Center for Structural Biochemistry, Department of Biosciences at Novum
and
Department of Medical Biochemistry and Biophysics
Karolinska Institutet, Stockholm, Sweden

# mPGES-1: A KEY REGULATOR OF FEVER AND NEONATAL RESPIRATORY DEPRESSION

Sipra Saha


Stockholm 2006

# ABSTRACT

Prostaglandins are potent lipid mediators, synthesized *de novo* from arachidonic acid (AA) upon cell activation. AA is oxidized by the cyclooxygenase isoenzymes (COX-1 and COX-2) to form $PGH_2$, the common substrate for downstream enzymes involved in prostaglandin biosynthesis. COX-1 is constitutively expressed in most cells and regarded as housekeeping protein. In contrast, expression of COX-2 is markedly increased by pro-inflammatory cytokines at sites of inflammation. $PGH_2$ is converted into biologically active prostanoids like $PGD_2$, $PGE_2$, $PGF_{2\alpha}$, $TXA_2$ and $PGI_2$ by specific enzymes in a cell-specific manner. The isomerization of $PGH_2$ to $PGE_2$, a potent mediator of pain and inflammation, is specifically catalyzed by human microsomal prostaglandin E synthase-1 (mPGES-1), a member of the MAPEG (**m**embrane **a**ssociated **p**roteins in **e**icosanoid and **g**lutathione metabolism) superfamily. High levels of $PGE_2$ have been found in numerous disease states.

Human mPGES-1 was expressed as an N-terminal-histidine-tagged protein in *E. coli*. The membrane bound enzyme was solubilized using Triton X-100 and purified to apparent homogeneity using a combination of hydroxyapatite and immobilized metal affinity chromatography. Purified mPGES-1 exhibited high glutathione (GSH)-dependent catalytic activity for the conversion of both $PGH_2$ to $PGE_2$ and, $PGG_2$ to 15-hydroperoxy-$PGE_2$. Moreover, mPGES-1 also exhibited GSH-dependent peroxidase activity towards cumene hydroperoxide and 5-HpETE as well as low but significant glutathione transferase activity, possibly reflecting a relationship to other members of the MAPEG family. A 10 Å projection map of mPGES-1 determined using electron crystallography as well as hydrodynamic studies of mPGES-1-Triton X-100 complex, independently demonstrated the trimeric organization of mPGES-1.

The role of mPGES-1 in endotoxin-induced fever, as well as aseptic, cytokine-dependent, inflammation-induced fever was investigated. In response to intraperitoneal injection of lipopolysaccharide (LPS), wildtype DBA/1lacJ mice developed a robust fever with markedly increased $PGE_2$ levels in the cerebrospinal fluid (CSF) and significant LPS-induced mPGES-1 activity in membrane fractions isolated from brain tissues. In contrast, the mPGES-1 knockout mice did not develop fever and, the $PGE_2$ levels in the CSF did not differ significantly from the saline-treated wildtype mice, suggesting a critical role for mPGES-1 in the development of endotoxin-induced fever. In a cytokine-dependent fever model, subcutaneous injection of turpentine induced biphasic fever in wildtype mice, whereas mPGES-1 knockout mice displayed a core body temperature similar to the saline-treated wildtype mice, indicating that mPGES-1 activity was indispensable for the induction of cytokine-dependent fever. mPGES-1 did not, however, mediate hyperthermia induced by psychological stress.

The role of mPGES-1 in neonatal respiratory depression was investigated using 9-day old DBA/1lacj mice. Wildtype mice treated with IL-1β exhibited a reduced respiratory frequency during normoxia as well hyperoxia compared to saline treated mice. This effect of IL-1β was attenuated in mPGES-1 knockout mice. Moreover, IL-1β treatment induced apneas, irregular breathing pattern and reduced the anoxic survival of the wildtype mice, and these effects were attenuated in mice lacking mPGES-1. Both IL-1β and hypoxia treatment synergistically induced a rapid 4-fold mPGES-1 activity in the brainstem of wildtype mice compared to the saline treatment. These results suggest a central role for mPGES-1 in the regulation of neonatal breathing.

Taken together, these findings provide further support of mPGES-1 as an attractive target for the development of anti-inflammatory and anti-pyretic drugs. It is also possible that such drugs could be used in neonates at risk for respiratory suppression.

ISBN 91-7140-750-2

**Matricharane samarpayami**

**In memory of my Parents**

List of Publications

The present thesis is based on the following original publications.

I. Thorén S, Weinander R, **Saha S**, Jegerschöld C, Pettersson PL, Samuelsson B, Hebert H, Hamberg M, Morgenstern R, Jakobsson P-J (2003). Human microsomal prostaglandin E synthase-1: purification, functional characterization, and projection structure determination. J. Biol Chem., 278(25): 22199-209

II. Engblom D, **Saha S**, Engström L, Dahlström M, Audoly LP, Jakobsson P-J, Blomqvist A (2003). Microsomal prostaglandin E synthase-1 is the central switch during immune induced pyresis. Nature Neuroscience., 6(11): 1137-38

III. **Saha S**, Engström L, Mackerlova L, Jakobsson P-J, Blomqvist A (2005). Impaired febrile responses to immune challenge in mice deficient in microsomal prostaglandin E synthase-1. Am.J. Physiol Regul Integr Comp Physiol., 288(5): R1100-7

IV. Hofstetter, A, **Saha S**, Siljehav, V, Jakobsson P-J, Herlenius E. Prostaglandin $E_2$ mediates respiratory depression induced by interleukin-1β and hypoxia. Manuscript

Publications not included in the thesis

- Ek M, Engblom D, **Saha S**, Blomqvist A, Jakobsson PJ, Ericsson-Dahlstrand A (2001). Inflammatory response: pathway across the blood-brain barrier. Nature., 410(6827): 430-1
- Engblom D, Ek M, **Saha S**, Ericsson-Dahlstrand A, Jakobsson P-J, Blomqvist A (2002). Prostaglandins as inflammatory messengers across the blood-brain barrier. J Mol Med., 80(1): 5-15
- Engblom D, Ek M, Andersson IM, **Saha S**, Dahlström M, Jakobsson P-J, Ericsson-Dahlstrand A, Blomqvist A (2002). Induction of microsomal prostaglandin E synthase in the rat brain endothelium and parenchyma in adjuvant-induced arthritis. J Comp Neurol., 452(3): 205-14
- Trebino CE, Stock JL, Gibbons CP, Naiman BM, Wachtmann TS, Umland JP, Pandher K, Lapointe JM, **Saha S**, Roach ML, Carter D, Thomas NA, Durtschi BA, McNeish JD, Hambor JE, Jakobsson P-J, Carty TJ, Perez JR, Audoly LP (2003). Impaired inflammatory and pain responses in mice lacking an inducible prostaglandin E synthase. Proc Natl Acad Sci U S A., 100(15): 9044-9
- Degousee N, Angoulvant D, Fazel S, Stefanski E, **Saha S**, Iliescu K, Lindsay TF, Fish JE, Marsden PA, Li RK, Audoly LP, Jakobsson P-J and Rubin BB (2006). C-Jun N-terminal kinase mediated stabilization of microsomal prostaglandin $E_2$ synthase-1 mRNA regulates delayed microsomal prostaglandin $E_2$ synthase-1expression and prostaglandin $E_2$ biosynthyesis by cardiomyocytes. JBC, article in press


Published and printed by Karolinska University Press
Box 200, SE-171 77 Stockholm, Sweden

ISBN 91-7140-750-2

# CONTENTS

# LIST OF ABBREVIATIONS

| | |
|---|---|
| AA | Arachidonic acid |
| BBB | Blood brain barrier |
| CDNB | 1-chloro-2,4-dinitrobenzene |
| CNS | Central nervous system |
| COX | Cyclooxygenase |
| cPGES | Cytosolic Prostaglandin E synthase |
| $cPLA_2$ | Cytosolic phospholipase $A_2$ |
| CSF | Cerebrospinal fluid |
| DP | Prostaglandin D receptor |
| EP | Prostaglandin E receptor |
| FLAP | Five lipoxygenase activating protein |
| FP | Prostaglandin F receptor |
| GSH | Reduced glutathione |
| IL-1α | Interleukin 1 alpha |
| IL-1β | Interleukin 1 beta |
| IL-1ra | Interleukin 1 receptor antagonist |
| $iPLA_2$ | Cytosolic $Ca^{2+}$-independent phospholipase $A_2$ |
| IP | Prostacyclin I receptor |
| LPS | Lipopolysaccharide |
| LTC4S | Leukotriene C4 synthase |
| MAPEG | Membrane associated proteins in eicosanoid and glutathione metabolism |
| MGST | Microsomal glutathione transferase |
| mPGES | Microsomal prostaglandin E synthase |
| NSAIDs | Non steroidal anti-inflammatory drugs |
| NTS | Nucleus of the solitary tract |
| $PGD_2$ | Prostaglandin $D_2$ |
| PGDH | Prostaglandin dehydrogenase |
| $PGE_2$ | Prostaglandin $E_2$ |
| $PGF_{2\alpha}$ | Prostaglandin $F_2$ alpha |
| $PGG_2$ | Prostaglandin $G_2$ |
| $PGH_2$ | Prostaglandin $H_2$ |
| $PGI_2$ | Prostaglandin $I_2$ (Prostacyclin) |
| PGT | Prostaglandin transport |
| POA | Preoptic area |
| Pre-BötzC | Pre-Bötzinger complex |
| RA | Rheumatoid arthritis |
| RP-HPLC | Reverse phase-high performance liquid chromatography |
| RT-PCR | Reverse transcriptase-Polymerase chain reaction |
| RVLM | Rostral ventrolateral medulla |
| SDS-PAGE | Sodium dodecyl sulphate-Polyacrylamide gel electrophoresis |
| $sPLA_2$ | Secretory phospholipase $A_2$ |
| TLR-4 | Toll like receptor 4 |
| TNF-α | Tumor necrosis factor alpha |
| $TXA_2$ | Thromboxane $A_2$ |

# INTRODUCTION

## BRIEF HISTORY

In 1930, Ulf Von Euler of Sweden described prostaglandins from human semen. Since prostate gland was the organ from which the substance was isolated, Von Euler named the substance as 'Prostaglandin' (379). Prior to this identification, Battez and Boulet in 1913 discovered that extracts from the human prostate gland could lower blood pressure and contract the urinary bladder in dogs (14). Similar research led by two gynecologists, Kurzrok and Lieb showed that human semen contracted and relaxed human uterus during artificial insemination (175). The research on prostaglandins was continued and the E and F series of prostaglandins were isolated by Bergström and Sjövall (19). Soon after, the structures of these compounds were solved by mass spectrometry (18). In 1964, Bergström and van Dorp independently observed that incubation of homogenates of the sheep vesicular gland with [$^3$H]-labeled arachidonic acid resulted in an enzymatic conversion to $PGE_2$ and thus established that prostaglandins originate from C20 polyunsaturated fatty acids (17, 357). Subsequently, Hamberg and Samuelsson detected and isolated an endoperoxide by short-time incubations of arachidonic acid with the microsomal fraction of homogenates of sheep vesicular glands and the endoperoxide was later termed prostaglandin $H_2$ ($PGH_2$) (113, 115).

In the 5$^{th}$ century BC, Hippocrates, a Greek physician found that a bitter powder extracted from willow bark could provide relief from pain and fever. The active-extract of the bark, called *salicin*, was isolated in its crystalline form in 1828 by Henri Leroux, a French pharmacist. Few years later Raffaele piria; an Italian chemist isolated the acid in the pure state. In 1971 Sir John Vane discovered that the analgesic effect of aspirin was the result of its inhibition of prostaglandin biosynthesis (358). In honor to their discoveries in the field of prostaglandins and related bioactive substances and the mechanism of action of aspirin, Sune Bergström, Bengt Samuelsson and Sir John Vane were awarded the 1982 Nobel Prize in Physiology or Medicine.

Prostaglandins are important mediators of various physiological processes such as regulation of gastrointestinal, renal and blood homeostasis. On the other hand, they also act as potent mediators of inflammation and fever. Two distinct cyclooxygenase isoenzymes were discovered, Cyclooxygenase-1 (COX-1), the constitutive form and Cyclooxygenase-2 (COX-2), the inducible enzyme (69, 173, 219, 383, 389). COX-2 specific inhibitors were developed in order to reduce the side effects caused by non-specific COX inhibitors. However, in 2004, Vioxx$^{TM}$, a COX-2 specific inhibitor was withdrawn by Merck from the market following several investigations showing increased risk of cardiovascular related deaths (315, 343).

## EICOSANOIDS

Eicosanoids are a class of polyunsaturated hydrophobic molecules that act as autocrine and paracrine mediators. The term 'eicosanoids' (evolved from Greek word *eicosi* meaning 20) is used collectively to denote fatty acid molecules with 20 carbon atoms. The eicosanoid family constitutes prostaglandins, leukotrienes, thromboxanes, lipoxins and other related compounds. Certain fatty acids such as linoleic acids (18:2 cis-$\Delta^9$, $\Delta^{12}$) and α-linolenic acids (18:3 cis-$\Delta^9$, $\Delta^{12}$, $\Delta^{15}$) are essential in the human diet. Humans can easily make monounsaturated fatty acids with a double bond at ω-9 positions, but do not have the enzyme machinery necessary to introduce a double bond beyond ω-9 position of the fatty acid chain. However, arachidonic acid (20:4, ω6) and dihomo-γ-linolenic acids (20:3, ω6) can be synthesized from linoleic acid. Figure 1 illustrates different precursors of eicosanoids.

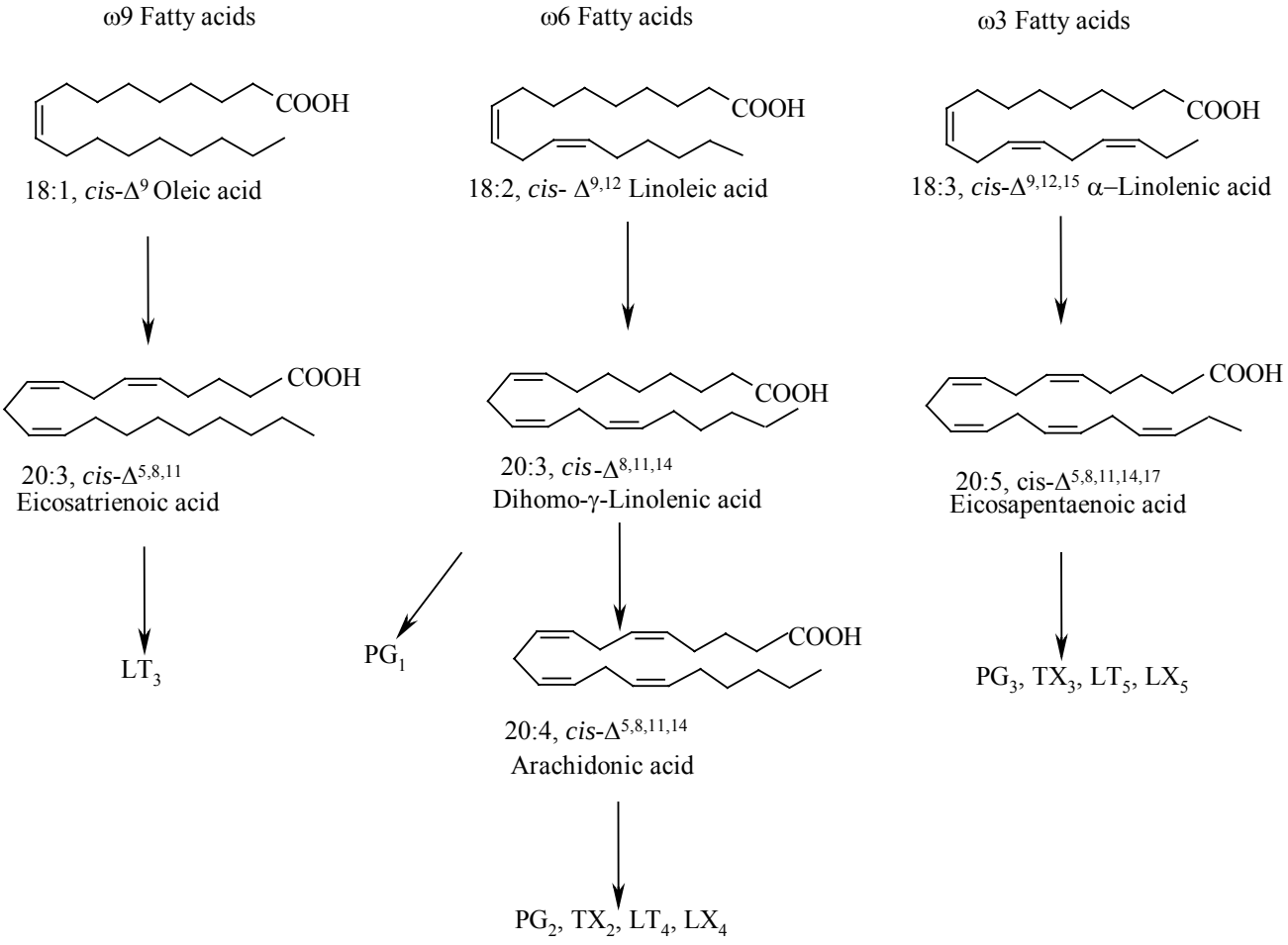


**Figure 1.** Precursors of eicosanoids.

## PHOSPHOLIPASES RELEASE ARACHIDONIC ACID

Among the various fatty acids, arachidonic acid is the most abundant in human cells. This $C_{20}$ polyunsaturated fatty acid is predominantly bound to the *sn*-2 position of membrane phospholipids (138). The level of free arachidonic acid under normal physiological condition is very low. However, different stimulatory agents trigger the release of arachidonic acid by phospholipases. Availability of free arachidonic acid is essential for the biosynthesis of eicosanoids and it is a rate-limiting step in this process. Phospholipases and acyl-CoA transferases determine the concentration of free arachidonic acid through hydrolysis and re-esterification of phospholipids (105). A number of different enzymes have so far been identified with phospholipase activity (11, 172) and present in different isoforms in different cell types (68). The superfamily of the phospholipase enzymes is divided into four classes: secretory ($sPLA_2$), cytosolic $Ca^{2+}$-dependent ($cPLA_2$), cytosolic $Ca^{2+}$-independent ($iPLA_2$) and platelet-activating factor (PAF) acetyl hydrolase (172).

### Phospholipase $A_2$

*$sPLA_2$s*

This group of secretory phospholipase $A_2$ consists of 14-19 kDa secreted enzymes and comprise the largest group of phospholipase enzymes. These enzymes contain a highly conserved $Ca^{2+}$ binding loop and a catalytic site. The presence of at least 6 conserved disulphide bonds contribute to the high degree of stability of these enzymes (172). $sPLA_2$s hydrolyze the ester bond at the *sn*-2 position of glycerophospholipids in the presence of mM concentrations of $Ca^{2+}$ with no strict fatty acid selectivity (172). The genes for the different subgroups of $sPLA_2$s enzymes are clustered on the same chromosome locus and are often referred to as the group II subfamily. Several of the $sPLA_2$ have been reported to take part in a number of biological processes such as inflammation and host defense. Secretory $PLA_2$-IIa is the most widely distributed isozyme in human. The expression of $sPLA_2$-IIA is markedly induced by pro-inflammatory stimuli (260) and downregulated by anti-inflammatory cytokines or glucocorticoids in a wide variety of cells and tissues (246). Using $sPLA_2$-IIA transgenic mice the anti-bacterial and atherosclerotic properties of $sPLA_2$ have been studied (177, 340). Indeed, $sPLA_2$-IIA possesses the strongest bactericidal activities among the $sPLA_2$ members and several lines of recent evidences suggest that the anti-bacterial function of $sPLA_2$-IIA appears to be the primary physiological function (160). Transgenic over-expression of $sPLA_2$-IIA in mouse testis leads to infertility due to the impairment of spermatogenesis indicating abnormal expression of this enzyme in male genital organs may cause developmental defects (95).

$sPLA_2$-IID is structurally similar to $sPLA_2$-IIA and constitutively expressed in the immune and digestive organs and upregulated by pro-inflammatory stimuli in some restricted tissues (139). $sPLA_2$-IIE, which is another $sPLA_2$-IIA related enzyme, is expressed constitutively in several tissues at low levels and also upregulated by pro-inflammatory stimuli (325). Similarly, $sPLA_2$-IIF is expressed in the testis of adult mice and also detected in low levels in various human tissues. This enzyme is thought to be involved in the regulation of developmental process as high levels of expression is found in mouse embryo (238, 356).

$sPLA_2$-III is an unusually large protein (55 kDa) among the $sPLA_2$ family. It is expressed in the kidney, heart, liver and skeletal muscles. $sPLA_2$-V is mainly detected in the human heart and lungs (48) and its expression is also found to be induced by pro-

inflammatory stimuli (300). sPLA$_2$-X is synthesized as a zymogen and the removal of the N-terminal propeptide produces an active mature enzyme (116).

*Cytosolic $Ca^{2+}$-dependent phospholipase $A_2$*

The cPLA$_2$ family consists of three isozymes, cPLA$_2\alpha$, cPLA$_2\beta$ and cPLA$_2\gamma$. cPLA$_2\alpha$ is constitutively expressed in most cells and tissues and its expression is induced under certain conditions (196). cPLA$_2\alpha$ shows remarkable selectivity toward phospholipids containing arachidonic acid at the *sn*-2 position (57). It also possesses *sn*-1 lysophospholipase activity and weak transacylase activity (202). In contrast, cPLA$_2\beta$ and cPLA$_2\gamma$ possess less specificity towards the fatty acid selectivity and, in fact, the *sn*-1 hydrolysis by these enzymes is more potent compared to *sn*-2 hydrolysis (278). Submicromolar concentration of $Ca^{2+}$ is required for the translocation of cPLA$_2\alpha$ from the cytosol to the nuclear membrane which is necessary for the release of arachidonic acid (289). cPLA$_2\alpha$ has been reported to play a role in cellular proliferation, transformation and oncogenesis in certain cell types. cPLA$_2\alpha$ deficient mice showed markedly reduced airway anaphylactic response (352), significantly reduced experimental Parkinsonian syndrome (157) and delayed onset of labor (29, 352) demonstrating its role in maintaining both physiological functions and pathophysiological reactions.

*Cytosolic $Ca^{2+}$-independent phospholipase $A_2$*

iPLA$_2$ is classified as group VI PLA$_2$ and two enzymatically active forms namely iPLA$_2$-VIA and -VIB have been identified. iPLA$_2$ exists in several splice variants (181, 331). iPLA$_2$-VIA and -VIB are ubiquitously expressed in various tissues and are fully active in the absence of $Ca^{2+}$. The iPLA$_2$ protein is about 85 kDa in size and contains lipase consensus sequence and ATP binding motif (2). Besides their roles as housekeeping genes, such as maintenance of phospholipids homeostasis, iPLA$_2$-VIA seems also involved in stimulus coupled AA release (243).

*PAF acetyl hydrolase*

PAF acetyl hydrolase (PAF-AH) specifically catalyzes the hydrolysis of phospholipids containing an acetyl group at *sn*-2 position and it degrades PAF, a potent inflammatory mediator. Two types of PAF-AH have been identified, a 45 kDa secreted form with potent anti-inflammatory properties (342) and an intracellular form containing three subunits of 29, 30 and 45 kDa (121). These two forms show significant sequence identity (~ 41%) and classified as group VII enzymes. High levels of the plasma type enzyme is expressed in thymus and tonsil, while the intracellular enzyme is abundant in liver and kidney (121, 342).

# METABOLISM OF ARACHIDONIC ACID

In mammals, oxygenation of free arachidonic acid is carried out by three different enzymatic pathways. The cyclooxygenase pathway produces prostaglandins and thromboxanes via production of the intermediate prostaglandin endoperoxides $PGG_2$ and $PGH_2$. The lipoxygenase pathway produces leukotrienes and certain hydroperoxy acids (HpETEs). The cytochrome P-450 pathway produces a series of epoxy and hydroxyl-acid-derivatives (313). In figure 2, the cyclooxygenase and 5-lipoxygenase pathways are briefly illustrated.

