Carrageenan-induced paw edema in rat elicits a predominant PGE$_2$ response in the central nervous system associated with the induction of microsomal prostaglandin E$_2$ synthase-1

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Running title: Induction of mPGES-1 in the CNS during peripheral inflammation
Summary:

Peripheral inflammation involves an increase in cyclooxygenase-2 (COX-2) mediated prostaglandin (PG) synthesis in the central nervous system (CNS) which contributes to allodynia and hyperalgesia. In the present study we have determined the changes in prostanoid tissue levels and in expression of terminal prostanoid synthases in both the CNS and inflamed peripheral tissue during carrageenan-induced paw inflammation in the rat. Prostanoid levels were measured by LC-MS and enzyme expression at the RNA level by qPCR analysis during both the early (1 to 6 h) and late (12 and 24 h) phases of the inflammatory response. In the paw, the early phase was associated with increases in PGE₂ and thromboxane (TX)B₂ levels and with a peak of COX-2 expression that preceded that of microsomal prostaglandin E₂ synthase-1 (mPGES-1). COX-2 and mPGES-1 remained elevated during the late phase and PGE₂ continued to further increase through 24 h. The cytosolic PGE₂ synthase (cPGES) showed a small transient increase during the early phase while mPGES-2 expression was not affected by inflammation. In the cerebrospinal fluid (CSF), elevated levels of PGE₂, 6-keto-PGF₁α, PGD₂, and TXB₂ were detected during the early phase. PGE₂ levels also increased in the spinal cord and, to a lesser extent, in the brain, and remained elevated in both the CSF and spinal cord during the late phase. The expression of mPGES-1 was strongly up-regulated in brain and spinal cord during inflammation whereas no change was detected for the expression of cPGES, mPGES-2, COX-1 and terminal PGD, TX or PGI synthases. The results show that the carrageenan-induced edema in the paw elicits an early phase of COX-2 induction in the CNS leading to an increase synthesis in PGD₂, 6-keto-PGF₁α and TXB₂ in addition to the major PGE₂ response. The data also indicate that the up-regulation of mPGES-1 contributes to COX-2 mediated PGE₂ production in the CNS during peripheral inflammation.
Introduction

Carrageenan-induced inflammation in the rat paw represents a classical model of edema formation and hyperalgesia which has been extensively used in the development of nonsteroidal anti-inflammatory drugs (NSAIDs)\(^1\) and selective COX-2 inhibitors. Several lines of evidence indicate that COX-2 mediated increase in PGE\(_2\) production in the CNS contributes to the severity of the inflammatory and pain responses in this model. COX-2 is rapidly induced in the spinal cord and other regions of the CNS following carrageenan injection in the paw (1). The administration of selective COX-2 inhibitors, but not COX-1 inhibitors, reduce the levels of PGE\(_2\) in the CSF and hyperalgesia (2-5). In addition, it has been shown that the intrathecal administration of PGE\(_2\) potentiates carrageenan-induced inflammation (6) and that the direct microinjection of PGE\(_2\) in the brain causes hyperalgesia (7). Selective COX-2 inhibitors can also inhibit peripheral pain responses when given intrathecally (3,8,9) whereas a selective COX-1 inhibitor has no effect (10). The central effects of PGE\(_2\) appears to be mediated via the EP\(_3\) receptor based on observations that the microinjection of an agonist of the EP\(_3\) receptor in the brain directly causes hyperalgesia (7) and the inflammatory responses are strongly reduced in the mice deficient in the EP\(_3\) receptor (11).

Altogether these studies indicate that the central production of PGE\(_2\) mediated by COX-2 during inflammation contributes to nociception and hyperalgesia at the site of peripheral inflammation. However, the role of the different prostanoid synthases in inflammation and the identity of the critical prostanoids involved in the inflammatory processes have not been well defined. In addition to PGE\(_2\), PGI\(_2\) has also been proposed to represent an important mediator of inflammation based on the reduced edema and pain response in the IP receptor knock-out mice.
Clinical studies have indicated that selective COX-2 inhibitors inhibit the production of urinary PGI₂ metabolites in addition to those of the PGE₂ pathway (14) indicating that COX-2 plays a role in the synthesis of both prostanoid mediators in humans.

