HIGHLIGHTED TOPIC | A Physiological Systems Approach to Human and Mammalian Thermoregulation

Effects of LPS stimulation on the expression of prostaglandin carriers in the cells of the blood-brain and blood-cerebrospinal fluid barriers

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Submitted 3 October 2005; accepted in final form 25 November 2005

Kis, Bela, Toyohi Isse, James A. Snipes, Lei Chen, Hiroshi Yamashita, Yoichi Ueta, and David W. Busija. Effects of LPS stimulation on the expression of prostaglandin carriers in the cells of the blood-brain and blood-cerebrospinal fluid barriers. J Appl Physiol 100: 1392–1399, 2006. First published December 1, 2005; doi:10.1152/japphysiol.01259.2005.—Prostaglandins produced in cerebral endothelial cells (CECs) are the final signal transduction mediators from the periphery to the brain during fever response. However, prostaglandins are organic anions at physiological pH, and they enter cells poorly using simple diffusion. Several transporters have been described that specifically transport prostaglandins across cell membranes. We examined the expression of the two principal prostaglandin carriers, prostaglandin transporter (PGT), and multidrug resistance-associated protein 4 (MRP4) in cells of the blood-brain barrier and in choroid epithelial cells in vitro as well as in vivo in rat brain in control conditions and after lipopolysaccharide (LPS) challenge. We detected PGT in primary cultures of rat CECs, astrocytes, pericytes, and choroid epithelial cells. LPS stimulation had no effect on the expression level of PGT in these cells; however, after LPS stimulation the polarized, dominantly luminal, expression pattern of PGT significantly changed. MRP4 is also expressed in CECs, and its level was not influenced by LPS treatment. In rat brain, PGT was highly expressed in the supraoptic and paraventricular nuclei of the hypothalamus, in the ependymal cell layer of the third ventricle, and in the choroid plexus. LPS treatment increased the expression of PGT in the supraoptic and paraventricular nuclei. Our results suggest that PGT and MRP4 likely play a role in transporting prostaglandins through the blood-brain and blood-cerebrospinal fluid barriers and may be involved in the maintenance of prostaglandin homeostasis in the brain and in the initiation of fever response.

The elevation of body temperature that occurs during fever is prostaglandin E2, which acts on EP3 type prostaglandin receptors in the hypothalamus (44, 47). There are several theories as to how peripheral cytokines can activate the central mechanism of fever; such theories include the existence of carrier-mediated transport of cytokines into the brain (2), the entrance of cytokines to the brain through areas devoid of blood-brain barrier (BBB) (45), and the role of neuronal afferents (especially the vagus nerve) to signal the thermoregulatory center (18). Although it is possible that all these mechanisms are involved in fever induction to some extent, it is more likely that cytokine-induced cerebral endothelial prostaglandin production is the dominant player in the signal transduction from the periphery to the thermoregulatory center (3, 47). Prostaglandins produced and released by the cerebral endothelium also play an important role in the regulation of cerebrovascular tone and permeability (7) and in the cerebrovascular response to brain injury (12, 17).

Prostaglandins are organic anions with pKₐ values of ~5. At physiological pH, they exist as charged molecules (40). Thus the plasma membrane is impermeable to prostaglandins, and they enter cells poorly using simple diffusion (4, 8). However, 3H-labeled prostaglandin E₂ has been detected in the brain immediately after intravenous injection (14), and systemic administration of prostaglandins quickly initiated fever, sleep induction, and a change in the regulation of food intake (14, 35). These experiments suggest that a carrier system should exist that transports prostaglandins from the blood to the brain through the BBB and likely the same system transports prostaglandins from cerebral endothelial cells (CECs) to the brain during the fever response.