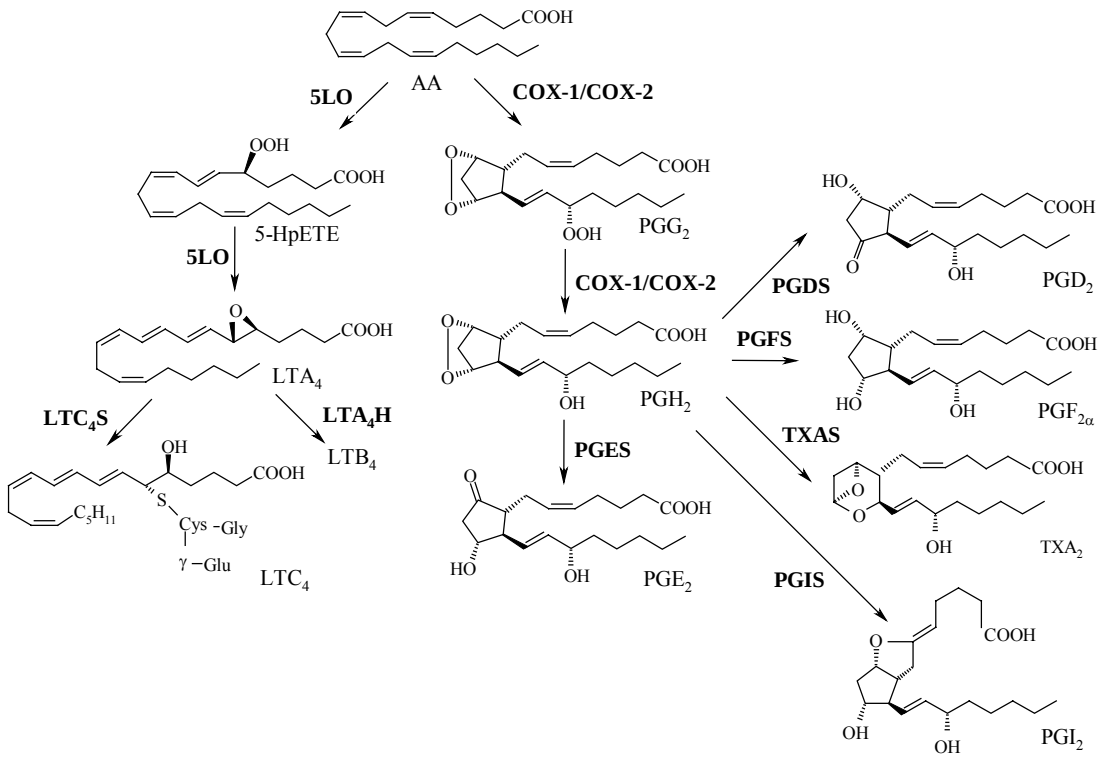


**Figure 2.** An overview of arachidonic acid metabolic pathway. The intermediate products of arachidonic acid such as $PGH_2$ and Leukotriene $A_4$ produced by the cyclooxygenase and the 5-lipoxygenase enzymes respectively are further metabolized by specific enzymes to produce leukotrienes and prostaglandins.

## BIOSYNTHESIS OF PROSTAGLANDINS

Prostaglandins represent a group of potent lipid mediators, formed by most cells in our body and act as local hormones. They are synthesized *de novo* from membrane-released arachidonic acid upon cell-stimulation, caused by mechanical stress, cytokines, growth factors or hormones. In activated cells, phospholipase $A_2$-derived arachidonic acid is metabolized to $PGH_2$ sequentially in a two-step reaction by either of the two cyclooxygenase isoforms (Figure 2). $PGH_2$ is the common substrate for several different downstream enzymes and accordingly converted to biologically active prostanoids *i.e.* $PGE_2$, $PGD_2$, $PGF_{2\alpha}$, $TXA_2$ or $PGI_2$ by their respective enzymes in a cell-specific manner. Once the prostaglandins are formed, they are released from the cells and bind to receptors specific for each prostanoid to exert their biological functions. In many cases only one prostanoid is produced in a given cell type. For

example, thromboxane $A_2$ synthase is present in platelets, prostacyclin synthase is present in endothelial cells and $PGF_{2\alpha}$ synthase is present in the uterus. Furthermore, two types of $PGD_2$ synthases have been identified in brain and mast cells. Microsomal prostaglandin E synthase-1, which specifically catalyzes the conversion of $PGH_2$ into $PGE_2$, is expressed mainly in activated cells involved in inflammation (143), including endothelial cells. Two more enzymes, the cytosolic PGE synthase (cPGES) (334) and the membrane bound PGE synthase-2 (mPGES-2) (332) have also been identified with PGE synthase activity. However, these enzymes are constitutively expressed in most cells and believed to constitute housekeeping genes.

**Cyclooxygenases**

Cyclooxygenase, also referred to as prostaglandin endoperoxide H synthase, is a membrane bound, heme-containing, bis-oxygenase and peroxidase. COX enzymes contain two distinct active sites, one cyclooxygenase and one peroxidase site that catalyze two sequential reactions. The cyclooxygenase reaction includes a double dioxygenation of arachidonic acids and formation of the ring structure in the carbon skeleton leading to the formation of $PGG_2$, a 15-hydroperoxy endoperoxide intermediate. In the next step, the peroxidase reaction carries out the reduction of $PGG_2$ to form the 15-hydroxyl endoperoxide compound, $PGH_2$ (113, 115). There exist two isoforms of COX, referred to as COX-1 and COX-2. In general, COX-1 is constitutively expressed in most organs and believed to be responsible for homeostatic functions. In contrast, expression of the COX-2 transcript is barely detectable in most quiescent cells, but is markedly upregulated by inflammatory stimuli (125). Both enzymes are located in the luminal surface of the endoplasmic reticulum and on the inner and outer membranes of the nuclear envelope (235). COX-1 and COX-2 enzymes share 60% identity in their amino acid sequence and nearly superimposable in overall folding (204, 279).

*COX-1 and -2: gene structure, expression and function*

In 1976, Miyamoto *et al* reported the purification of the COX-1 enzyme from bovine vesicular gland microsomes and demonstrated the enzyme to be a membrane-bound heme containing protein (227). The COX-1 cDNA was cloned from sheep vesicular glands (69, 219, 389) and soon after, the primary gene structure of the enzyme was characterized (390). The gene is located on the human chromosome 9q32-q33.3 and approximately 25 kb in size (166). The gene contains 11 exons and transcribed as 2.8 kb mRNA which is translated to form a protein with an apparent molecular mass of 68 kDa (169). There are several putative transcriptional regulatory elements (Sp1, AP-2, NF-IL-6 and GATA) in the promoter region of the COX-1 gene. However, the COX-1 promoter does not possess a TATA or CAAT box and rich in GC sequences, consistent with the features of the housekeeping gene. The two Sp1 sites contribute to constitutive expression of COX-1 (385). In addition to the constitutive expression of COX-1, increased expression has also been observed in several cell systems although not coupled to stimulus-induced expression (239, 347).

A second isoform of COX *i.e.* COX-2 was discovered independently by Xie *et al* (383) and Kubuju *et al* (173). The COX-2 gene is about 8 kb long, composed of 10 exons and located on human chromosome 1q25.2-q25.3. The mRNA is about 4.1-4.5 kb and encodes a protein of about 68 kDa (125). The promoter region of the COX-2

gene contains a TATA box as well as various transcription elements, such as NF-IL6, AP-2, Sp-1, NF-κB, CRE/E-box (220). NF-κB-dependent transcription of COX-2 has been shown to be suppressed by high concentrations of NSAIDs, which block IκB kinase activation (388). A number of studies have demonstrated stimulus induced expression of COX-2 enzyme in various inflammatory diseases (176, 374). Pro-inflammatory stimuli often has also increased the stability of COX-2 mRNA and proteins which requires the presence of an AU rich region (ARE) found within the 3' untranslated region of COX-2 mRNA (77, 185, 309). The induction of COX-2 is markedly downregulated by anti-inflammatory glucocorticoids, even though the COX-2 promoter does not contain a glucocorticoid response element (GRE). The mechanism for glucocorticoid-mediated downregulation of COX-2 induction has been demonstrated to involve suppression of the AP-1 and NFκB-dependent transcription (7, 301) as well as destabilization and degradation of COX-2 mRNA and protein (76, 254).

*Pathophysiological functions: lessons from mice deficient in COX-1/COX-2*

In order to better characterize the physiological and pathological functions of each COX isoform *in vivo*, mice deficient in either COX-1 or COX-2 were generated (180). Despite the thought that inhibition of COX-1 was associated with ulcerative effects of NSAIDs, genetic deficiency of COX-1 did not increase spontaneous gastric ulceration in mice (180, 198). COX-1 deficiency in female mice did not impede conception or fetal development but significantly prolonged gestation period and thereby reduced the offspring survival. In contrast, female mice deficient in COX-2 showed reproductive abnormalities with defects in ovulation and implantation (194). Although COX-1 has been thought to be the isoform primarily responsible for the maintenance of physiological processes in kidney, the genetic deficiency of COX-1 failed to produce an identifiable renal pathology. In contrast, COX-2 deficient mice showed severe developmental defects in the kidney. The contribution of each COX isoform in the development of inflammation was studied by applying the tumor promoter, 12-*O*-tetradecanoylphorbol-13-acetate (TPA) topically to the ear to induce edema. The level of edema induced by TPA was not significantly different between wildtype, COX-1 and COX-2 deficient mice. These findings were surprising, in particular for the COX-2 deficient mice, since TPA is a potent inducer of COX-2 expression and expected to mediate, at least in part, the inflammatory response to TPA (173). Furthermore, the role of COX isoforms in the development of intestinal tumorigenesis was studied using the *Min* (multiple intestinal neoplasia) mice model. *Min* mice have a chemically induced mutation in the *Apc* (*adenomatous polyposis coli*) gene, which results in a 100% incidence of neoplasia (267, 311). The *Min* mice deficient in either COX-1 or COX-2 showed 80% reduction in intestinal tumorigenesis (55) suggesting the involvement of both COX-1 and COX-2 enzyme in intestinal tumorigenesis. In a collagen induced arthritis (CIA) model, COX-2 deficient mice showed reduced incidence and severity of the disease compared to the wildtype and COX-1 deficient mice (245). A recent development in the area of cyclooxygenase research shows that disruption of COX-1 and COX-2 gene in mouse has effects both in the severity and nociception of diseases in a sex specific manner (52). In a chronic Freund's adjuvant-induced arthritis and inflammatory pain model, both COX-1 and COX-2 knockout females displayed less severe bone destruction and inflammation compared to the male knockouts.

*COX and NSAIDs*

Over the past years non-steroid anti-inflammatory drugs have been widely used to treat various types of chronic and acute pain. COX-1 and COX-2 are the major targets for NSAIDs. The classical NSAIDs inhibit both COX-1 and COX-2 isoenzymes producing therapeutic as well as adverse effects. Therapeutic doses of classical NSAIDs often lead to gastrointestinal bleeding and inhibition of mucoprotective prostaglandins. In 1999, Warner *et al* reported a full *in vitro* analysis of COX-1 &-2 selectivities for a wide range of NSAIDs and COX-2 selective compounds and discovered that inhibition of COX-1 underlies the gastrointestinal toxicity of NSAIDs (360) which also reconfirmed earlier observations (226). Using a carrageenan-induced inflammatory rat model, Seibrt *et al* have demonstrated that expression of COX-2 mRNA was upregulated in response to carrageenan and a selective COX-2 inhibitor blocked the resultant edema formation (308). The structural basis for the selective inhibition of COX-2 was explained from the structures of COX-2 and selective COX-2 inhibitors determined at 3.0-2.5Å resolution. This structure study also demonstrated some of the conformational changes associated with time dependent inhibition of COX-2 (174). In 1999, rofecoxib and celecoxib were the first two drugs in this new class of selective COX-2 inhibitors to be approved for use (42). In 2001, valdecoxib was another addition to this class of selective COX-2 inhibitors (270). These new generation of COX-2 inhibitors were widely used to treat inflammatory diseases such as rheumatoid arthritis (104, 152, 256), osteoarthritis (37, 179, 294), as well as neurodegenerative diseases including Alzheimer's disease (103) and Parkinson's disease (335, 336). Many forms of cancers were shown to be associated with overexpression of COX-2 and COX-2 specific inhibitors were used in attempts to lower the progression of these diseases (118, 267, 310, 327). However, in addition to the anti-inflammatory effect, COX-2 specific inhibitors were found to trigger cardiovascular disease such as myocardial infarction and stroke (170) and in September 2004, Vioxx was withdrawn from the market due to the increased risk of myocardial infarction found among the group taking 25mg/day rofecoxib (343). These side effects caused by COX-2 specific inhibitotrs bring limitations to the use of these drugs in general and patients with less sensitivity to gastrointestinal side effects are today treated with non-specific COX inhibitors.

*COX-3*

A third isoform of COX enzyme was thought to exist with the observation that acetaminophen, a drug with potent anti-pyretic action but very-weak anti-inflammatory activity reduces the levels of prostaglandin metabolites in urine (30). In 2003, Chandrasekharan *et al* reported the identification of a splice variant of COX-1, named COX-3 and cloned into baculovirus (43). The COX-3 mRNA is transcribed from COX-1 gene and retains intron-1 and therefore the COX-3 protein is also called COX-1b. RT-PCR of canine cerebral cortex RNA, as well as analysis of Northern blots indicated that COX-3 mRNA is present in the brain region at about 5% of the level of COX-1 mRNA. In human, COX-3 mRNA is transcribed as 5.2 kb transcript and abundantly expressed in cerebral cortex and heart. Recently the rat COX-3 mRNA from cerebral endothelial cells was cloned and a vector containing the rat COX-3 cDNA was transfected to COS 7 cells (314). Western blot analysis using an affinity-purified antibody against COX-3 protein demonstrated highest expression in heart, kidney and neuronal tissues. Notably, the COX-3 protein did not show any cyclooxygenase activity and thus the physiological significance for the existence of COX-3 is not known.

## PROSTANOID SYNTHASES

### Prostaglandin E synthases

Prostaglandin E synthases are enzymes involved in the catalyzation of cyclooxygenase derived $PGH_2$ to $PGE_2$. In 1999, Jakobsson *et al* for the first time reported cloning and expression of recombinant human microsomal prostaglandin E synthase-1 (mPGES-1) (143). Following this report, orthologs of this protein were cloned from several animal species (212, 242, 299). In addition one cytosolic (334) as well as another microsomal PGE synthase (332) have been identified. There are also various cytosolic glutathione-S-transferases with PGE synthase activity *albeit* with very low catalytic efficiency (20). This section will focus on the current understanding of different forms of PGE synthase enzymes.

*Identification of Microsomal Prostaglandin E synthase-1 and the MAPEG members*

mPGES-1 constitutes a member of the MAPEG (membrane associated proteins in eicosanoid and glutathione metabolism) superfamily (142). mPGES-1 is a glutathione dependent membrane bound protein. The human enzyme was initially discovered as an expressed sequence tag (EST) clone by database searches based on the amino acid sequence of microsomal glutathione S transferase 1 (MGST1) (142). The homologous EST clones were characterized and found to code for a novel protein with 38% sequence identity to MGST1 and hence the protein was referred to as MGST1-like 1 (MGST1-L1). The gene coding for MGST1-L1 was also independently identified as a p53 induced gene (PIG12), but no function was described (280). MGST1-L1 was subsequently found to specifically catalyze the conversion of $PGH_2$ to $PGE_2$ and henceforth referred to as mPGES-1 (143). Based on amino acid sequence, size, hydropathy profile (represented in figure 3) and membrane localization, mPGES-1 was classified as a member of the MAPEG superfamily (142). The six human protein members of the MAPEG superfamily are 16-18 kDa integral membrane proteins, including 5-lipoxygenase activating protein (FLAP), leukotriene $C_4$ synthase ($LTC_4S$), MGST1, MGST2, MGST3 and mPGES-1. Multiple sequence alignment demonstrates six conserved amino acid sequence in the human members. MGST1, -2 and -3 possess glutathione transferase and glutathione-dependent peroxidase activities, possibly demonstrating the roles of these enzymes in detoxification of xenobiotics and protection against oxidative stress. MGST1 is highly expressed in liver and acts on various lipid hydroperoxides (233, 236). FLAP, $LTC_4S$ and MGST2 form a group of enzyme involved in the biosynthesis of leukotrienes. FLAP has been demonstrated to present the substrate to 5-lipoxygenase (5-LO) and shown to be necessary for 5-LO activity (78, 211, 223). $LTC_4S$ specifically conjugates the 5-LO product $LTA_4$ with reduced glutathione to produce $LTC_4$ (178, 369). MGST2 and MGST3 were also found to possess $LTC_4S$ activity, however, they have broader substrate specificity (140, 141). The MAPEG superfamily also includes several plant members (*Arabidopsis thaliana*, *Oryza sativa* and *Ricinus communis*), fungi (*Aspergillus nidulans*) and bacterial members (*Synechosystis*, *Escherichia coli* and *Vibrio cholerae*).

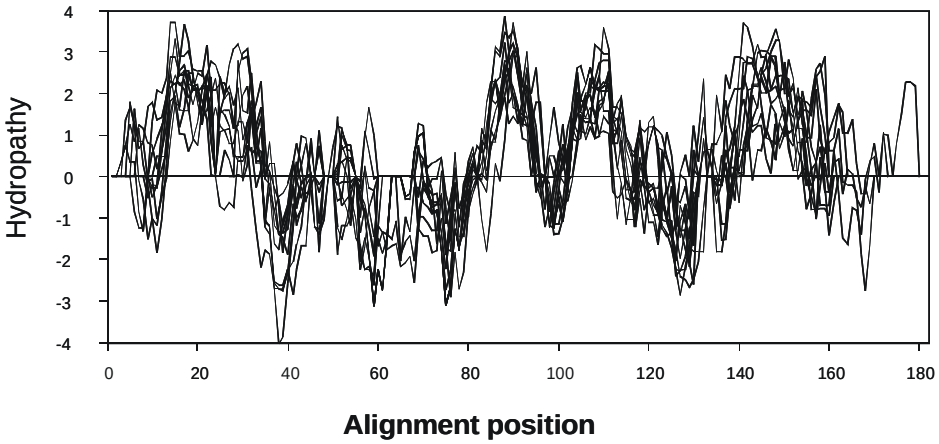


**Figure 3.** Hydropathy plots of the human MAPEG members based on multiple sequence alignments.

*Structure and enzymatic properties*

mPGES-1 has been characterized as an inducible, glutathione-dependent membrane bound enzyme (143). The primary structures of human, rat and mouse mPGES-1 demonstrated a high degree of amino acid sequence homology (> 80%). mPGES-1 also shows significant homology with other proteins in the MAPEG superfamily including MGST1, MGST2, MGST3, FLAP and $LTC_4$ synthase with the highest homology being found with MGST1 (38%). The hydropathy plot in figure 3 suggests that all MAPEG proteins have similar three dimensional and membrane-spanning topographic properties (142). All MAPEG proteins have similar molecular masses ranging from 14-18 kDa and all, except FLAP, have pI values of 10-11. Amino acid sequence alignment of the MAPEG family members demonstrates a strictly conserved $Arg^{110}$ residue. Murakami *et al* have demonstrated that replacement of $Arg^{110}$ by Ser abrogated the catalytic activity of mPGES-1 implying an essential role of this residue in protein function (242). Although $Tyr^{117}$ is also a conserved amino acid in all human MAPEG enzymes, this residue did not seem to play an essential role for the enzymatic activity of mPGES-1 or MGST1 (242, 368). mPGES-1 activity is inhibited by the COX-2 inhibitor NS-398 with $IC_{50}$ value of 20 μM *in vitro*. In addition, $LTC_4$ and sulindac sulphide are also weak inhibitors of mPGES-1 activity with $IC_{50}$ values of 5 μM and 80 μM respectively (339). 15-deoxy-$\Delta^{12,14}$-$PGJ_2$ and other fatty acids have also been recently described as inhibitors of mPGES-1 with similar $IC_{50}$ value (0.3 μM) (284).

The gene of mPGES-1 maps to chromosome 9q34.3 (94) and spans about 15 kb, divided into three exons. The putative promoter of the human mPGES-1 gene is GC-rich, lacks a TATA box and contains binding sites for C/EBPα and β, two AP-1 sites, two tandem GC boxes, two progesterone receptors and three GRE elements, two cAMP response elements, and six serum response elements (SRE) (248). A recent investigation demonstrated that the suppression of IL-1β-induced mPGES-1 activity by curcumin (an anti-cancer and anti-inflammatory agent) was mediated by the inhibition of transcriptional activity of EGR-1 (230). Recently Degousee *et al* described the role of Jun N-terminal kinase (JNK) for mPGES-1 expression in rat neonatal cardiomyocytes. By chromatin immunoprecipitation analyses and nuclear run off assays there was no evidence of increased transcriptional activity, however the IL-1β and LPS induction of mPGES-1 mRNA was found to depend on mRNA stability (67).

Under normal physiological conditions, the expression of mPGES-1 is very low; however, inflammatory stimuli promote an increase in the levels of mPGES-1 mRNA and protein in various tissues. Marked elevation of mPGES-1 usually occurs concomitantly with the induction of COX-2 (242) although discordant expression has been described (392, 393). Furthermore, coordinate upregulation of COX-2 and mPGES-1 and the subsequent attendant $PGE_2$ biosynthesis are reversed simultaneously to basal levels by glucocorticoids (339). When COX-2 and mPGES-1 were cotransfected into HEK293 cells, considerable amounts of $PGE_2$ was produced both from exogenous and endogenous AA relative to cells transfected with either enzyme alone, suggesting a coupling between COX-2 and mPGES-1 (242). However, a recent study showed that deletion of the mPGES-1 gene resulted in 80-90% decrease in basal, COX-1-dependent $PGE_2$ production in stomach and spleen, demonstrating the ability of mPGES-1 to couple also with COX-1 (31) as suggested previously by Dieter *et al* (71). The potential involvement of mPGES-1 in various pathological conditions will be elaborated later in the discussion section.

*Mice deficient in the mPGES-1 gene*

mPGES-1 deficient mice were generated by targeted homologous recombination in DBA/1lacJ embryonic stem cells and the phenotype was evaluated in several models of acute and chronic inflammation (345). These mice were born healthy and could not be distinguished from the wildtype littermates in general behavior, appearance and body weight or tissue histology. In a model of inflammatory pain, which was induced by intraperitoneal injection of dilute acetic acid, mPGES-1 deficient mice showed 40% decreased pain perception that was comparable to the response demonstrated by NSAID-treated wildtype mice. mPGES-1 was also found to play a major role in the pathogenesis of collagen induced arthritis (CIA), an experimental animal model of inflammatory arthritis, which in many ways resemble human rheumatoid arthritis (RA) (380). Macrophages derived from mPGES-1 knockout mice did not produce $PGE_2$ after LPS stimulation (348). In addition, inflammatory pain hypersensitivity was demonstrated to be much milder in mPGES-1 deficient mice compared to the wildtype littermates (148). The involvement of $PGE_2$ in neuropathy pain was demonstrated by use of mPGES-1 knockout mice (206). In a recent study Oshima *et al* have shown that transgenic mice expressing both COX-2 and mPGES-1 develop hyperplastic gastric tumors with inflammatory histopathology (268). As $PGE_2$ produced by mPGES-1 is involved in inflammation and because preterm deliveries are associated with infection combined with inflammation, the role of mPGES-1 was evaluated in preterm delivery using mPGES-1 knockout mice in LPS-induced preterm labor model (171). It was found that in the mPGES-1 knockout mice, LPS induced the expression of mPGES-2 in the myometrium and the fetal membrane and the duration of labor in mPGES-1 knockout mice was not statistically different from the wildtype mice. These results provide an indication that inhibition of mPGES-1 is not sufficient to prevent preterm labor as mPGES-2 might compensate the role of mPGES-1. In a very recent study by Hartney *et al* the impact of sustained alterations in $PGE_2$ pathways on changes in airway resistance was investigated (119). In this study, mice with a genetic deletion of 15-hydroxy prostaglandin enzyme that caused elevation of $PGE_2$ levels in lungs, presented attenuated airways responsiveness to metacholine as measured by lung resistance. All these data reveal a dual role played by mPGES-1; $PGE_2$ formed by

induced expression of mPGES-1 is mostly involved in pain and inflammatory processes, whereas $PGE_2$ produced under basal expression of mPGES-1 maintains certain physiological functions.