PGE₂ is synthesized by the sequential reactions catalyzed by COX which converts arachidonic acid to PGH₂ and by PGE synthase which converts PGH₂ to PGE₂. Several proteins that possess variable levels of PGE synthase activity have been identified: mPGES-1 (15), glutathione transferases (16), cPGES/p23 (17) and mPGES-2 (18,19). The mPGES-1 is considered to be the major form implicated in COX-2 mediated PGE₂ production based on co-transfection experiments in cultured cells indicating a better coupling with COX-2 than with COX-1 for the production of PGE₂ (20) and on the inducibility of the enzyme in response to pro-inflammatory mediators (15,21,22), and in various models of inflammation and fever in vivo (23-26). Furthermore, the mPGES-1 has been shown to co-localize with COX-2 in endothelial cells of the brain vasculature after induction with IL-1β (23,27). The profile of the mPGES-1 knockout mice is strongly supportive for a role of this enzyme in the production of inflammatory PGE₂ production with a marked reduction of LPS-stimulated PGE₂ synthesis in peritoneal macrophages (28) and a reduction in inflammation in the collagen-induced arthritis model (29).

Most of the studies on the regulation of mPGES-1 in vivo have been performed using models of IL-1 or LPS induced pyresis in which the expression of the enzyme has been shown to increase in the CNS and in certain peripheral tissues (23-25,27,28,30,31). Recently, mPGES-1 has also been shown to be inducible during a chronic model of adjuvant-induced arthritis (26,30). In the present study we report on the up-regulation of mPGES-1 in the CNS during the acute model of carrageenan-induced inflammation of the paw in rat, providing further evidence for the role of mPGES-1 in the increased synthesis of PGE₂ associated with inflammatory responses.
Materials and Methods

Model of carrageenan-induced edema in rat—All procedures for in vivo experiments were approved by the Animal Care Committee at the Merck Frosst Centre for Therapeutic Research (Kirkland, Quebec, Canada) and were performed according to guidelines established by the Canadian Council on Animal Care. Paw edema was induced in Sprague-Dawley rats (150-200 g) (Charles River Laboratories, St-Constant, Quebec, Canada) by injection of 100 ul of 1% carrageenan (lambda carrageenan, type IV, Sigma-Aldrich) diluted in saline in the left hind foot pad (31). Paw volumes were determined using a water plethysmometer (Ugo Basil, Italy). At time 0, 1, 3, 5, 6, 12 and 24 hours, rats were euthanized by carbon dioxide inhalation. The tissues (brain, spinal cord and soft paw tissue) were flash frozen in liquid nitrogen and kept at -80°C until processing. The CSF was collected and kept at -80°C for prostanoid analyses.

Preparation of tissue extracts—Frozen tissues were pulverized in liquid nitrogen using a mortar and pestle, homogenized in 5 volumes of ice-cold phosphate-buffered saline, pH 7.4, (2.5 mM GSH, 2 mM DTT and 1X of Complete Protease Inhibitor mixture (Roche Diagnostics GmbH, Manheim, Germany) with a tissue tearer (polytron PRO 200) and sonicated on an ice bath for 12 s (Kontes, Micro ultrasonic cell disrupter, output 70%). The tissue homogenates were centrifuged at 1,000 x g, 4°C for 10 min and the resulting supernatant was further centrifuged at 100,000 x g, 4°C for 90 min. The pellet (microsomal fraction) was resuspended in the cold homogenization buffer and the supernatant kept as the soluble fraction. Protein levels were determined using a Bradford protein assay (Bio-Rad Protein Assay, Bio-Rad, Hercules, CA). Fractions were kept at -80°C until further analysis.
Measurements of prostanoids by LC-MS—PGE$_2$, PGF$_{2\alpha}$, PGD$_2$, 6-keto-PGF$_{1\alpha}$ and TXB$_2$ in the soluble fractions were analyzed by liquid chromatography-mass spectrometry (LC-MS). PG standards and deuterated PGs were purchased from Cayman Chemical (Ann Arbor, MI); the non-deuterated PGs were purchased as solids.

Samples (100 µl) for LC-MS analysis were protein precipitated by the addition of acetonitrile (150 µL) containing deuterated prostaglandins (2 ng/ml) as internal standards (d4-PGE$_2$ served as internal standard for both PGE$_2$ and PGD$_2$). Samples were vortexed and centrifuged (3,000 x g, 10 min) and the supernatant transferred to a new 96-well plate. The plates were sealed using a TopSeal™ microplate heat sealing film (Packard Instrument B.V., Meriden, CT, USA). Samples were injected (50 µl) onto a 4.6 x 150 mm YMC ODS-A column using a Shimadzu SIL-HTc autosampler and LC-10ADVP pumps. Prostaglandins were eluted at 1 ml/min using a linear gradient from 10 to 90% acetonitrile vs. 0.1% formic acid over 10 min.