It is also of interest that prostaglandins are not readily catabolized in the brain of mammals (34). 15-Hydroxyprostaglandin dehydrogenase, the rate-limiting enzyme of prostaglandin catabolism, is expressed in the brain during gestation but not after birth (26, 34, 37). To be inactivated, prostaglandins have to be excreted from the brain. The primary means of removing prostaglandins from the central nervous system (CNS) is transport across the choroid plexus and possibly the BBB (13). Therefore, to understand the physiological role of...
prostaglandins in the CNS, it is important to examine how peripheral prostaglandins pass through the BBB, how prostaglandins are released from CECs during acute-phase response, and how prostaglandins are cleared from the brain.

Several transporters have been described that specifically transport prostaglandins. The first-cloned and best-characterized prostaglandin carrier is prostaglandin transporter (PGT), which belongs to the superfamily of organic anion-transporting polypeptides (OATPs) (20, 40). PGT is expressed in various tissues and is especially abundant in the lung. The preference in PGT-mediated prostaglandin uptake rates in transfected HEK293 cells showed the following profile: prostaglandin E1 \(\approx\) prostaglandin F1 \(\approx\) prostaglandin E2 \(\approx\) prostaglandin F2\(\alpha\) \(>\) thromboxane B2 \(>\) 6-keto-prostaglandin F1\(\alpha\), which correlates well with the rank order of prostaglandin clearance by the lung (20). The PGT catalyzes prostaglandin-lactate exchange, and because the lactate gradient is outwardly directed in most cells, the PGT most likely mediates uptake rather than release of prostaglandins (8).

In contrast to the PGT, it was shown recently that multidrug resistance-associated protein (MRP) 4 (MRP4) actively transports prostaglandins out of the cells (38). The family of MRPs belongs to the superfamily of ATP-binding cassette transporters. MRPs are ATP-driven export pumps that mediate the transport of organic anions out of the cells (30). They are also involved in mediating resistance of tumor cells against chemotherapeutic drugs (6). An interesting pharmacological aspect of the MRP4-mediated prostaglandin transport is that it is inhibited by several nonsteroidal anti-inflammatory drugs, like indomethacin and ibuprofen, at therapeutically relevant concentrations (38). Therefore, in addition to inhibiting cyclooxygenase (COX)-1 and -2, these drugs might also act by inhibiting prostaglandin release (38).

The purpose of our study was to examine the expression of the two principal prostaglandin carriers PGT and MRP4 in cells of the BBB and choroid epithelial cells in basal conditions and after lipopolysaccharide (LPS) stimulation in vitro in primary cultures of cells as well as the in vivo expression of PGT in rat brain in control condition and after LPS challenge.

**MATERIALS AND METHODS**

**Animals.** Wistar rats were obtained from Harlan (Indianapolis, IN). All animal experiments were approved by the Animal Care and Use Committee of Wake Forest University School of Medicine.

**Rat CEC culture.** Primary rat CECs were isolated as previously described (24, 25) and were seeded onto collagen type IV- and fibronectin-coated dishes. The endothelial culture medium consisted of Dulbecco’s modified Eagle’s medium (DMEM; Gibco-BRL, Grand Island, NY) supplemented with 20% fetal bovine plasma-derived serum (Animal Technologies, Tyler, TX), 2 mM glutamine, 1 ng/ml basic fibroblast growth factor, 50 \(\mu\)g/ml endothelial cell growth supplement (BD Biosciences, Bedford, MA), 100 \(\mu\)g/ml heparin, 5 \(\mu\)g/ml vitamin C, and antibiotics. Confluent cultures (4th to 5th day in vitro) consisted of more than 95% of rat CECs verified by positive immunohistochemistry for von Willebrand factor and negative immunocytochemistry for glial fibrillary acidic protein and \(\alpha\)-smooth muscle actin.

**Rat cerebral astrocyte culture.** Rat cerebral astrocyte cultures were prepared as previously described (21). Cortical pieces from postnatal day 1 neonatal Wistar rats were mechanically dissociated in astrocyte culture medium (DMEM supplemented with 10% fetal bovine serum and antibiotics). Dissociated cells were seeded into cell culture flasks. To obtain type I astroglia, confluent cultures were shaken at 37°C overnight. The purity of astrocytes was checked by immunostaining for glial fibrillary acidic protein, and the cells were used at passage 2.