### Microsomal PGE synthase 2

mPGES-2 was expressed and purified from microsomes of bovine heart (363, 364). The corresponding monkey protein was recently cloned, expressed and purified (332). Interestingly, no sequence homology was found between the two membrane bound PGE synthase enzymes. The gene for mPGES-2 is localized to human chromosome 9q33-34 in proximity to the genes for mPGES-1, COX-1 and lipocalin-type PGDS (332). The cDNA encodes a 33 kDa protein with a consensus region of glutaredoxin and thioredoxin. Recombinant mPGES-2 can use different reducing agents, for instance dithiotheritol, glutathione or β-mercaptoethanol for its PGE synthase activity. Purified mPGES-2 displayed a $V_{max}$ and $K_m$ value of 3.3 $\mu mol \cdot min^{-1} \cdot mg^{-1}$ and 28 μM, respectively, for $PGH_2$ with a pH optimum of 6-7 (332). Northern blot analysis demonstrated that mPGES-2 mRNA was mainly localized in various regions of the brain and heart but not in genital organs as compared to mPGES-1. mPGES-2 is initially produced as a golgi membrane-associated protein followed by spontaneous cleavage of the N-terminal hydrophobic domain, leading to the formation of a truncated mature protein that is distributed in the cytosol (240). Cotransfection of mPGES-2 with either COX isozyme to HEK293 cells demonstrated that mPGES-2 could be coupled with both COX-1 and COX-2 (240). Although mPGES-2 is constitutively expressed in many cells and the expression was not induced by inflammatory stimuli, a marked elevation of this enzyme was demonstrated in human colorectal cancer tissues and cell lines (240). However, the role of mPGES-2 in cancer remains to be investigated. Crystallization of mPGES-2 reveals that it forms a homo dimer and attached to the lipid membrane by anchoring the N-terminal section (386).

### Cytosolic PGE synthase

Cytosolic PGE synthase (cPGES) is a glutathione-requiring enzyme, constitutively expressed in a variety of cells. cPGES is a 23 kDa protein and identical to p23, a heat shock protein 90 (Hsp90)-binding protein (334). cPGES is highly conserved among animal species (> 95%) and its gene consists of 8 exons (241). Although cPGES is expressed ubiquitously and constitutively in most cells, lipopolysaccharide (LPS) treatment evoked a three-fold increase in activity in the cytosol of rat brain (334). Co-transfection of cPGES and COX-1 in a human embryonic kidney cell line (HEK293) resulted in a ten-fold increase in $PGE_2$ formation, relative to the cells transfected with COX-1 alone, suggesting a functional coupling between these enzymes (334). Furthermore, IL-1 administration in the mouse cortex via intraparenchymal microinjection led to an increase in $PGE_2$ with concomitant elevated expression of cPGES as well as COX-2 and mPGES-1 (232). Recombinant cPGES/p23 was purified from *E. coli* and the $K_m$ value of this enzyme for $PGH_2$ was found to be 14 μM, which was comparable to other cytosolic terminal prostaglandin synthases. In a follow-up study it was shown that Hsp90 significantly affects the $PGE_2$-biosynthetic activity of cPGES (333). Furthermore, in the presence of ATP and $Mg^{2+}$, cPGES and Hsp90 formed a complex and the PGES activity of recombinant cPGES was increased by association with Hsp90 (333). Moreover, incubation of the rat fibroblastic 3Y1 cells

with Hsp90 inhibitors reduced the interaction of cPGES and Hsp90 to a basal level, accompanied by ablation of $PGE_2$ generation. In a more recent study, it has been proposed that functional cPGES exists in cells as a multicomponent complex containing Hsp90 and casein kinase II (CK-II) (159). According to this study, under normal culture conditions, only a small fraction of cPGES pool is complexed with CK-II and Hsp90 and undergoes steady state phosphorylation possibly through serum stimulation. However, activation of CK-II by upstream signals triggers dual phosphorylation of $Ser^{113}$ and $Ser^{118}$ on cPGES and promotes the recruitment of cPGES into the Hsp90 complex which finally leads to full activation of cPGES. This provides evidence that the functional aspect of cPGES is controlled by a protein kinase in cooperation with molecular chaperone.

*Functional Coupling between Cyclooxygenases and PGE synthases*

mPGES-1 and COX-2 enzymes have been found to be coexpressed in several tissues (242, 322, 339) indicating a functional coupling of these two enzymes in the downstream pathway of arachidonic acid metabolism to produce $PGE_2$. In line, rat peritoneal macrophages stimulated with LPS for various periods exhibited marked increase in $PGE_2$ production with concomitant induction of COX-2 (214). Co-expression experiments clearly demonstrated functional coupling of mPGES-1 with COX-2 in marked preference to COX-1 (242). In this study, human kidney cells stably cotransfected with COX-2 and mPGES-1 accumulated a large amount of $PGE_2$ in the culture supernatant compared to cells transfected with either enzyme alone. In support of this, COX-2 and mPGES-1 were colocalized in symptomatic atherosclerotic plaques and provided the evidence that expression of these enzymes in activated macrophage is associated with acute ischemic syndrome (56). In a more recent study, mPGES-1 protein expression was found to be stimulated following IL-1β challenge in gastric tissue and both COX-2 and mPGES-1 immunoreactivity was observed in inflammatory and messenchymal cells of the same ulcer bed section (107). In line, COX-2 and mPGES-1 were coordinately upregulated in synovial cells from patients with rheumatoid arthritis resulting in an abundant production of $PGE_2$ at the site of inflammation (163). Similar coordinate upregulation of these two inducible enzymes were demonstrated in rat adjuvant induced arthritis model (58). More studies have provided evidence and strengthened the coupling of these two inducible enzymes. In rats, LPS-treated microglia exhibited co-expression and induction of mPGES-1 and COX-2 proteins (137). Both *in vivo* and *in vitro* experiments demonstrated the upregulation of mPGES-1 and COX-2 proteins in LPS-treated microglial cells leading to a 100-fold increase of $PGE_2$ production compared to control cells. On the other hand, functional coupling of cPGES and COX-1 was observed in HEK293 cells as demonstrated by a high conversion of exogenous AA to $PGE_2$ in cells cotransfected with COX-1 and cPGES as compared to cells transfected with either COX alone (334). Furthermore, COX-1-cPGES-mediated $PGE_2$ biosynthesis was recently shown to play a role in spinal nociceptive processing during the early phase of formalin evoked pain response (130). When rats were infused intrathecally with cPGES antisense oligonucleotides, which reduced the endogenous cPGES expression, the nociceptive behavior was significantly decreased.

Several lines of investigations also suggest an existence of alternative coupling between COX isozymes and the PGE synthases. Intracerebral injection of IL-1β

induces COX-2 mediated expression of mPGES-1 and cPGES (232). Schneider *et al* have demonstrated colocalization of COX-1, COX-2 and mPGES-1 to mediate $PGE_2$ biosynthesis in kidney (304). Similarly, in several cell lines, mPGES-2 was found to promote $PGE_2$ biosynthesis by coupling to both COX-1 and COX-2 (240). Recently, $PGE_2$ production in the murine mammary gland shown to be dependent on the functional pairing of mPGES-1 and COX-1 (44). In support, mPGES-1 and COX-1 mRNA were upregulated in several ovarian cancer cell lines and contributed to the overproduction of $PGE_2$. Moreover, the $PGE_2$ synthesis in these cell lines was inhibited by SC-560, a COX-1 specific inhibitor, in a dose-dependent manner and neither NS-398 nor rofecoxib significantly suppressed $PGE_2$ production in these cells (154).

**The μ class of GSTs**

Several cytyosolic GSTs have the capability to catalyze the conversion of $PGH_2$ to $PGE_2$, $PGD_2$ and $PGF_{2\alpha}$ non-specifically (45, 258, 350). Recombinant GST-μ2 and -μ3 expressed in bacteria catalyze the conversion of $PGH_2$ to $PGE_2$ specifically. The apparent $K_m$ values of GST-μ2 and -μ3 for the conversion of $PGH_2$ to $PGE_2$ were estimated to be 140 μM and 1500 μM respectively. Human GST-μ2 and -μ3 are mainly localized in the brain among the various tissues analyzed whereas the rat GST-μ3 is expressed in the thalamus and hypothalamus (146). These enzymes display very low $K_{cat}/K_m$ values and thus are not significant for $PGE_2$ production in humans.

**PGF synthase**

The F series of PGs are widely distributed in various organs of mammals and exhibit varieties of activities, including contraction of the pulmonary arteries. $PGF_{2\alpha}$ can be formed by three different enzymatic pathways; from $PGD_2$ by $PGD_2$-11-ketoreductase, from $PGE_2$ by $PGE_2$-9-ketoreductase and from $PGH_2$ by 9,11-endoperoxide reductase (361). The PGF synthase that catalyzes the formation of 9α,11β-$PGF_2$ from $PGD_2$ ($PGD_2$ 11-ketoreductase activity) in the presence of NADPH was first purified from bovine lung (362). This enzyme belongs to the aldo-keto reductase-family based on broad substrate specificity, molecular weight and high amino acid sequence homology with other members of this family (377). A recent study demonstrated that $PGF_{2\alpha}$ metabolism was high during mid-pregnancy and post-labor (89). The two stereoisomers that are formed *in vivo* ($PGF_{2\alpha}$ and 9α,11β-$PGF_2$) are potent smooth muscle contractors (271, 277). A study by Wu *et al* demonstrated that PGF synthase mRNA expression was decreased during betamethasone-induced premature labor in endometrium and maternal placenta but remain unchanged in fetal placenta and myometrium (382). An additional enzyme was recently identified in bovine endometrium and possessed higher PGF synthase activity compared to the previously discovered enzymes (208).

**PGD synthase**

Prostaglandin $D_2$ is a major prostaglandin produced in the central nervous system and involved in the regulation of sleep-awake responses, mediated through DP receptors (123). PGD synthase catalyzes the isomerization of $PGH_2$ to $PGD_2$ in presence of sulfhydryl compounds. Two distinct types of PGD synthase have been identified: the lipocalin-type (L-PGDS) and the hematopoietic-type (H-PGDS). L-PGDS and H-PGDS are quite different from each other in terms of amino acid sequence, tertiary

structure, evolutionary origin, cellular distribution, chromosomal localization and also functional relevance (353). The primary structure of L-PGDS revealed that the enzyme is a member of the lipocalin gene family, which is composed of various secretory proteins, involved in binding and transport of small hydrophobic molecules. L-PGDS is localized in the central nervous system and male genital organs of various mammals as well as in the human heart and it is secreted into the cerebrospinal fluid, seminal plasma and the blood stream (82, 351). H-PGDS is a cytosolic enzyme and a member of the GST family. H-PGDS is widely distributed in peripheral tissues and mainly localized in antigen presenting cells, mast cells, megakaryocytes and Th2 lymphocytes (150). Several studies indicate that $PGD_2$ plays a major role in the resolution phase of inflammation that is mediated partly by its non enzymatic degradation product 15-deoxy $\Delta^{12,14}$ $PGD_2$ (101, 102). In support of this, a recent study shows that endotoxin-induced inflammation causes sequential stimulation of an immediate and short lasting mPGES-1 and a delayed induction of H-PGDS in mouse heart (305). An enhanced expression of PPARγ was also observed in these cells and the levels of expression peaked earlier than that of H-PGDS which was thought to mediate the anti-inflammatory effects of $PGD_2$ metabolites.

**Thromboxane synthase**

Thromboxane $A_2$ synthase (TXAS), a membrane bound hemoprotein, catalyzes the isomerization of $PGH_2$ to form thromboxane $A_2$ ($TXA_2$). TXAS activity was first described in platelets (250) and the enzyme was later purified to homogeneity as a 60 kDa hemoprotein with spectroscopic characteristics of a cytochrome P450 enzyme (122). $TXA_2$ has been implicated as an autocrine or paracrine lipid mediator in a variety of pathophysiological processes (257). $TXA_2$ exerts a vasoconstrictor effect by serving as an agonist of the thromboxane receptor (TP) on the vascular smooth muscle cell membranes. Significant effect of TXAS on platelet aggregation was also demonstrated (114). The putative effect of $TXA_2$ on thrombosis was demonstrated by the clinical effectiveness of aspirin in the treatment of acute coronary syndromes. Aspirin, at low doses, selectively inhibits $TXA_2$ formation without changing the basal prostacyclin biosynthesis (33, 273).

**Prostacyclin synthase**

Prostacyclin synthase (PGIS) catalyzes the conversion of prostaglandin $H_2$ ($PGH_2$) to prostacyclin ($PGI_2$) and has been purified from solubilized microsomal fractions of porcine and bovine aorta and identified as a hemoprotein. PGIS has been characterized as a member of the cytochrome P-450 family (70). $PGI_2$ is a strong vasodilator that inhibits growth of vascular smooth muscle cells, and the most potent endogenous inhibitor of platelet aggregation. Recently, the human PGIS was cloned, expressed in baculovirus- insect cell system and the recombinant human protein was purified (355). The $V_{max}$ and $K_m$ values of the purified PGIS for $PGH_2$ were 15 $\mu mol \cdot min^{-1} \cdot mg^{-1}$ and 30 μM, respectively. Immunohistochemical study demonstrated the localization of PGIS in epithelial cells, smooth muscle cells and endothelial cells (135). PGIS is primarily expressed in vascular endothelial and smooth muscle cells. Recent investigations, however, have shown that PGIS is also expressed in non-vascular cells such as neurons, oviducts, embryonic cells, and cancer cells, and its functions have extended to neuroprotection (195), reproduction (136), and colon cancer growth (35, 111). PGIS has been shown to be involved in embryo implantation and decidualization.

PGIS and COX-2 were found to be colocalized in maternal-embryo interface as well as in embryo trophoblasts and both the enzymes were coordinately upregulated with generation of high levels of $PGI_2$ (193).

## PROSTANOID RECEPTORS

The biological activities of prostanoids are mediated through specific G protein-coupled receptors. There are at least 9 known prostaglandin receptor forms in mouse and human, as well as several additional splice variants with divergent carboxy termini (249). Four of the receptor subtypes bind $PGE_2$ (EP1-4), two bind $PGD_2$ (DP1 and CRTH2), and the receptors FP, IP, and TP bind $PGF_{2\alpha}$, $PGI_2$, and $TXA_2$ respectively. These prostanoid receptors have been cloned from various species, including human and their distinct binding properties as well as signal transduction pathways have been characterized by analyses of cells expressing each receptor. These receptors are rhodopsin-type receptors with seven transmembrane domains. Based on homology and signaling attributes, the prostaglandin receptors belong to three clusters. The IP, DP1, EP2 and EP4 belong to the relaxant receptors, signaling through $G_s$ mediated increase in intracellular cAMP and the contractile receptors EP1, FP and TP form a second group that signal through $G_q$ mediated increase in intracellular calcium. The EP3 receptor is regarded as an inhibitory receptor coupling through $G_i$ mediated decrease in cAMP. Among the EP receptors, EP3 was the first EP receptor to be cloned (324), followed by cloning of EP1 (98), EP4, (which was originally misdesignated as EP2) (133) and EP2 receptors (287). Most of the GPCRs are localized on the plasma membrane while some are situated on the nuclear envelope (21). Investigation of the expression patterns revealed that the EP receptors are present in major subsets of cells involved in adaptive immune response including T, B and dendritic cells (341) suggesting a pivotal role of $PGE_2$ in modulating immune system. It has been demonstrated that enhanced pain perception in LPS-treated mice is mediated through either one or both the EP3 and IP receptor, while endotoxin-induced fever is mediated through the EP3 receptor only (349). The human IP receptor was first cloned from lung and megakaryocyte cDNA libraries (28, 153). IP receptor is expressed in the spinal cord and implicated in spinal pain transmission in response to peripheral inflammation (79). The FP receptor was originally cloned from human kidney, uterus and placental cDNA libraries (1). Consistent with the functions of $PGF_{2\alpha}$, FP receptor has been found to be expressed in corpus luteum, ocular tissues and ventricular myocytes (237, 296). The human TP receptor was the first eicosanoid receptor to be cloned (128) and two splice variants of TP were found in human. The mRNA for both splice variants was shown to express in platelets, placenta, vascular smooth muscle, brain and small intestine (221). The $PGD_2$ receptor DP is widely expressed in hematological and non-hematological cells (34, 120). CRTH2 was initially cloned as a Th2-selective surface molecule (127) and differs from DP in biological functions and cellular distributions (99). CRTH2 has been specifically identified in hematological tissues such as Th2 cells, cytotoxic T cells, eosinophils, basophils, mast cells and monocytes (317). Several lines of evidences indicate both pro-inflammatory (97, 215, 317) and anti-inflammatory role of DP and CRTH2 receptors (4, 50).

## PROSTAGLANDIN TRANSPORT

$PGH_2$ is synthesized from arachidonic acid by cyclooxygenase-1 or -2 in the lumen of the endoplasmic reticulum (ER) (235) and believed to diffuse through the ER membrane to the cytosol where it is converted to more polar prostanoids by terminal enzymes (293, 330). Prostaglandin's efflux across the plasma membrane to the extracellular compartment is driven by pH and the membrane potential (306). The first prostaglandin carrier characterized was the rat prostaglandin transporter (rPGT) which was identified as an 'organic anion transporter' (OATP) (149). Subsequently, human (hPGT) (203) and the mouse (mPGT) transporters (283) were cloned and characterized. PGT exhibits a broad tissue mRNA expression in rat, human and mouse (307, 329). The carrier-mediated epithelial transport of prostaglandins has been demonstrated by northern blot detection of PGT mRNA in epithelial tissues (149). In a recent investigation, expression of two principal prostaglandin carriers, *i.e.* the prostaglandin transporter (PGT) and the multidrug resistance-associated protein 4 (MRP4) were examined both *in vitro* and *in vivo* in cells of blood brain barrier and choroids epithelial cells in rat brain after LPS challenge (155). Both PGT and MRP4 were found to express in cerebral epithelial cells (CEC) under basal conditions and the levels of expression in these cells were not influenced by LPS treatment. In rat brain, PGT was highly expressed in supraoptic and paraventricular nuclei of the hypothalamus and the expression was induced by LPS treatment. This indicates a potential role of PGT and MRP4 in transporting prostaglandins through blood brain barrier (BBB). Recently a new class of PGT inhibitors was developed by screening a library of small molecules. This allowed to study the mechanism of $PGE_2$ influx and efflux and supported the hypothesis that $PGE_2$ efflux occurs by simple diffusion (51). Computer modeling, using standard algorithms, indicated that the deduced PGT polypeptide resembled a standard 12 membrane-spanning transporter.

## PROSTAGLANDIN CATABOLISM

Biological inactivation of prostaglandins and related eicosanoids is carried out mainly by 15-hydroxyprostaglandin dehydrogenases (15-PGDHs) (5). Two types of 15-PGDHs have been identified. The type I enzyme is $NAD^+$-dependent and primarily utilizes prostaglandins and related eicosanoids as substrates (145). The type II enzyme uses both $NAD^+$ and $NADP^+$ as cofactors and exhibits broader substrate specificity (197). In contrast to type I, the type II enzyme possesses a higher $K_m$ towards prostaglandins and hence is not believed to catabolize prostaglandins. The type I 15-PGDH catalyzes the initial oxidation of the 15(S)-hydroxyl group followed by a reduction of the $\Delta^{13}$ double bond to 15-keto-13,14-dihydro prostaglandins catalyzed by the 13-keto prostaglandin reductase (6, 326). In a recent investigation, the levels of type I 15-PGDH was compared in normal and tumor tissues. 15-PGDH was found to be present in high levels in human and mouse large intestine whereas the expression and activity of this enzyme was significantly downregulated in several colorectal carcinoma cell lines (10). Moreover, genetic disruption of 15-PGDH completely blocked the production of urinary $PGE_2$ metabolite. 15-PGDH was also found to be underexpressed in human lung tumors (75). In addition, mice injected with A549 cells expressing wildtype 15-PGDH displayed a significant decrease in tumor growth compared to mice injected with control A549 cells. This study also demonstrated that overexpression of

15-PGDH induced apoptosis in A549 cells. These studies suggest a potential role of 15-PGDH in tumor suppression by decreasing the levels of proliferative $PGE_2$.

Carbonyl reductase is another prostaglandin-inactivating enzyme that possesses 9-keto-reductase activity and thus inactivates $PGE_2$ by converting it to $PGF_{2\alpha}$ (338). Both 15-PGDH and carbonyl reductase are widely distributed in peripheral tissues but are weakly expressed in mammalian brain (87, 372). A recent finding shows a negative correlation between the expression of carbonyl reductase and tumor progression and angiogenesis (328). In non-small cell lung cancer high expression of carbonyl reductase was demonstrated to be a significant factor to predict a favorable prognosis.

# FEVER

## INTRODUCTION

Fever is a central nervous system (CNS) elicited response to infectious diseases and regarded as the hallmark of infection. Clinically, fever can be defined as an elevation in core body temperature caused by a raised thermoregulatory set point. Thus, fever differs from hyperthermia that does not necessarily depend on a raised temperature set point. Following a peripheral infection, a number of brain-mediated signs of illness are generated that include fever and activation of the hypothalamic-pituitary-adrenal (HPA) axis. HPA axis activation results in the production of corticosteroids in the adrenal medulla that have immune modulatory effects. Previous studies have suggested both beneficial and injurious effects of fever. However, until recent investigations, the potential benefits of the febrile response have not been tested experimentally. In 1980, Vaughn *et al* have shown that blocking fever in rabbits infected with *Pasteurella multocida* resulted in decreased survival (365). Several lines of investigations suggested that febrile response is also present among small organisms such as beetles and leeches, indicating fever as phylogenetically well conserved adaptive response to infection (158). The positive effects of fever has been thought to be mediated by increased T-cell proliferation and cytotoxicity, increased bioactivity of inflammatory cytokines, enhanced neutrophil motility and chemotaxis and promotion of lymphocyte delivery to endothelial venules (88, 158). Traditionally, fever has been thought to be initiated by pyrogenic cytokines acting on the preoptic area (POA) of the anterior hypothalamus in the brain. Infact, a variety of endogenous substances and drugs seem to affect temperature regulation by altering the activity of hypothalamic neurons and perhaps, the best examples of such substances are the pyrogenic cytokines. These substances are released by phagocytic leukocytes in response to a wide array of stimuli and have the capacity to raise the thermoregulatory set point. Traditionally pyrogens have been divided into two general categories: exogenous pyrogen (originate outside the body such as microbes, toxins or other product of microbial origin) and endogenous pyrogen (derived from host cells such as cytokines) (207). The endogenous pyrogen was found to be identical to the leukocyte activating factor (LAF) and most commonly known as interleukin 1 (IL-1). The role of individual cytokines in the generation of fever will be discussed in this section.