Detection of prostaglandins was achieved using a Sciex API-4000 triple quadrupole mass spectrometer. Analysis was carried out using negative ion electrospray with 1 ml/min entering the source. The source was operated at 500 °C, electrospray voltage was -4500 V, and gas 1 and gas 2 were 60 and 50, respectively. Each prostaglandin was optimized individually for parent mass and fragment mass sensitivity as shown in Table 1. The limit of detection ranged from 16 to 200 pg/ml depending on the prostaglandin.

Western Blot analyses—Aliquots containing 25 or 50 µg of protein were loaded on SDS-PAGE using 4-20% gradient gels (Novex, Invitrogen) and transferred electrophoretically to polyvinylidene difluoride membranes using a Novex immunoblot apparatus according to manufacturer instructions. Immunodetection was performed using COX-2 (1/300 dilution) and
mPGES-1 (1/300) purified antibodies (Cayman Chemical). Chemiluminescence detection was performed using Fuji Film LAS-1000 charge-couple device and Image Gauge software for quantitative analysis or by film exposure (Kodak BioMax MR,Eastman Kodak company, Rochester, N.Y.) using BIO-RAD GS-800 Densitometer and Quantity One software for analysis.

Analyses of mRNA by qPCR—The mRNA isolation and quantification were performed as previously described (26) with some modifications. Briefly frozen tissues were pulverized in liquid nitrogen using mortar and pestle, homogenized in TRIzol reagent (Canadian Life Technologies, Burlington, Ont., Canada) and total RNA was isolated according to manufacturer's instructions. A clean-up of the RNA was performed using the RNeasy mini kit (Qiagen, Mississauga, Ont., Canada) as described in the manufacturer's instruction and including a Dnase 1 treatment (Rnase-Free Dnase set from Qiagen). The quality of mRNA was evaluated using RNA 6000 Nano assay and Agilent 2100 Bioanalyser (Agilent technologies, Waldbronn, Germany).

Reverse transcription of RNA (50 ng) was performed using Taqman transcription reagents (PE Biosystems, Branchburg, NJ) using 1X Taqman reverse transcription buffer, 5.5 mM magnesium chloride, 500 µM dNTP, 2.5 µM random hexamers, 0.4 U/µl Rnase inhibitors and 1.25 U/µl Multiscribe reverse transcriptase. Real-time quantitative PCR for mPGES-1, COX-2, COX-1, cPGES, mPGES-2 and PGIS was performed using probes and primers as fully described by Claveau et al. (26). The oligonucleotide sequences used for PGDS and TXS probes and primers were as follow: PGDS probe, 5’-ACGCGTACTCATCGTAGTCTGTTTCTACCA-3’; PGDS forward primer, 5’-CCCGGACAGTACACCTACAACAG-3’; PGDS reverse primer, 5’-TGGTGCCCTTGCTGAACAG-3’; TXS probe, 5’-TCGATGCCAAAGGCCCACACTGG-3’;
TXS forward primer, 5’-CCAGAGGTGTACTGCTTTTTACC-3’; TXS reverse primer, 5’-GGAGCATCTGGGAGGTCAC-3’.

Statistical analysis—Each n value corresponds to a different animal and is indicated in the figure legend. Standard one-way analysis of variance was used to compare the multiple groups. The data were log-scaled so that underlying assumptions of equal variance and normality were better satisfied, and expressions of effects could be made in terms of percent change. The means and standard errors for each group were estimated from the analysis of variance, which pools the data variability from the 14 groups under the common assumption that the population variances are equal. All follow-up comparisons were deemed statistically significant at the 0.05 level unless noted otherwise.

Results

Time course of edema formation—Carrageenan-induced edema in the hind foot pad was used as a model to determine the temporal relationships between edema formation, prostanoid synthesis and expression of selected terminal prostanoid synthases both at the site of peripheral inflammation and in the CNS. Under the conditions used for carrageenan-induced inflammation, swelling of the paw occurred rapidly after the injection of carrageenan with an increase in volume of 1.5-fold at 1 h, which reached a maximum at 6 h (2.7-fold) and remained elevated until the last measurement at 24 h (Fig. 1).

