Behavioral/Systems/Cognitive

A Novel Antipyretic Action of 15-Deoxy-$\Delta^{12,14}$-Prostaglandin J_2 in the Rat Brain

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Fever is an important part of the host defense response, yet fever can be detrimental if it is uncontrolled. We provide the first evidence that 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2), an endogenous ligand for peroxisome proliferator-activated receptor γ (PPARγ), can attenuate the febrile response to lipopolysaccharide (LPS) in rats via an action on the brain. Furthermore, we show that PPARγ is expressed in the hypothalamus, an important locus in the brain for fever generation. In addition, 15d-PGJ_2 and its synthesizing enzyme (PGD_2 synthase) were present in rat cerebrospinal fluid, and their levels were enhanced in response to systemic injection of LPS. The antipyretic effect of 15d-PGJ_2 was associated with reduction in LPS-stimulated cyclooxygenase-2 expression in the hypothalamus but not in p44/p42 mitogen-activated protein kinase phosphorylation or in the expression of the PPARγ.

Thus it is likely that there is a parallel induction of an endogenous prostanoid pathway in the brain capable of limiting deleterious actions of the proinflammatory prostaglandin E_2-dependent pathway.

Key words: **stress; central autonomic pathways; hypothalamus; fever; lipopolysaccharide; PPARγ**

Introduction

Fever is a regulated increase in core temperature (Kluger, 1991) that can be initiated by a multitude of proinflammatory cytokines such as interleukin-1β, interleukin-6, and TNFα (Luheshi and Rothwell, 1996). These proinflammatory cytokines activate *de novo* synthesis of cyclooxygenase-2 (COX-2) in the endothelial and perivascular cells in the fever-controlling region of the hypothalamus (Schiltz and Sawchenko, 2002). Induced COX-2 (Lacroix and Rivest, 1998; Matsumura et al., 1998) generates prostaglandin E_2 (PGE_2) (Yamagata et al., 2001), which activates neurons in the preoptic region of the hypothalamus to cause heat conservation and fever generation (Lipton et al., 1973; Scammell et al., 1996). Although the mechanisms of fever genesis have been studied extensively, our understanding of the molecular determinants of fever resolution is still incomplete. Clinical and experimental observations have shown that the increase in body temperature seldom exceeds an upper limit even during a prolonged fever. Furthermore, such a fever can normally resolve even in the absence of exogenous administration of antipyretic drugs (Kozak et al., 2000; Tatro, 2000; Aronoff and Neilson, 2001). This consistently observed phenomenon suggests the existence of an endogenous antipyretic system that prevents the body from reaching an excessively high temperature that would be harmful (Greisman and Mackowiak, 2002). We and others have explored several endogenous antipyretic systems that include vasopressin (Veale et al., 1981; Pittman et al., 1998), α-melanocyte-stimulating hormone (Murphy et al., 1983), glucocorticoids (Morrow et al., 1993), the P-450-dependent epoxygenase pathway (Kozak et al., 2000), and anti-inflammatory cytokines such as IL-10 (Leon, 2002; Cartmell et al., 2003) and IL-1 receptor antagonist (Cartmell et al., 1998). Although the mechanism of endogenous antipyretics is mostly unknown, it is well established that a target of exogenous antipyretic therapy is COX-2 (Tatro, 2000).

In addition to PGE_2 genesis, cyclooxygenation of arachidonic acid during inflammation also leads to the formation of prostaglandin D_2 (PGD_2) and its metabolite 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2) (Hirata et al., 1988; Shibata et al., 2002). PGD_2 formation, and consequently 15d-PGJ_2 production, results from an isomerization of PGH_2 by lipocalin-type PGD_2 synthase (PGD_2-S) in rat brain (Urade et al., 1985; Beuckmann et al., 2000). *In vitro* studies in various non-neuronal tissues showed that after binding with high affinity to its nuclear receptor peroxisome proliferator-activated receptor γ (PPARγ) (Forman et al., 1995; Kliewer et al., 1995), 15d-PGJ_2 alters the nuclear factor κB (NF-κB)-dependent pathways (Gilroy et al., 1999; Rossi et al., 2000; Straus et al., 2000), reduces the expression of lipopolysaccharide (LPS)-activated proinflammatory cytokines such as interleukin-1β and TNFα (Simonin et al., 2002), and attenuates the expression and activity of COX-2 induced by LPS (Inoue et al., 2000; Maggi et al., 2000; Simonin et al., 2002).

Because COX-2 plays a major role in LPS fever (Lacroix and Rivest, 1998; Matsumura et al., 1998; Li et al., 1999), we hypothesized that 15d-PGJ_2 may act in the brain to attenuate LPS-induced fever and reduce COX-2 expression in a fever-controlling region in rat brain. The questions addressed in this *in vivo* study are as follows. (1) Is the 15d-PGJ_2 receptor (i.e., PPARγ) expressed in the hypothalamus? (2) Does 15d-PGJ_2 af-

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fect LPS fever? (3) Can 15d-PGJ_2 affect COX-2 expression in the hypothalamus of LPS-treated rats? (4) Is 15d-PGJ_2 produced in the brain during fever?

