Cloning, Expression, and Up-regulation of Inducible Rat Prostaglandin E Synthase during Lipopolysaccharide-induced Pyresis and Adjuvant-induced Arthritis*

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We have cloned and expressed the inducible form of prostaglandin (PG) E synthase from rat and characterized its regulation of expression in several tissues after in vivo lipopolysaccharide (LPS) challenge. The rat PGE synthase is 80% identical to the human enzyme at the amino acid level and catalyzes the conversion of PGH2 to PGE2 when overexpressed in Chinese hamster ovary K1 (CHO-K1) cells. PGE synthase activity was measured using [3H]PGH2 as substrate and stannous chloride to terminate the reaction and convert all unreacted unstable PGH2 to PGF2α before high pressure liquid chromatography analysis. We assessed the induction of PGE synthase in tissues from Harlan Sprague-Dawley rats after LPS-induced pyresis in vivo. Rat PGE synthase was up-regulated at the mRNA level in lung, colon, brain, heart, testis, spleen, and seminal vesicles. Cyclooxygenase (COX)-2 and interleukin 1β were also up-regulated in these tissues, although to different extents than PGE synthase. PGE synthase and COX-2 were also up-regulated to the greatest extent in a rat model of adjuvant-induced arthritis. The RNA induction of PGE synthase in lung and the adjuvant-treated paw correlated with a 3.8- and 16-fold induction of protein seen in these tissues by immunoblot analysis. Because PGE synthase is a member of the membrane-associated proteins in eicosanoid and glutathione metabolism (MAPEG) family, of which leukotriene (LT) C4 synthase and 5-lipoxygenase-activating protein are also members, we tested the effect of LTC4 and the 5-lipoxygenase-activating protein inhibitor MK-886 on PGE synthase activity. LTC4 and MK-886 were found to inhibit the activity with IC50 values of 1.2 and 3.2 μM, respectively. The results demonstrate that PGE synthase is up-regulated in vivo after LPS or adjuvant administration and suggest that this is a key enzyme involved in the formation of PGE2 in COX-2-mediated inflammatory and pyretic responses.

Prostaglandin (PG) E2 is a major prostanoid derived from PGH2 that can be generated by either degradation of PGH2 or by a reaction catalyzed by PGE synthase (1). PGH2 is formed by the bio-oxygenation of arachidonic acid catalyzed by either isoform cyclooxygenase (COX)-1 or COX-2 and serves as the precursor to all prostanoid products formed, including prostaglandins, prostacyclin, and thromboxanes (2–4). Prostanoids have diverse biological functions including the maintenance of vascular and kidney homeostasis, relaxation and contraction of smooth muscle, regulation of gastrointestinal secretion and motility, and induction of sleep, pain, and inflammation (5). Within all these varied roles, data on COX-2 regulation of expression and the pharmacological effects of selective COX-2 inhibitors have delineated that COX-2 is the major isoform responsible for synthesis of inflammatory and pyretic prostanoids (2–4, 6). In addition, studies with a specific monoclonal antibody to PGE2 indicated that the major prostanoid that contributes to inflammation is PGE2 (7). Therefore, it is compelling to suggest that an inducible PGE synthase may provide a novel therapeutic target for arthritis and pain downstream of COX-2 activity.

PGE synthase is a member of the membrane-associated proteins in eicosanoid and glutathione metabolism (MAPEG) superfamily, which consists of six human proteins with divergent functions (8). The initial discovery of this family came to fruition through work on the leukotriene pathway. Leukotrienes are derived from arachidonic acid through the 5-lipoxygenase pathway (9) and act as potent mediators of inflammation and bronchoconstriction (10). The initial discovery of the MAPEG family was initiated upon the cloning of LTC4 synthase, which was found to be 31% identical to 5-lipoxygenase-activating protein (an arachidonate transfer protein required for leukotriene biosynthesis) (11, 12). The search for new members led to the discovery of three novel proteins (microsomal glutathione transferases [MGSTs]) 2 and 3 (13, 14) and PGE synthase and one preexisting enzyme (MGST1). Four members of this family can conjugate glutathione to lipophilic substrates; however, one of these enzymes, LTC4 synthase, conjugates glutathione specifically to LTA4 to form the potent bronchoconstrictive leukotriene C4. The best-characterized member is MGST1, which is involved in cellular detoxification of various xenobiotics (15). MGST2 and MGST3 can both conjugate glutathione to LTA4 whereas only MGST2 can conjugate glutathione to the classical glutathione transferase substrate 1-chloro-2,4-dinitrobenzene. Both MGST2 and MGST3 also possess a glutathione-dependent peroxidase activity with hydroperoxy fatty acid substrates (14). The final member of this family, PGE synthase, reverse transcription; PCR, polymerase chain reaction; CHO, Chinese hamster ovary.
which has the highest sequence identity to MGST1, could not conjugate glutathione to either LTA4 or 1-chloro-2,4-dinitrobenzene and, interestingly, was found to possess PGE synthase activity (1).