Increased PGE₂ and TXB₂ levels in inflamed paw—The soft tissues of the paws were collected at different times after carrageenan or saline injection and were profiled for their content in PGE₂, PGD₂, PGF₂α, 6-keto-PGF₁α and TXB₂ by LC-MS analysis. PGE₂ levels in the paw were found to increase in two phases (Fig 2A). An initial 2- to 4-fold increase over the
measurements at t=0 and saline controls was observed from 1 to 6 h after the carrageenan injection. PGE$_2$ levels continued to increase with time, reaching 6- and 8-fold increases over their respective saline controls at 12 and 24 h.

Among the other prostanoids, the most noticeable changes were observed for TXB$_2$ with a modest but significant increase of about 2- to 4-fold at 3-5 h, similar to that of PGE$_2$ at these time points (Fig. 2B). In contrast to PGE$_2$, however, the increase was transient and levels of TXB$_2$ returned to near baseline at the 12 and 24 h time points. Basal levels of PGD$_2$ (0.05-0.08 ng/mg protein) and 6-keto-PGF$_1_\alpha$ (0.1-0.4 ng/mg protein) were detected but did not show any significant increase during inflammation as compared to controls (data not shown). The content in PGF$_2_\alpha$ was low throughout the time course (small peak of 0.04 ng/ml at 3 h post carrageenan; data not shown).

**Expression of terminal synthases in the inflamed paw**—The expression of the various prostanoid synthases was measured by real time quantitative PCR. The data are expressed as the relative expression of mRNA as compared to saline control paw at t=0 (and using 18S rRNA to normalize). Both COX-2 and mPGES-1 mRNAs were elevated in inflamed paws during the whole time course, with the peak of COX-2 mRNA (5-fold at 1 h versus t=0) preceding that of mPGES-1 (60-fold at 5 h) (Fig 3A). At 24 h the mPGES-1 mRNA was still 4-fold higher and statistically different from the saline injected control.

In contrast to COX-2 and mPGES-1, the mRNAs of COX-1 and mPGES-2 did not increase during edema (Fig. 3B). The cPGES showed only very modest changes with a small peak of induction detected at 5 h (about 2-fold vs t = 0) (Fig. 3B).

The induction of COX-2 and mPGES-1 was also determined at the protein level using Western blot analysis (Fig. 4). No signal could be detected for COX-2 in the tissues from the
saline controls at any time point, nor at t = 0 or 1 h after carrageenan injection. An increase in COX-2 protein expression was detected at 3 h and a large accumulation in protein at 24 h. The induction of the mPGES-1 in the carrageenan-injected paw was evident at 5 h (5-fold) with a larger accumulation in protein at 12 and 24 h (Fig. 4). A low level of basal mPGES-1 protein was also detected at t=0 and was not altered with time in saline injected controls. The cPGES and mPGES-2 proteins were detected at all time points but were not affected significantly by the inflammatory response (data not shown).

**Characteristics of the PGE2 response in the CNS during inflammation of the paw**—In order to better define the CNS response during peripheral inflammation, we also investigated the effect of carrageenan-induced paw inflammation changes on the CNS prostanoid profile and on the expression of terminal prostanoid synthases in brain and spinal cord. PGE2 was barely detectable in the CSF from naïve or saline-injected control animals (< 0.02 ng/ml). A significant augmentation in PGE2 was measured at 3 h after carrageenan injection and reached a peak at 6 h representing at least a 50-fold increase (Fig. 5A). In contrast to the paw tissues where PGE2 levels continued to increase until 24 h, PGE2 levels in the CSF declined at 12 and 24 h, but still remained significantly elevated as compared to corresponding saline controls (Fig. 5A).

Low basal levels of PGE2 were detected in the extracts from spinal cord and brain tissues that were not altered with time in control animals injected with saline. Carrageenan injection in the paw caused a marked increase of PGE2 in the spinal cord with a maximum at 6 h (6-fold) and a time course (Fig. 5B) that was similar to that observed for the CSF. In the brain, the increase in PGE2 levels (2-fold increase at 3-6 h) was less pronounced than in the spinal cord (Fig. 5B).