Materials and Methods

Male Sprague Dawley rats bred in the University of Calgary vivarium were kept in temperature-controlled quarters under a normal 12 hr light/dark cycle (lights on at 7 A.M.). They were housed individually, and pellet chow and water were accessible *ad libitum*. All experimental protocols were approved by the University of Calgary Animal Care Committee and performed in accordance with the Canadian Council of Animal Care guidelines.

General animal preparations and surgery. Male rats weighing 250–270 gm were anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and implanted with a stainless steel guide tube (23 ga thin wall) that was positioned above the lateral ventricle (coordinates: 0.8 mm caudal to bregma; 1.6 mm lateral to midline; 2.6 mm ventral to the cortical surface) according to the stereotaxic atlas of Paxinos and Watson (1986). A precalibrated, battery-driven temperature transmitter (Mini-Mitter, Sunriver, OR) was inserted into the abdominal cavity of each rat. After at least 1 week of recovery, rats were transferred to an environmentally isolated and temperature-controlled (22°C) testing room and allowed to acclimatize to the environment for 1 d. Rats were anesthetized with urethane (1.5 gm/kg, i.p.) (Malkinson et al., 1988) and wrapped loosely in a blanket to facilitate heat retention. Under these conditions, we can elicit reproducible and dose-dependent febrile responses to both intraperitoneal and intracerebroventricular pyrogens (Malkinson et al., 1988; Komaromi et al., 1994; Chen et al., 1997). Rats were then given an infusion of either saline or 15d-PGJ_2 at a rate of 25 μl/hr for 2 hr. In preliminary experiments, a dose of 10 ng/μl of 15d-PGJ_2 did not affect LPS fever in a consistent manner. In the experiments reported here, a dose of 50 ng/μl, which is within the range of the *in vitro* effective doses of 15d-PGJ_2 (Jiang et al., 1998; Rossi et al., 2000), was used. All rats received an intraperitoneal injection of LPS at 30 min after the beginning of the intracerebroventricular infusion of either 15d-PGJ_2 or saline solutions to allow time for 15d-PGJ_2 to diffuse to its active site in the brain and initiate its signaling cascade. Core temperatures were monitored for 6 hr after LPS injection using a telemetry system (DataQuest II; Data Sciences Inc., St. Paul, MN) that automatically takes a reading every 5 min.

Protein extraction. Three hours after intraperitoneal injection of LPS in the presence of intracerebroventricular infusion of 15d-PGJ_2 as described above, another group of urethane-anesthetized rats were transcardially perfused with cold PBS containing (in mM): 137 NaCl, 2.7 KCl, 10 Na_2HPO_4, 1.8 KH_2PO_4, to remove the blood. A basal diencephalon region that consists of the preoptic area and the hypothalamus was quickly dissected and put in lysis buffer [MOPS: 20 mM Mg $(C_2H_3O_2)_2$: 4.5 mM KCl: 150 mM, 1% Triton] supplemented with protease inhibitor tablets (catalog #D68298; Roche Diagnostics, Mannheim, Germany) and a mixture of protease and phosphatase inhibitors (1 μM okadaic acid, catalog #495604, and 1 μM microcystin-LR, catalog #475815; both purchased from Calbiochem, La Jolla, CA; 1 mM sodium vanadate, catalog #S-6508, Sigma; 500 μM phenyl-methyl-sulfonyl-fluoride, catalog #837091, Boehringer Mannheim, Indianapolis, IN). After mechanical dissociation of the brain tissue, protein levels were assayed using a bicinchoninic acid protein assay (Pierce, Rockford, IL; reagent A, catalog #23223, and reagent B, catalog #23224). The proteins were then put in a sample buffer composed of 125 mM Tris-HCl (Sigma; T-1503), 3% SDS (Bio-Rad Laboratories, Richmond, CA; catalog #161–0301), 20% Glycerol (Sigma; G-5516), bromophenol blue (Sigma; B-5525), 20% β-mercapto-ethanol (BDH Ltd., Poole, UK; catalog #44143), boiled for 5 min, and stored at –20°C until Western blot analysis.

