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Transporter-mediated Prostaglandin E_2 Elimination across the Rat Blood-brain Barrier and Its Attenuation by the Activation of N-methyl-D-aspartate Receptors

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Summary: Prostaglandin (PG) E_2 is involved in neuroinflammation and neurotoxicity, and the cerebral PGE_2 concentration is increased in neurodegenerative diseases. Because the intracerebral concentration of L-glutamate (L-Glu) is reported to be also elevated in neurodegenerative diseases, it has been proposed that L-Glu affects PGE_2 dynamics in the brain, and thus exacerbates neural excitotoxicity. The purpose of this study was to investigate the effect of intracerebral L-Glu on PGE_2 elimination across the blood-brain barrier (BBB) in rats by using the intracerebral microinjection technique. [^{3}H]PGE_2 injected into the cerebral cortex was eliminated from the brain in rats, and the apparent brain-to-blood [^{3}H]PGE_2 efflux clearance was found to be 60.1 μL/(min·g brain). Intracerebral pre-administration of 50 mM L-Glu significantly inhibited [^{3}H]PGE_2 elimination across the BBB and this L-Glu-induced inhibition was abolished by co-administration of an intracellular Ca^{2+} chelator. The intracellular Ca^{2+} concentration is reported to be increased *via* N-methyl-D-aspartate (NMDA)-type L-Glu receptors (NMDAR) and [^{3}H]PGE_2 elimination was attenuated by intracerebral pre-administration of a mixture of NMDA and D-serine. Moreover, the co-administration of antagonists of NMDAR with L-Glu abolished the attenuation of PGE_2 elimination induced by intracerebral L-Glu administration. These results suggest that L-Glu attenuates BBB-mediated PGE_2 elimination *via* NMDAR-mediated processes.

Keywords: blood-brain barrier; calcium; L-glutamate; intracellular calcium ion; N-methyl-D-aspartate receptor; organic anion transporter; prostaglandin E_2

Introduction

Prostaglandin E_2 (PGE_2), one of the eicosanoids, is involved in the modulation of synaptic signaling, excitability, and neuroinflammatory responses in the central nervous systems (CNS) under physiological and pathological conditions.[1,2] Under normal conditions, the concentration of PGE_2 in the brain interstitial fluid (ISF) and cerebrospinal fluid (CSF) is maintained, at most, at 1 nM.[3] On the other hand, the level of PGE_2 in the brain is reported to be increased in various CNS diseases such as neural inflammation, seizure, and Alzheimer's disease and, thus, the neural excitatory signals and neurotoxicity are induced by the excess PGE_2 in the brain.[2,4] Therefore, it is conceivable that PGE_2 in the brain is related to the pathogenesis and progression of neural excitation and toxicity in patients with CNS diseases.

The cerebral accumulation of PGE_2 in the presence of CNS disease is caused by an imbalance in the rates of production and elimination of PGE_2 from the brain. PGE_2 is biosynthesized from arachidonate *via* cyclooxygenase and prostaglandin E synthetase (PGES), which are up-regulated under neuroinflammatory conditions.[5] Regarding the elimination of PGE_2 from the brain, the removal of PGE_2 from the brain ISF and CSF plays an important role in this process because the activity of 15-hydroxyprostaglandin dehydrogenase, an enzyme associated with PG catabolism, is reported to be almost undetectable in the brain.[6] The exchange of compounds between the circulating blood and brain/CSF is

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regulated by the blood-brain barrier (BBB) and blood-CSF barrier (BCSFB),[7,8] and the BBB and BCSFB are formed of brain capillary endothelial cells (BCEC) and choroid plexus epithelial cells, respectively. These cells express various organic anion transporters which contribute to compound transport across the BBB and BCSFB, such as organic anion transporter 3 (Oat3/Slc22a8), organic anion transporting polypeptide 1a4 (Oatp1a4/Slco1a4), prostaglandin transporter (Pgt/Slco2a1), and multidrug resistance-associated protein 4 (Mrp4/Abcc4).[9–13] Of these transporters, it has been reported that Oat3, Pgt, and Mrp4 accept PGE_2 as a substrate.[14–16] Our previous studies have revealed that PGE_2 is eliminated from the brain across the BBB *via* Mrp4 in mice,[17,18] while [^{3}H]PGE_2 in the circulating blood after intravenous administration is reported to be transported to the brain across the BBB in rats.[19] Regarding the carrier-mediated influx transport of PG at the BBB, Taogoshi *et al.* have reported that Oatp1a4 and/or Pgt are involved in PGE_1 influx transport at the BBB.[20] Taking these lines of evidence into consideration, it would seem that both brain-to-blood and blood-to-brain transport determine the net flux of PGE_2 transport at the BBB. As well as the BBB, the BCSFB contributes to the elimination of PGE_2 from the CSF *via* Pgt and Oat3 at the apical membrane of choroid plexus epithelial cells in rats.[14] Therefore, it is important to elucidate the *in vivo* net flux of PGE_2 transport at the BBB and compare the elimination capacities between the BBB and BCSFB in order to understand the role of the BBB in the modulation of PGE_2 concentration in the brain.