**Elevation in prostaglandin and TXB2 levels in the CNS during the early phase of inflammation**—The tissues from the CNS were also analyzed for their contents of the other
prostanoids. In the CSF, 6-keto-PGF$_{1\alpha}$, PGD$_2$, TXB$_2$ levels were all at the limit of detection of the LC-MS analysis for samples from untreated animals or from the controls collected at different times after saline injection. After carrageenan injection, 6-keto-PGF$_{1\alpha}$ was detected at elevated levels in the CSF at the 3, 5 and 6 h, while PGD$_2$ and TXB$_2$ showed increases at 6 h (Fig. 6A). PGF$_{2\alpha}$ could not be detected at any time point in the CSF. Only small changes for these prostanoids were detected in brain and spinal cord extracts. In the spinal cord, small transient increases (~2-fold) were observed at 6 h for 6-keto-PGF$_{1\alpha}$, PGD$_2$, TXB$_2$ and PGF$_{2\alpha}$ (data not shown). In the brain, PGD$_2$, PGF$_{2\alpha}$, TXB$_2$ and 6-keto-PGF$_{1\alpha}$ all showed minor increases at 3-6 h (1.5 to 2-fold) which reached statistical significance over t=0 and saline only for PGD$_2$ and PGF$_{2\alpha}$ (Fig. 6B).

A marked up-regulation of mPGES-1 but not of PGIS, TXS, and PGDS expression in the CNS during inflammation of the paw—Total brain and spinal cord extracts were prepared from animals treated with carrageenan for different periods of time and were analyzed by qPCR for expression of COX and PGE terminal synthases. As shown in Fig. 7A, mPGES-1 and COX-2 are both strongly induced in the spinal cord with maximal increases between 3 and 6 hours. In the brain, we observed an 8- to 10-fold induction of mPGES-1 at 3-6 h while the COX-2 showed only a small but significant 2-fold induction at 1-5 h (Fig. 7B). In contrast, there was no induction at the mRNA level for COX-1, cPGES or mPGES-2 in the spinal cord or in the brain (Fig. 7A,B). Microsomal fractions were prepared from spinal cord homogenates in order to enrich mPGES-1 protein for Western blot analysis. A protein band corresponding to mPGES-1 was detected at 6 and 24 h after the initiation of inflammation at about 3-times higher levels than in the control, indicating that the induction of mPGES-1 also occurs at the protein level (Fig. 7C). Tissues were also analyzed for PGIS mRNAs since deletion of the prostacyclin receptor
(IP) has suggested a role for prostacyclin in edema formation and pain (12,32). As shown in Fig. 8A, no significant induction of PGIS could be observed during the development of paw edema in brain, spinal cord or inflamed tissue from the paw. Similarly, PGDS and TXS expression in the brain and spinal cord did not show any significant changes during the inflammation response (Fig. 8B,C). These results confirm the involvement of COX-2 in the production of PGs in the CNS during inflammation and show that the selective induction of mPGES-1 is associated with the pronounced increase in PGE\textsubscript{2} levels.

**Discussion**

Both PGE\textsubscript{2} and PGI\textsubscript{2} have been implicated as mediators of the inflammatory and pain responses. In the present study, we seek to obtain more information on the role of these and other prostanoids by examining the changes in tissue levels of the major prostanoids and the expression of terminal prostanoid synthases in the CNS as compared to the site of peripheral footpad edema.

*Induction of COX-2 and mPGES-1 during sustained PGE\textsubscript{2} production in inflamed paws—*

Previous studies have shown that COX-2 is detected at elevated levels in paw tissues and in the CNS (33-35) following carrageenan-induced inflammation. In the current model, swelling of the paw progressively increased over the first 6 h and the edema persisted for at least 24 h. A very early induction of COX-2 occurred in the paw, with maximal mRNA expression at 1 h, together with an increased expression of mPGES-1 that peaked at 6 h and remained elevated over controls until the last 24 h time point. The sequential induction of COX-2 and mPGES-1 observed *in vivo* paralleled the *in vitro* data obtained with cell lines during stimulation of PGE\textsubscript{2} production (21,36). Both enzymes accumulate at the protein level in paws at later time points during the
maintenance of inflammation as PGE2 levels continued to increase. These data indicate the early phase of the modest increase in PGE2 might be primarily COX-2 dependent and that both elevated COX-2 and mPGES-1 contribute to a more sustained production and accumulation of PGE2 at the inflammation site. Of the three PGE2 synthases evaluated (cPGES, mPGES-1 and mPGES-2), only mPGES-1 mRNA was strongly up-regulated (a transient doubling of expression was detected for cPGES) as observed in the adjuvant-induced arthritis model in rat (26) and after IL-1β induced inflammation in mouse brain (37) although a small increase in cPGES protein could also be detected in these models. Although no induction was seen in the current model, mPGES-2 (designated as GBF-1) has been reported recently to be inducible in response to interferon-γ (38). It should be noted that the mPGES-1 protein was detected in extracts of normal paws and thus the constitutive expression of the enzyme might contribute to basal PGE2 levels as well as to the early phase of PGE2 production. Constitutive expression of mPGES-1 has also been reported previously in the mouse hypothalamus (39).