The cDNA for human PGE synthase has recently been cloned, and the enzyme has been shown to be inducible by IL-1β in the lung carcinoma-derived A549 cell line (1). The corresponding rat sequence has been cloned and shown to be up-regulated during β-amyloid treatment of rat brain (16). In the present study, we present the cloning, expression, and demonstration of activity for rat PGE synthase. In addition, to investigate the role that PGE synthase might play in an inflammatory process, we have determined the inducibility and tissue distribution of PGE synthase RNA in comparison with COX-2 in LPS-induced pyresis in rats and adjuvant-treated rat paws. We also present the first induction of PGE synthase at the protein level in the latter two models of inflammation.

MATERIALS AND METHODS

CHO-K1 cells were obtained from the American Type Culture collection. Cell culture media, serum, antibiotics, and LipofectAMINE were purchased from Life Technologies, Inc. Oligonucleotides and a polyclonal peptide antiserum to human PGE synthase (1) were obtained from Research Genetics (Huntsville, AL). Restriction enzymes, Pwo polymerase, ligase, and Complete protease mixture were obtained from Roche Molecular Biochemicals. PGE2, [3H]PGH2, PGG2, PGE2, PGD2, LTc4, and a partially purified PGE synthase antibody were purchased from Cayman Chemical Co. (Ann Arbor, MI). Glutathione and stannous chloride were obtained from Sigma and BDH, Inc., respectively.

[32P]dCTP was obtained from PerkinElmer Life Sciences, and the random primer kit for generating radiolabeled cDNA was obtained from Amersham Pharmacia Biotech.

Identification, Cloning, and Expression of Rat PGE Synthase—The full-length human PGE synthase protein sequence was used to perform a BLAST search of the GenBank expressed sequence tag rodent data base. A rat expressed sequence tag was identified with significant sequence identity to the human enzyme and with the accession number AI136526. The expressed sequence tag clone was obtained from RevertAid Plus (Life Technologies, Inc.). Cells were harvested 24–48 h after transfection into CHO-K1 cells using LipofectAMINE 2000 (Invitrogen). The clone was sequenced using an Applied Biosystems 373A automated sequencer and dye terminator reactions as described by the manufacturer’s instructions. The clone was full length and was therefore transferred into CHO-K1 cells using LipofectAMINE 2000 (Life Technologies, Inc.). Cells were harvested 24–48 h after transfection and resuspended in 15 mM Tris-HCl, pH 8.0, 0.25% sucrose, 0.1 mM EDTA, and 1 mM glutathione. Resuspended cells were sonicated four times for 30 s at 4°C using a Cole Parmer 4710 Ultrasonic Homogenizer at 70% duty cycle. Disrupted cells were subjected to centrifugation at 5,000 × g for 5 min, and the supernatant was subjected to ultrafiltration through a membrane delo 100,000 g x g for 1.5 h. The membrane pellet obtained was resuspended in 10 mM potassium phosphate (pH 7.0), 20% glycerol, 0.1 mM EDTA, and 1 mM glutathione. Both mock and human PGE synthase in pcDNA 3.1-transfected cells were prepared in a similar fashion. Protein concentrations were determined using the Coomassie protein assay (Pierce) as described by the manufacturer. Immunoblot Analysis—Protein samples were resolved by SDS-polyacrylamide gel electrophoresis using 4–20% gradient gels supplied by Bio-Rad Laboratories, and the gel was stained with Coomassie Brilliant Blue R-250. Western blotting was performed using micromolar Western blot Chemiluminescence Reagent (PerkinElmer Life Sciences) according to the manufacturer’s instructions. Detection and quantitative analysis were performed using a Fuji Film LAS-1000 charged-couple device and Image gauge software.

