for Oatp2). The PCR products were separated by electrophoresis in 2.0% agarose gel and visualized with ethidium bromide.

Analytical methods

Alkaline phosphatase (EC 3.1.3.1) was assayed as described previously (Shiraishi et al 2000). Protein concentration was measured by the method of Bradford (1976) with bovine serum albumin as a standard.

Data analysis

Statistical analysis was performed using the Kruskal–Wallis test followed by post-hoc analysis. A difference of $P < 0.05$ was considered statistically significant.

Results

The K_{in} of [^{3}H]PGE_1 (control, 6.6 nM) after a 15-s brain perfusion was 0.140 ± 0.008 (mL min-g^{-1}) and was only 5.1% of the K_{in} of [^{14}C]diazepam (2.77 ± 0.28 mL min-g^{-1}) (mean ± s.e.m., n = 7), which was used as a marker of the cerebral perfusate flow rate. The rate-limiting step in PGE_1 transport across the BBB is therefore the plasma membrane permeability of the cerebral capillaries. The calculated PS of [^{3}H]PGE_1 was 0.144 ± 0.008 (mL min-g^{-1}, mean ± s.e.m., n = 7), corresponding to that of [^{3}H]PGE_2 (0.101 mL min-g^{-1}) estimated by the intravenous administration to rats (Eguchi et al 1992).

Since PGs including PGE_1 contain a carboxyl side-chain in their structure, the effects of aliphatic monocarboxylic acids with different carbon chain lengths on [^{3}H]PGE_1 transport across the BBB were examined (Table 1). The transport of [^{3}H]PGE_1 was not affected by short straight-chain monocarboxylic acids such as butylic acid (C_4) and valeric acid (C_5), but was significantly inhibited by medium straight-chain monocarboxylic acids such as hexanoic acid (C_6), enanthic acid (C_7) and octanoic acid (C_8). More potent inhibitory effects on [^{3}H]PGE_1 uptake were observed with an increasing number of carbon atoms. Octanoic acid inhibited the transport of [^{3}H]PGE_1 in a concentration-dependent manner. In addition, valproic acid, an eight-carbon branched-chain monocarboxylic acid, decreased [^{3}H]PGE_1 transport across the BBB. Benzoic acid, however, an aromatic carboxylic acid, did not inhibit [^{3}H]PGE_1 transport across the BBB (97.3 ± 8.7% of control, mean ± s.e.m., n = 3). We next studied the effects of aliphatic dicarboxylic acids with 5–8 carbon atoms on [^{3}H]PGE_1 transport across the BBB (Table 2). [^{3}H]PGE_1 transport was not affected by any of the dicarboxylic acids used in this study: glutaric acid (C_5), adipic acid (C_6), pimelic acid (C_7) and suberic acid (C_8).

To examine possible transporter(s) involved in [^{3}H]PGE_1 transport across the BBB, the effects of substrates and/or inhibitors for several transporters on the PS product of [^{3}H]PGE_1 were investigated. First, *p*-aminohippuric acid (PAH), a substrate for organic anion transporter (OAT), was used. However, there was no significant decrease in [^{3}H]PGE_1 transport across the BBB by PAH at a concentration of 650 μM, which is 10 times as high as the Michaelis constant (K_m) for OAT3, 65 μM (Kusuhara et al 1999) (88.1 ± 16.8% of control, mean ± s.e.m., n = 3). Next, the effect of BCG, an inhibitor for PGT, on [^{3}H]PGE_1 transport across the BBB was examined. At a 10-fold higher concentration than its inhibition constant for PGT ($K_i = 3.5$ μM) (Kanai et al 1995), BCG inhibited the transport of [^{3}H]PGE_1 by about 70% (Table 3). The effects of digoxin and taurocholate, substrates for Oatp2, were also examined. The concentrations of digoxin and taurocholate in the perfusate were 2.5 and 350 μM, respectively, which are 10 times higher than the respective K_m for Oatp2 (Noé et al 1997). Both digoxin and taurocholate inhibited [^{3}H]PGE_1 transport across the BBB by approximately 50% (Table 3).