Carrageenan-induced edema of the paw causes an up-regulation of COX-2 and a general increase in prostanoids in the CNS during the early phase of inflammation—The up-regulation of expression of COX-2 mRNA in the CNS was rapid, detectable at the first one hour time point, and was more pronounced in the spinal cord than in whole brain tissue extracts. In both tissues the elevation of COX-2 was accompanied by an increase in PGE2 (2- to 5-fold) and a more modest increase in PGD2, PGF2α, TXB2 and 6-keto-PGF1α during the early phase. PGF2α and PGD2 have also been implicated in centrally mediated pain responses. It has been shown that the intrathecal administration of PGE2 and PGF2α at low doses produced hyperalgesia to mechanical stimuli in rats (40). These two prostanoids have also been reported to cause touch-evoked allodynia in mice (41,42), but with much weaker effects in rats (40) which may reflect species
differences in prostanoid responses. In mice, there is strong evidence that PGD$_2$ plays a role in nociception (43) and is essential for PGE$_2$ induced alldynia (44). In the present study with rats, PGD$_2$ was the most abundant prostanoid in brain extracts, consistent with previous reports (45), and the increase in PGD$_2$ in the brain during the early phase of inflammation was statistically significant vs both t=0 and saline controls. Although the level of stimulation was very low, the maximal levels reached in the spinal cord during the early phase were 1.3 ± 0.3 ng/mg protein for PGD$_2$ and 0.28 ± 0.04 ng/mg protein for PGF$_{2\alpha}$ as compared to 0.6 ± 0.1 ng/mg protein for PGE$_2$. Thus it is possible that both PGD$_2$ and PGF$_{2\alpha}$, in addition to PGE$_2$, contribute to pain responses in carrageenan-induced inflammation. In the CSF, where the amounts of prostanoids are extremely low in untreated animals, marked increases in PGD$_2$, TXB$_2$ and 6-keto-PGF$_{1\alpha}$ were detected during the early phase. These prostanoids were also found to increase in the CNS after kainate induced COX-2 expression (46) and neuronal COX-2 overexpression (47). All these observations are consistent with the present data suggesting that COX-2 plays a role in the activation of prostanoid synthetic pathways other than the PGE$_2$ pathway in the CNS. Although 6-keto-PGF$_{1\alpha}$, TXB$_2$ and PGD$_2$ were all found to increase in the CSF, no increase in the corresponding terminal synthases could be detected. These data are consistent with the up-regulation of COX-2 resulting in an increased synthesis of the PGH$_2$ substrate available for each of the prostacyclin, TX and PGD synthetic pathways during the acute phase of inflammation.

**Peripheral inflammation causes a pronounced elevation of PGE$_2$ and the selective induction of mPGES-1 in the CNS**—As compared to the other prostanoids measured, PGE$_2$ showed the largest induction in the spinal cord and reached the highest levels in the CSF among the prostanoids tested (by about 3-fold) during the early phase. In contrast to the large accumulation of PGE$_2$ observed for the paw, PGE$_2$ levels in the CNS decreased after the peak at
6 h (but were still slightly higher in spinal cord and in the CSF than in the corresponding saline controls at 24 h). The present data indicate that this elevation in PGE₂ correlated with the marked and selective induction of mPGES-1 in the spinal cord. In whole brain extracts, a large increase of mPGES-1 also occurred, but the increases in PGE₂ and COX-2 were less pronounced.

COX-2 induction in the CNS has been proposed to result from the afferent neuronal input and from an increase in circulating of pro-inflammatory cytokines. Strong evidence that IL-1β, whose levels are highly up-regulated in the paw and in the CSF following injection of the paw with Freund’s adjuvant, represents a major mediator of COX-2 induction in the CNS (8). The inducibility of mPGES-1 has been extensively characterized in rat brain following treatment with IL-1β or LPS (23,24,27,48,49) and during adjuvant-induced arthritis (30). In these studies, the peak of expression of COX-2 preceded that of mPGES-1 following the induction of fever with LPS or the intravenous administration of IL-1β (8,24,27). The characteristics of the present model are thus consistent with an IL-1 mediated process where both COX-2 and mPGES-1 are rapidly induced in sequence to contribute the maximal PGE₂ production (at 6 h) and elevated levels throughout the inflammation response. PGE₂ in the spinal cord can contribute to potentiation of peripheral edema (6), to the enhanced neuron hyperexcitability (50) and hyperalgesia (7,33).