The level of L-glutamate (L-Glu), one of the excitatory neurotransmitters, is also increased in patients with CNS diseases involving neuroexcitation and neurotoxicity, such as convulsions and Alzheimer's disease.[21,22] The concentration of L-Glu in the brain ISF and CSF is maintained at a few μM under normal conditions.[23,24] In various neuroexcitatory diseases, it has been reported that the concentration of L-Glu in the brain is raised to ~10 mM as the average concentration in neuroexcitatory diseases because L-Glu (~100 mM) in synaptic vesicles of the presynaptic terminal is actively released into the synaptic cleft.[25–27] The local excess release of L-Glu leads to an increase in the intracellular concentration of calcium ions ($[Ca^{2+}]_i$) *via* the activation of L-Glu receptors, such as N-methyl-D-aspartate (NMDA)-type L-Glu receptors (NMDA-R) and metabotropic L-Glu receptors (mGluR).[21] It has been reported that the activation of NMDA-R increases the extracellular concentration of PGE_2 because of the up-regulation of PGE_2-synthesizing enzymes.[28] Hence, there is a possibility that the concentration of PGE_2 in the brain is affected by the intracerebral accumulation of L-Glu and thus the activation of L-Glu receptors. However, changes in the inactivation of PGE_2 in the brain, namely the elimination of PGE_2 in the brain across the BBB, by an intracerebral excess of L-Glu has not yet been elucidated.

The purpose of this study was to investigate the brain-to-blood efflux transport of PGE_2 across the BBB by combining brain efflux index (BEI) and brain slice uptake studies in rats. Moreover, the alteration of PGE_2 efflux transport at the BBB and associated factors by intracerebral excess of L-Glu was evaluated by a BEI study after pre-administration of L-Glu and compounds which are agonists and antagonists for L-Glu receptors.

Materials and Methods

Animals: Adult male Wistar rats (150–250 g) were purchased from Japan SLC (Hamamatsu, Japan). They were maintained in a controlled environment and all experiments were approved by the Animal Care Committee, University of Toyama.

Reagents: [5,6,8,11,12,14,15-^{3}H(N)]Prostaglandin E_2 ([^{3}H]PGE_2, 119 Ci/mmol) was obtained from Perkin-Elmer Life and Analytical Sciences (Boston, MA). Mannitol, D-[1-^{14}C] ([^{14}C]D-mannitol, 55 mCi/mmol) was purchased from American Radiolabeled Chemicals (St. Louis, MO). Benzylpenicillin, D-Serine (D-Ser), and PGE_2 were purchased from Wako Pure Chemical Industries (Osaka, Japan). 1,2-bis(2-aminophenoxy)-ethane-N,N,N′,N′-tetraacetic acid tetrakis(acetoxymethyl ester) (BAPTA-AM), D-(−)-2-amino-5-phosphonopentanoic acid (D-AP5), (5R,10S)-(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]-cyclohepten-5,10-iminehydrogen maleate (MK-801), NMDA, and *trans*-1-aminocyclopentane-1,3-dicarboxylic acid (t-ACPD) were purchased from Sigma-Aldrich (St. Louis, MO). Oseltamivir acid, Ro64-0802, was obtained from Toronto Research Chemicals (North York, Canada). All other chemicals were commercial products of analytical grade. The K_m or IC_{50} values of compounds for organic anion transporters are summarized in **Supplementary Table S1**.[9,14–16,29–33]

Brain efflux index study: *In vivo* rat BBB-mediated efflux transport was evaluated using an intracerebral microinjection technique: the BEI method.[34] The detailed procedure is described in the **Supplemental materials**.

In pre-administration studies, a compound solution (50 μL) of L-Glu or agonists of L-Glu receptors, in the presence or absence of NMDA-type L-Glu receptor antagonists (MK-801 and D-AP5, 1 mM) or calcium chelator (BAPTA-AM, 100 μM), at the indicated concentration in extracellular fluid (ECF) buffer, with or without 0.2% DMSO, was injected into the Par2 region 5 min before microinjection of [^{3}H]PGE_2.[17,35] As a control, ECF buffer, with or without 0.2% DMSO, was injected. The concentration of intracerebrally-injected agonists and antagonists was more than 10-fold higher than the reported EC_{50} and IC_{50} values of these compounds for targeted L-Glu receptors.[36,37]

The BEI value was defined according Eq. (1), and the percentage of [^{3}H]PGE_2 remaining in the ipsilateral cerebrum (100 − BEI) was determined using Eq. (2):

$$\mathrm{BEI}\,(\%) = \frac{[^3\mathrm{H}]PGE_2 \text{ undergoing efflux at the BBB}}{[^3\mathrm{H}]PGE_2 \text{ injected into the brain}} \times 100 \quad (1)$$

$$100 - \mathrm{BEI}\,(\%) = \frac{[^3\mathrm{H}]PGE_2/[^{14}\mathrm{C}]\text{D-mannitol amount ratio in the brain}}{[^3\mathrm{H}]PGE_2/[^{14}\mathrm{C}]\text{D-mannitol concentration ratio in the injectate}} \times 100 \quad (2)$$

To evaluate the effect on [^{3}H]PGE_2 elimination across the BBB, the BEI value of [^{3}H]PGE_2 at 20 min was determined in the presence or absence of compounds.

The apparent elimination rate constant (k_{eff}) was determined by fitting a semilogarithmic plot of the percentage of [^{3}H]PGE_2 remaining in the ipsilateral cerebrum (100 − BEI) *versus* time using a non-linear least-squares regression analysis program. The apparent brain-to-blood efflux clearance of [^{3}H]PGE_2 across the BBB, $CL_{BBB,eff}$, can be obtained from Eq. (3):

$$CL_{BBB,eff} = k_{eff} \times V_{d,brain} \quad (3)$$

where $V_{d,brain}$ represents the distribution volume determined by an *in vitro* brain slice uptake study as described below.

***In vitro* rat brain slice uptake:** The [^{3}H]PGE_2 uptake study using brain slices was performed as described previously with