Western blot analysis. Protein extracts from each individual rat (30 μg per well) were separated on 10% SDS-PAGE (15% SDS-PAGE for PGD_2-S detection) using a constant current of 30 mA per gel of 1.2 mm of thickness. Molecular weights markers (Bio-Rad; catalog #161–0372) were used in each individual gel. At the end of separation, the proteins were transferred onto nitrocellulose membrane for 2 hr under a constant current (1.2 mA/cm^2 of gel surface) using a transfer buffer containing 20% methanol, 50 mM Trizma base (Sigma), 40 mM glycine (Sigma), and 0.04% SDS (Bio-Rad). Membranes were then incubated overnight at 4°C with 10% fat-free milk in Tris-buffered saline containing Tween 20 (TBS-T) composed of 20 mM Trizma-base (Sigma), 0.15 M sodium chloride (Fisher Scientific), and 0.1% polyoxyethylene-sorbitan monolaurate (Sigma). Immunostaining for the target proteins was performed as described previously (Mouihate et al., 2002). Well characterized rabbit antibodies for COX-2 (Cayman Chemical Company; catalog #160126) (Mouihate et al., 2002; Mouihate and Pittman, 2003), PPARγ (detects both γ1 and γ2 forms; Santa Cruz Biotechnology; catalog #sc-7196) (Bordji et al., 2000; Wang et al., 2002), phospho-p44/p42 mitogen-activated protein kinase (MAPK) antibody (Cell Signaling Technology; catalog #9106S) (Bolshakov et al., 2000), and goat antibody for PGD_2-S (Santa Cruz Biotechnology; catalog #sc-14825) (Urade et al., 1985) were used at 1:2000, 1:1000, 1:1000, and 1:1000, respectively. After 2 hr of incubation with primary antibodies, the membrane was washed with TBS-T and incubated with either goat anti-rabbit IgG conjugated with horseradish peroxidase (COX-2, PPARγ, phospho-p44/p42 MAPK; 1:4000) (Santa Cruz Biotechnology; sc-2004) or donkey anti-goat IgG conjugated with horseradish peroxidase (for PGD_2-S; 1:2000) (Santa Cruz Biotechnology; catalog #sc-2020) for 1 hr at room temperature. A chemiluminescence substrate was applied to the membrane (ECL Kit, Amersham Biosciences, Arlington, IL), and protein bands were visualized using Kodak X-Omat film (Eastman Kodak Company, Rochester, NY). In these experiments, actin served as housekeeping protein. Therefore, after detection of either COX-2 or PPARγ bands, membranes were stripped with mercaptoethanol (BDH Ltd.) and reblotted with rabbit anti-actin antibody (Sigma; catalog #A2066) at 1:10,000 dilution and processed as described above.

Cerebrospinal fluid collection and enzyme immunoassay. A group of 30 male rats were injected with either saline or LPS (200 μg/kg or 400 μg/kg, i.p.). At different time points after injection, rats were anesthetized with urethane, and the neck muscles were reflected to gain access and withdraw a CSF sample (~140 μl) from the cisterna magna. CSF samples were quickly frozen and stored at −70°C until use. 15d-PGJ_2 levels were assayed using an enzyme immunoassay kit (Assay Designs, Ann Arbor, MI) according to the manufacturer's instructions. This kit has a sensitivity of 36.8 pg/ml and intra-assay and inter-assay precisions of 6 and 13%, respectively. A fraction of CSF was also used for Western blot. A volume of 40 μl of CSF of each rat was mixed with 20 μl of sample buffer (the composition is described above). These CSF samples were then boiled for 5 min and stored at −20°C until immunoblotting. An equal volume of 10 μl of each CSF sample was separated on 15% SDS-PAGE and processed for PGD_2-S detection as described above.

Data analysis. Temperature readings at 10 min intervals (average of two consecutive 5 min readings) were calculated as net deviation from the mean baseline temperature. Data are presented as means ± SE. Fever data were subjected to two-way ANOVA followed by Student–Newman–Keuls *post hoc* comparisons to identify significant difference at various time points.

For immunoblots, the area under the intensity profile curve of a given band was quantified using Quantity One Quantitation software (Bio-Rad). The ratio of optical value densities of (protein of interest)/actin were calculated. These ratios values were then normalized to their corresponding values in control rats (saline-injected rats). Immunoblot data were compared using either a paired *t* test (two experimental groups) or ANOVA (three or more experimental groups). Enzyme immunoassay data were compared using Kruskal–Wallis analysis of variance on ranks followed by Dunn's multiple comparison test. Significance was accepted at the 0.05 level.

Results

15d-PGJ_2 attenuates LPS-induced fever

To test whether 15d-PGJ_2 affects body temperature in the absence of any other stimulus, rats received intraperitoneal saline in conjunction with an intracerebroventricular infusion of either saline or 15d-PGJ_2 (50 ng/μl at a rate of 25 μl/hr) for 2 hr. Basal body temperature was similar in both saline- and 15d-PGJ_2-treated rats [36.23 ± 0.41 (n = 5) and 36.5 ± 0.25 (n = 6),