RESULTS

Induction of Rat PGE Synthase during Pyresis

In Vivo Induction of PGE Synthase—All procedures used in the in vivo assays were approved by the Animal Care Committee at the Merck Frosst Center for Therapeutic Research (Kirkland, Quebec, Canada) according to guidelines established by the Canadian Council on Animal Care.

Harlan Sprague-Dawley rats were injected with a single i.v. bolus of 0.12 mg/kg LPS or saline vehicle control, and 7 h after infusion, the rats were sacrificed, and tissues were perfused with saline and dissected. The adjuvant-induced arthritis model was performed with two groups of Harlan Sprague-Dawley rats each and an intradermal injection of 0.5 mg of Mycobacterium butyricum in mineral oil into the left hind foot pad as described previously (17). The tissues were flash-frozen in liquid nitrogen and used for RNA preparation. mRNA was isolated from the tissues using the kit reagents of the Fast Track 2.0 mRNA Isolation Kit (Invitrogen). RNA concentration was quantified by spectrophotometry. mRNA (0.1 μg) was reverse-transcribed into cDNA with random hexamers using kit reagents and following the manufacturer’s recommended conditions (GeneAmp PCR Reaction Kit; PerkinElmer Life Sciences). The RT reaction was incubated in a thermal cycler (GeneAmp PCR System 9600, Perkin Elmer Cetus) at 62°C for 1 h and 94°C for 5 min and then cooled to 4°C. Half of the reverse-transcribed cDNA product (10 μl) was amplified by PCR in a 100 μl reaction. The reaction contained 1 μl of reverse hexamers (Roche Molecular Biochemicals), and either 0.3 μM primers (PGE synthase) or 0.2 μM primers (β-actin, COX-2, and IL-1β). The PCR reaction was incubated at 94°C for 5 min and then amplified for 25–50 cycles using the reaction conditions as follows: 94°C for 30 s, 65°C for 30 s, and 72°C for 1 min. After amplification, the reactions were incubated at 72°C for 7 min and then cooled to 4°C. Synthetic DNA amplimers for PGE synthase were as follows: sense, 5′-ATGATTCTTCCGTGTTTGGTGATGGAGG-3′; and antisense, 5′-ACAGATTTGGGGGCACTTTCCCCAGA-3′. Synthetic primers for β-actin were ordered from CLONTECH. Synthetic DNA amplimers for COX-2 were as follows: sense, 5′-GAGGATCAAGATGTTGACCAAG-3′; and antisense, 5′-AACGCTTGTGCGTACTGTTG-3′. Synthetic DNA amplifiers for IL-1β were as follows: sense, 5′-GCACCTCTTCCTTGTCCATC-3′; and antisense, 5′-CTGATGACAGCACTTTGGGGAA-3′. Reverse transcription-PCR products were analyzed by 1% (w/v) agarose gel electrophoresis. PGE synthase RT-PCR products were transferred to nitrocellulose membrane and analyzed by Southern blot. The full-length PGE synthase cDNA was labeled with [α-32P]dCTP using the [32P]dCTP labeling kit (Amersham Pharmacia Biotech). Hybridization was performed in 5× sodium citrate (pH 7.0) and the superhybridization solution of sodium sperm DNA for 18 h at 42°C. Blots were washed to a final stringency of 0.5× SSC, 0.5% SDS at 65°C followed by autoradiography at −80°C. cDNA from PGE synthase- or COX-2-transformed bacteria was used as a template, with PGE synthase and COX-2 primers respectively, and served as a positive control. Comparative analysis between the amount of PCR product and the amount of initial template demonstrated that the method was linear over the entire range of the conditions utilized for each amplification. Detection and quantitative analysis were performed using a Fuji Film LAS-1000 charge-coupled device and Image gauge software.