**Rat cerebral microvascular pericyte culture.** Pure cultures of rat cerebral pericytes were obtained by prolonged culture of primary rat CEC preparations, as previously described (21). Pericyte survival and proliferation were favored by selective culture conditions, using uncoated dishes, and DMEM supplemented with 10% fetal bovine serum and antibiotics. Pericytes were characterized by their large size and branched morphology, positive immunostaining for \(\alpha\)-smooth muscle actin, and absence of von Willebrand factor and glial fibrillary acidic protein staining.

**Rat choroidal epithelial cell culture.** Rat choroidal epithelial cell cultures were prepared from 2-wk-old (20–30 g) Wistar rats as previously described (23). A pool of choroidal tissues was incubated with collagenase type 2 (350 \(\mu\)U/ml, Worthington, Lakewood, NJ) and DNase (10 \(\mu\)g/ml, Sigma, St. Louis, MO) for 1 h at 37°C. After digestion, cells were dissociated by gentle trituration and were plated in culture medium (DMEM supplemented with 10% fetal bovine serum, 2 mM l-glutamine, 10 ng/ml epidermal growth factor (GIBCO-BRL), 50 \(\mu\)g/ml endothelial cell growth supplement (Sigma), and antibiotics) onto uncoated culture dishes and cultured for 40 min to allow attachment of contaminating cells, mostly fibroblasts. The unattached epithelial cells were collected and cultured in Primaria dishes (BD Biosciences). The cultures reach confluence in 6–7 days.

**LPS treatment of cultured cells.** When the cultures reached confluence, the cells were incubated with 100 ng/ml LPS (Sigma, Escherichia coli 026:B6) in the regular culture media at 37°C for different periods of time. After the incubations, cells were washed twice with phosphate-buffered saline (PBS). Total RNA was isolated by SV Total RNA Isolation System (Promega, Madison, WI). For Western blot analysis, proteins were isolated from samples using NP-40 lysis buffer supplemented with proteinase inhibitors (1 \(\mu\)g/ml aprotinin, 50 \(\mu\)g/ml phenylmethylsulfonyl fluoride, and 1 \(\mu\)g/ml leupeptin) as described previously (22). For each series of experiments, cells were from at least three independent cultures.

**RT-PCR.** RT-PCR experiments were carried out in an Eppendorf Mastercycler thermocycler (Brinkmann Instruments, Westbury, NY). From each sample, 300 pg of total RNA were reverse transcribed and amplified using AccessQuick single-tube coupled RT-PCR system (Promega) with gene specific primers targeting rat PGT or rat MRP4 cDNAs. \(\beta\)-Actin primers (Promega) were also included into the RT-PCR reaction to normalize our RT-PCR results with an expected product length of 285 base pairs. The sequence for the PGT sense primer was 5’-GAGCAATGTCCTCACCACAATTCG-3’ and for the antisense it was 5’-GGCTCGCGCAAAGTGTCCAC-3’. The expected PCR product length of 443 base pairs (GenBank NM_022667). The sequences for the MRP4 sense primer was 5’-GGCACCTGAAGTCAGCAAAACG-3’ and for the antisense it was 5’-GTCGCTGTCAAT-AGTGG-3’ with the expected product length of 229 base pairs (GenBank NM_133411). In our preliminary experiments, we used different cycle numbers and different amounts of total RNA to titrate the optimal condition for RT-PCR amplification to be in the linear range. In control experiments, when reverse transcription was omitted, no amplification was observed. All the PCR products were identified by the size and the sequence. Automated DNA sequencing of the PCR products was performed on an ABI Prism 377 DNA sequencer (Applied Biosystems, Foster City, CA). For quantitative analysis, the bands were scanned in a Foto/Analyst Investigator PC System using PC Image 5.0 software (Fotodyne Hartland, WI), and the densities of the bands were quantitated by using Image J 1.3.1 software (National Institutes of Health, Bethesda, MD).