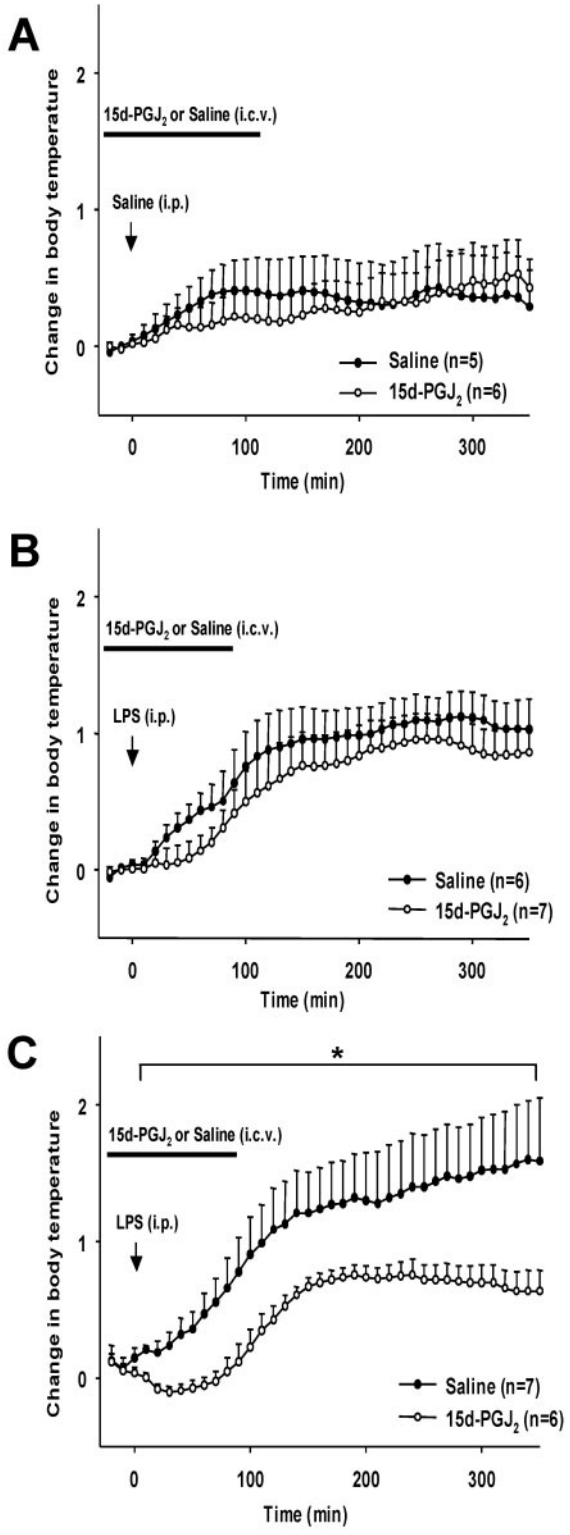

Figure 1. 15d-PGJ$_2$ effects on LPS-induced fever. Rats were infused intracerebroventricularly with either saline or 15d-PGJ$_2$ (50 ng/μl) for 2 hr at a rate of 25 μl/hr and concomitantly received one of the following intraperitoneal injections: saline (*A*), LPS at a dose of 200 μg/kg (*B*), and LPS at a dose of 400 μg/kg (*C*). Data are presented as means ± SEM; "n" represents the number of rats per group. *$p < 0.05$.

respectively]. As shown in Figure 1*A*, in the absence of any pyrogen (intraperitoneal saline), the body temperature of 15d-PGJ$_2$-treated rats (intracerebroventricular) was not significantly different from the control group (intracerebroventricular saline).

A second series of rats were infused as above with either saline ($n = 6$) or 15d-PGJ$_2$ (50 ng/μl; $n = 7$) for 2 hr (at a rate of 25 μl/hr). Both rat groups had similar basal body temperature (saline group: 36.03 ± 0.31; 15d-PGJ$_2$ group: 36.34 ± 0.33). At the 30th min of the intracerebroventricular infusion, which corresponds to time 0, all rats received an injection of LPS (200 μg/kg, i.p.). LPS induced a progressive and sustained rise in core temperature of ~1°C in both the control and 15d-PGJ$_2$-treated groups (Fig. 1*B*). 15d-PGJ$_2$ had no significant effect on the febrile response, although the mean temperature of the 15d-PGJ$_2$-treated rats was consistently lower than the saline control.

In a different rat group, either saline or 15d-PGJ$_2$ (50 ng/μl) was infused as in the experimental design described above. Basal body temperature was similar in both control and 15d-PGJ$_2$-treated rats [36.22 ± 0.3 ($n = 7$) and 36.55 ± 0.26 ($n = 6$), respectively]. Figure 1*C* shows that LPS at a dose of 400 μg/kg induces a progressive and sustained rise in core temperature of ~1.5°C in control rats (intracerebroventricular saline, for 2 hr). This LPS-induced fever was significantly reduced to ~0.7°C by the infusion of 15d-PGJ$_2$ (50 ng/μl, i.c.v.).

15d-PGJ$_2$ reduces COX-2 expression in the hypothalamus of LPS-treated rats

Because of the pivotal role of COX-2 in LPS fever, the effect of 15d-PGJ$_2$ on hypothalamic COX-2 expression *in vivo* was explored. In this experiment, rats were injected intracerebroventricularly with either saline or 15d-PGJ$_2$ (50 ng/μl) as described previously. They then all received an injection of LPS (400 μg/kg, i.p.), and COX-2 expression in the hypothalamus was assessed at 3 hr after LPS injection. As shown in Figure 2*A*, there is less COX-2 [at the expected molecular weight of 72 kDa (Mouihate and Pittman, 2003)] protein expressed in the hypothalamus of a 15d-PGJ$_2$-treated rats, whereas the amount of actin protein remains constant. Densitometric analysis showed that 15d-PGJ$_2$ significantly reduced COX-2 expression in the hypothalami of LPS-treated rats (Fig. 2*B*).

PPARγ is expressed in the rat hypothalamus

Rats received an intracerebroventricular infusion of either saline or 15d-PGJ$_2$ (50 ng/μl) as described above. Three hours after LPS injection (400 μg/kg, i.p.), hypothalamic proteins were processed for immunodetection of PPARγ. Figure 3*A* displays two immunoreactive bands at ~50 kDa as described by Bordji et al. (2000) and Wang et al. (2002). The high molecular weight band was more abundant. Both PPARγ-immunoreactive bands were unaffected by intracerebroventricular infusion of 15d-PGJ$_2$ to LPS-treated rats. We also determined whether LPS alone would affect PPARγ expression. As shown in Figure 3*B*, LPS (400 μg/kg, i.p.) did not affect the expression levels of PPARγ in the hypothalamus as compared with control rats (intraperitoneal saline).