PGE Synthase Assay—Micromolar membranes from mock-transfected CHO-K1 cells or rat PGE synthase-transfected CHO-K1 cells were diluted into 0.1 M potassium phosphate, pH 7.0, and 2.5 mM reduced glutathione. The reaction was initiated with 10 μM PGH2 and 0.2 μM of [3H]PGH2 (100 μCi/mmole) and terminated with an equal volume of acetonitrile/H2O/acetic acid (35:65:0.1%) containing 1 mg/ml stainable chondroitin. Samples were analyzed by reverse phase HPLC using a Waters Nova-Pak C18 column (3.9 × 150 mm, 4 μm particle size) and a Waters 625 HPLC system with a Beckman 171 radiodetector. Radioiodinated standards, [3H]PGF2α (Amersham Pharmacia Biotech), [3H]PGE2 (PerkinElmer Life Sciences), and [3H]PGD2 (PerkinElmer Life Sciences) were utilized for determining the separation by reverse phase HPLC and for quantitation of product formation of PGF2α.
Induction of Rat PGE Synthase with in Vivo LPS Challenge—Prostaglandin E<sub>2</sub> levels have been shown to be elevated upon LPS challenge in rat brain with a concomitant induction of cyclooxygenase-2 (18). We designed a study to compare the inducibility of PGE synthase, COX-2, and IL-1β in Harlan Sprague-Dawley rats after LPS treatment. Harlan Sprague-Dawley rats were treated with 0.12 mg/kg LPS by a single i.v. bolus, and 7 h after challenge, various tissues were collected for analyses by quantitative PCR. This dose of LPS causes a significant elevation of body temperature of rats (from 36.4 °C to 38.5 °C). Characterization of eight major tissues from vehicle- or LPS-treated rats by RT-PCR followed by Southern blot analysis demonstrated that lung, brain, heart, spleen, and seminal vesicles contain increased mRNA levels of PGE synthase as compared with the vehicle-treated control animals (Figs. 4 and 5). RT-PCR was also performed for COX-2 and IL-1β, and the results were compared with those for PGE synthase, whereas the mock-transfected cells showed no detectable signal.

The rat PGE synthase membrane preparation was tested for enzymatic activity using [3H]PGH<sub>2</sub> as substrate and separation of the reaction products by reverse phase HPLC. The assay was comprised of 10 μg/ml membrane protein in 100 mM potassium phosphate, pH 7.0, and 2.5 mM glutathione and initiated with 10 μM PGH<sub>2</sub> and 0.2 μCi of [3H]PGH<sub>2</sub> (100 μCi/μmol). The reaction was terminated with 1 mg/ml stannous chloride in HPLC running buffer to quantitatively convert the remaining PGH<sub>2</sub> substrate to PGF<sub>2α</sub>. The conversion of PGH<sub>2</sub> to PGE<sub>2</sub>, with product formation for the rat enzyme being 3.6-fold higher than the activity reported for the human microsomal PGE synthase, was rapid, and the reaction reached a plateau after 1 min, mainly due to substrate depletion (Fig. 3).

Product accumulation obtained under these conditions was analyzed using authentic [3H]PGE<sub>2</sub>, [3H]PGF<sub>2α</sub>, and [3H]PGD<sub>2</sub> standards. The retention times of the prostaglandin products were verified using [3H]PGE<sub>2</sub>, [3H]PGF<sub>2α</sub>, and [3H]PGD<sub>2</sub> standards.

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and not clearly detectable COX-2 induction in spleen and seminal vesicle. COX-2 was also present in non-LPS-treated rat brain, and this observation is consistent with constitutive expression of COX-2 in this tissue (19). IL-1β was significantly up-regulated in lung, spleen, and seminal vesicle, and a slight induction was also detected in brain and heart. The lung seems to be very responsive to LPS administration, with a significant induction of PGE synthase, COX-2, and IL-1β mRNA.

Prostaglandins play an important role in the maintenance of the integrity of the gastrointestinal mucosa. We have therefore analyzed tissues of the GI tract from these vehicle- and LPS-treated rats (Fig. 5). In vehicle-treated animals, PGE synthase mRNA was detectable in the stomach, but low or undetectable signals were seen in colon, ileum, and jejunum. Upon LPS stimulation, significant up-regulation of PGE synthase was only detected in the colon. An extra sample from myeloproliferative tissue, the thymus, was included, and a slight induction of PGE synthase was detected. COX-2 mRNA levels were detected in the colon, ileum, jejunum, and stomach but were similar in both vehicle- and LPS-treated animal tissues, whereas up-regulation in the thymus was detected for COX-2 upon LPS challenge. IL-1β was detectable in all LPS-treated tissues.