**Western blotting.** Western blot analysis was performed as described previously (22). An equal amount of protein for each sample was separated by 10% SDS-PAGE and transferred onto a nitrocellulose membrane (Bio-Rad Laboratories, Hercules, CA). Membranes were incubated in a blocking buffer (Tris-buffered saline, 0.1% Tween 20, and 5% skimmed milk powder) for 1 h at room temperature, and then
the blots were incubated with rabbit polyclonal anti-rat PGT (1:1,000, Alpha Diagnostic International, San Antonio, TX) primary antibodies overnight at 4°C. The membranes were then washed three times in Tris-buffered saline with 0.1% Tween 20 and then incubated for 1 h in the blocking buffer with anti-rabbit IgG (1:50,000; Jackson Immuno Research, West Grove, PA) conjugated to horseradish peroxidase. The final reaction products were visualized using enhanced chemiluminescence (SuperSignal West Pico, Pierce, Rockford, IL) and recorded on X-ray film. For quantitative analysis, the bands were scanned in a Foto/Analyst Investigator PC System using PC Image 5.0 software (Fotodyne Hartland, WI), and the densities of the bands were quantitated by using Image J 1.3.1 software (National Institutes of Health, Bethesda, MD).

PGT immunocytochemistry on cultured CECs. To improve BBB properties of primary rat CECs in vitro, the CECs were cocultured with astrocytes. CECs were seeded onto collagen type IV- and fibronectin-coated Transwell inserts (diameter 12 mm, pore size 3 μm; BD Biosciences), which were placed into 12-well plates containing confluent layers of astrocytes. The induction of BBB phenotype of CEC monolayer was proved by high transendothelial electrical resistance (TEER) measured with an EVOM resistance meter (World Precision Instruments, Sarasota, FL). When TEER reached at least 200 Ω·cm² (when CECs were cultured without astrocytes the TEER was <150 Ω·cm²), cultures were washed with PBS, fixed in 3.7% formaldehyde, and permeabilized with 0.1% saponin in PBS. Blocking of nonspecific binding sites was achieved by incubating the cells for 30 min at room temperature in PBS containing 1% normal goat serum (Vector Labs, Burlingame, CA) and 0.1% saponin. After blocking, the rat CECs were incubated with polyclonal rabbit anti-rat PGT primary antibody (1:200; Alpha Diagnostic International) at room temperature for 60 min, and then they were incubated with Alexa fluor 546 goat anti-rabbit secondary antibody (1:1,000; Molecular Probes, Eugene, OR) at room temperature for 30 min. Confocal images of cellular fluorescence were acquired on a Zeiss LSM 510 laser scanning microscope (excitation wavelength = 543 nm, emission wavelength > 560 nm; Zeiss, Jena, Germany).

PGT immunohistochemistry on brain slices. On the day of the experiments between 9:00 AM and 10:00 AM, male Wistar rats (body weight 200 ± 20 g) were injected intraperitoneally with either LPS (250 μg/kg, E. coli 026:B6, Sigma) or sterile saline (n = 6 in both groups). The core body temperature was monitored with a rectal laser scanning microscope (excitation wavelength 543 nm, emission wavelength 605-675 nm; Laser Microscopy, St. Louis, MO) and recorded on X-ray film. For quantitative analysis, the bands were scanned in a Foto/Analyst Investigator PC System using PC Image 5.0 software (Fotodyne Hartland, WI), and the densities of the bands were quantitated by using Image J 1.3.1 software (National Institutes of Health, Bethesda, MD).