To examine the expression of PGT and Oatp2 mRNA in the BBB, RT-PCR analysis was performed with the

Table 1 Effects of monocarboxylic acids on the PS product of [^{3}H]PGE_1. The PS products for [^{3}H]PGE_1 in the brain were determined after in-situ perfusion for 15 s without or with various monocarboxylic acids at a concentration of 20 mM, except for octanoic acid (2 or 20 mM)

PS product of [^{3}H]PGE_1 (mL min-g^{-1})	
Control	0.139 ± 0.006
Butyric acid (C_4)	0.111 ± 0.018
Valeric acid (C_5)	0.127 ± 0.002
Hexanoic acid (C_6)	0.091 ± 0.006*
Enanthic acid (C_7)	0.060 ± 0.004*
Octanoic acid (C_8) (2 mM)	0.078 ± 0.009*
Octanoic acid (C_8) (20 mM)	0.040 ± 0.003*
Valproic acid	0.058 ± 0.007*

Each value is expressed as the mean ± s.e.m. of results from three to five rats. *$P < 0.05$, significantly different from the value for the control.

Table 2 Effects of dicarboxylic acids on the PS product of [^{3}H]PGE_1

PS product of [^{3}H]PGE_1 (mL min-g^{-1})	
Control	0.144 ± 0.009
Glutaric acid (C_5)	0.133 ± 0.018
Adipic acid (C_6)	0.117 ± 0.023
Pimelic acid (C_7)	0.127 ± 0.019
Suberic acid (C_8)	0.143 ± 0.006

The PS products for [^{3}H]PGE_1 in the brain were determined after in-situ perfusion for 15 s without or with various dicarboxylic acids at the concentration of 20 mM. Each value is expressed as mean ± s.e.m. of results from three to five rats.

Table 3 Effects of bromocresol green (BCG), digoxin and taurocholate on the PS product of [^{3}H]PGE$_1$

PS product of [^{3}H]PGE$_1$ (mL min-g^{-1})	
Control (0.1% ethanol)	0.134 ± 0.014
BCG	0.035 ± 0.004*
Digoxin	0.063 ± 0.007*
Taurocholate	0.063 ± 0.012*

The PS products for [^{3}H]PGE$_1$ in the brain were determined after in-situ perfusion for 15 s with 0.1% ethanol (control), 35 μM BCG, 2.5 μM digoxin or 350 μM taurocholate. Each value is expressed as mean $\pm$ s.e.m. of results from three to four rats. *$P < 0.05$, significantly different from the value for control.

specific primers for each transporter. The purity of the brain capillary-rich fraction was evaluated by measuring the specific activity of alkaline phosphatase, highly expressed in brain capillaries (Williams et al 1980; Lawrenson et al 1999). The specific activity of alkaline phosphatase in the brain capillary-rich fraction was about 3.3-fold higher than that in whole brain homogenate. Purification of the brain capillary by our isolation method was also confirmed by microscopy (data not shown). By PCR amplification with reverse transcription of total RNA from the brain capillary-rich fraction, products of expected size (444 bp for PGT mRNA, 562 bp for Oatp2 mRNA) were detected (Figure 1). In addition, their band intensities in the brain capillary-rich fraction were stronger than those in the whole brain (Figure 1). No band was detected when PCR was carried out without reverse transcription of total RNA from the brain capillary-rich fraction and the whole brain.