### Cytokine signaling across the blood-brain barrier

Cytokines are highly inducible, secreted proteins mainly produced by immunocompetent cells. The primary function of cytokines is the regulation and the coordination of immune responses. Several lines of investigations demonstrate that cytokines and their receptors are expressed in the central nervous system where they are produced by glial or neuronal cell types. In the CNS, cytokines are believed to function as possible trophic factors, which can directly affect neuronal function and also participate in local inflammatory processes. The pro-inflammatory cytokines interleukin-1 (IL-1), interleukin-6 (IL-6) and the tumor necrosis factor alpha (TNF-$\alpha$) have been investigated mostly for their pyrogenic action (41, 74, 244). The role for endogenous IL-10 in the regulation of fever has been investigated in a number of studies (186, 188). Circulating cytokines bind directly to most cells and trigger an acute

phase reaction. However, the tight seal of cells that line the blood vessels in the brain, known as the BBB, protect the brain from potentially harmful and neuroactive substances. The lipophobic property and relatively large size of the cytokine molecules prevent their entry through the BBB. This raises the question, how the circulating pyrogens signal to the brain. Several theories have been proposed for the action of peripheral cytokines to develop fever (25, 252). The existence of a carrier mediated transport into the brain has been reported for IL-1α, IL-1β (12, 13) and also for IL-1 receptor antagonist (IL-1ra) (112) but not for IL-10 (151). This mechanism seems to be mediated by low capacity saturable transporters that could account for a modest elevation of central cytokine levels over an extended period of time, possibly sufficient to have a biological effect on the receptor of the hypothalamic neurons.

Cytokines might also reach the brain through areas devoid of BBB, particularly in the circumventricular organ at the *organum vascolosum laminae terminalis* (OVLT) in the midline of POA. The peripheral cytokine activation of endothelial cells in the circumventricular organs might result in the release of putative neuroregulators, which then process the original signal to the POA (26). The action of the peripheral cytokines on the central thermoregulatory circuit might also be mediated by neuronal afferents (292).

**Interleukin 1 family**

The interleukin-1 (IL-1) family consists of IL-1α, IL-1β and IL-1ra (83). In humans, the gene encoding for these three proteins are located in a cluster on chromosome 2q14 (366). IL-1α is mainly cell associated and therefore IL-1β is believed to be the ligand most important for distant signaling (8). IL-1β is secreted after maturation of its precursor form (pro-IL-1β) by IL-1β caspase 1 (40, 72). IL-1ra is a secreted protein acting as a negative regulator of IL-1α and IL-1β actions. IL-1α, IL-1β and IL-1ra all bind to the same IL-1 receptors: the type I and type II receptors. The type I IL-1 receptor (IL-1RI) is a member of the Toll receptor family (218). The type II receptor is a decoy receptor without signaling properties and believed to be a negative regulator of IL-1 actions (60). LPS, a component of the gram negative bacterial cell wall, upregulates the expression of IL-1α, IL-1β and IL-1RI while causing downregulation followed by inhibition of IL1-RII (276). The IL-1β level is elevated in plasma and cerebrospinal fluid of several species following peripheral application of LPS (378), turpentine (229) and zymogen (205) leading to fever generation. Injection of IL-1β induces fever in rodents, rabbits and humans (9, 244, 251).

**IL-1 and LPS signaling**

IL1-1α, IL-1β and IL-1ra act through the IL-1 type I receptor, a hetero-dimer of the IL-1RI and its accessory protein IL1RAcP. Binding of these ligands induce the recruitment of the cytosolic adaptor protein, MyD88 (myeloid differentiation factor 88) (36) and initiates the activation of the IL-1 receptor-associated kinase (IRAK)-1 and TNF receptor-associated factor (TRAF)-6 pathway. This subsequently leads to the activation of NF-κB, which stimulates the transcription of multiple genes such as COX-2 and mPGES-1 causing the production of potent inflammatory mediator $PGE_2$. LPS is a potent pyrogen and strong inducers of pro-inflammatory cytokines. Investigation by Lien *et al* showed that LPS acts predominantly through the Toll-like receptor 4 (TLR-4) (192). LPS by binding to the TLR-4 can activate the same transduction signal as IL-

1β, utilizing the IRAK/TRAF pathway. Thus LPS can act directly to generate the fever response by activating the same signaling pathway as IL-1 but through an independent receptor. These findings showed the presence of two major peripheral pyrogens, the endogenous IL-1 and the exogenous LPS, which can induce febrile response independent of each other but through the same intracellular mechanism. A schematic representation of IL-1 and LPS action is shown in figure 4.

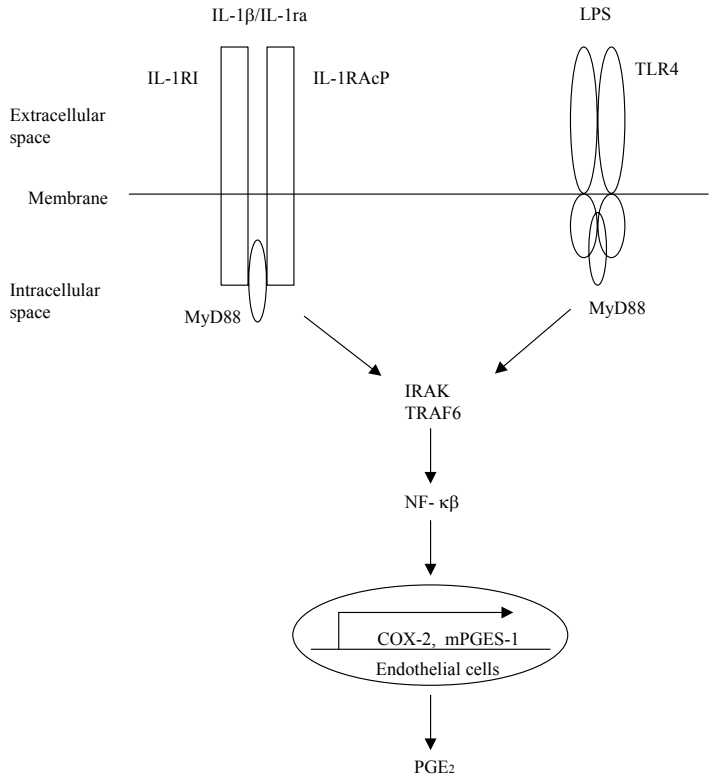


**Figure 4.** Schematic representation of IL-1β and LPS signaling.

## Interleukin 6

IL-6 is an important mediator of host responses to disease. Together with IL-1β, IL-6 is considered as a major pyrogen (384). There have been multiple reports of elevated IL-6 levels in a variety of human diseases and in human body fluid such as plasma, cerebrospinal fluid and joint fluids (59). The human gene of IL-6 is localized on chromosome 7p15-p21 (91) and upon inflammatory stimuli the gene is transcribed in different cell types including monocytes, synoviocytes, vascular endothelial cells and fibroblasts. In the CNS, IL-6 is synthesized by glial cells and neurons (96, 210). IL-6 binds to a specific surface receptor complex consisting of specific cytokine binding subunits, IL-6R α chain and a signal transducing protein gp130 (391). Injection of LPS into a subcutaneous air pouch in rats resulted in an increased concentration of bioactive IL-6 in the plasma (222) and the rise in body temperature was abolished by pre-treatment of IL-6 antiserum (39). In addition, IL-6 knockout mice did not develop fever

neither in response to intraperitoneal administration of low dose of LPS or IL-1β nor to intracerebroventricular (i.c.v.) injection of IL-1β (41). Instead, i.c.v. injection of IL-6 resulted in an elevation of body temperature in these mice suggesting that IL-6 is a necessary component of fever response to both endogenous IL-1β and exogenous LPS.

**Interleukin 10**

Interleukin 10 has been recognized as one of the anti-inflammatory cytokines that counteract inflammatory responses. IL-10 was originally known as cytokine synthesis inhibitory factor (CSIF) as it displayed the ability to suppress the production of IL-2 and interferon-γ (IFN-γ) by T-helper cells as well as it inhibits cytokine production by activated macrophages (92). IL-10 is produced by a variety of cell types namely Th2 lymphocytes, monocytes, macrophages and other cell types (66). IL-10 was also demonstrated to inhibit the formation of endogenous pyrogens such as IL-1, IL-6 and TNF α (65). The gene for human IL-10 is located on chromosome 1q32.1 and active as a 37 kDa homo-dimer. The anti-inflammatory role of IL-10 is evident from the study showing the ability of IL-10 to inhibit the formation of endogenous pyrogens such as IL-1, IL-6 and TNF (65). In line, co-administration of rat recombinant IL-10 led to the reduction of the strength and duration of fever induced by LPS in rats (38). The same study showed that neutralization of IL-10 by a specific antiserum increased the duration of febrile response and augmented the formation of pro-inflammatory cytokines at the site of the inflammatory stimulation. Mice injected intraperitoneally with recombinant murine IL-10 or rats treated i.c.v. with IL-10 are resistant to LPS induced fever (188). Moreover, IL-10 null mice responded to a low dose of LPS with an increased and prolonged fever, accompanied by elevated plasma levels of IL-6. A high dose of LPS in these mice caused a profound long lasting hypothermia leading to enhanced mortality (188).

**Tumor Necrosis Factor**

TNF-α is the principal mediator of acute inflammation in response to gram-negative bacteria. This cytokine is mainly produced by LPS-activated mononuclear phagocytes (359). Antigen-activated T cells, natural killer cells and mast cells also secret TNF-α (15, 61). In human, the TNF-α gene is located on the short arm of chromosome 6 (318). TNF-α is synthesized as a membrane bound homo-trimer protein which is subsequently cleaved by TNF-α converting enzyme (TACE) to produce the secreted form (81). There are two transmembrane signaling receptors, type I and type II, through which the effect of TNF-α is mediated (47, 124) and the third soluble receptor acts as an antagonist of TNF activity (126). The role of TNF-α in fever is more complex since TNF-α possesses both pyrogenic and anti-pyretic effects (74, 200). In support of the anti-pyretic effect of TNF-α, it was demonstrated that LPS-induced fever in rat was enhanced by systemic injection of TNF-α neutralizing antiserum (199) and LPS induced fever in rats was attenuated by treatment of a non-pyrogenic dose of TNF-α (156). In favor of pyrogenic effect of TNF-α and in contrast to previous findings, Cooper *et al* have shown that a neutralizing TNF-α antiserum attenuates fever observed after turpentine injection in rats (62). A study involving TNF receptor knockout mice demonstrated that both the wildtype and TNF receptor knockout mice displayed similar febrile response to LPS, supporting the anti-pyretic effect of TNF (189).

### mPGES-1 expression in the brain endothelial cells

Elevated levels of $PGE_2$ in the brain triggers CNS activity in the generation of fever. In 1970, Milton and Wendlandt reported that injection of $PGE_1$ into the third ventricle resulted in an elevation of body temperature in cats (224). Following this report, much attention has been focused on PGEs in fever generation. Since $PGE_2$ is the predominant form of PGE in the brain, it is currently considered as the principal mediator of fever (27). However, it was not clear until recently, where and how $PGE_2$ is formed in the brain under pathological conditions such as fever. Using the *in situ* hybridization technique, Ek *et al* have demonstrated that IL-1β treatment of rats led to an upregulation of COX-2, mPGES-1 and IL-1R in a majority of endothelial cells of the BBB, suggesting a coupled system for production of $PGE_2$ in these cells (85). Another study by Yamagata *et al* demonstrated that after LPS challenge, mPGES-1 and COX-2 were coexpressed in the perinuclear region in a subset of brain endothelial cells (387). These findings suggest a functional link between COX-2 and mPGES-1 and their relevance to fever.

### Role of EP receptors in fever

$PGE_2$ acts by interacting with one of the four EP receptor subtypes (EP1-EP4). The sensitive site where $PGE_2$ produces fever is located within the region of the brain containing the OVLT and the surrounding preoptic area (POA) (375). In rats, the distribution of EP1-EP4 receptor mRNA was apparent in the region of OVLT/POA and at least three EP receptors EP1, EP3 and EP4 were found to have distinctively different distribution patterns (263). The involvement of EP1 receptor in mediating IL-1β fever has been suggested since hyperthermia induced by central injection of $PGE_2$ was blocked by a specific EP1 receptor antagonist (259, 261). Studies involving EP receptor knockout mice have demonstrated that mice lacking the EP3 receptor failed to show a febrile response to either i.c.v. injection of IL-1β or to intravenous (i.v.) LPS (354). It has also been showed that i.c.v. injection of $PGE_2$ induces fever in EP1, EP2 and EP4 receptor deficient mice but not in EP3 receptor deficient mice, indicating a potential role mediated by only EP3 receptor in fever development. Furthermore, Oka *et al* have studied the febrile response to different doses of intraperitoneally injected LPS in EP1 and EP3 receptor deficient mice (262). While EP1 receptor deficient mice showed febrile response to LPS, at doses of 1, 10 and 100 μg /kg body weight, mice deficient in the EP3 receptor displayed a normal circadian temperature, once again confirming the critical involvement of EP3 receptor in endotoxin induced fever. In addition, EP3 receptor deficient mice did not develop fever in response to subcutaneous injection of turpentine, whereas, the EP1 receptor deficient mice demonstrated a similar febrile response as wildtype mice. These data strongly demonstrate the involvement of the EP3 receptor also in aseptic inflammation induced fever development although a role for EP1 receptor was also suggested.

## ASEPTIC INFLAMMATION-INDUCED FEVER

Turpentine (a viscous liquid distilled from pine tree) injected subcutaneously in an experimental model of local aseptic inflammation induces robust acute phase responses (APR) consisting of fever, anorexia, cachexia and acute phase protein production. In

studies using cytokine deficient mice, IL-1β and IL-6 were shown to be necessary for the development of turpentine-induced fever (167, 168). Pre-treatment of mice with a monoclonal antibody to IL-1R1 attenuates several of the acute phase responses to turpentine (100, 264). In addition, fever was shown to be completely abolished in IL-1β and IL-1R1 knockout mice after subcutaneous turpentine injection (187). Previous investigations using IL-1β knockout mice showed that subcutaneous turpentine injection to wildtype mice resulted in significant increase in plasma IL-6 levels which was not the case in IL-1β knockout mice (100, 394). Interestingly, fever induction by turpentine was abolished only in IL-1β knockout mice but not in IL-1α knockout mice, indicating that IL-1α deficiency in the febrile response can be compensated for by the presence of IL-1β but not vice versa (134).

# NEONATAL RESPIRATORY DEPRESSION

## INTRODUCTION

Sudden infant death syndrome (SIDS) is a major cause of death between one month and one year of age among infants in industrialized nations and developed countries (22, 217). The definition for SIDS was established in 1969 as ‘‘the sudden death of any infant or young child, which is unexpected by history, and in which a thorough postmortem examination fails to demonstrate an adequate cause of death’’ (16). The definition was revised in 1989 to ‘‘the sudden death of an infant under one year of age which remains unexplained after a thorough case of investigation, including performance of a complete autopsy, examination of the death scene and review of the clinical history’’ (291, 376). The major risk factors that contribute to the occurrence of SIDS are bacterial infections, in particular infection of the respiratory tract (23). Pathological and epidemiological data indicate that viral infections are also important triggering factor of acute hypoxia and death in several SIDS victims (93). In about 80% of the SIDS victims, bacterial or mild viral infection preceded death (282). Genetical, developmental, and environmental factors could also contribute to the severity of inflammatory responses to infection. Apnea, the medical term for cessation of breathing, is another major cause of infant death. Apnea is also a common symptom in infants with infection and septicemia (213). Infants at the age of two to four months are more susceptible to the detrimental effect of infection because of the presence of an immature immune system and a decrease in maternal antibodies (23, 285). Pro-inflammatory cytokines such as IL-1β produced during acute phase immune responses have been proposed to serve a critical mediator of apnea and SIDS (109). Figure 5 briefly illustrates a hypothetical pathway by which an infection may lead to apnea and SIDS. The involvements of cytokine in the pathogenesis of SIDS became evident from the study in which half of the infants investigated for SIDS contained a higher concentration of IL-6 in their cerebrospinal fluid, similar to those dying from infectious diseases such as meningitis or septicemia (367).

## RESPIRATORY CONTROL CENTRE

Breathing movements start episodically in uterus and are continuous after birth. However, although ready to function at birth; the mammalian breathing control system is immature and undergoes a prolonged period of postnatal maturation.

Three different breathing patterns are generated which include eupnea (the normal resting pattern), sighing (larger inspiratory efforts overlying on eupneic breaths) and gasping (short inspiratory efforts with high amplitude interspersed with long expiratory pauses). One of the central questions in the control of breathing is how and where in the brain the respiratory rhythm is generated. Le Gallois proposed that the source, the *noeud vital* for the respiratory rhythm exists at the pontomedullary junction within the brainstem (184). Recent investigations by Wenninger *et al* have addressed a number of issues concerning generation of respiratory rhythm (370, 371). These data together with previous investigations (147, 288, 312) indicate a necessary role for pre-Bötzinger complex (pre-BötzC) neurons in the generation of normal relaxed respiratory rhythm. The pre-BötzC is a discrete region located ventral to the caudal end of the compact part of nucleus ambiguus, within the ventral respiratory column of the

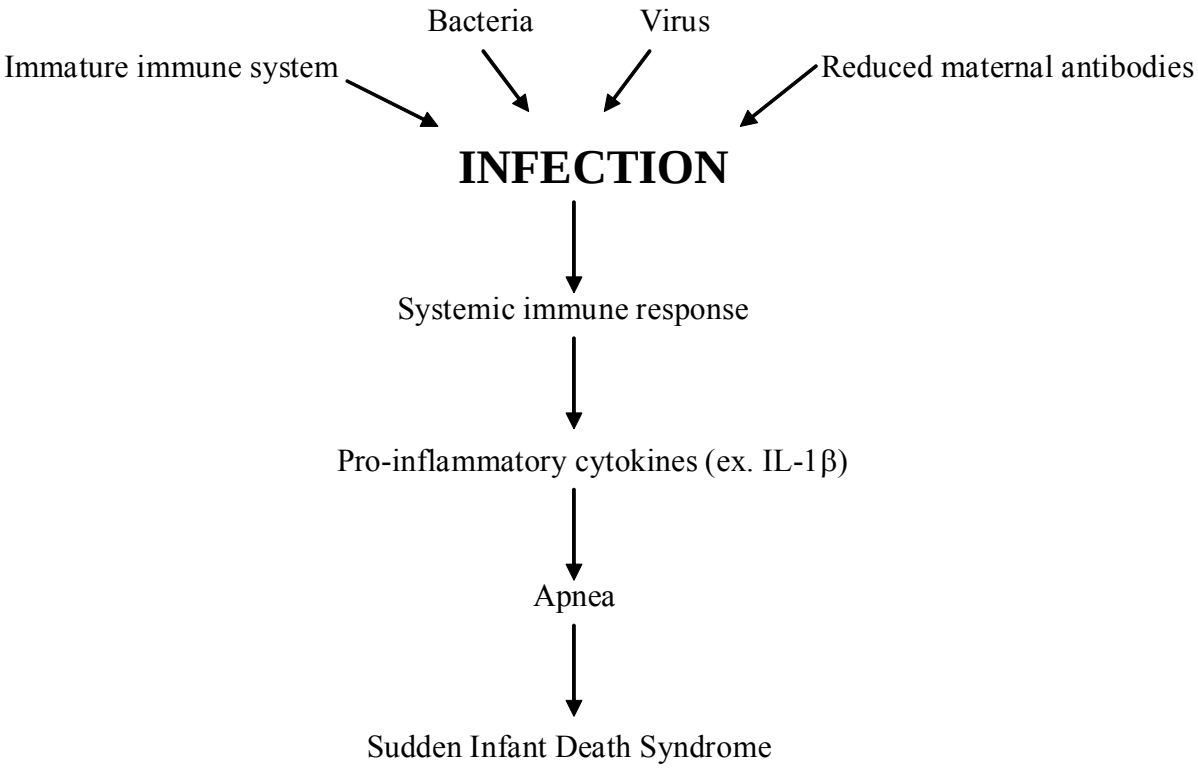


**Figure 5.** Schematic representation of a potential pathway by which pro-inflammatory cytokines such as IL-1β released due to an infection may lead to apnea and SIDS in newborn infants.

ventrolateral medulla (3). A number of *in vitro* and *in vivo* studies have subsequently reinforced the concept that pre-BötzC is important in the generation of normal respiratory rhythm. Feldman *et al* have shown that blocking of the synaptic transmission in the pre-BötzC in rats or cats abolishes respiratory activity (90). In contrast to these findings, a very recent report has provided evidence that pre-BötzC neurons are critical for the neurogenesis of gasping but not for the eupneic rhythm (272). Gray *et al* reported that neuron expressing neurokinin 1 receptor (NK1R) are abundant in pre-BötzC (106) and Wenninger *et al* observed that a small reduction of NK1R expressing neurons in pre-BötzC induces abnormal breathing pattern in goats (370). However mice lacking NK1R continue to breathe indicating that NK1R *per se* are not critical for breathing (337). Further research in this area shows that ventral medulla is not the only site at which genesis of respiratory rhythm occurs. It has been demonstrated that lesions, neurotoxins, neurochemical stimulations and blocking of pontine portions of the rhombencephalon induce modification in respiratory pattern suggesting a potential role of pontine region in respiratory rhythm generation (323).

## INFECTION, PROSTAGLANDINS AND APNEA

Pro-inflammatory cytokine IL-1β has been proposed to play a critical role in mediating infection and hypoxia in newborn babies. Several lines of investigations have shown that IL-1β and TNF-α reduce the normoxic breathing frequency in rodents (265, 346). In addition, IL-1β markedly reduced the number of breathing efforts and the ability to autoresuscitate during anoxic challenge in mice compared to the vehicle treatment

(131). However, in isolated brainstem IL-1β has no direct effect on respiratory rhythm generation indicating an underlying mechanism mediating the effect of IL-1β in respiratory depression (265). Previous studies have shown that $PGE_1$ and $PGE_2$ cause apnea in newborn swine (319) and newborn human infants with cyanotic heart disease (253). The incidence of central apnea in preterm infants was also shown to be related to endogenous PGE concentration (129). Several studies in animal models have verified the widespread existence of $PGE_2$ receptors in the central nervous system including the neurons in the nucleus of the solitary tract (NTS) and rostral ventrolateral medulla (RVLM) (84). Using dual *in situ* hybridization technique Ek *et al* have demonstrated the colocalization of COX-2, mPGES-1 and IL-1 receptor mRNA in the endothelial cells of rat brain treated with IL-1β (85). It may therefore possible that peripheral administration of IL-1β upregulates the expression of COX-2 and mPGES-1 mRNA centrally. As a consequence, the downstream signal molecule $PGE_2$ is produced in the endothelial cells of the BBB and crosses the blood brain barrier because of its small size and lipophilic property or by a transport mechanism and acts on EP3 receptor to exert its effect. In an intermittent hypoxia (IH) model of sleep-disordered breathing, Li *et al* have observed induction of COX-2 mRNA and upregulation of COX-2 protein, resulting in substantial increase in $PGE_2$ production (191). Furthermore, treatment of these animals with NS-398, a selective COX-2 inhibitor blocked the IH-induced $PGE_2$ elevation suggesting a critical contribution of COX-2 associated with hypoxic episode during sleep.

# AIMS OF THE PRESENT INVESTIGATION

The general aims of this study were as follows:

- To biochemically characterize the human microsomal prostaglandin E synthase-1 (Paper I)
- To investigate the role of mPGES-1 in lipopolysaccharide mediated fever in mice (Paper II)
- To investigate the role of mPGES-1 in cytokine dependent fever in mice (Paper III)
- To investigate the mechanism behind cytokine induced respiratory depression in neonates (Paper IV)

# METHODOLOGY

The methods used in the present investigation are described below. Detailed descriptions of the other methods can be found in the original papers.

## PAPER I

### mPGES-1 over-expression

BL21DE3 *E. coli* cells containing the plasmid pSP19T7LT with an inserted mPGES-1 were grown in terrific broth at 37 $^{o}$C. Cells were grown until $Abs_{600}$ reached 0.55-0.65 AU. 3 mM isopropyl-β-thiogalactopyranoside (IPTG) was added to the growing cells at this point and the cells were allowed to grow for another 3 hr. The cells were harvested and the pellet was washed with PBS and stored at -20 $^{o}$C.

### Preparation of membrane fraction

Cell pellets were allowed to thaw on ice and resuspended in TSEG (Tris, sucrose, EDTA, Glutathione) buffer. The cells were lysed by repeated freezing (in $CO_2$-ice-ethanol bath) and thawing (in a waterbath at 37 $^{o}$C) and sonicated to disrupt the membranes. The cell debris was separated by a short centrifugation (10 min at 5000 x $g_{av}$). The supernatant containing the cytosol and the membrane fraction was subjected to ultracentrifugation (1hr at 250,000 x $g_{av}$). The pellet containing the membrane fraction was stored at -80$^{o}$C.