**Induction of PGE Synthase in the Rat Adjuvant Arthritis Model**—The rat adjuvant arthritis model is used extensively as a pharmacological model of clinical arthritis and has a major prostaglandin component. Rats injected with adjuvant will begin demonstrating edema and hyperalgesia within several days of induction of disease, and this process is diminished in the presence of cyclooxygenase-2 inhibitors such as rofecoxib (6). We have utilized this model to detect PGE synthase, and 5 days after adjuvant treatment, a significant increase in the inducible PGE synthase was detected (Fig. 6). As seen in Fig. 6A, no PGE synthase is detected in the naive (vehicle-treated) rat paw.

**Quantitative Analysis of PGE Synthase Expression**—We have also quantitated the RNA induction of PGE synthase and COX-2 in the tissues from LPS-treated rats and adjuvant-treated paws. Normalizing for β-actin expression, the lung and the adjuvant-treated paw had the most significant induction of PGE synthase with 7- and 20-fold increases, respectively, as compared with tissues from vehicle-treated animals (Fig. 7A). COX-2 was also elevated 2.6-fold in lung and 6.5-fold in the adjuvant-treated paw (Fig. 7A). The remaining tissues that contained up-regulated PGE synthase (Fig. 5) RNA (testes, spleen, seminal vesicles, colon, and thymus) showed a 2- to 3-fold induction over non-LPS-exposed tissues, and the brain
and heart contained a slightly higher increase of PGE synthase (5-6-fold). COX-2 mRNA was induced 2- to 5.8-fold in brain, heart, testes, spleen, and seminal vesicles, with the highest induction (as a ratio of β-actin expression) observed in heart and brain. Because RNA induction may not always correlate with protein expression, we analyzed PGE synthase protein expression by immunoblot. Protein expression was examined in lung tissue from LPS-treated animals and adjuvant-treated paws (Fig. 7B) because these tissues contained the highest induction and levels of PGE synthase mRNA (Figs. 6 and 7A). The most significant protein induction detected was in the rat adjuvant-treated paw with a 16-fold increase of PGE synthase protein as compared with the naïve paw. This is in concordance with the 20-fold increase in mRNA (Fig. 7A) obtained from rat paws treated in a similar fashion. A 3.8-fold induction of PGE synthase protein was also detected in lung tissue (Fig. 7B) from LPS-treated animals, which is within 2-fold of the RNA induction (7-fold) obtained from similar tissues (Fig. 7A). This is the first reported evidence of PGE synthase protein in two major models of inflammation, LPS-induced pyresis and adjuvant-induced arthritis.

Inhibition of PGE Synthase—As described earlier, PGE synthase is a member of the MAPEG family, which also contains 5-lipoxygenase-activating protein (FLAP) and LTC₄ synthase. We analyzed several inhibitors of FLAP such as MK-886 and the reaction product of LTC₄ synthase, and we found that LTC₄ and MK-886 inhibit rat PGE synthase with IC₅₀ values of 1.2 and 3.2 μM, respectively (Fig. 8). In contrast, the cyclooxygenase inhibitors indomethacin and acetaminophen were inactive up to concentrations of 100 and 1000 μM, respectively, as inhibitors of rat PGE synthase.

DISCUSSION

Induction of prostaglandin synthesis through the induction of the COX-2 enzyme is widely accepted as the mechanism responsible for prostanoid mediated pain, fever, and inflammation (2–6). The major prostanoids implicated in these pathophysiological conditions are PGI₂ and PGE₂. PGI₂ has been implicated because the knockout of the prostacyclin receptor results in decreased paw swelling in the carrageenan-induced acute inflammatory model in mice (20). A monoclonal antibody to PGE₂ also demonstrates efficacy in a carrageenan rat paw model of inflammation (7). The role of the prostaglandin E receptor is more difficult to define because there are four identified receptors for PGE₂ (5). One approach to assess the role of PGE₂ in various models would be to evaluate the regulation of the enzyme directly involved in its synthesis, PGE synthase. We have now cloned the rat enzyme and examined its induction along with COX-2 in the rat upon LPS challenge. Using a new assay for PGE synthase activity in which the remaining PGH₂ at the end of the reaction is converted to PGF₂α with stannous chloride, rat PGE synthase was found to be active when ex-
pressed in CHO cells. The rat PGE synthase is 80% identical to the human enzyme.