15d-PGJ$_2$ effects on the phosphorylation status of p44/p42 MAPK

Because *in vitro* expression PPARγ can be enhanced by its own ligands through an activation of p44/p42 MAPK, this experiment was designed to explore whether this prostaglandin can alter phosphorylation status of p44/p42 MAPK in the hypothalami of LPS-treated rats. Three hours after LPS injection, hypothalamic proteins were processed for immunodetection of the phosphorylated p44/p42 MAPK. Our experiment revealed two immunoreactive bands at the expected molecular weights of 44 and 42 kDa (Bolshakov et al., 2000) (Fig. 4*A*). Densitometric analysis

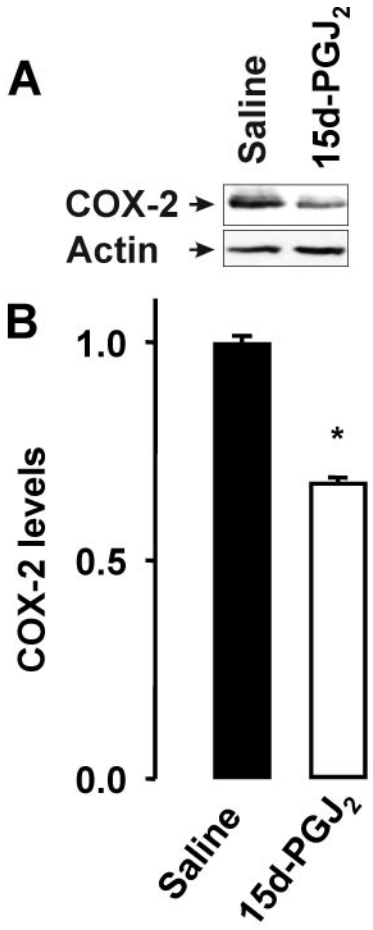

Figure 2. 15d-PGJ_2 effects on induced COX-2. Proteins were extracted from hypothalami of rats that received LPS injection (400 μg/kg, i.p.) and intracerebroventricular infusion of either saline or 15d-PGJ_2 as described above. They were separated by electrophoresis, and COX-2 and actin were immunodetected as shown in a representative blot (*A*). Optical density analysis of COX-2 is shown in *B*. $*p < 0.05$; $n = 3$ for each data point.

showed that 15d-PGJ_2 treatment does not affect the phosphorylation levels of p44/p42 MAPK (Fig. 4*B*).

LPS effects on 15d-PGJ_2 and PGD_2-S levels in the CSF

This experiment was designed to test whether 15d-PGJ_2 and its upstream synthesizing enzyme PGD_2-S are present in cerebrospinal fluid and whether their levels are affected after an immune challenge. Rats received an intraperitoneal injection of either saline or LPS (200 or 400 μg/kg). One or 2 d after LPS injection, CSF samples were collected separately from each rat brain, and 15d-PGJ_2 levels were measured. As shown in Figure 5, the basal level of 15d-PGJ_2 in rat CSF is 185.1 $\pm$ 39.36 pg/ml. One day after intraperitoneal injection, LPS at a dose of 400 μg/kg but not at a dose of 200 μg/kg significantly enhanced 15d-PGJ2 levels in the CSF (881.2 $\pm$ 228.2 and 319.4 $\pm$ 78.78 pg/ml, respectively). Two days after LPS injection, mean values of 15d-PGJ_2 were increased but were not significantly different from control group (762.3 $\pm$ 230.8 pg/ml for LPS at 200 μg/kg; 568.4 $\pm$ 209.6 pg/ml for LPS at 400 μg/kg).

Because 15d-PGJ_2 is a metabolite of PGD_2 and PGD_2 genesis depends on PGD_2-S, CSF samples were also processed for immunodetection of PGD_2-S levels. A PGD_2-S-immunoreactive band at the expected molecular weight of 26 kDa (Urade et al., 1985) was present in all CSF samples (Fig. 6). Densitometric analysis showed that PGD_2-S levels in rat CSF were significantly enhanced after intraperitoneal injections of LPS at doses of both 200 μg/kg (Fig. 6*A*) and 400 μg/kg (Fig. 6*B*) as compared with control rats. It is noteworthy that at 1 d after LPS injection, PGD_2-S levels are significantly higher in CSF of rats that received a dose of 400 μg/kg of LPS when compared with that of rats injected with a dose of 200 μg/kg.

Discussion

Several *in vitro* studies have established a role for 15d-PGJ_2 in attenuating expression of proinflammatory related genes (Maggi et al., 2000; Rossi et al., 2000; Straus et al., 2000; Clark, 2002). To the best of our knowledge, this is the first *in vivo* study to show that 15d-PGJ_2 possesses antipyretic properties. When injected into the brain (intracerebroventricular injection), this prostaglandin attenuated LPS fever and reduced COX-2 expression in the hypothalamic regions of LPS-treated rats. Furthermore, the PPARγ receptor for 15d-PGJ_2 was also present in the hypothalamus and was unchanged during fever development. These data suggest that 15d-PGJ_2 most likely affects LPS fever at the CNS level by acting on COX-2 expression in the fever-controlling region of the hypothalamus.