### Purification of human recombinant $his_6$-tagged mPGES-1

Membrane bound $his_6$-mPGES-1 was solubilized in 4% Triton X-100, either from membrane fraction or from whole cell lysate. The solubilized fraction was mixed with hydroxyapatite and the unbound fraction containing mPGES-1 was collected by a short centrifugation. The supernatant was filtered through a 0.45 μM filter, adjusted to pH 8 and immediately loaded onto the pre-equilibrated nickel affinity column on fast protein liquid chromatography (FPLC). The Ni-column was washed with 60 mM imidazole buffer until the absorption maxima returned to base line. Finally $his_6$-mPGES-1 was eluted with 350 mM imidazole. The eluted peak from the nickel column was immediately desalted using a PD10 column.

### Electron Crystallography

Purified recombinant human $his_6$-mPGES-1 protein at a concentration of 1 mg/ml containing 1% Triton X-100 was mixed with bovine liver lecithin (BLL) at LPR (lipid to protein molar ratio) 9. This mixture was dialyzed for 8 days at room temperature. Crystals were checked by negative staining in a transmission electron microscope (Philips CM 120) operated at 120 kV. Specimens were kept frozen during data collection and electron micrographs were recorded on Kodak SO-163 film using the same microscope. The data was subjected to several steps of image processing.

### Hydrodynamic studies

Hydrodynamic studies were performed to determine the molecular mass of $his_6$-mPGES-1. The sedimentation coefficient of the mPGES-1-Triton X-100 complex was determined by density sucrose-gradient ultracentrifugation. Purified mPGES-1 and marker proteins with known sedimentation coefficients were added on top of 5-20 % sucrose gradient containing 2 mM GSH and 1% Triton X-100. The samples were then subjected to ultracentrifugation at 160,000 x $g_{av}$ for 45 h at 20°C. Fractions were collected from the bottom of the tube. Protein content, refractive index and mPGES-1 activity was determined in each fraction. The sedimentation coefficient of $his_6$-mPGES-1 was calculated from the plot of protein content, refractive index and mPGES-1 activity using the known sedimentation coefficients of the marker proteins.

The partial specific volume of the $his_6$-mPGES-1-Triton X-100 complex was determined by density equilibrium centrifugation. Purified mPGES-1 was applied on top of 20-50% sucrose gradient containing 1 mM GSH and 1% Triton X-100 and centrifuged for 96 hr at 246,000 x $g_{av}$ at 20°C. Fractions were collected from the bottom of the centrifuge tube and the refractive index was plotted against the mPGES-1 activity. The partial specific volume of the mPGES-1-Triton X-100 complex was determined from the refractive index plot of sucrose in the fraction with the highest PGES activity.

Stoke's radius was determined by gel filtration exclusion chromatography. Purified mPGES-1 was loaded on a Sepharyl S-300 HR column together with marker proteins. Samples were collected and the PGES activity and absorbance was measured at 280 and 405 nm, respectively. From a plot of known Stokes radii of the marker proteins and the square root of $-\log K_{av}$ values, the Stokes radius of mPGES-1 Triton X-100 was calculated. The molecular weight of the mPGES-1-Triton X-100 complex was determined by substituting the values of sedimentation coefficient, partial specific volume and Stokes radius into the Svedberg equation (**Paper I, Eq.1**).

In another experiment, we measured the protein content and UV absorption of mPGES-1 peak fraction from the nickel column, and calculated the amount of bound detergent in the mPGES-1-Triton X-100 complex.

### mPGES-1 activity assay

mPGES-1 activity was measured on membrane fractions of *E. coli* expressing human mPGES-1, purified protein (**Paper I**) and in the microsomal fraction of mice brain (**Paper II, Paper IV**), following the method developed by Thorén *et al* (339). Briefly, samples were incubated with 10 μM $PGH_2$ for 1 min on ice or at 37°C. The reaction was terminated by adding acidified $FeCl_2$ solution. The samples were immediately subjected to solid phase extraction; $PGE_2$ was eluted with acetone, evaporated under nitrogen flow and finally dissolved in 33% acetonitrile. An aliquot of the sample was analyzed by RP-HPLC combined with on-line UV detection at 195 nm. The amount of $PGE_2$ in each sample was calculated after subtracting the non-enzymatic $PGE_2$ formation obtained in the buffer control.

### SDS-PAGE and Western Blot

All samples were diluted to appropriate protein concentration in 0.1 M potassium inorganic phosphate (KPi) buffer, mixed with SDS containing loading buffer and boiled for 2 min. Proteins were separated on 4-15% SDS-PAGE and electroblotted onto

a PVDF membrane. After blocking with 5% non-fat dry milk, the membranes were incubated with primary antibody at 4°C, overnight. After few washing steps, the membranes were incubated with secondary antibody, conjugated with Horseradish peroxidase. Immunoreactivity was detected with enhanced chemiluminescense (ECL-plus kit, Amersham Biosciences, Sweden).

## PAPER II AND III

### Animals

Wildtype and mPGES-1 knockout mice were generated by breeding heterozygotes littermates of the DBA/1lacj strain as previously reported (345). Animals were kept one per cage in a pathogen free facility at an ambient temperature of 27± 1°C and on a 12 hr light/dark cycle (lights on at 7 a.m.) with food and water available *ad libitum*. All experimental procedures were performed during the early phase of the light cycle.

### Intraperitoneal injection of LPS and IL-1β

The mice were briefly restrained and injected intraperitoneally with LPS (2 μg dissolved in 100 μl 0.9% NaCl, Sigma 0111:B4) or IL-1β (600 μg in 100 μl 0.9% NaCl, Pierce Chemical Company, Sweden). Control mice were injected with 100 μl saline (0.9% NaCl).

### Subcutaneous injection of turpentine

The mice were briefly anaesthetized with isoflurane and given a subcutaneous injection of 150 μl commercially purified turpentine (Riedel-de Haën, VWR, Stockholm, Sweden) in the left thigh. Control mice were injected with 150 μl saline.

### Telemetric temperature recording

This method was used to measure the core temperature of mice. At least one week prior to the fever study, mice were implanted with transmitters in the intraperitoneal cavity. The one week period allows the mice to be acclimatized to the transmitter. A receiver that transmits the signals on-line to the connected computer was placed beneath the cage of each mouse. Recordings were started at least 1 hr prior to injection and data were obtained every 2 min throughout the entire observation period.

### Enzyme immunoassay (EIA)

The amount of $PGE_2$ in the cerebrospinal fluid (CSF) of the control and LPS-treated wildtype and mPGES-1 knockout mice was measured by UV spectroscopy at 405 nm, using the $PGE_2$ metabolite EIA kit from Cayman Chemical.

### Circadian changes in core temperature and motor activity

Core temperature and motor activity were monitored for two consecutive days. Animal's motor activity was quantitatively assayed from the change in position of the transmitter in relation to the receiver and the speed with which movement occurred.

### Cage exchange induced stress response

Cage exchange induced stress was evoked by exchanging the home cage of two mice. Control mice were just lifted up and replaced in the same cage.

## PAPER IV

### Animals

9 days old inbred DBA/1lacj and C57Bl/6 mice were used. All newborn pups were accompanied by their mother prior to the experiment and kept under standardized conditions with food and water provided *ad libitum*.

### Whole-body plethysmography

Understrained whole body plethysmography implemented in the present investigation is a non-invasive alternative method compared to spirometry and pneumotachography in smaller animals. This method is based on the principle that the warming and humidification of inspired air results in an increased pressure within the plethysmography chamber. This method of respiratory evaluation depends on the fluctuation in air flow superimposed upon the baseline air flow through the plethysmograph chamber are result of the animal's own respiratory effort.

### Respiratory activity measurement in response to intraperitoneal treatment of IL-1β and i.c.v. injection of $PGE_2$

Mice were injected intraperitoneally with IL-1β or saline. At 70 min after injection, each mouse was placed in the plethysmograph chamber. Respiratory activity of the mouse was recorded in response to 4 min normoxia followed by 1 min of hyperoxia (100% $O_2$). The animals were then subjected to a 5 min recovery period with 100% normoxia which was followed by a 5 min anoxic exposure. Finally 100% $O_2$ was administered for 8 min. Skin temperature was measured before and after experimentation.

i.c.v. injection of $PGE_2$ or saline was performed after briefly anesthetizing the mice with isoflurane. Immediately thereafter the mice were put in the plethysmograph chamber and subjected to 100% normoxia for 14 min allowing them more time to recover from the anesthesia. The mice were then subjected to 1 min hyperoxia; 5 min normoxia followed by 5 min anoxic exposure and 8 min of 100% $O_2$ administration. Skin temperature was measured throughout the experimentation.

# RESULTS

Reference to figures in this section refers to the figures in the papers included in the thesis.

## Paper I

*Solubilization and purification of recombinant human mPGES-1*

We have found that buffer containing 4% Triton X-100 solubilized most of the mPGES-1 from the membrane fraction. Addition of 1 mM GSH and 10% glycerol in the solubilization buffer found to induce the mPGES-1 activity (**fig. 2A**) as well as preserved the enzymatic activity for a longer time period (**fig. 2B**). Infact, a pure enzyme preparation stored at -20$^{o}$C did not show any loss of activity when assyed after 6 months compared to the freshly purified enzyme. When the purity of 350 mM imidazole peak was verified by protein electrophoresis, we found that this peak displayed a single band with a molecular weight of about 18 kDa on the silver stained SDS-PAGE gel (**fig. 4A**) and Western blot analysis demonstrated mPGES-1 immunoreactivity of the purified protein (**fig. 4B**). The amount of purified protein recovered was up to 2% of the total membrane protein. The purified protein was highly active and demonstrated a high specific conversion of $PGH_2$ to $PGE_2$.

*Quaternary structure of mPGES-1*

Purified $his_6$-mPGES-1 in 1% Triton X-100 was successfully crystallized using the crystallization conditions similar to those applied for MGST1. Triton X-100 used for solubilization of mPGES-1 from the bacterial membrane was coincidentally the same detergent suitable for 2-D crystallization. A 10 Å projection structure of mPGES-1 obtained after several image processing steps demonstrated a trimeric organization of the protein in the crystal (**fig. 8**).

*Hydrodynamic studies*

Hydrodynamic studies were performed on the mPGES-1-Triton X-100 complex. From the sedimentation coefficient, partial specific volume and Stokes radius (4.1 S, 0.891 $cm^3/g$ and 5.09 nm, respectively), we calculated the molecular mass of the mPGES-1-Triton X-100 complex to 215,000. From the detergent binding experiment, we found that 2.8g Triton X-100 was bound/g protein. Based on these findings, a trimer of mPGES-1 would weigh 53,700 and thus bind 150,400 units of Triton X-100. The resulting weight of the protein detergent complex was close to the calculated molecular mass of mPGES-1-Triton X-100 complex and thus agrees best with a trimeric quaternary structure.

*Steady state kinetics of mPGES-1*

Purified human recombinant $His_6$-mPGES-1 showed Michaelis-Menten rate behavior toward $PGH_2$ in presence of GSH (**fig. 6A**). The purified enzyme exhibited an apparent $K_m$ and $V_{max}$ value of 160 μM and 170 $\mu mol \cdot min^{-1} \cdot mg^{-1}$, respectively for $PGH_2$. In

addition to the PGE synthase activity, purified mPGES-1 also catalyzed the conversion of $PGG_2$ to 15-hydroperoxy-$PGE_2$. The $V_{max}$ was 250 $\mu mol \cdot min^{-1} \cdot mg^{-1}$ and apparent $K_m$ for $PGG_2$ was almost identical to that demonstrated for $PGH_2$ (**table I&II**). mPGES-1 also catalyzed the reduction of 15-hydroperoxy-$PGE_2$ and cumene hydroperoxide, however, at a very low rate (**table II**). Moreover, purified mPGES-1 catalyzed a small but significant CDNB-GSH conjugating activity with a specific activity of 0.81 $\mu mol \cdot min^{-1} \cdot mg^{-1}$.

**Paper II**

The role of mPGES-1 for endotoxin-induced fever in mice was investigated. We performed intraperitoneal injections of bacterial wall lipopolysaccharide to mice deficient in the gene encoding for mPGES-1 and their wildtype littermates. In response to LPS injection, wildtype mice displayed a robust and sustained fever, which started at about 1.5 hr after injection and persisted for about 5 hr (**fig. 1**). In contrast, mPGES-1 knockout mice did not develop LPS-induced fever. However, an initial restrain-induced hyperthermia was seen in mice irrespective of genotype and treatment. Analysis of the $PGE_2$ concentration in the CSF showed a marked increase of the $PGE_2$ levels after immune challenge of the wildtype mice (**fig. 2a**). In comparison, mPGES-1 knockout mice exhibited similar levels of $PGE_2$ as saline-treated mice. We have also analyzed $PGE_2$ synthesizing capacity in the membrane fraction of the brain. While mPGES-1 activity was increased in response to immune challenge in the wildtype mice, mPGES-1 knockout mice displayed a low activity similar to that seen in saline-treated wildtype mice. By RT-PCR, we confirmed that the knockout mice were devoid of mRNA encoding mPGES-1 whereas wildtype mice displayed a strong immune elicited induction of mPGES-1 mRNA. In contrast, mPGES-2 was not upregulated following the immune challenge. Finally, by i.c.v. injection of $PGE_2$ we showed that mPGES-1 deficient mice, but not the EP3 receptor knockout mice, retain intact capacity to develop fever to the same degree as the wildtype mice.

**Paper III**

We have shown previously that intraperitoneal injection of LPS induces fever in wildtype DBA/1lacJ mice. LPS is a potent pyrogen and a strong inducer of pro-inflammatory cytokines. However, a recent discovery showed that LPS acts predominantly by binding to the Toll-like Receptor 4 (TLR-4), implying that LPS can act on brain directly, the fever response thus being independent of cytokine signaling. Therefore, we examined the febrile response in an animal model that is dependent on intact cytokine signaling such as the aseptic inflammation induced by subcutaneous injection of turpentine.

We have observed similar core body temperature and motor activity in wildtype and mPGES-1 knockout mice. In response to subcutaneous injection of turpentine, the wildtype mice showed a biphasic febrile response (**fig. 5**). However, the mPGES-1 knockout mice did not develop fever in response to turpentine and displayed a core temperature similar to that demonstrated by the saline injected wildtype mice. Nevertheless, turpentine-induced local inflammation was observed in both wildtype and mPGES-1 knockout mice demonstrated by complete lack of motor activity in these mice starting soon after the injection, in comparison to the control mice displaying normal motor activity (**fig. 6**). The response of the mPGES-1 knockout mice and their

wildtype littermates to i.p. injection of IL-1β was very similar to that demonstrated by i.p. injection of LPS (**fig. 7 and Paper II fig. 1**). In order to investigate the role of mPGES-1 in psychological stress-induced hyperthermia, we have used a cage exchange-induced stress model. Both the mPGES-1 knockout mice and the wildtype littermates responded with similar hyperthermic reaction to cage exchange-induced stress which is significantly higher compared to the hyperthermic response displayed by the control mice of either genotype (**fig. 3)**.

**Paper IV**

*IL-1β induced respiratory depression in wildtype mPGES-1 mice during normoxia and hyperoxia*

Using flow plethysmography, we monitored the respiratory activity in 9 days old mPGES-1 wildtype and knockout mice in response to IL-1β or saline treatment. The basal respiratory frequency was reduced in IL-1β treated wildtype mice between 70-80 min of peripheral administration (**fig. 1a**). In contrast, IL-1β had no such effect in the heterozygote or mPGES-1 knockout mice. IL-1β also lowered the respiratory frequency during 1 min hyperoxia in wildtype mice compared to those treated with vehicle. However, this inhibitory effect of IL-1β was not observed in mPGES-1 knockout mice. In a complementary study using 9 days old C57Bl/6 mice lacking EP3 receptor, we found that IL-1β could not alter the basal ventilation or the hyperoxic response compared to vehicle treatment.

*IL-1β reduced anoxic survival in wildtype mice but not mPGES-1$^{-/-}$ or EP3R$^{-/-}$ mice*

9 days old DBA/1lacj and C57Bl/6 mice were exposed to 5 min anoxia (100% $N_2$) at 80 min after IL-1β or vehicle treatment. Ventilation was investigated during this period. All mice exhibited a characteristic biphasic response with an initial increase in ventilation (hyperpnia) followed by a hypoxic ventilatory depression (gasping response). Total number of gasps in the wildtype mice was significantly reduced by IL-1β treatment compared to vehicle-injected mice (**fig. 2d**). However, mice with either single or both allele deletion of mPGES-1 gene did not display any change in the gasping response to IL-1β treatment compared to vehicle injected mice. The survival of IL-1β treated mPGES-1 wildtype mice after hypoxic apnea was significantly reduced compared to those treated with vehicle (**fig. 2e**). In comparison, IL-1β treated mPGES-1 knockout mice demonstrated a higher survival rate after anoxia. Survival rate of the heterozygote mice was comparable to the mPGES-1 knockout mice. IL-1β treated EP3 receptor knockout mice did not show any alteration to anoxic respiratory response compared to vehicle treated mice. Interestingly, C57Bl/6 mice strain displayed reduced sensitivity to anoxia compared to the DBA/1lacj strains. All DBA/1lacj mice terminated their gasping response during the 5 min anoxic period while the C57Bl/6 mice continued the gasping response beyond the 5 min anoxia. 66% of C57Bl/6 mice survived to anoxia exposure compared to 55% of DBA/1lacj mice.

*$PGE_2$ induced apneas and irregular breathing pattern in EP3 wildtype mice*

To investigate if $PGE_2$ plays a central role in modulating respiratory behavior, C57Bl/6 and DBA/1lacj mice were injected i.c.v. with $PGE_2$. Ventilatory response was examined at 10 min following injection. $PGE_2$ induced significantly greater number of

apneic events and irregular breathing patterns during normoxia and hyperoxia in wildtype EP3 mice compared to the vehicle treatment. $PGE_2$ administration also reduced respiratory frequency in the wildtype but not in EP3 knockout mice during normoxia as well as hyperoxia. However, the apneic events and irregular breathing patterns displayed by $PGE_2$ treated mPGES-1 wildtype mice during normoxia and hyperoxia was not significantly different from control mice. When mice were exposed to 5 min anoxia at 20 min after i.c.v. injection of $PGE_2$ or saline, all mice exhibited an initial hyperpnic period followed by a gasping response. However, the duration of hyperpnia was prolonged in EP3 knockout mice compared to the wildtype EP3 mice. $PGE_2$ treated wildtype EP3 mice displayed lower respiratory frequency during hyperpnea compared to the vehicle treated mice and EP3 knockout mice.

*mPGES-1 enzyme activity was induced by IL-1β and hypoxia*

mPGES-1 activity was measured in the brainstem and cortex of IL-1β or saline-treated wildtype and knockout mice. We have observed that irrespective of treatment, brainstem displayed higher activity compared to corresponding cortex (**fig. 4**). Mice with targeted deletion of mPGES-1 gene demonstrated abrogated mPGES-1 activity. Basal level of mPGES-1 activity was observed in saline-treated wildtype mice. Wildtype mPGES-1 mice treated with IL-1β and exposed to hypoxia displayed 4 times higher enzymatic activity compared to the control mice as well as significantly higher activity compared to either treatment alone. A longer exposure (180 min) of the wildtype mice to IL-1β induced a three-fold increase in mPGES-1 activity compared to control mice. The IL-1β induced mPGES-1 activity displayed by wildtype EP3 mice was comparable to wildtype mPGES-1 mice.

# DISCUSSION

## mPGES-1: BIOCHEMICAL CHARACTERIZATION (PAPER I)

Previous attempts to purify GSH-dependent mPGES-1 protein were partially successful, the main obstacle being the instability of the protein, which lost activity soon after purification (231). We have successfully purified the recombinant human mPGES-1 enzyme that was over-expressed as an N-terminal 6-histidine tagged ($his_6$) fusion protein in *E. coli* BL21DE3 cells. Kinetic characterization of the purified protein displayed a high $K_m$ value for $PGH_2$ (160 μM). Independent from our study, a report describing purification of mPGES-1, expressed in a baculovirus-insect cell system, demonstrated a lower apparent $K_m$ for $PGH_2$ (14 μM) (269). A possible explanation for these discrepancies in $K_m$ could be the differences in post-transcriptional processing between prokaryotic and eukaryotic systems. Another explanation could be the different detergents used for the solubilization of mPGES-1 during purification. $PGH_2$ is a lipophilic substrate and it is reasonable to believe that different types of detergent might considerably influence the binding of $PGH_2$ to the active site of mPGES-1. In line with our findings, membrane fraction from *E. coli* expressing mouse mPGES-1 also demonstrated high $K_m$ value for $PGH_2$ (183). However, a true comparison of the $K_m$ values is not possible since the lipid composition in the membrane fraction may provide a different microenvironment for mPGES-1 enzymatic activity compared to the detergent solubilized enzyme. Nevertheless, the purified enzyme displayed a very high catalytic activity for the formation of $PGE_2$ demonstrating the specificity of this enzyme for $PGE_2$ biosynthesis. In addition to PGE synthase activity, purified human mPGES-1 also catalyzed GSH dependent conversion of $PGG_2$ to 15-hydroperoxy-$PGE_2$ and glutathione-dependent peroxidase activity for cumene hydroperoxide, 5-HpETE and 15-hydroperoxy-$PGE_2$. 15-hydroperoxy-$PGE_2$ can also be reduced non-enzymatically to $PGE_2$ in presence of GSH (298). The alternative pathway for the biosynthesis of $PGE_2$ from $PGG_2$ via the intermediate formation of 15-Hydroperoxy-$PGE_2$ might represent physiological importance. However, more *in vivo* investigations are required in order to establish the physiological relevance of 15-hydroperoxy-$PGE_2$ production.

The projection map of purified mPGES-1 demonstrated a homo-trimeric structure and it resembles the structure of MGST1 suggesting that the fold of PGES is a left-handed four-helix bundle as shown for MGST1 (**fig. 6**) (132, 303). Furthermore, hydrodynamic studies performed on the purified protein also independently demonstrated the trimeric organization of mPGES-1. This is in line with the studies on the closely related MAPEG member MGST1, which has been demonstrated to be a trimer (32). Recently, the projection map determined by electron crystallography of recombinant human $LTC_4$ synthase, another MAPEG protein, at a resolution of 4.5 Å also demonstrated trimeric organization of the enzyme (302) although the active form of the enzyme was previously suggested to constitute a homo-dimer (255).