The coinduction of PGE synthase with COX-2 and IL-1β demonstrates that in vivo this enzyme is up-regulated under proinflammatory conditions such as LPS-induced pyresis and adjuvant-induced arthritis. Interestingly, the highest induction of rat PGE synthase was seen in the lung and in the adjuvant-treated paw. Aerosolized PGE₂ has been demonstrated to enhance respiratory function when administered before antigen challenge (21), and this increased PGE synthase may be a method of maintaining significant oxygenation in the lungs for tissues undergoing damage after LPS provocation. The significant induction of PGE synthase in adjuvant-induced arthritis is consistent with the important role that prostaglandins play in this inflammatory model (22, 23). Most striking is the induction of PGE synthase protein in this model, which correlates well with the mRNA induction. The protein detection in the paw and lung tissues and the similarity of induction as compared with RNA levels suggest concordance of PGE synthase mRNA expression with protein expression in these tissues. During preparation of the manuscript for this article, another study confirming the LPS-stimulated induction of PGE synthase has been published (24). This recent study has also demonstrated preferential coupling of inducible PGE synthase with COX-2 as opposed to COX-1. This further strengthens the role of PGE synthase as a therapeutic target for inflammation.

The kidney is also an important target tissue for prostaglandins, and they play an important role in regulating renal hemodynamics. We have not detected either COX-2 or PGE synthase in the kidney, but this is not unexpected because COX-2 is localized mainly to one specific region, the macula densa (25, 26). In situ hybridization would be required for more precise determinations of mRNA induction of COX-2 and PGE synthase in the kidney and other tissues that express these enzymes in localized regions.

It is well established that nonsteroidal anti-inflammatory drugs cause GI lesions, and GI ulceration is a major clinical side effect of nonsteroidal anti-inflammatory drugs that non-selectively inhibit COX-1 and COX-2 (27–29). These GI side effects have been attributed to prostanooids derived from COX-1, which cause ulcerations in mucosal blood flow, and changes in mucous secretion and bicarbonate and tumor necrosis factor α production. The importance of prostaglandins as cytoprotectants in the GI tract has been described previously (30). PGE synthase is constitutively expressed in the stomach, and its role in cytoprotection and its relationship to COX-1 and COX-2 in GI tissues remain to be investigated. Also, because COX-2 has been implicated to be a mediator of colonic tumors (31), the induction of PGE synthase in this tissue and its link to COX-2 provide impetus to examine its expression in colon tumors as compared with normal colonic epithelium (32). The constitutive mRNA expression of COX-2 in several GI tissues is in contrast to the undetectable level of COX-2 protein (33) in these tissues, and this may result from induction during manipulation of tissues, or it may suggest tight translational control of protein expression.

PGE synthase is a member of the MAPEG family, which includes FLAP and LTC₄ synthase. Comparison of the hydropathy plots of these three proteins demonstrates an identical putative membrane topography for all three of these family members, although the sequence identity of PGE synthase with FLAP and LTC₄ synthase is less than 20% at the amino acid level. MK-886, which is a potent inhibitor of leukotriene biosynthesis (IC₅₀ = 100 nM (34)), was also found to inhibit PGE synthase with a moderate potency (IC₅₀ = 3.2 μM). MK-886 is also a weak inhibitor of LTC₄ synthase with an IC₅₀ of 11 μM (35). Interestingly as depicted in Fig. 9, the region of FLAP that is essential for binding compounds such as MK-886 (36, 37) is highly conserved in both LTC₄ synthase and PGE synthase. In fact, the negative charge of the aspartate or a glutamate at position 62 of FLAP is essential for binding MK-886 analogues. MK-886 appears to inhibit leukotriene biosynthesis by binding to an arachondate binding site on FLAP (38). The presence of a consensu amino acid sequence and sensitivity to indole inhibitors of the MK-886 series for FLAP, LTC₄ synthase, and PGE synthase suggest that this region might also be involved in the binding of eicosanoids for each of these proteins. The motif ERXXXAXXXXD/E could represent a consensus sequence for interaction with arachidonic acid and/or several of its oxygenation products.

The EP₃ knockout has elegantly demonstrated that this receptor, along with PGE₂, is the major mechanism of LPS- and IL-1β-induced pyresis (39). The induction of PGE synthase in the brain during LPS-induced pyresis and in the paw in adjuvant-induced arthritis suggests that this enzyme may have an important function in the initiation of pyresis, pain, and inflammation. The development of selective inhibitors of PGE synthase and a mouse deletion of this gene will provide substantial input in the role of PGE₂ as compared with other prostanooids in the initiation of inflammatory responses.

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