15d-PGJ_2 has been studied extensively since its discovery as an endogenous specific ligand for PPARγ. The most known and widely studied aspect of 15d-PGJ_2 actions is its anti-inflammatory function. Indeed, 15d-PGJ_2 attenuates immune activation of several proinflammatory molecules in various culture systems (for review, see Straus and Glass, 2001). LPS activation of proinflammatory genes coding for interleukin-1β, inducible nitric oxide, TNFα, and COX-2 was attenuated substantially in the presence of 15d-PGJ_2 (Petrova et al., 1999; Maggi et al., 2000; Drew and Chavis, 2001; Janabi, 2002; Simonin et al., 2002). Interestingly, these same genes are involved in fever development. This study is the first to show that 15d-PGJ_2 reduces LPS fever and that 15d-PGJ_2 is present in the rat CSF and can be additionally generated when rats are immune challenged. These increased levels of 15d-PGJ_2 are likely to be brought about by LPS-induced increase in PGD_2-S level or activity, or both.

Evidence exists that 15d-PGJ_2 can be produced within the CNS. In fact, the 15d-PGJ_2 precursor PGD_2 (Shibata et al., 2002) is actively produced and released in cerebrospinal fluid (Urade et al., 1985, 1993). Furthermore, our data demonstrate that LPS increases the levels of 15d-PGJ_2 and, as reported previously, PGD_2-S in the CSF (Ishizaka et al., 2001). It is questionable whether 15d-PGJ2 plays a role in acute defervescence, because our urethane-anesthetized model is probably not ideal for studying defervescent mechanisms. Nonetheless, the presence of elevated levels of 15d-PGJ_2 in the CSF 1–2 d after LPS raises the possibility that this prostaglandin may play a role in modeling the CNS response to peripheral inflammation at these times. In preliminary experiments, we did not observe a significant increase in the basal levels of 15d-PGJ_2 in the CSF 3 and 6 hr after LPS injection (data not shown). This apparent temporal discrepancy between fever defervescence and increased levels of 15d-PGJ_2 may be attributable to the quick degradation and conversion of 15d-PGJ_2 to its metabolites (Shibata et al., 2002). Local release and action of 15d-PGJ_2 within the fever-sensitive area to cause acute defervescence cannot be excluded (Mong et al., 2003).

The 15d-PGJ_2 attenuates the hypothalamic expression of COX-2, a key enzyme in fever generation, thus raising the possibility that COX-2 may be a preferential target in 15d-PGJ_2-induced antipyresis. The reduced COX-2 expression by 15d-PGJ_2 is most likely brought about by an alteration of the NF-κ B pathway (Rossi et al., 2000) because this pathway leads to COX-2

activation (Straus et al., 2000; Straus and Glass, 2001). Interestingly, PGD$_2$-S colocalizes with COX-2 in arachnoid tissue, in epithelial cells of the choroid plexus, and in perivascular microglial cells (Beuckmann et al., 2000), suggesting that COX-2-containing cells may also produce 15d-PGJ$_2$. Thus 15d-PGJ$_2$ may act in an intracellular (intracrine) manner to downregulate COX-2 and subsequently reduce fever response. It is also possible that 15d-PGJ$_2$ could affect the fever response by acting on PGE$_2$ receptors or cytokines and their receptors (i.e., IL-1β receptors), or both, in the fever-controlling regions of the hypothalamus. We have found, however, that the hypothalamic expression of E-type prostaglandin receptor, a necessary receptor for fever development (Ushikubi et al., 1998), is stable after LPS treatment (Mouihate et al., 2002).

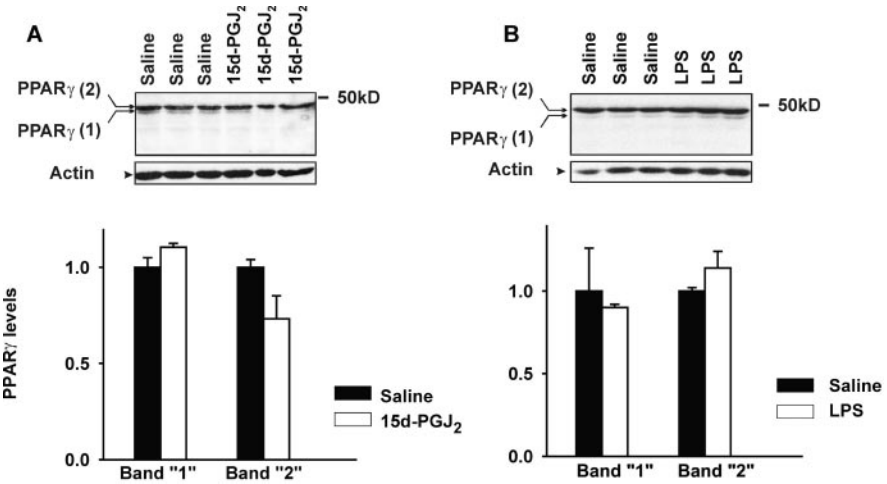

Figure 3. PPARγ expression in the hypothalamus. *A*, Rats received LPS injection (400 μg/kg, i.p.) and intracerebroventricular infusion of either saline or 15d-PGJ$_2$ (50 ng/μl) for 2 hr at a rate of 25 μl/hr. *B*, Rats received an injection of either saline or LPS (400 μg/kg, i.p.). Hypothalamic proteins were separated by electrophoresis. PPARγ- and actin-immunoreactive bands were detected (top panels). Optical density analysis of the PPARγ is shown in bottom panels (n = 3 for each treatment group).