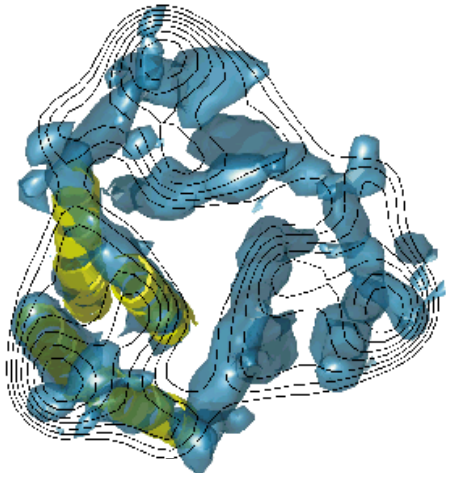

**Figure 6.** Overlap between the MGST1 3D structure and the projection map of mPGES-1.

## mPGES-1 IN FEVER DEVELOPMENT (PAPER II & III)

Several pro-inflammatory cytokines such as IL-1β and IL-6 act as endogenous pyrogens signalling through respective receptors (73). Since the core temperature is regulated centrally, it has been assumed that these cytokines are released into the systemic circulation and transported to the preoptic area of the anterior hypothalamus. However, the entry of these cytokines into the brain is restricted by the presence of BBB, although saturable transport system, transport through OVLT and binding of cytokines to peripheral nerves have been reported. Until recently the mechanism of fever generation was not clear. Recent developments in knockout mice technology provide a unique tool to study fever and unravel the underlying mechanism. The most recent and widely accepted concept of fever mechanism is the formation of $PGE_2$ in the brain endothelial cells in response to immune stimuli and because of its small size and lipophilic property entry of $PGE_2$ through the BBB is facilitated. $PGE_2$ by binding to EP3 receptor in the POA of anterior hypothalamus elevates the thermal set point resulting in fever generation (a simplified diagram is represented in figure 7). Using mPGES-1 knockout mice we have recently shown that mPGES-1 is necessary for endotoxin induced fever development (**Paper II, fig. 1**) (86). Our investigation also demonstrated that the fever generating signal of $PGE_2$ is mediated through EP3 receptors since mice deficient in EP3 receptor did not develop fever in response to i.c.v. injection of $PGE_2$ (**Paper II, fig. 3**). In line with this data, previous investigation using EP receptor knockout mice has also demonstrated that only mice lacking EP3 receptor failed to develop fever in response to LPS, IL-1β and $PGE_2$ (354). Moreover, co-induction and co-expression of COX-2 and mPGES-1 in the endothelial cells of rat brain in response to LPS demonstrates a functional link between the two enzymes for the biosynthesis of $PGE_2$ (387). Importantly, using *in situ* hybridization histochemistry, Ek *et al* have shown that upregulation of COX-2 mRNA expression peaked at 1 hr of IL-1β injection in rat while expression of mPGES-1 mRNA peaked at 3 hr thus demonstrating a coordination between up-stream protein expression and $PGE_2$ biosynthesis (85). By RT-PCR analysis of mPGES-1 and mPGES-2 in brain tissue we have shown that only mPGES-1 mRNA was upregulated in response to LPS treatment in wildtype mice (**Paper II, fig. 2C**). In contast, mPGES-2 mRNA showed constitutive

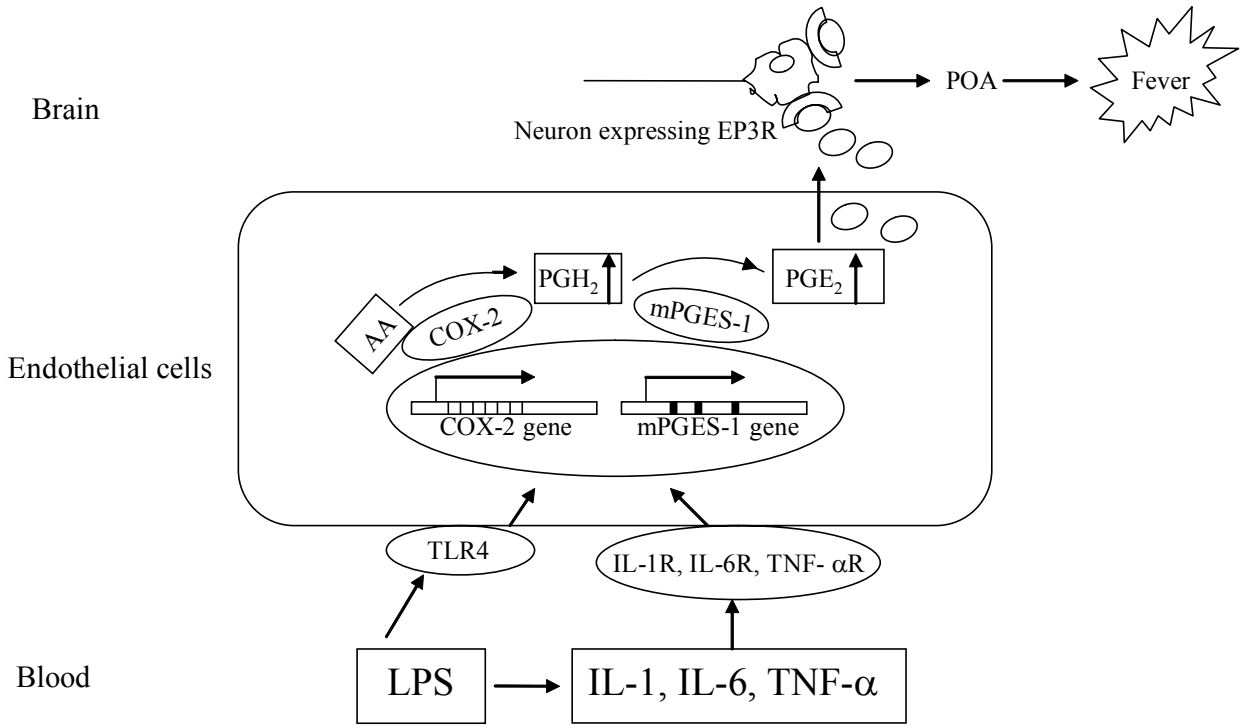


**Figure 7.** Schematic representation of the COX-2-mPGES-1-EP3 mediated pathway for the generation of LPS/IL-1β induced fever.

expression which did not upregulate by LPS treatment. This data gives a clear indication that endotoxin induced $PGE_2$ formation is dependent on mPGES-1 expression and the absence of mPGES-1 enzyme is not compensated by mPGES-2 for the synthesis of $PGE_2$. Analysis of $PGE_2$ levels in the CSF showed that wildtype mice injected with LPS contained significantly higher $PGE_2$ compared to the LPS treated mPGES-1 knockout mice. Interestingly, Yamagata *et al* have also detected similar $PGE_2$ levels in the CSF of LPS treated rats and pre-treatment of rats with NS-398 completely suppressed the $PGE_2$ levels which again confirms the COX-2-mPGES-1 pathway for the induced biosynthesis of $PGE_2$ in fever generation (387). These findings are in support of the model where cytokines exert their effects on the BBB mediated by $PGE_2$.

LPS is known to elicit a cascade of cytokine synthesis, including the formation of IL-1β, IL-6 and TNF-α. Functional IL-1 type 1 receptors are also expressed on endothelial cells, which express mPGES-1 (164). In addition to activating cytokines, LPS has been shown to exert effect directly by binding to TLR-4 (**fig. 4**) (53). In order to investigate if mPGES-1 is essential for cytokine-dependent fever generation, we used mPGES-1 knockout mice in an aseptic, cytokine-dependent fever model. Previous investigation using knockout mice lacking IL1-R1 has shown an essential role of IL1-R1 in APR such as fever, lethargy and anorexia to local inflammation caused by turpentine (187). However, intraperitoneal injection of a low or high dose of LPS induced similar APR in wildtype and IL1-R1 knockout mice. In our turpentine-induced fever model, wildtype mice showed a biphasic fever response to subcutaneous injection of turpentine. In contrast, the mPGES-1 deficient mice did not develop fever and displayed a temperature pattern similar to that of saline injected wildtype mice (**Paper III**, **fig. 5**) (295). However, both wildtype and the mPGES-1 knockout mice displayed

similar core body temperature throughout the light and dark cycle. Thus, in contrast to the dependence on mPGES-1 for the inflammation induced febrile response that was demonstrated in the aseptic fever model, mPGES-1 did not seem to be critical for regulating normal circadian temperature variations. In support, investigation using EP1 and EP3 receptor knockout mice showed that the baseline temperature in these mice did not differ from wildtype mice (262). In our stress-induced fever model, both the wildtype and the mPGES-1 knockout mice demonstrated similar febrile responses suggesting an mPGES-1 independent mechanism (**paper III**, **fig. 3**). In contrast to the findings demonstrated in our stress-induced fever model, Morimoto *et al* have shown that psychological stress elevated the plasma $PGE_2$ levels in rat (234), indicating the involvement of other PGE synthase enzymes in stress-induced fever development. The response displayed by the wildtype and the mPGES-1 deficient mice to intraperitoneal injection of IL-1β was very similar to that demonstrated in intraperitoneal LPS injection (**paper III**, **fig. 7 & paper II**, **fig. 1**), suggesting a similar phenomena involved in both processes.

## mPGES-1 IN NEONATAL RESPIRATORY DEPRESSION (PAPER IV)

Several factors have been attributed to the pathology of sudden infant death syndrome. Pro-inflammatory cytokines such as IL-1β has been proposed to play an active role in mediating infection and apnea. However, the entry of IL-1β into the brain is restricted since the larger size and lipophobic nature doesn't allow it to cross the BBB. Several lines of evidences show that $PGE_2$ has prominent respiratory depressant effects in the perinatal period in humans, sheeps and pigs (108, 190, 201). Moreover, intracarotid treatment of $PGE_2$ in 5-day old lambs caused hypoventilation due to a decrease in breathing frequency. $PGE_2$ also caused hypoxemia, hypercapnia, and increased the incidence of short apneas (117). Hoch *et al* have observed a significant correlation between urinary PGE metabolite concentration and number of central apneas in neonates (129). In our latest investigation we have demonstrated that intraperitoneal treatment of mice with IL-1β suppresses the central respiration and autoresuscitation by activation of mPGES-1. The positive correlation between increase activity of mPGES-1 and incidence of apneic events in the wildtype mice suggests a potential role of this enzyme in respiratory depression during hypoxic events. By direct application of $PGE_2$ to the brainstem-spinal cord preparation, we showed that the central respiratory rhythm generation was inhibited in the wildtype EP3 mice. In contrast, these effects were not apparent in mice lacking EP3 receptor. The possible underlying mechanism in respiratory depression might be the activation of mPGES-1 results in induced production of $PGE_2$, which, by binding to EP3 receptors modulates the activity of the neurons involved in respiratory rhythm generation. Previous findings have demonstrated the presence of NK1R expressing neurons in the pre-BötzC area (370). However, it remains to be investigated if EP3 receptors are also colocalized in the neurons expressing NK1R. Measurement of $PGE_2$ levels in the CSF will provide more information about the endogenous release of $PGE_2$ and its involvement in causing respiratory depression. Our results also demonstrated that similar to IL-1β, severe hypoxia in the wildtype mice can also activate mPGES-1 suggesting a similar pathway leading to respiratory depression during neonatal period. These effects of IL-1β and hypoxia were absent in mPGES-1 knockout mice and deletion of mPGES-1 gene demonstrated a positive effect on the survival of mice during anoxic events.

We have observed that wildtype mice exposed to 5 min hypoxia displayed significantly higher mPGES-1 activity compared to the control mice. The mechanism behind such a rapid (about 20 min between hypoxic exposure and sacrifice) activation of mPGES-1 in response to hypoxia is not known. One could speculate that post-transcriptional modification of the constitutively expressed mPGES-1 might be one potential mechanism. A previous investigation has demonstrated that the presence of IL-1α regulated stability of COX-2 mRNA and sustained COX-2 protein production in a human cell system (290). A very recent investigation has also demonstrated that in rat neonatal cardiomyocytes, both IL-1β and LPS stimulated mPGES-1 mRNA expression and protein synthesis by stabilization of mPGES-1 mRNA (67). Whether short term hypoxia exposure could also lead to stabilize both COX-2 and mPGES-1 mRNA and subsequently enhance the production of $PGE_2$ is subject of future investigations.

## mPGES-1 IN PATHOPHYSIOLOGY

### Pain

Prostaglandins, in particular $PGE_2$ and $PGI_2$ are key mediators of both central and peripheral pain sensitization (297). The importance of $PGE_2$ in inflammatory pain has been demonstrated by using selective anti-$PGE_2$ antibodies that inhibit pain sensitization, edema and hyperalgesia in rats (228, 281). Peripheral nociceptor terminals are sensitized by COX-2 derived $PGE_2$ and produce localized pain hypersensitivity (216). Peripheral inflammation also affects the neighboring tissue and causes pain hypersensitivity as well as muscle and joint pain, fever, fatigue and anorexia (64). These illness symptoms were thought to involve signaling by nerve impulses originating at the site of injury through the spinal cord to the brain (381). However, accumulating evidences show that $PGE_2$ is produced in the spinal cord in response to peripheral tissue injury and inflammation and thus $PGE_2$ is suggested in contributing nociceptive transmission and amplification of sensory outflow from the spinal cord (225, 247). In fact, a recent investigation demonstrated the crucial role mediated by mPGES-1 derived $PGE_2$ in developing anorexia in response to peripheral infection and neuroinflammation in mice (274). Moreover, Ek *et al* (85) and Yamagata *et al* (387) have independently shown that the pro-inflammatory cytokine, IL-1β, upregulates COX-2 and mPGES-1 expression in brain endothelial cells. These cells posses a coupled enzyme system resulting in induced production of $PGE_2$ during peripheral infections which provide signals to the brain. Studies using prostanoid receptor knockout mice showed that IP and EP3 are the major receptors involved in mediating enhanced acetic acid-induced writhing response which is a model of acute inflammatory pain, in LPS-treated mice (349). In line, Trebino *et al* (345) have demonstrated reduced pain sensitivity in mice deficient in mPGES-1. Furthermore, a recent study by Kamei *et al* (148) have also shown reduced pain sensitivity and inflammation in mPGES-1 deficient mice, altogether implicating a major contribution of mPGES-1 in inflammatory pain.

### Inflammation

Inflammation is the classic local response to tissue injury for instance, by microorganisms with the typical symptoms of pain, swelling, redness, heat generation and loss of function. Use of NSAIDs inhibit prostaglandin formation at inflammatory

sites and thus reduces the pathological symptoms related to inflammation (358). Large quantities of $PGE_2$ are formed at the site of inflammation, which mediate the pathological features of such conditions (182). Pro-inflammatory stimuli, such as IL-1β, TNF-α and LPS induce the expression of COX-2 and mPGES-1 both *in vitro* (322) and *in vivo* (212, 387). Trebino *et al* have shown that mPGES-1 plays a major role in both acute and chronic inflammation in mice (345). Furthermore, a recent study by Westman *et al* (373) have demonstrated that mPGES-1 is over-expressed in synovial tissues from patients with rheumatoid arthritis (RA). These data support the involvement of mPGES-1 in the development of inflammation.

**Rheumatoid arthritis**

Rheumatoid arthritis is a prototype of inflammatory arthritis characterized by chronic and erosive synovitis of peripheral joints. NSAIDs, glucocorticoids as well as disease-modifying anti-rheumatic drugs (DMARDs) have been clinically used for the medical treatment of RA (275). RA is regarded as an autoimmune disease and there is a strong association with a number of autoantibodies (320). COX-2 and mPGES-1 are coordinately upregulated by IL-1β in the perinuclear membrane in rheumatoid arthritis synovial fibroblasts (RASFs), causing an increased $PGE_2$ production at the site of inflammation (163). Although the synovial fibroblasts derived from RA patients were demonstrated to constitutively express cPGES and mPGES-2, the expression of these enzymes were not upregulated in response to IL-1β stimuli. Moreover, $PGE_2$ enhances mPGES-1 expression associated with an increase of cAMP levels via EP2 and EP4 receptors in IL-1β stimulated RASFs (162). A recent study showed that in early passages of RA-derived synovial cells, the COX-1 gene is induced by IL-1α and thus, suggest the involvement of COX-1 in an acute phase inflammation of RA patients (266). However, IL-1α-induced COX-1 expression was not observed in the RA-derived synovial cells after five passages. In addition to contributing to the pathogenesis of RA, mPGES-1 expression was induced by IL-1β in the synovial fibroblasts from patients with osteoarthritis (OA). Evidence also shows that IL-1β and TNF-α stimulation triggered the expression of mPGES-1 in the articular chondriocytes of OA patients (161). A current investigation also focused on the effect of TNF-α blockers in the remission of RA (165). TNF blockers did not show significant down regulation of COX-2 and mPGES-1 in the synovial tissue from RA patients before and after treatment. In contrast, local administration of steroids decreased the expression of COX-2 and mPGES-1 in the synovial tissues of patients with inflammatory arthritis including RA. Altogether these evidences suggest mPGES-1 as an additional target for the treatment of inflammatory arthritis including RA and OA.

**Cancer**

The first evidence of a potential relationship between COX-2 and human cancers was reported in 1994, when COX-2 mRNA levels were found to be markedly elevated in colorectal carcinomas (80). COX-2 and the downstream enzyme mPGES-1 were found to be upregulated in many forms of cancer, including colorectal, breast, prostate, colon and lung cancer cells (46, 110, 392, 393). There are clinical, biochemical and epidemiological evidences for the importance of COX-2 derived $PGE_2$ in the development of colorectal cancer and possibly other cancers (374). Experimental and

epidemiologic studies have demonstrated that NSAIDs are effective in the prevention of human cancers (35, 54, 286, 310).

The potential role of COX-2 and mPGES-1 in colorectal carcinogenesis was strengthened by gene targeting studies. In a model of familial adenomatous polyposis (FAP), it was shown that disruption of the COX-2 gene results in a reduction in the number of intestinal polyps (267). In a similar study, using the same model system, it was demonstrated that EP2 and not the other EP receptor subtypes substantially affected the polyposis phenotype (316) suggesting that $PGE_2$ signalling mediated through EP2 receptor contributes essential role in intestinal polyposis. In a human embryonic kidney cell line HEK293, transfection of both mPGES-1 and COX-2 resulted in an aggressive cell growth and aberrant morphology, suggesting a functional coupling of these enzymes in the development of tumors (242). However, the degree of expression of COX-2 and mPGES-1 was different in various tumors. For example, in non-small lung cancer and colorectal adenomas, marked differences in the extent of up-regulation of mPGES-1 and COX-2 were observed in individual tumors, suggesting different regulatory mechanisms (392, 393). In contrast to these findings, a transgenic mice model overexpressing mPGES-1 in the alveolar and airways epithelial cells did not show any significant difference in tumor multiplicity or size compared to the control mice (24) suggesting that overproduction of $PGE_2$ is not sufficient in the induction of lung tumors. In this context one might speculate that although over-expression of mPGES-1 will lead to induction of $PGE_2$ production but these cells may not express sufficient EP receptor subtype that will subsequently binds the excess $PGE_2$ in order to mediate the effect in inducing tumorigenesis. Further investigation is needed to verify the role of inducible PGES-1 in the pathogenesis of lung cancer. Evaluation of COX-2 and mPGES-1 protein expression in gastric cancer shows that compared to non-neoplastic adjacent tissues, these inducible proteins were expressed more in cancer tissues and gradually increased with the progression of gastric lesions with the highest expression observed in dysplasia (144). In summary, increasing bodies of evidences show the potential involvement of mPGES-1 in many different cancer types, including breast, lung, intestinal, gastric and others, suggesting mPGES-1 as a potential additional target for the treatment of cancer.

## mPGES-1 AS A DRUG TARGET

NSAIDs have been used for centuries in the treatment of common cold, headache and fever as well as in rheumatoid arthritis, atherosclerosis and cancer. Although COX-2 specific inhibitors posses less gastrointestinal toxicity, as compared to the non-specific COX inhibitors, severe unwanted side effects still exist (63). For example, specific COX-2 inhibition can lead to edema and elevated blood pressure due to altered excretion of sodium (321), and there are reports of cardiovascular morbidity following the use of these drugs (343). Inhibition of mPGES-1 is probably a better alternative to combat these diseases and allow the production of anti-inflammatory metabolites. For instance, cells expressing PGD synthase might shunt COX-2 derived $PGH_2$ to $PGD_2$, which can further be non-enzymatically metabolized to produce the anti-inflammatory metabolite 15-deoxy-$\Delta^{12,14}$-$PGJ_2$ (209). However, the amounts of these metabolites produced *in vivo* are not yet known and needs further investigations. Since mPGES-1 deficient mice demonstrate a normal physiology (behavior, weight, reproduction etc.), inhibition of mPGES-1 would theoretically not interfere with any homeostatic

functions although the role of mPGES-1 in kidney models needs to be investigated. Furthermore, accumulating evidences suggest mPGES-1 as an important determinant in pain, inflammation, cancers and neurological disorders and thus selective inhibition of $PGE_2$ biosynthesis by specific inhibition of mPGES-1 seems to be a promising therapeutic target.

## CONCLUSION

We have characterized the recombinant human mPGES-1, purified the protein to apparent homogeneity and prepared 2-D crystals. We have demonstrated by two independent methods, electron microscopy and hydrodynamic studies, that purified recombinant human 6-histidine tagged mPGES-1 constitutes a homo-trimer. In addition to PGE synthase activity, purified mPGES-1 possessed several other activities, like GSH-dependent peroxidase and GSH-transferase activities, probably representing the evolutionary relationship to other MAPEG members. In an endotoxin-induced fever model, mPGES-1 was found to be critical in mediating fever. Several studies have demonstrated co-expression of mPGES-1 and COX-2 in the brain endothelial cells, suggesting that these enzymes are functionally coupled. mPGES-1 was also found to play a major role in a local, aseptic, inflammation-induced fever development in mice. We have also demonstrated that mPGES-1 mediates Il-1β induced respiratory depression in neonatal mice and there seems to be a positive correlation between $PGE_2$ levels and incidence of apneic events in human infants. There are now increasing evidences for the role of mPGES-1 in a number of pathological conditions and diseases like RA, cancer, atherosclerosis and neurodegenerative disorders. Taken together, the results from our studies and others suggest that mPGES-1 is an important enzyme involved in the pathogenesis of various diseases.

## FUTURE PERSPECTIVE

Despite a substantial amount of research showing the involvement of mPGES-1 in various inflammatory diseases, a number of questions remain unanswered. The development of mPGES-1 knockout mice has enabled a few steps forward in the understanding of the physiological and pathophysiological functions of mPGES-1. However, if this understanding can be extended and applicable in human beings remain a major issue to be solved. The most downstream position of mPGES-1 in the arachidonic acid metabolic cascade presents this enzyme as a potent and selective target as a novel anti-inflammatory drug. Determination of the 3-dimensional structure of mPGES-1 at atomic resolution would provide some basis for better understanding of the mechanism of catalysis and hopefully assorting rational drug design. The effect of genetic disruption of the mPGES-1 gene has provided the evidence for the shunting of prostaglandins towards the major formation of $TxB_2$ (a stable derivative of $TxA_2$) in murine macrophages (344), although the major shunting of $PGH_2$ *in vivo* seems to be $PGI_2$ (49). The redirection of $PGH_2$ metabolism by inhibition of mPGES-1 and the physiological effect of the altered prostanoid metabolic profile will have profound importance for the pharmacological effects of future drug candidates.

# ACKNOWLEDGEMENTS

I take the opportunity to express my sincere gratitude to a number of people for their constant support and inspiration that enabled me to reach this point.

Prof. Hans Hebert for providing a new turn in my research carrier. I am grateful for giving me the opportunity to pursue my Ph.D at KI, your excellent scientific advice, positive attitude and your enthusiastic view in my research. A special thanks to Peter for your friendship and all the practical helps we needed at the beginning of our Stockholm life, Philip for your help and practical guidance, Caroline for your unlimited patience for the search of good crystals, Anna-Karin and Kimberly for the help and understanding during teaching at Södertörn Högskola. Thanks to Passi, Ronny, Björn, Urban, Ulrika, Thomas, Martin and Hans Emlund for the nice discussions during group meetings, Kristina and Ylva for administrative helps.

I would like to thank Prof. Bengt Samuelsson for an excellent scientific and working environment at MBB.
Thanks to Per-Johan Jakobsson, my main supervisor, for your constant support and guidance, valuable thought provoking discussions and providing me with excellent research projects. Thanks for teaching me the art of biochemical techniques and always making me feel confident through your positive thinking. A special thanks to Staffan for sharing your knowledge and experience in the prostaglandin field and especially our discussions about all aspects of life and for your valuable input to my thesis. Thanks to Rolf for making me friendly with FPLC, Pelle for helping attitude and sharing knowledge in enzyme kinetics, Karina for our good friendship, Gudrun for keeping a good atmosphere in the lab, Sven for your interest about mPGES-1 and sharing your results, Hanna for our short project discussions.
Thanks to Marina and Marie for helping out with the genotyping.
I would like to thank Prof. Jesper Haeggström and Olof Rådmark for always keeping good spirit and excellent scientific advises during morning meetings. A special thanks to Anders Wetterholm for critical reading of my thesis and helping out to overcome any problematic situation (life-saver). Thanks to Eva for being so friendly and for sharing your experience of plantation and Min for all the practical helps, saved a lot of time. Thanks to former officemate Hong for the discussions about our scientific and family life.
Thanks to Fredrik, Peter, Tove, Filippa, Maria, Hiromi, Lena, Ulrika, Mathias, Vildan, Anthony, Marika and former officemates Cecelia, Cattis and Mattias for all the interesting chats and evening parties.
A special thanks to Al for proofreading the abstract of my thesis.
Thanks to Prof. Mats Hamberg for your interest in my research and scientific advice. Thanks to Gunvor for the practical help and Anneli for administrative help.
A big thanks to Angelica, Sussane, Agneta, Sören, Jan and Svante for your generous help. Thanks to all the staffs at Scheele's animal facility for taking a good care of our animals.
I would like to thank all the people at Biolipox.