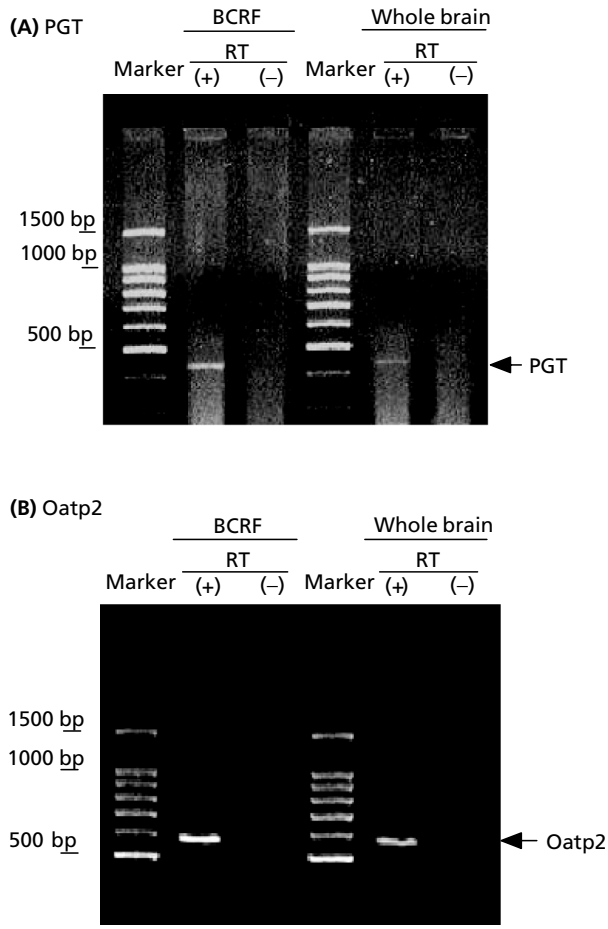

Figure 1 RT-PCR amplification of PGT (A) and Oatp2 (B) mRNA in rat brain capillary-rich fraction (BCRF) and whole brain. Total RNA from rat BCRF or whole brain was reverse-transcribed and first-strand cDNA synthesized was amplified with a set of specific primers described in the text. The PCR products with or without reverse transcription were separated by electrophoresis through a 2.0% agarose gel and stained by ethidium bromide.

Discussion

The purpose of the present study was to characterize the BBB transport of PGE$_1$ using an in-situ brain perfusion technique. The results show that PGE$_1$ transport across the BBB is inhibited by medium straight-chain monocarboxylic acids (C_6–C_8), but not by short straight-chain monocarboxylic acids (C_4, C_5) and dicarboxylic acids (C_5–C_8). Furthermore, the transport of PGE$_1$ was significantly decreased by an inhibitor for PGT, BCG, and substrates for Oatp2 such as digoxin and taurocholate. In addition, RT-PCR revealed PGT mRNA as well as Oatp2 mRNA is expressed in the brain capillaries.

Monocarboxylic acids such as acetic acid and lactic acid are well known to be transported via a carrier-mediated system at the BBB (Oldendorf 1973; Terasaki et al 1991; Kido et al 2000). Monocarboxylate transporter 1 (MCT1) is expressed in the luminal and abluminal membranes of capillary endothelial cells (Gerhart et al 1997). It has therefore been suggested that MCT1 plays an important role in the transport of various monocarboxylic acids, including lactate, across the BBB. Since PGE$_1$ as well as other PGs have a carboxylic side-chain, the effects of various monocarboxylic acids on PGE$_1$ transport across the BBB were examined in this study. Medium-chain monocarboxylic acids (hexanoic acid, enanthic acid and octanoic acid) significantly inhibited [^{3}H]PGE$_1$ transport across the BBB, but the transport of [^{3}H]PGE$_1$ was not affected by short-chain monocarboxylic acids such as butyric acid, a substrate for MCT1 (Tamai et al 1999; Kido et al 2000). In addition, benzoic acid, which is transported by MCT1 (Tamai et al 1999; Kido et al 2000), did not inhibit [^{3}H]PGE$_1$ transport across the BBB. Taken together, it is likely that MCT1 is not involved in the transport of PGE$_1$ across the BBB.

An anticonvulsant drug, valproic acid, a branched-chain monocarboxylic acid, is reported to inhibit the transport of monocarboxylic acids at the BBB (Terasaki et al 1991; Kido et al 2000). In contrast to other MCT1 inhibitors, valproic acid decreased [^{3}H]PGE$_1$ transport across the BBB. Adkison and Shen (1996) reported that

the uptake of [^{3}H]valproic acid was inhibited by medium-chain monocarboxylic acids such as hexanoic acid and octanoic acid, but not by short-chain monocarboxylic acids. Thus, the effects of monocarboxylic acids on [^{3}H]valproic acid transport across the BBB are in accordance with those on [^{3}H]PGE_1 transport observed in this study. In this study, dicarboxylic acids, including glutaric acid and adipic acid, significantly increased the brain uptake of [^{3}H]valproic acid, suggesting the involvement of an anion exchanger, such as OAT subtype 1 (Adkison & Shen 1996). However, any dicarboxylic acids used in this study had no significant effect on [^{3}H]PGE_1 transport. In addition, PAH, a substrate for the OAT family, did not inhibit [^{3}H]PGE_1 uptake at the BBB, whereas it is reported that [^{3}H]valproic acid uptake at the BBB was decreased by PAH (Adkison & Shen 1996). The transport characteristics of PGE_1 across the BBB are therefore similar but not the same as those of valproic acid.