RESULTS

PGT protein was expressed in all cell types we examined. The PGT expression level was lower in CECs than in astrocytes, pericytes, and choroid epithelial cells (Fig. 1A). LPS treatment stimulated COX-2 expression in all cell types, but it had no effect on the expression of PGT (Figs. 1, B–D, and 2A). MR4 was also detected in CECs, and its expression level was not influenced by LPS treatment (Fig. 2, B and C).

Our PGT immunocytochemistry showed almost homogenous staining in the cytoplasm in horizontal sections of CECs (Fig. 3A). However, confocal microscopy z-axis reconstruction of vertical sections of the cells revealed intense staining at or close to the luminal plasma membrane, whereas there was no significant staining in the basolateral half of CECs (Fig. 3B). This polarized staining pattern disappeared 3 and 6 h after LPS stimulation; staining was observed both in the apical and basolateral site of CECs (Fig. 3B).

PGT immunohistochemistry on brain slices demonstrated strong PGT expression in the supraoptic and paraventricular nuclei of the hypothalamus, in the ependymal cell layer of the third ventricle, and in the choroid plexus of rat brain (Fig. 4). PGT expression in the supraoptic and paraventricular nuclei of LPS-treated rats was stronger compared with control rats (Fig. 4). We could not find increased PGT expression in the ependymal layer (Fig. 4) and in the choroid plexus in LPS-treated rats (data not shown).

DISCUSSION

The main findings of our experiments are the following. 1) PGT was expressed in primary cultures of rat CECs, astrocytes, pericytes and choroid epithelial cells. 2) LPS treatment had no effect on the expression level of PGT in these cells; however, our confocal microscopy z-axis reconstruction revealed that the polarized, dominantly luminal expression pattern of PGT was altered after LPS stimulation. 3) MR4 was also expressed in CECs and its level was not influenced by LPS treatment. 4) PGT was highly expressed in the supraoptic and paraventricular nuclei of the hypothalamus, in the ependymal cell layer of the third ventricle and in the choroid plexus of rat brain. 5) LPS treatment increased the expression of PGT in the supraoptic and paraventricular nuclei.

Two principal barriers, the BBB and the blood-cerebrospinal fluid (CSF) barrier (BCSFB) separate the brain from the circulating blood and are responsible for maintaining the stability of the brain parenchymal microenvironment by strictly controlling the movement of molecules, and cells (11, 27, 28, 39). The BBB is formed by the brain capillaries, and it is a functional unit of cells united by tight junctions. In addition, glial endfeet cover 99% of the abluminal surface of microvascular CECs. Pericytes, on the other hand, are embedded in the capillary basement membrane (39). To fulfill the barrier function, CECs have special structural and biochemical characteristics. Tight intercellular junctions and low numbers of pinoctytic vesicles limit paracellular and transendothelial trafficking (39). Additionally, the presence of enzymatic barrier and efflux transporters, including P-glycoprotein, MRPs, and sev-
eral other transporters, restricts the entrance of toxic substances into the CNS (27, 28, 39). The BBB phenotype of CECs is induced primarily by contact with the astroglial environment (39). The choroid plexus is a highly vascularized structure present in the brain ventricles, and it is the primary source of active CSF production (10). It consists of fenestrated capillaries

Fig. 1. Prostaglandin transporter (PGT) and cyclooxygenase-2 (COX-2) expression in rat cerebral endothelial cells, astrocytes, pericytes, and choroid epithelial cells. A: representative Western blot for PGT expression in primary cultures of rat cerebral endothelial cells (CECs), astrocytes, cerebral microvascular pericytes and choroid epithelial cells. B: representative Western blot for PGT and COX-2 expressions in primary cultures of rat astrocytes, cerebral microvascular pericytes, and choroid epithelial cells 0, 3, and 6 h after lipopolysaccharide (LPS; 100 ng/ml) stimulation. C: representative Western blot for PGT and COX-2 expression in primary cultures of rat CECs at different time points after LPS (100 ng/ml) stimulation. D: densitometric analysis of the Western blot results of PGT and COX-2 expression in primary cultures of rat CECs at different time points after LPS (100 ng/ml) stimulation. Values are means ± SE; n = 8 dishes; cells are from 4 independent cultures. *P < 0.05.