I am also thankful to Prof. Ralf Morgenstern for the scientific advice. Richard, Johan, Louise and Miyuki for the fruitful discussions and sharing nice time during MAPEG meetings.

A special thanks to Prof. Anders Blomqvist for giving me the opportunity to work with the fever projects in your lab at Linköping. David, Linda, Ludmilla, Johan, Louise, Anna-Maria, Anna and Camilla for your help and support.

Thanks to Eric Herlenius, my cosupervisor for your enthusiasm and giving me the opportunity to work with the respiratory project. Despite of your night shifts at the clinic and million of applications (more than one) to write during weekends, you always found time to discuss about our latest project. A special thanks to Annika for the cooperation in the project and your excellent understanding power. Veronica for the helps with running initial experiments, Linda and Johan for the practical helps. Thanks to Ruth for all the helps at ALB. Kristin Löfgren and Josephine for your help with animal work.

Thanks to Prof. Barry Rubin, Toronto, for the collaboration with the rat cardiomyocyte project.

Thanks to our family friends:
Swami Shivanandaji for your endless affection and valuable advises. Dr. Tapan Das for your encouragement and interest in my research. Ghoshalda and boudi for inspiration.
Rajiv Kumar for becoming a close family friend and your concern in our scientific as well as family life. Bidisa and Munu for your love and our friendship. Ranada, Tabassum for having a great time together in Röntgenvägen 1. Boudi, Puja and Ulrika for your nice company. Safiqda for our nice chat during dinner. Mahboobda, Iladi and Spondon for helping out with almost everything and sharing moments of joy and sorrow. Bulubhai, Ruluapa, Anisha and Tomali for the enjoyable moments we share together during weekends. Praveen, Ruma and Anushri for sharing our news and views about day to day life. Koustuv and Madhumita for nice hospitality. Amit, Divya, Adi and Anandita for being a nice neighbour.

Thanks to my in-laws family for your love and understanding. Tuski, Dimpi, Gora, Subha, Deep and Titli for your affection. Dada and boudi for love and care and Ipsha for counting days to our India visit.

Last but not least, I thank my husband Priya for your endless love and support, and teaching me to keep patience and my son Aritra for your faith that mamma is the best and also for correcting my Swedish pronunciations.

# REFERENCES


1. **Abramovitz M, Boie Y, Nguyen T, Rushmore TH, Bayne MA, Metters KM, Slipetz DM, and Grygorczyk R.** Cloning and expression of a cDNA for the human prostanoid FP receptor. *J Biol Chem* 269: 2632-2636, 1994.
2. **Akiba S and Sato T.** Cellular function of calcium-independent phospholipase A2. *Biol Pharm Bull* 27: 1174-1178, 2004.
3. **Alheid GF, Gray PA, Jiang MC, Feldman JL, and McCrimmon DR.** Parvalbumin in respiratory neurons of the ventrolateral medulla of the adult rat. *J Neurocytol* 31: 693-717, 2002.
4. **Angeli V, Staumont D, Charbonnier AS, Hammad H, Gosset P, Pichavant M, Lambrecht BN, Capron M, Dombrowicz D, and Trottein F.** Activation of the D prostanoid receptor 1 regulates immune and skin allergic responses. *J Immunol* 172: 3822-3829, 2004.
5. **Anggard E.** The biological activities of three metabolites of prostaglandin E 1. *Acta Physiologica Scandinavica* 66: 509-510, 1966.
6. **Anggard E, Larsson C, and Samuelsson B.** The distribution of 15-hydroxy prostaglandin dehydrogenase and prostaglandin-delta 13-reductase in tissues of the swine. *Acta Physiologica Scandinavica* 81: 396-404, 1971.
7. **Auphan N, DiDonato JA, Rosette C, Helmberg A, and Karin M.** Immunosuppression by glucocorticoids: inhibition of NF-kappa B activity through induction of I kappa B synthesis. *Science* 270: 286-290, 1995.
8. **Auron PE, Warner SJ, Webb AC, Cannon JG, Bernheim HA, McAdam KJ, Rosenwasser LJ, LoPreste G, Mucci SF, and Dinarello CA.** Studies on the molecular nature of human interleukin 1. *J Immunol* 138: 1447-1456, 1987.
9. **Avitsur R, Pollak Y, and Yirmiya R.** Administration of interleukin-1 into the hypothalamic paraventricular nucleus induces febrile and behavioral effects. *Neuroimmunomodulation* 4: 258-265, 1997.
10. **Backlund MG, Mann JR, Holla VR, Buchanan FG, Tai HH, Musiek ES, Milne GL, Katkuri S, and DuBois RN.** 15-Hydroxyprostaglandin dehydrogenase is down-regulated in colorectal cancer. *J Biol Chem* 280: 3217-3223, 2005.
11. **Balsinde J, Winstead MV, and Dennis EA.** Phospholipase A(2) regulation of arachidonic acid mobilization. *FEBS Lett* 531: 2-6, 2002.
12. **Banks WA and Kastin AJ.** Blood to brain transport of interleukin links the immune and central nervous systems. *Life Sci* 48: PL117-121, 1991.
13. **Banks WA, Kastin AJ, and Broadwell RD.** Passage of cytokines across the blood-brain barrier. *Neuroimmunomodulation* 2: 241-248, 1995.
14. **Battez G and Boulet L.** Action de l'extrait de prostate humaine sur la vessie et sur la pression artérielle. *CR Soc Biol Paris* 74: 8, 1913.
15. **Baumgartner RA, Deramo VA, and Beaven MA.** Constitutive and inducible mechanisms for synthesis and release of cytokines in immune cell lines. *J Immunol* 157: 4087-4093, 1996.
16. **Beckwith JB.** Defining the sudden infant death syndrome. *Arch Pediatr Adolesc Med* 157: 286-290, 2003.
17. **Bergström S, Danielsson H, and Samuelsson B.** The enzymatic formation of prostaglandin $E_2$ from arachidonic acid. Prostaglandin and related factors 32. *Biochim Biophys Acta* 90: 207-210, 1964.
18. **Bergström S, Ryhage R, Samuelsson B, and Sjövall J.** Prostaglandins and related factors. 15. The structures of prostaglandin $E_1$, $F_{1\alpha}$ and $F_{1\beta}$. *J Biol Chem* 238: 3555-3564, 1963.
19. **Bergström S and Sjövall J.** The isolation of prostaglandin E from sheep prostate glands. *Acta Chem Scand* 14: 1701-1705, 1960.
20. **Beuckmann CT, Fujimori K, Urade Y, and Hayaishi O.** Identification of mu-class glutathione transferases M2-2 and M3-3 as cytosolic prostaglandin E synthases in the human brain. *Neurochem Res* 25: 733-738, 2000.

21. **Bhattacharya M, Peri KG, Almazan G, Ribeiro-da-Silva A, Shichi H, Durocher Y, Abramovitz M, Hou X, Varma DR, and Chemtob S.** Nuclear localization of prostaglandin E2 receptors. *Proc Natl Acad Sci U S A* 95: 15792-15797, 1998.
22. **Blackwell CC, Moscovis SM, Gordon AE, Al Madani OM, Hall ST, Gleeson M, Scott RJ, Roberts-Thomson J, Weir DM, and Busuttil A.** Cytokine responses and sudden infant death syndrome: genetic, developmental, and environmental risk factors. *J Leukoc Biol* 78: 1242-1254, 2005.
23. **Blackwell CC and Weir DM.** The role of infection in sudden infant death syndrome. *FEMS Immunol Med Microbiol* 25: 1-6, 1999.
24. **Blaine SA, Meyer AM, Hurteau G, Wick M, Hankin JA, Murphy RC, Dannenberg AJ, Geraci MW, Subbaramaiah K, and Nemenoff RA.** Targeted over-expression of mPGES-1 and elevated PGE2 production is not sufficient for lung tumorigenesis in mice. *Carcinogenesis* 26: 209-217, 2005.
25. **Blatteis CM.** The afferent signalling of fever. *J Physiol* 526 Pt 3: 470, 2000.
26. **Blatteis CM.** Role of the OVLT in the febrile response to circulating pyrogens. *Prog Brain Res* 91: 409-412, 1992.
27. **Blatteis CM and Sehic E.** Circulating pyrogen signaling of the brain. A new working hypothesis. *Ann N Y Acad Sci* 813: 445-447, 1997.
28. **Boie Y, Rushmore TH, Darmon-Goodwin A, Grygorczyk R, Slipetz DM, Metters KM, and Abramovitz M.** Cloning and expression of a cDNA for the human prostanoid IP receptor. *J Biol Chem* 269: 12173-12178, 1994.
29. **Bonventre JV, Huang Z, Taheri MR, O'Leary E, Li E, Moskowitz MA, and Sapirstein A.** Reduced fertility and postischaemic brain injury in mice deficient in cytosolic phospholipase A2. *Nature* 390: 622-625, 1997.
30. **Botting RM.** Mechanism of action of acetaminophen: is there a cyclooxygenase 3? *Clin Infect Dis* 31 Suppl 5: S202-210, 2000.
31. **Boulet L, Ouellet M, Bateman KP, Ethier D, Percival MD, Riendeau D, Mancini JA, and Methot N.** Deletion of microsomal prostaglandin E2 (PGE2) synthase-1 reduces inducible and basal PGE2 production and alters the gastric prostanoid profile. *J Biol Chem* 279: 23229-23237, 2004.
32. **Boyer TD, Vessey DA, and Kempner E.** Radiation inactivation of microsomal glutathione S-transferase. *J Biol Chem* 261: 16963-16968, 1986.
33. **Braden GA, Knapp HR, and FitzGerald GA.** Suppression of eicosanoid biosynthesis during coronary angioplasty by fish oil and aspirin. *Circulation* 84: 679-685, 1991.
34. **Breyer RM, Bagdassarian CK, Myers SA, and Breyer MD.** Prostanoid receptors: subtypes and signaling. *Annu Rev Pharmacol Toxicol* 41: 661-690, 2001.
35. **Buchanan FG, Chang W, Sheng H, Shao J, Morrow JD, and DuBois RN.** Up-regulation of the enzymes involved in prostacyclin synthesis via Ras induces vascular endothelial growth factor. *Gastroenterology* 127: 1391-1400, 2004.
36. **Burns K, Martinon F, Esslinger C, Pahl H, Schneider P, Bodmer JL, Di Marco F, French L, and Tschopp J.** MyD88, an adapter protein involved in interleukin-1 signaling. *J Biol Chem* 273: 12203-12209, 1998.
37. **Cannon GW, Caldwell JR, Holt P, McLean B, Seidenberg B, Bolognese J, Ehrich E, Mukhopadhyay S, and Daniels B.** Rofecoxib, a specific inhibitor of cyclooxygenase 2, with clinical efficacy comparable with that of diclofenac sodium: results of a one-year, randomized, clinical trial in patients with osteoarthritis of the knee and hip. Rofecoxib Phase III Protocol 035 Study Group. *Arthritis Rheum* 43: 978-987, 2000.
38. **Cartmell T, Ball C, Bristow AF, Mitchell D, and Poole S.** Endogenous interleukin-10 is required for the defervescence of fever evoked by local lipopolysaccharide-induced and Staphylococcus aureus-induced inflammation in rats. *J Physiol* 549: 653-664, 2003.
39. **Cartmell T, Poole S, Turnbull AV, Rothwell NJ, and Luheshi GN.** Circulating interleukin-6 mediates the febrile response to localised inflammation in rats. *J Physiol* 526 Pt 3: 653-661, 2000.
40. **Cerretti DP, Hollingsworth LT, Kozlosky CJ, Valentine MB, Shapiro DN, Morris SW, and Nelson N.** Molecular characterization of the gene for human interleukin-1 beta converting enzyme (IL1BC). *Genomics* 20: 468-473, 1994.

41. **Chai Z, Gatti S, Toniatti C, Poli V, and Bartfai T.** Interleukin (IL)-6 gene expression in the central nervous system is necessary for fever response to lipopolysaccharide or IL-1 beta: a study on IL-6-deficient mice. *J Exp Med* 183: 311-316, 1996.
42. **Chan CC, Boyce S, Brideau C, Charleson S, Cromlish W, Ethier D, Evans J, Ford-Hutchinson AW, Forrest MJ, Gauthier JY, Gordon R, Gresser M, Guay J, Kargman S, Kennedy B, Leblanc Y, Leger S, Mancini J, O'Neill GP, Ouellet M, Patrick D, Percival MD, Perrier H, Prasit P, Rodger I, and et al** Rofecoxib [Vioxx, MK-0966; 4-(4'-methylsulfonylphenyl)-3-phenyl-2-(5H)-furanone]: a potent and orally active cyclooxygenase-2 inhibitor. Pharmacological and biochemical profiles. *J Pharmacol Exp Ther* 290: 551-560, 1999.
43. **Chandrasekharan NV, Dai H, Roos KL, Evanson NK, Tomsik J, Elton TS, and Simmons DL.** COX-3, a cyclooxygenase-1 variant inhibited by acetaminophen and other analgesic/antipyretic drugs: cloning, structure, and expression. *Proc Natl Acad Sci U S A* 99: 13926-13931, 2002.
44. **Chandrasekharan S, Foley NA, Jania L, Clark P, Audoly LP, and Koller BH.** Coupling of COX-1 to mPGES1 for prostaglandin E2 biosynthesis in the murine mammary gland. *J Lipid Res* 46: 2636-2648, 2005.
45. **Chang M, Hong Y, Burgess JR, Tu CP, and Reddy CC.** Isozyme specificity of rat liver glutathione S-transferases in the formation of PGF2 alpha and PGE2 from PGH2. *Arch Biochem Biophys* 259: 548-557, 1987.
46. **Chang SH, Liu CH, Conway R, Han DK, Nithipatikom K, Trifan OC, Lane TF, and Hla T.** Role of prostaglandin E2-dependent angiogenic switch in cyclooxygenase 2-induced breast cancer progression. *Proc Natl Acad Sci U S A* 101: 591-596, 2004.
47. **Chen G and Goeddel DV.** TNF-R1 signaling: a beautiful pathway. *Science* 296: 1634-1635, 2002.
48. **Chen J, Engle SJ, Seilhamer JJ, and Tischfield JA.** Cloning and recombinant expression of a novel human low molecular weight Ca(2+)-dependent phospholipase A2. *J Biol Chem* 269: 2365-2368, 1994.
49. **Cheng Y, Wang M, Yu Y, Lawson J, Funk CD, and Fitzgerald GA.** Cyclooxygenases, microsomal prostaglandin E synthase-1, and cardiovascular function. *J Clin Invest* 116: 1391-1399, 2006.
50. **Chevalier E, Stock J, Fisher T, Dupont M, Fric M, Fargeau H, Leport M, Soler S, Fabien S, Pruniaux MP, Fink M, Bertrand CP, McNeish J, and Li B.** Cutting edge: chemoattractant receptor-homologous molecule expressed on Th2 cells plays a restricting role on IL-5 production and eosinophil recruitment. *J Immunol* 175: 2056-2060, 2005.
51. **Chi Y, Khersonsky SM, Chang YT, and Schuster VL.** Identification of a new class of prostaglandin transporter inhibitors and characterization of their biological effects on prostaglandin E2 transport. *J Pharmacol Exp Ther* 316: 1346-1350, 2006.
52. **Chillingworth NL, Morham SG, and Donaldson LF.** Sex differences in inflammation and inflammatory pain in cyclooxygenase-deficient mice. *Am J Physiol Regul Integr Comp Physiol* in press, 2006.
53. **Chow JC, Young DW, Golenbock DT, Christ WJ, and Gusovsky F.** Toll-like receptor-4 mediates lipopolysaccharide-induced signal transduction. *J Biol Chem* 274: 10689-10692, 1999.
54. **Chu AJ, Chou TH, and Chen BD.** Prevention of colorectal cancer using COX-2 inhibitors: basic science and clinical applications. *Front Biosci* 9: 2697-2713, 2004.
55. **Chulada PC, Thompson MB, Mahler JF, Doyle CM, Gaul BW, Lee C, Tiano HF, Morham SG, Smithies O, and Langenbach R.** Genetic disruption of Ptgs-1, as well as Ptgs-2, reduces intestinal tumorigenesis in Min mice. *Cancer Res* 60: 4705-4708, 2000.
56. **Cipollone F, Prontera C, Pini B, Marini M, Fazia M, De Cesare D, Iezzi A, Ucchino S, Boccoli G, Saba V, Chiarelli F, Cuccurullo F, and Mezzetti A.** Overexpression of functionally coupled cyclooxygenase-2 and prostaglandin E synthase in symptomatic atherosclerotic plaques as a basis of prostaglandin E(2)-dependent plaque instability. *Circulation* 104: 921-927, 2001.
57. **Clark JD, Lin LL, Kriz RW, Ramesha CS, Sultzman LA, Lin AY, Milona N, and Knopf JL.** A novel arachidonic acid-selective cytosolic PLA2 contains a Ca(2+)-dependent translocation domain with homology to PKC and GAP. *Cell* 65: 1043-1051, 1991.

58. **Claveau D, Sirinyan M, Guay J, Gordon R, Chan CC, Bureau Y, Riendeau D, and Mancini JA.** Microsomal prostaglandin E synthase-1 is a major terminal synthase that is selectively up-regulated during cyclooxygenase-2-dependent prostaglandin E2 production in the rat adjuvant-induced arthritis model. *J Immunol* 170: 4738-4744, 2003.
59. **Coceani F, Lees J, Mancilla J, Belizario J, and Dinarello CA.** Interleukin-6 and tumor necrosis factor in cerebrospinal fluid: changes during pyrogen fever. *Brain Res* 612: 165-171, 1993.
60. **Colotta F, Re F, Muzio M, Bertini R, Polentarutti N, Sironi M, Giri JG, Dower SK, Sims JE, and Mantovani A.** Interleukin-1 type II receptor: a decoy target for IL-1 that is regulated by IL-4. *Science* 261: 472-475, 1993.
61. **Conti P, Dempsey RA, Reale M, Barbacane RC, Panara MR, Bongrazio M, and Mier JW.** Activation of human natural killer cells by lipopolysaccharide and generation of interleukin-1 alpha, beta, tumour necrosis factor and interleukin-6. Effect of IL-1 receptor antagonist. *Immunology* 73: 450-456, 1991.
62. **Cooper AL, Brouwer S, Turnbull AV, Luheshi GN, Hopkins SJ, Kunkel SL, and Rothwell NJ.** Tumor necrosis factor-alpha and fever after peripheral inflammation in the rat. *Am J Physiol* 267: R1431-1436, 1994.
63. **Crofford LJ, Lipsky PE, Brooks P, Abramson SB, Simon LS, and van de Putte LB.** Basic biology and clinical application of specific cyclooxygenase-2 inhibitors. *Arthritis Rheum* 43: 4-13, 2000.
64. **Dantzer R, Bluthe RM, Gheusi G, Cremona S, Laye S, Parnet P, and Kelley KW.** Molecular basis of sickness behavior. *Ann N Y Acad Sci* 856: 132-138, 1998.
65. **de Waal Malefyt R, Abrams J, Bennett B, Figdor CG, and de Vries JE.** Interleukin 10(IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. *J Exp Med* 174: 1209-1220, 1991.
66. **de Waal Malefyt R, Yssel H, Roncarolo MG, Spits H, and de Vries JE.** Interleukin-10. *Curr Opin Immunol* 4: 314-320, 1992.
67. **Degousee N, Angoulvant D, Fazel S, Stefanski E, Saha S, Iliescu K, Lindsay TF, Fish JE, Marsden PA, Li RK, Audoly LP, Jakobsson PJ, and Rubin BB.** c-Jun N-terminal kinase mediated stabilization of microsomal prostaglandin E2 synthase-1 mRNA regulates delayed microsomal prostaglandin E2 synthase-1 expression and prostaglandin E2 biosynthesis by cardiomyocytes. *J Biol Chem* in press, 2006.
68. **Dennis EA.** The growing phospholipase A2 superfamily of signal transduction enzymes. *Trends Biochem Sci* 22: 1-2, 1997.
69. **DeWitt DL and Smith WL.** Primary structure of prostaglandin G/H synthase from sheep vesicular gland determined from the complementary DNA sequence. *Proc Natl Acad Sci U S A* 85: 1412-1416, 1988.
70. **DeWitt DL and Smith WL.** Purification of prostacyclin synthase from bovine aorta by immunoaffinity chromatography. Evidence that the enzyme is a hemoprotein. *J Biol Chem* 258: 3285-3293, 1983.
71. **Dieter P, Scheibe R, Jakobsson PJ, Watanabe K, Kolada A, and Kamionka S.** Functional coupling of cyclooxygenase 1 and 2 to discrete prostanoid synthases in liver macrophages. *Biochem Biophys Res Commun* 276: 488-492, 2000.
72. **Dinarello CA.** Biologic basis for interleukin-1 in disease. *Blood* 87: 2095-2147, 1996.
73. **Dinarello CA.** Cytokines as endogenous pyrogens. *J Infect Dis* 179 Suppl 2: S294-304, 1999.
74. **Dinarello CA, Cannon JG, Wolff SM, Bernheim HA, Beutler B, Cerami A, Figari IS, Palladino MA, Jr., and O'Connor JV.** Tumor necrosis factor (cachectin) is an endogenous pyrogen and induces production of interleukin 1. *J Exp Med* 163: 1433-1450, 1986.
75. **Ding Y, Tong M, Liu S, Moscow JA, and Tai HH.** NAD+-linked 15-hydroxyprostaglandin dehydrogenase (15-PGDH) behaves as a tumor suppressor in lung cancer. *Carcinogenesis* 26: 65-72, 2005.
76. **Dixon DA, Kaplan CD, McIntyre TM, Zimmerman GA, and Prescott SM.** Post-transcriptional control of cyclooxygenase-2 gene expression. The role of the 3'-untranslated region. *J Biol Chem* 275: 11750-11757, 2000.
77. **Dixon DA, Tolley ND, King PH, Nabors LB, McIntyre TM, Zimmerman GA, and Prescott SM.** Altered expression of the mRNA stability factor HuR promotes cyclooxygenase-2 expression in colon cancer cells. *J Clin Invest* 108: 1657-1665, 2001.