Recently, some researchers have provided information that PGs are recognized by specific transporters, especially those belonging to the organic anion transporting polypeptide (Oatp for rat and mouse; OATP for human) family. PGT is the first cloned transporter that transports PGs (Kanai et al 1995; Lu et al 1996). Rat and human PGTs are considered to be members of the Oatp/OATP family based on their structural homology (Schuster 1998; Adachi et al 2003). PGT is expressed in various tissues and is especially abundant in the lung. PGT-mediated uptake rates in Hela cells showed the following profile: $PGE_1 \sim PGE_2 \sim PGF_{2\alpha} > TxB_2 \gg$ 6-keto $PGF_{1\alpha}$, which is well correlated with the rank order of PG clearance by the lung (Kanai et al 1995). It is therefore suggested that PGT plays an important role in the pulmonary clearance of PGs. PGT is expressed in the brain (Kanai et al 1995; Lu et al 1996), but so far it has been unclear whether or not PGT is present in the BBB, as far as we know. In this study, RT-PCR analysis indicated the existence of PGT mRNA in the BBB. In addition, BCG, one of the most potent inhibitors for PGT (Kanai et al 1995), markedly inhibited [^{3}H]PGE_1 transport across the BBB. Thus, PGT may be partly involved in PGE_1 transport across the BBB.

Rat Oatp2, which was isolated from the brain (Noé et al 1997), is shown to be highly expressed in both the luminal and the abluminal membranes of rat brain capillary endothelial cells by in-situ hybridization histochemistry and immunofluorescence microscopy (Gao et al 1999). Oatp2 mRNA was also detected in the brain capillary-rich fraction by RT-PCR analysis in this study. Recently, it has been reported that Oatp2 is responsible for BBB transport of its substrates such as dehydroepiandrosterone sulfate, [D-penicillamine2,5]-enkephalin and estradiol-17β-glucuronide (Asaba et al 2000; Dagenais et al 2001; Sugiyama et al 2001). Digoxin and taurocholate, substrates for Oatp2, significantly decreased [^{3}H]PGE_1 transport across the BBB. These observations suggest that Oatp2 may also be involved in PGE_1 transport through the BBB.

Besides PGT and Oatp2, it has been reported that several members of the Oatp family, such as Oatp1 (Jacquemin et al 1994), Oatp3 (Walters et al 2000), Oatp11/rOATP-D (Adachi et al 2003), Oatp14 (Sugiyama et al 2003) and moat1 (Nishio et al 2000), are expressed in rat brain. Among these Oatps, Oatp1 and Oatp14 do not transport PGs such as PGE_2, PGD_2 and $PGF_{2\alpha}$ (Kanai et al 1996; Sugiyama et al 2003). However, Oatp3, Oatp11/rOATP-D and moat1 transport PGs, including PGE_1 and PGE_2 (Cattori et al 2001; Adachi et al 2003; Nishio et al 2000). It is therefore possible that these transporters may be also involved in PGE_1 transport across the BBB. Further studies are needed to clarify all the transporters involved and the contribution of each transporter in PGE_1 transport across the BBB.

Conclusions

We have demonstrated that the transport of PGE_1 across the BBB is mediated by some specific transport systems. In addition, we found that PGT mRNA, as well as Oatp2 mRNA, is expressed in the BBB. It is suggested that members of the Oatp family, such as PGT and Oatp2, may be involved in the transport of PGE_1 from the blood circulation into the brain across the BBB. These observations could provide useful information regarding the efficient delivery of PGE_1, which may be useful for the treatment of neurodegenerative disorders, into the brain across the BBB.

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