Prostacyclin levels are elevated in the CNS during the early phase carrageenan-induced paw edema without significant up-regulation of PGIS–Studies with the IP receptor KO mice have suggested a role for prostacyclin in this model (12). We have not detected any significant increase in the stable prostacyclin metabolite 6-keto-PGF₁α in inflamed paws. In another model of inflammation in rat (adjuvant-induced arthritis), 6-keto-PGF₁α was found to show a transient increase in the paw (26) and to be elevated in the spinal cord during chronic inflammation (51).
In the early phase of carrageenan-induced edema we observed small increases in 6-keto-PGF$_{1\alpha}$ in the brain and the spinal cord comparable to those observed for the other prostanoids. Interestingly, the increase in 6-keto-PGF$_{1\alpha}$ in the CSF appears to occur earlier than that of PGD$_2$ and TXB$_2$. In whole extracts of brain, spinal cord or paw tissues, no major change of PGIS expression (< 2-fold) was detected, in contrast to mPGES-1. The increase in 6-keto-PGF$_{1\alpha}$ detected in the CNS thus appears to reflect the increase in COX-2 activity leading to an increase in PGH$_2$ substrate availability for the different prostaglandins, prostacyclin and TX pathways. It has been shown recently that the expression of the IP receptor increases in the spinal cord and that the IP receptor agonist cicaprost induces mechanical hyperalgesia in inflamed paw (52), suggesting of a role for PGI$_2$ in acute inflammation.

In summary, we have found that the induction of COX-2 that occurs in the CNS during carrageenan-induced paw inflammation leads to an increase in PGs, prostacyclin and TX in the early phase and to a large increase in PGE$_2$ production associated with selective up-regulation of mPGES-1. The results of this study provide further evidence for the implication of mPGES-1 as an important terminal enzyme for COX-2-mediated synthesis of inflammatory PGE$_2$, not only at the site of inflammation but also in the CNS. The data from this study, along with other observations on the induction of the mPGES-1 during fever and adjuvant-induced arthritis, and on the profile of the mPGES-1 KO mice showing reduced sensitivity to collagen-induced arthritis are all consistent with the proposals that mPGES-1 plays a role in inflammatory responses and represents a potential therapeutic target for novel anti-inflammatory agents.

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Reference List


Footnotes

1 The abbreviations used are: COX, cyclooxygenase; CNS, central nervous system; CSF, cerebrospinal fluid; cPGES, cytosolic prostaglandin E2 synthase; LPS, lipopolysaccharide; mPGES microsomal prostaglandin E2 synthase; PG, prostaglandin; PGDS, lipocalin type prostaglandin D synthase; PGIS prostacyclin synthase; TX, thromboxane; TXS, thromboxane synthase.

Figure legends

Figure 1. Time course of carrageenan-induced edema in the rat paw. Results are expressed as paw volumes (means ± SE, n=4 animals) determined at different times after injection of carrageenan (or saline) in the left hindfoot pad.

Figure 2. Increase in the levels of prostanoids in paw tissues during carrageenan-induced edema. The contents in PGE2 (A) and TXB2 (B) were determined in soluble extracts prepared from the soft tissues of the paw collected at different times after carrageenan administration. Analyses were performed by LC-MS. Data are reported as means ± SE (n= 5 or 6 for carrageenan samples; n=2 for saline controls). * Different from both carrageenan at t=0 and corresponding saline-injected control at p< 0.05.

Figure 3. Real-time quantitative PCR analysis of the expression of the mRNAs of mPGES-1, COX-2, COX-1, cPGES and mPGES-2 in paw tissues during carrageenan-induced edema. At the different time points total RNA was isolated from paw soft tissues, reverse-transcribed and analyzed by real-time quantitative PCR analysis. The levels of mRNA are expressed relative to that of the saline control at t=0, after normalization to 18S rRNA. Data are reported as means ± SE (n=5 or 6 for carrageenan samples; n=2 for saline). * Significantly
different from both the value at $t=0$ and the corresponding saline-injected control (data not shown) at $p<0.05$.