Fig. 2. Effect of LPS stimulation on PGT and multidrug resistance-associated protein 4 (MRP4) mRNA expressions in rat CECs. Confluent primary cultures of rat CECs were treated with LPS (100 ng/ml) for different periods of time. After the incubation, total RNA were isolated from the cells and RT-PCR was performed to detect mRNAs of PGT and MRP4. A: representative gel electrophoresis of RT-PCR products for PGT (443 bp) and β-actin (285 bp) in cultured rat CECs of 3 similar experiments. M, molecular weight marker. B: representative gel electrophoresis of RT-PCR products for MRP4 (229 bp) in cultured rat CECs of 3 similar experiments. M, molecular weight marker. C: densitometric analysis of the RT-PCR results of PGT and MRP4 expression in primary cultures of rat CECs at different time points after LPS (100 ng/ml) stimulation. The expression values of PGT and MRP4 were normalized by the expression level of β-actin. Values are mean ± SE; n = 4 and 3 dishes, respectively, cells are from 4 independent cultures.
surrounded by epithelial cells. Because of the fenestrated capillaries, blood components have free access to the basolateral membranes of the choroid epithelial cells; however, the presence of tight junctions between the epithelial cells prevents free exchange of compounds providing a barrier between the CSF and the blood circulation. In addition, similar to CECs, the choroid epithelium has metabolic enzymes and efflux transport systems to facilitate the elimination of xenobiotics and endogenous wastes from the CSF to the circulating blood (11, 27, 28).

The transport of prostaglandins through the BBB and BCSFB has two important aspects. First, prostaglandins are important signal molecules from the periphery to the brain during the acute phase response. Second, because prostaglandins are ineffectively metabolized in the CNS, accumulation of prostaglandins in the extracellular fluids of the brain would be expected to have adverse effects; therefore, their removal across the BBB and BCSFB is essential (26, 34, 37).

We found PGT expression in the cells of the BBB and also in choroid epithelial cells. This transporter appears to function as a prostaglandin/lactate exchanger, and its driving force is the concentration gradient of lactate and prostaglandins between the extra- and intracellular compartments (8). Because CECs engage in substantial aerobic glycolysis, they would generate a sufficient outwardly directed lactate gradient to drive the prostaglandin/lactate exchanger toward active prostaglandin uptake into the cells similar to that observed in renal collecting ducts and Madin-Darby canine kidney (MDCK) cells (15). This uptake may result in intracellular metabolism of prostaglandins or may be part of a transport process across the CECs, as in MDCK cells (15). We found that PGT was localized at or close to the luminal membrane. The same subapical or apical membrane expression of PGT was observed in rat and mouse kidney tubules, in prostate, in seminal vesicle, and in MDCK cells (3, 15). Expression of apical membrane PGT in MDCK cells induced a 100-fold increase in the apical-to-basolateral prostaglandin E₂ flux (15). In agreement with these findings, Moore et al. (33) demonstrated that CECs grown on micropore filters release prostaglandins from both apical and basal surfaces and the ratio of basal to apical release is as high as 4:1. In addition, bromocresol green, an inhibitor of PGT, strongly decreased ³H-labeled prostaglandin E₁ transport from the blood across the BBB (42). It is likely that PGT is one of the carrier mechanisms that mediate prostaglandin uptake from the blood to the CECs, but the mechanism of prostaglandin release at the abluminal plasma membrane is unknown. MRP4 was described as a prostaglandin efflux transporter (38), and we did find MRP4 expression in CECs. In brain microvesSEL endothelial cells MRP4 and MRP5 transcripts were reported to be higher than MRP1, an isoform that is highly expressed in the brain (48).