In addition to altering the NF-κ B pathway and consequently the expression of COX-2, 15d-PGJ$_2$ can alter the p44/p42 MAPK pathway and enhance the expression of PPARγ (Prusty et al., 2002). In this study, neither PPARγ receptor expression in the hypothalamus nor the active form of p44/p42 MAPK was affected by intracerebroventricular injection of 15d-PGJ$_2$ into brains of immune-challenged rats. These data support the notion that the effects of 15d-PGJ$_2$ on the febrile response do not require the p44/p42 MAPK-dependent pathway.

PPARγ mRNA is transiently expressed in relatively high levels in the CNS during rat embryonic development (Braissant and Wahli, 1998). In adult rats, however, small amounts of PPARγ mRNA were detected by *in situ* hybridization in the hippocampus and cerebellum (Braissant et al., 1996). There has been no report of its presence in hypothalamic structures. Because mRNA expression does not always equate to protein expression (Tropea et al., 2001), we used Western blot with a well characterized PPARγ antibody (Bordji et al., 2000; Wang et al., 2002) to detect whether the protein was present. This is the first demonstration that PPARγ is expressed in the hypothalamic region of the rat brain and that such expression was unaffected by intraperitoneal LPS injection. Despite its established role in attenuating proinflammatory responses (Heneka et al., 2000; Sastre et al., 2003), PPARγ may not be the only receptor involved in mediating the effects of 15d-PGJ$_2$ because this prostaglandin can also alter NF-κ B activation in a PPARγ-independent manner (Straus et al., 2000), probably through PPARδ receptor subtype (Welch et al., 2003; Woods et al., 2003). *In vitro* studies would be required to determine whether either receptor binding properties or post-receptor signaling mechanisms remain similarly unaltered.

It was curious that the antipyretic effect of 15d-PGJ$_2$ was more apparent when higher doses of LPS were injected. This effect is unlikely to be accounted for by an increase in the sensitivity to 15d-PGJ$_2$, because LPS does not affect expression levels of hypothalamic PPARγ receptor (Fig. 3*B*). It is more likely that a higher dose of LPS was needed to induce enhanced endogenous intracellular levels of 15d-PGJ$_2$, which when combined with exogenous 15d-PGJ$_2$ could exert a more powerful antipyretic effect (Fig. 5) (Ishizaka et al., 2001). Alternatively, threshold levels of inflammation may be required before 15d-PGJ$_2$ becomes effective. In support of this, we were able to detect greater quantities of 15d-PGJ$_2$ and PGD$_2$-S diffusing into the CSF at the higher does of LPS.

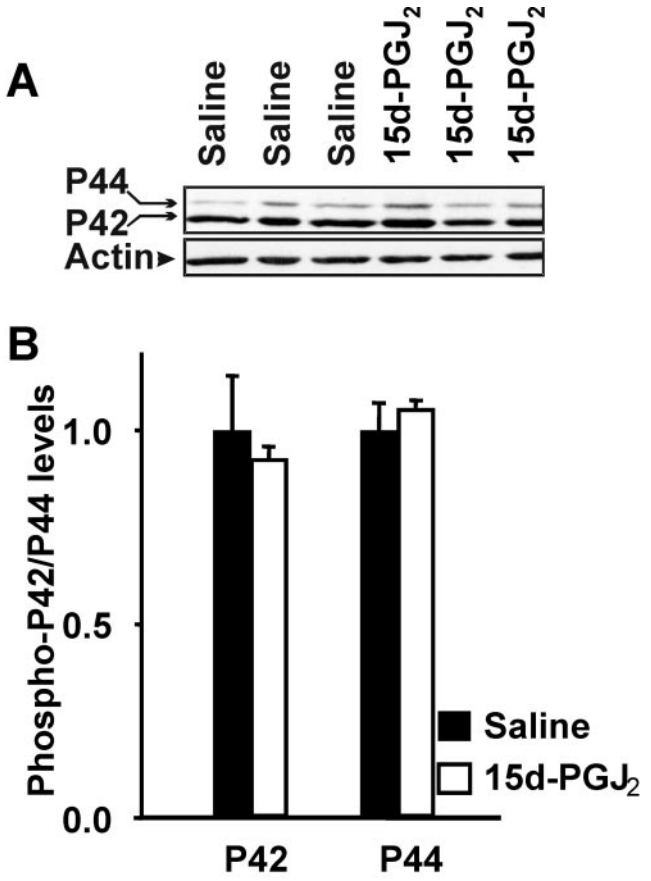

Figure 4. 15d-PGJ$_2$ and the p44/p42 MAPK. Rats received LPS injection (400 μg/kg, i.p.) and intracerebroventricular infusion of either saline or 15d-PGJ$_2$ as described above. Proteins were extracted from their hypothalami and separated by electrophoresis. The phosphorylated p44/p42 MAPK- and actin-immunoreactive bands are shown in *A*. Optical density analysis of p44/p42 MAPK is shown in *B* ($n = 3$ for each treatment group).