Figure 4. Increases in the expression of COX-2 and mPGES-1 proteins in the inflamed paw following the administration of carrageenan. Fifty $\mu$g of protein of the microsomal fraction from extracts of paw tissues collected at the indicated times after the injection of saline (s) or carrageenan (c) were analyzed by immunoblot for the mPGES-1 or COX-2 protein.

Figure 5. Increase in the level of PGE$_2$ in CSF, spinal cord and brain extracts during carrageenan-induced paw edema. PGE$_2$ levels in the CSF are expressed in ng/ml as mean ± SE (n=4) (A). PGE$_2$ levels in brain (n=5 for carrageenan samples; n=2 for saline) and spinal cord (n=3 for carrageenan; n=1 for saline) are expressed in ng/mg of protein of soluble extracts (B). * Different from both $t=0$ and saline controls at $p<0.05$.

Figure 6. Variation in prostanoid levels of the CSF and brain during carrageenan-induced paw edema. The tissue content in 6-keto-PGF$_{1\alpha}$, PGD$_2$, TXB$_2$ and PGF$_{2\alpha}$, was determined by LC-MS. Data are reported as means ± SE for CSF (n=4) (A) and for brain soluble extracts (n=5) (B). * Significantly different from both $t=0$ and saline controls (data not shown) at $p<0.05$.

Figure 7. Real-time quantitative PCR analysis of the expression of the mRNA of mPGES-1, COX-2, COX-1, cPGES and mPGES-2 in the CNS during carrageenan-induced paw edema. Data are reported as ratios (means ± SE) to the corresponding saline controls at $t=0$ for (A) the spinal cord (n=3) and (B) brain tissues (n=5). * Significantly different from both $t=0$ and
saline controls (data not shown) at p<0.05. (C) Immunoblot analysis of mPGES-1 in microsomal fractions from spinal cords of carrageenan-treated animals collected at time 0, 6 and 24 h. Purified rat mPGES-1 was used as standard.

Figure 8. Expression of PGIS, PGDS and TXS during carrageenan-induced paw edema. The expression of mRNA was monitored by real-time quantitative PCR analysis in selected tissues collected at different time points after carrageenan injection. Data are reported as means ± SE for the spinal cord (n=3), brain (n=5) or paw soft tissues (n=5 or 6). No significant difference was detected when compared to both t=0 and the corresponding saline controls (data not shown).

Table 1. MS conditions for the analyses of prostaglandins and TXB₂.

<table>
<thead>
<tr>
<th>Prostaglandin</th>
<th>Q1 Mass</th>
<th>Q3 Mass</th>
<th>DP Voltage</th>
<th>Collision Energy</th>
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<tr>
<td>PGE₂ and PGD₂</td>
<td>351.3</td>
<td>315.1</td>
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<td>-16</td>
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<tr>
<td>d4-PGE₂</td>
<td>355.3</td>
<td>319.1</td>
<td>-70</td>
<td>-16</td>
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<td>PGF₂α</td>
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<td>309.0</td>
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<td>-26</td>
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<tr>
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<td>313.0</td>
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<td>-26</td>
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<td>-36</td>
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<tr>
<td>d4-6-keto-PGF₁α</td>
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<tr>
<td>TXB₂</td>
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<td>-28</td>
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<tr>
<td>d4-TXB₂</td>
<td>373.1</td>
<td>168.8</td>
<td>-65</td>
<td>-28</td>
</tr>
</tbody>
</table>
Figure 3
Figure 4
**Figure 5**

A

- **Carrageenan**
- **Saline**

Time (h)

PGE2 (ng/ml)

B

- **Spinal cord-carrageenan**
- **Spinal cord-saline**
- **Brain-carrageenan**
- **Brain-saline**

PGE2 (ng/mg protein)

Time (h)
Figure 6

A

Prostanoids levels in CSF (ng/ml)

- 6-keto-PGF$_{1\alpha}$
- PGD$_2$
- TXB$_2$

Time (h)

B

Prostanoids levels in brain (ng/mg protein)

- PGD$_2$
- PGF$_{2\alpha}$

Time (h)
Figure 7
Figure 8
Carrageenan-induced paw edema in rat elicits a predominant PGE2 response in the central nervous system associated with the induction of microsomal prostaglandin E2 synthase-1

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