It has been known for 30 yr that the choroid plexus actively transports prostaglandins and is a major contributor to the removal of prostaglandins from the brain (5, 13). We detected

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**Fig. 3.** Intracellular localization of PGT protein in cultured rat CECs. A: immunostaining of primary cultures of rat CECs with anti-rat PGT antibody. Nuclear counterstaining was done with To-Pro-3 (Molecular Probes). Scale bar, 10 μm. B: confocal microscopy z-axis reconstruction of PGT immunostaining in vertical sections of cultured rat CECs in control conditions and 3 and 6 h after LPS (100 ng/ml) treatment. Scale bar represents 10 μm.
PGT expression both in vivo and in vitro in choroid epithelial cells. Our immunohistochemical staining on brain slices found strong expression of PGT in choroid plexus, but it did not demonstrate preferential apical or basolateral localization of PGT in choroid epithelial cells. MRP4 expression was demonstrated in the basolateral membrane of the choroid plexus epithelium (29). Basolateral localization places MRP4 in proximity to the fenestrated capillaries of the choroid plexus, where it can efflux prostaglandins and toxic metabolites from the brain and prevent uptake of anionic substances from the blood. (29).

We used LPS stimulation to induce fever in vivo and to mimic the acute-phase response in vitro. Because prostaglandin production in CECs is a crucial step in fever development (31, 47), we expected significant changes in the expression of PGT and MRP4 at least in CECs and choroid epithelial cells. However, LPS had no effect on the expression level of PGT and MRP4 in our experiments. Our findings are consistent with the results on cultured human umbilical vein endothelial cells where LPS, interleukin-1β and tumor necrosis factor-α did not significantly induce PGT, but shear stress did upregulate its expression (32, 43). The only significant change that we observed after LPS stimulation was the change in PGT localization in CECs. The polarized, dominantly luminal expression pattern of PGT in CECs disappeared, and PGT immunoreactivity was apparent throughout the cytoplasm. If we assume that the localization of PGT at or close to the luminal membrane is important in the blood-to-brain transport of prostaglandins, the disappearance of this localization pattern might suggest reduced prostaglandin transport to the brain, which might have a protective fever reducing effect.

Our in vivo experiments showed that PGT is highly expressed in the supraoptic and paraventricular nuclei of the hypothalamus, in the ependymal cell layer of the third ventricle, and in the choroid plexus of rat brain. This finding is similar to the results of Adachi et al. (1). After LPS-induced fever, we found an increased expression of PGT in the supraoptic and paraventricular nuclei. Because prostaglandins involved in mediating the effects of LPS on hypothalamus, which play important roles in the neuroimmune stress response, we hypothesize that the LPS-induced PGT expression may be involved in this process (19, 41).
In addition to PGT and MRP4 there are several other carriers detected in the brain, including OATP2, OATP3, OATP-D, and multispecific OAT1, all of which can transport prostaglandins (1, 16, 36, 46). Therefore, these transporters can also play a significant role in the regulation of prostaglandin homeostasis in the CNS. Although our experiments provide important new information, it is also clear that additional studies are needed to explore the significance and contribution of each transporter to elucidate their role in the physiology of thermoregulation and fever.

ACKNOWLEDGMENTS

The authors gratefully thank Nancy Busija for critical reading of the manuscript.

GRANTS

The work in the authors’ laboratories was supported by Grant-in-Aid for Japan Society for the Promotion of Science Fellows (Nos. 98260, S-02167, and S-05146 for B. Kis), for Scientific Research (B) (No. 16390061 for Y. Ueta), and for Exploratory Research (No. 16650962 for Y. Ueta) from the Ministry of Education, Culture, Sports, Science and Technology, Japan; by grants from the National Institutes of Health (HL-30260, HL-66074, HL-65380, HL-77731, and DK-62372 for D. W. Busija); and by an American Heart Association Bagher Foundation Award (0270114N for D. W. Busija).

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