The possibility that centrally injected 15d-PGJ$_2$ can cross the blood–brain barrier and act on peripheral immune targets is un-

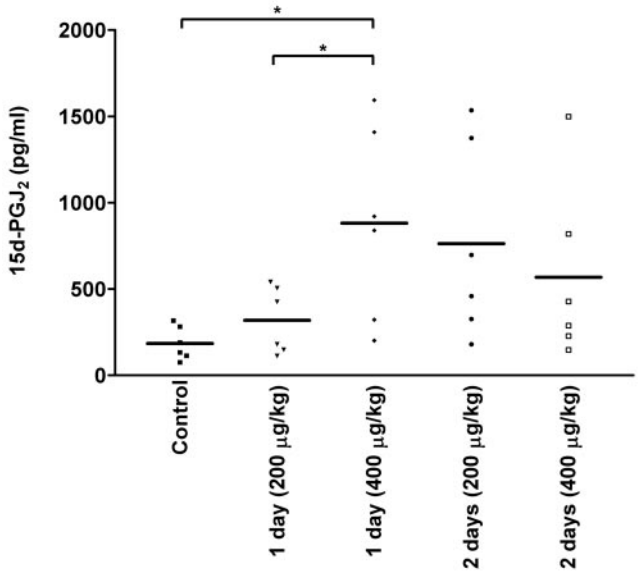

Figure 5. LPS effects on CSF levels of 15d-PGJ_2. Rats received either saline (control) or LPS injection (200 or 400 μg/kg, i.p.), and CSF was collected at either 1 or 2 d after injections. CSF levels of 15d-PGJ_2 were determined using an enzyme immunoassay. $n = 6$ animals at each time point; $^*p < 0.05$.

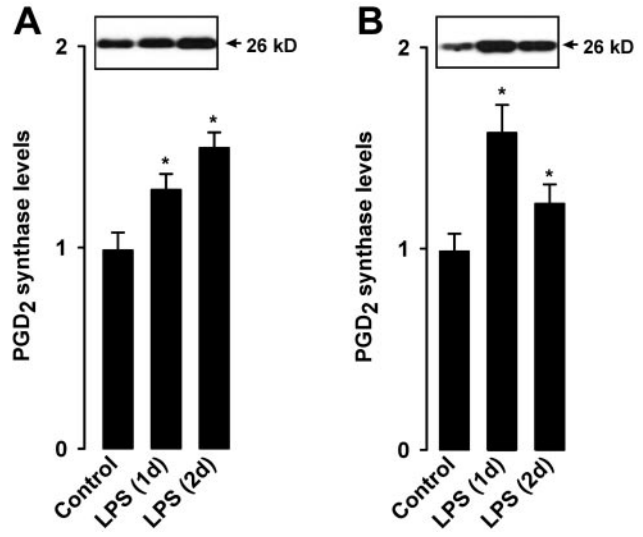

Figure 6. LPS effect on CSF levels of PGD_2-S. Rats received either saline (control) or LPS injection at a dose of 200 μg/kg (*A*) or 400 μg/kg (*B*). CSF was collected at either 1 d (LPS 1d) or 2 d (LPS 2d) after LPS injections and separated by electrophoresis. PGD_2-S was immunodetected (top panels), and its optical density analysis is shown in the bottom panels. $n = 6$ animals at each time point; $^*p < 0.05$.

likely. Prostaglandins containing a cyclopentenone ring (such as 15d-PGJ_2) are inactivated after a single passage through the lung (Horton and Jones, 1969; Papanicolaou and Meyer, 1972; Narumiya et al., 1999). Even if all the 15d-PGJ_2 were to escape into the peripheral circulation and not be inactivated, its concentration (0.2–0.4 μM) would still be less than what has been found to be effective *in vitro* (Jiang et al., 1998; Rossi et al., 2000). Therefore, it is more likely that centrally applied 15d-PGJ_2 exerts its antipyretic effect by acting locally in the CNS.

The physiological significance of the presence of 15d-PGJ_2 in the CNS is poorly understood. The potential role of 15d-PGJ_2 in thermoregulation is a very interesting avenue. 15d-PGJ_2 may act through a short and locally activated loop to bring down the immune-induced activation of cyclooxygenases and terminate deleterious effects of prolonged inflammatory processes (Straus and Glass, 2001). Whether this feedback loop is active under all conditions or whether it is temporally dissociated from the proinflammatory prostaglandin pathway activation remains to be seen. Now that we have shown that PPARγ is present in the fever-controlling region of rat brain, that its ligand 15d-PGJ_2 is active on fever response, and that peripheral inflammatory stimuli increase levels of 15d-PGJ_2 in CSF, this sets the stage to investigate a possible role for the endogenous 15d-PGJ_2 actions in both short-term and chronic neuro-immune responses.

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