78. **Dixon RA, Diehl RE, Opas E, Rands E, Vickers PJ, Evans JF, Gillard JW, and Miller DK.** Requirement of a 5-lipoxygenase-activating protein for leukotriene synthesis. *Nature* 343: 282-284, 1990.
79. **Doi Y, Minami T, Nishizawa M, Mabuchi T, Mori H, and Ito S.** Central nociceptive role of prostacyclin (IP) receptor induced by peripheral inflammation. *Neuroreport* 13: 93-96, 2002.
80. **Eberhart CE, Coffey RJ, Radhika A, Giardiello FM, Ferrenbach S, and DuBois RN.** Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. *Gastroenterology* 107: 1183-1188, 1994.
81. **Eck MJ and Sprang SR.** The structure of tumor necrosis factor-alpha at 2.6 A resolution. Implications for receptor binding. *J Biol Chem* 264: 17595-17605, 1989.
82. **Eguchi Y, Eguchi N, Oda H, Seiki K, Kijima Y, Matsu-ura Y, Urade Y, and Hayaishi O.** Expression of lipocalin-type prostaglandin D synthase (beta-trace) in human heart and its accumulation in the coronary circulation of angina patients. *Proc Natl Acad Sci U S A* 94: 14689-14694, 1997.
83. **Eisenberg SP, Brewer MT, Verderber E, Heimdal P, Brandhuber BJ, and Thompson RC.** Interleukin 1 receptor antagonist is a member of the interleukin 1 gene family: evolution of a cytokine control mechanism. *Proc Natl Acad Sci U S A* 88: 5232-5236, 1991.
84. **Ek M, Arias C, Sawchenko P, and Ericsson-Dahlstrand A.** Distribution of the EP3 prostaglandin E(2) receptor subtype in the rat brain: relationship to sites of interleukin-1-induced cellular responsiveness. *J Comp Neurol* 428: 5-20, 2000.
85. **Ek M, Engblom D, Saha S, Blomqvist A, Jakobsson PJ, and Ericsson-Dahlstrand A.** Inflammatory response: pathway across the blood-brain barrier. *Nature* 410: 430-431, 2001.
86. **Engblom D, Saha S, Engstrom L, Westman M, Audoly LP, Jakobsson PJ, and Blomqvist A.** Microsomal prostaglandin E synthase-1 is the central switch during immune-induced pyresis. *Nat Neurosci* 6: 1137-1138, 2003.
87. **Ensor CM and Tai HH.** 15-Hydroxyprostaglandin dehydrogenase. *J Lipid Mediat Cell Signal* 12: 313-319, 1995.
88. **Evans SS, Wang WC, Bain MD, Burd R, Ostberg JR, and Repasky EA.** Fever-range hyperthermia dynamically regulates lymphocyte delivery to high endothelial venules. *Blood* 97: 2727-2733, 2001.
89. **Farina M, Ribeiro ML, Weissmann C, Estevez A, Billi S, Vercelli C, and Franchi A.** Biosynthesis and catabolism of prostaglandin F2alpha (PGF2alpha) are controlled by progesterone in the rat uterus during pregnancy. *J Steroid Biochem Mol Biol* 91: 211-218, 2004.
90. **Feldman JL, Mitchell GS, and Nattie EE.** Breathing: rhythmicity, plasticity, chemosensitivity. *Annu Rev Neurosci* 26: 239-266, 2003.
91. **Ferguson-Smith AC, Chen YF, Newman MS, May LT, Sehgal PB, and Ruddle FH.** Regional localization of the interferon-beta 2/B-cell stimulatory factor 2/hepatocyte stimulating factor gene to human chromosome 7p15-p21. *Genomics* 2: 203-208, 1988.
92. **Fiorentino DF, Zlotnik A, Mosmann TR, Howard M, and O'Garra A.** IL-10 inhibits cytokine production by activated macrophages. *J Immunol* 147: 3815-3822, 1991.
93. **Fleming KA.** Viral respiratory infection and SIDS. *J Clin Pathol* 45: 29-32, 1992.
94. **Forsberg L, Leeb L, Thoren S, Morgenstern R, and Jakobsson P.** Human glutathione dependent prostaglandin E synthase: gene structure and regulation. *FEBS Lett* 471: 78-82, 2000.
95. **Fox N, Song M, Schrementi J, Sharp JD, White DL, Snyder DW, Hartley LW, Carlson DG, Bach NJ, Dillard RD, Draheim SE, Bobbitt JL, Fisher L, and Mihelich ED.** Transgenic model for the discovery of novel human secretory non-pancreatic phospholipase A2 inhibitors. *Eur J Pharmacol* 308: 195-203, 1996.
96. **Frei K, Malipiero UV, Leist TP, Zinkernagel RM, Schwab ME, and Fontana A.** On the cellular source and function of interleukin 6 produced in the central nervous system in viral diseases. *Eur J Immunol* 19: 689-694, 1989.
97. **Fujitani Y, Kanaoka Y, Aritake K, Uodome N, Okazaki-Hatake K, and Urade Y.** Pronounced eosinophilic lung inflammation and Th2 cytokine release in human

lipocalin-type prostaglandin D synthase transgenic mice. *J Immunol* 168: 443-449, 2002.

98. **Funk CD, Furci L, FitzGerald GA, Grygorczyk R, Rochette C, Bayne MA, Abramovitz M, Adam M, and Metters KM.** Cloning and expression of a cDNA for the human prostaglandin E receptor EP1 subtype. *J Biol Chem* 268: 26767-26772, 1993.
99. **Gallant MA, Samadfam R, Hackett JA, Antoniou J, Parent JL, and de Brum-Fernandes AJ.** Production of prostaglandin D(2) by human osteoblasts and modulation of osteoprotegerin, RANKL, and cellular migration by DP and CRTH2 receptors. *J Bone Miner Res* 20: 672-681, 2005.
100. **Gershenwald JE, Fong YM, Fahey TJ, 3rd, Calvano SE, Chizzonite R, Kilian PL, Lowry SF, and Moldawer LL.** Interleukin 1 receptor blockade attenuates the host inflammatory response. *Proc Natl Acad Sci U S A* 87: 4966-4970, 1990.
101. **Gilroy DW, Colville-Nash PR, Willis D, Chivers J, Paul-Clark MJ, and Willoughby DA.** Inducible cyclooxygenase may have anti-inflammatory properties. *Nat Med* 5: 698-701, 1999.
102. **Gilroy DW, Lawrence T, Perretti M, and Rossi AG.** Inflammatory resolution: new opportunities for drug discovery. *Nat Rev Drug Discov* 3: 401-416, 2004.
103. **Giovannini MG, Scali C, Prosperi C, Bellucci A, Pepeu G, and Casamenti F.** Experimental brain inflammation and neurodegeneration as model of Alzheimer's disease: protective effects of selective COX-2 inhibitors. *Int J Immunopathol Pharmacol* 16: 31-40, 2003.
104. **Goldenberg MM.** Etanercept, a novel drug for the treatment of patients with severe, active rheumatoid arthritis. *Clin Ther* 21: 75-87; discussion 71-72, 1999.
105. **Goppelt-Struebe M, Koerner CF, Hausmann G, Gemsa D, and Resch K.** Control of prostanoid synthesis: role of reincorporation of released precursor fatty acids. *Prostaglandins* 32: 373-385, 1986.
106. **Gray PA, Rekling JC, Bocchiaro CM, and Feldman JL.** Modulation of respiratory frequency by peptidergic input to rhythmogenic neurons in the preBotzinger complex. *Science* 286: 1566-1568, 1999.
107. **Gudis K, Tatsuguchi A, Wada K, Futagami S, Nagata K, Hiratsuka T, Shinji Y, Miyake K, Tsukui T, Fukuda Y, and Sakamoto C.** Microsomal prostaglandin E synthase (mPGES)-1, mPGES-2 and cytosolic PGES expression in human gastritis and gastric ulcer tissue. *Lab Invest* 85: 225-236, 2005.
108. **Guerra FA, Savich RD, Wallen LD, Lee CH, Clyman RI, Mauray FE, and Kitterman JA.** Prostaglandin E2 causes hypoventilation and apnea in newborn lambs. *J Appl Physiol* 64: 2160-2166, 1988.
109. **Guntheroth WG.** Interleukin-1 as intermediary causing prolonged sleep apnea and SIDS during respiratory infections. *Med Hypotheses* 28: 121-123, 1989.
110. **Gupta RA and Dubois RN.** Colorectal cancer prevention and treatment by inhibition of cyclooxygenase-2. *Nat Rev Cancer* 1: 11-21, 2001.
111. **Gupta RA, Tan J, Krause WF, Geraci MW, Willson TM, Dey SK, and DuBois RN.** Prostacyclin-mediated activation of peroxisome proliferator-activated receptor delta in colorectal cancer. *Proc Natl Acad Sci U S A* 97: 13275-13280, 2000.
112. **Gutierrez EG, Banks WA, and Kastin AJ.** Blood-borne interleukin-1 receptor antagonist crosses the blood-brain barrier. *J Neuroimmunol* 55: 153-160, 1994.
113. **Hamberg M and Samuelsson B.** Detection and isolation of an endoperoxide intermediate in prostaglandin biosynthesis. *Proc Natl Acad Sci U S A* 70: 899-903, 1973.
114. **Hamberg M, Svensson J, and Samuelsson B.** Thromboxanes: a new group of biologically active compounds derived from prostaglandin endoperoxides. *Proc Natl Acad Sci U S A* 72: 2994-2998, 1975.
115. **Hamberg M, Svensson J, Wakabayashi T, and Samuelsson B.** Isolation and structure of two prostaglandin endoperoxides that cause platelet aggregation. *Proc Natl Acad Sci U S A* 71: 345-349, 1974.
116. **Hanasaki K, Ono T, Saiga A, Morioka Y, Ikeda M, Kawamoto K, Higashino K, Nakano K, Yamada K, Ishizaki J, and Arita H.** Purified group X secretory phospholipase A(2) induced prominent release of arachidonic acid from human myeloid leukemia cells. *J Biol Chem* 274: 34203-34211, 1999.

117. **Hansen-Flaschen JH.** Dyspnea in the ventilated patient: a call for patient-centered mechanical ventilation. *Respir Care* 45: 1460-1464, discussion 1464-1467, 2000.
118. **Harris RE, Alshafie GA, Abou-Issa H, and Seibert K.** Chemoprevention of breast cancer in rats by celecoxib, a cyclooxygenase 2 inhibitor. *Cancer Res* 60: 2101-2103, 2000.
119. **Hartney JM, Coggins KG, Tilley SL, Jania LA, Lovgren AK, Audoly LP, and Koller BH.** Prostaglandin E2 protects lower airways against bronchoconstriction. *Am J Physiol Lung Cell Mol Physiol* 290: L105-113, 2006.
120. **Hata AN and Breyer RM.** Pharmacology and signaling of prostaglandin receptors: multiple roles in inflammation and immune modulation. *Pharmacol Ther* 103: 147-166, 2004.
121. **Hattori K, Adachi H, Matsuzawa A, Yamamoto K, Tsujimoto M, Aoki J, Hattori M, Arai H, and Inoue K.** cDNA cloning and expression of intracellular platelet-activating factor (PAF) acetylhydrolase II. Its homology with plasma PAF acetylhydrolase. *J Biol Chem* 271: 33032-33038, 1996.
122. **Haurand M and Ullrich V.** Isolation and characterization of thromboxane synthase from human platelets as a cytochrome P-450 enzyme. *J Biol Chem* 260: 15059-15067, 1985.
123. **Hayaishi O and Urade Y.** Prostaglandin D2 in sleep-wake regulation: recent progress and perspectives. *Neuroscientist* 8: 12-15, 2002.
124. **Hehlgans T and Mannel DN.** The TNF-TNF receptor system. *Biol Chem* 383: 1581-1585, 2002.
125. **Herschman HR.** Prostaglandin synthase 2. *Biochim Biophys Acta* 1299: 125-140, 1996.
126. **Himmler A, Maurer-Fogy I, Kronke M, Scheurich P, Pfizenmaier K, Lantz M, Olsson I, Hauptmann R, Stratowa C, and Adolf GR.** Molecular cloning and expression of human and rat tumor necrosis factor receptor chain (p60) and its soluble derivative, tumor necrosis factor-binding protein. *DNA Cell Biol* 9: 705-715, 1990.
127. **Hirai H, Tanaka K, Yoshie O, Ogawa K, Kenmotsu K, Takamori Y, Ichimasa M, Sugamura K, Nakamura M, Takano S, and Nagata K.** Prostaglandin D2 selectively induces chemotaxis in T helper type 2 cells, eosinophils, and basophils via seven-transmembrane receptor CRTH2. *J Exp Med* 193: 255-261, 2001.
128. **Hirata M, Hayashi Y, Ushikubi F, Yokota Y, Kageyama R, Nakanishi S, and Narumiya S.** Cloning and expression of cDNA for a human thromboxane A2 receptor. *Nature* 349: 617-620, 1991.
129. **Hoch B and Bernhard M.** Central apnoea and endogenous prostaglandins in neonates. *Acta Paediatr* 89: 1364-1368, 2000.
130. **Hofacker A, Coste O, Nguyen HV, Marian C, Scholich K, and Geisslinger G.** Downregulation of cytosolic prostaglandin E2 synthase results in decreased nociceptive behavior in rats. *J Neurosci* 25: 9005-9009, 2005.
131. **Hofstetter AO and Herlenius E.** Interleukin-1beta depresses hypoxic gasping and autoresuscitation in neonatal DBA/1lacJ mice. *Respir Physiol Neurobiol* 146: 135-146, 2005.
132. **Holm PJ, Morgenstern R, and Hebert H.** The 3-D structure of microsomal glutathione transferase 1 at 6 A resolution as determined by electron crystallography of p22(1)2(1) crystals. *Biochim Biophys Acta* 1594: 276-285, 2002.
133. **Honda A, Sugimoto Y, Namba T, Watabe A, Irie A, Negishi M, Narumiya S, and Ichikawa A.** Cloning and expression of a cDNA for mouse prostaglandin E receptor EP2 subtype. *J Biol Chem* 268: 7759-7762, 1993.
134. **Horai R, Asano M, Sudo K, Kanuka H, Suzuki M, Nishihara M, Takahashi M, and Iwakura Y.** Production of mice deficient in genes for interleukin (IL)-1alpha, IL-1beta, IL-1alpha/beta, and IL-1 receptor antagonist shows that IL-1beta is crucial in turpentine-induced fever development and glucocorticoid secretion. *J Exp Med* 187: 1463-1475, 1998.
135. **Huang JC, Goldsby JS, Arbab F, Melhem Z, Aleksic N, and Wu KK.** Oviduct prostacyclin functions as a paracrine factor to augment the development of embryos. *Hum Reprod* 19: 2907-2912, 2004.
136. **Huang JC, Wun WS, Goldsby JS, Matijevic-Aleksic N, and Wu KK.** Cyclooxygenase-2-derived endogenous prostacyclin enhances mouse embryo hatching. *Hum Reprod* 19: 2900-2906, 2004.

137. **Ikeda-Matsuo Y, Ikegaya Y, Matsuki N, Uematsu S, Akira S, and Sasaki Y.** Microglia-specific expression of microsomal prostaglandin E2 synthase-1 contributes to lipopolysaccharide-induced prostaglandin E2 production. *J Neurochem* 94: 1546-1558, 2005.
138. **Irvine RF.** How is the level of free arachidonic acid controlled in mammalian cells? *Biochem J* 204: 3-16, 1982.
139. **Ishizaki J, Suzuki N, Higashino K, Yokota Y, Ono T, Kawamoto K, Fujii N, Arita H, and Hanasaki K.** Cloning and characterization of novel mouse and human secretory phospholipase A(2)s. *J Biol Chem* 274: 24973-24979, 1999.
140. **Jakobsson PJ, Mancini JA, and Ford-Hutchinson AW.** Identification and characterization of a novel human microsomal glutathione S-transferase with leukotriene C4 synthase activity and significant sequence identity to 5-lipoxygenase-activating protein and leukotriene C4 synthase. *J Biol Chem* 271: 22203-22210, 1996.
141. **Jakobsson PJ, Mancini JA, Riendeau D, and Ford-Hutchinson AW.** Identification and characterization of a novel microsomal enzyme with glutathione-dependent transferase and peroxidase activities. *J Biol Chem* 272: 22934-22939, 1997.
142. **Jakobsson PJ, Morgenstern R, Mancini J, Ford-Hutchinson A, and Persson B.** Common structural features of MAPEG -- a widespread superfamily of membrane associated proteins with highly divergent functions in eicosanoid and glutathione metabolism. *Protein Sci* 8: 689-692, 1999.
143. **Jakobsson PJ, Thoren S, Morgenstern R, and Samuelsson B.** Identification of human prostaglandin E synthase: a microsomal, glutathione-dependent, inducible enzyme, constituting a potential novel drug target. *Proc Natl Acad Sci U S A* 96: 7220-7225, 1999.
144. **Jang TJ.** Expression of proteins related to prostaglandin E2 biosynthesis is increased in human gastric cancer and during gastric carcinogenesis. *Virchows Arch* 445: 564-571, 2004.
145. **Jarabak J and Fried J.** Comparison of substrate specificities of the human placental NAD- and NADP-linked 15-hydroxyprostaglandin dehydrogenases. *Prostaglandins* 18: 241-246, 1979.
146. **Johnson JA, el Barbary A, Kornguth SE, Brugge JF, and Siegel FL.** Glutathione S-transferase isoenzymes in rat brain neurons and glia. *J Neurosci* 13: 2013-2023, 1993.
147. **Johnson SM, Koshiya N, and Smith JC.** Isolation of the kernel for respiratory rhythm generation in a novel preparation: the pre-Botzinger complex "island". *J Neurophysiol* 85: 1772-1776, 2001.
148. **Kamei D, Yamakawa K, Takegoshi Y, Mikami-Nakanishi M, Nakatani Y, Oh-Ishi S, Yasui H, Azuma Y, Hirasawa N, Ohuchi K, Kawaguchi H, Ishikawa Y, Ishii T, Uematsu S, Akira S, Murakami M, and Kudo I.** Reduced pain hypersensitivity and inflammation in mice lacking microsomal prostaglandin e synthase-1. *J Biol Chem* 279: 33684-33695, 2004.
149. **Kanai N, Lu R, Satriano JA, Bao Y, Wolkoff AW, and Schuster VL.** Identification and characterization of a prostaglandin transporter. *Science* 268: 866-869, 1995.
150. **Kanaoka Y and Urade Y.** Hematopoietic prostaglandin D synthase. *Prostaglandins Leukot Essent Fatty Acids* 69: 163-167, 2003.
151. **Kastin AJ, Akerstrom V, and Pan W.** Interleukin-10 as a CNS therapeutic: the obstacle of the blood-brain/blood-spinal cord barrier. *Brain Res Mol Brain Res* 114: 168-171, 2003.
152. **Katori M, Majima M, and Harada Y.** Possible background mechanisms of the effectiveness of cyclooxygenase-2 inhibitors in the treatment of rheumatoid arthritis. *Inflamm Res* 47 Suppl 2: S107-111, 1998.
153. **Katsuyama M, Sugimoto Y, Namba T, Irie A, Negishi M, Narumiya S, and Ichikawa A.** Cloning and expression of a cDNA for the human prostacyclin receptor. *FEBS Lett* 344: 74-78, 1994.
154. **Kino Y, Kojima F, Kiguchi K, Igarashi R, Ishizuka B, and Kawai S.** Prostaglandin E2 production in ovarian cancer cell lines is regulated by cyclooxygenase-1, not cyclooxygenase-2. *Prostaglandins Leukot Essent Fatty Acids* 73: 103-111, 2005.
155. **Kis B, Isse T, Snipes JA, Chen L, Yamashita H, Ueta Y, and Busija DW.** Effects of LPS stimulation on the expression of prostaglandin carriers in the cells of the blood-brain and blood-cerebrospinal fluid barriers. *J Appl Physiol* 100: 1392-1399, 2006.

156. **Klir JJ, McClellan JL, Kozak W, Szelenyi Z, Wong GH, and Kluger MJ.** Systemic but not central administration of tumor necrosis factor-alpha attenuates LPS-induced fever in rats. *Am J Physiol* 268: R480-486, 1995.
157. **Klivenyi P, Beal MF, Ferrante RJ, Andreassen OA, Wermer M, Chin MR, and Bonventre JV.** Mice deficient in group IV cytosolic phospholipase A2 are resistant to MPTP neurotoxicity. *J Neurochem* 71: 2634-2637, 1998.
158. **Kluger MJ.** Fever: role of pyrogens and cryogens. *Physiol Rev* 71: 93-127, 1991.
159. **Kobayashi T, Nakatani Y, Tanioka T, Tsujimoto M, Nakajo S, Nakaya K, Murakami M, and Kudo I.** Regulation of cytosolic prostaglandin E synthase by phosphorylation. *Biochem J* 381: 59-69, 2004.
160. **Koduri RS, Gronroos JO, Laine VJ, Le Calvez C, Lambeau G, Nevalainen TJ, and Gelb MH.** Bactericidal properties of human and murine groups I, II, V, X, and XII secreted phospholipases A(2). *J Biol Chem* 277: 5849-5857, 2002.
161. **Kojima F, Naraba H, Miyamoto S, Beppu M, Aoki H, and Kawai S.** Membrane-associated prostaglandin E synthase-1 is upregulated by proinflammatory cytokines in chondrocytes from patients with osteoarthritis. *Arthritis Res Ther* 6: R355-365, 2004.
162. **Kojima F, Naraba H, Sasaki Y, Beppu M, Aoki H, and Kawai S.** Prostaglandin E2 is an enhancer of interleukin-1beta-induced expression of membrane-associated prostaglandin E synthase in rheumatoid synovial fibroblasts. *Arthritis Rheum* 48: 2819-2828, 2003.
163. **Kojima F, Naraba H, Sasaki Y, Okamoto R, Koshino T, and Kawai S.** Coexpression of microsomal prostaglandin E synthase with cyclooxygenase-2 in human rheumatoid synovial cells. *J Rheumatol* 29: 1836-1842, 2002.
164. **Konsman JP, Vigues S, Mackerlova L, Bristow A, and Blomqvist A.** Rat brain vascular distribution of interleukin-1 type-1 receptor immunoreactivity: relationship to patterns of inducible cyclooxygenase expression by peripheral inflammatory stimuli. *J Comp Neurol* 472: 113-129, 2004.
165. **Korotkova M, Westman M, Gheorghe KR, af Klint E, Trollmo C, Ulfgren AK, Klareskog L, and Jakobsson PJ.** Effects of antirheumatic treatments on the prostaglandin E2 biosynthetic pathway. *Arthritis Rheum* 52: 3439-3447, 2005.
166. **Kosaka T, Miyata A, Ihara H, Hara S, Sugimoto T, Takeda O, Takahashi E, and Tanabe T.** Characterization of the human gene (PTGS2) encoding prostaglandin-endoperoxide synthase 2. *Eur J Biochem* 221: 889-897, 1994.
167. **Kozak W, Kluger MJ, Soszynski D, Conn CA, Rudolph K, Leon LR, and Zheng H.** IL-6 and IL-1 beta in fever. Studies using cytokine-deficient (knockout) mice. *Ann N Y Acad Sci* 856: 33-47, 1998.
168. **Kozak W, Poli V, Soszynski D, Conn CA, Leon LR, and Kluger MJ.** Sickness behavior in mice deficient in interleukin-6 during turpentine abscess and influenza pneumonitis. *Am J Physiol* 272: R621-630, 1997.
169. **Kraemer SA, Meade EA, and DeWitt DL.** Prostaglandin endoperoxide synthase gene structure: identification of the transcriptional start site and 5'-flanking regulatory sequences. *Arch Biochem Biophys* 293: 391-400, 1992.
170. **Krotz F, Schiele TM, Klauss V, and Sohn HY.** Selective COX-2 inhibitors and risk of myocardial infarction. *J Vasc Res* 42: 312-324, 2005.
171. **Kubota K, Kubota T, Kamei D, Murakami M, Kudo I, Aso T, and Morita I.** Change in prostaglandin E synthases (PGESs) in microsomal PGES-1 knockout mice in a preterm delivery model. *J Endocrinol* 187: 339-345, 2005.
172. **Kudo I and Murakami M.** Phospholipase A2 enzymes. *Prostaglandins Other Lipid Mediat* 68-69: 3-58, 2002.
173. **Kujubu DA, Fletcher BS, Varnum BC, Lim RW, and Herschman HR.** TIS10, a phorbol ester tumor promoter-inducible mRNA from Swiss 3T3 cells, encodes a novel prostaglandin synthase/cyclooxygenase homologue. *J Biol Chem* 266: 12866-12872, 1991.
174. **Kurumbail RG, Stevens AM, Gierse JK, McDonald JJ, Stegeman RA, Pak JY, Gildehaus D, Miyashiro JM, Penning TD, Seibert K, Isakson PC, and Stallings WC.** Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents. *Nature* 384: 644-648, 1996.
175. **Kurzrok R and Lieb CC.** Biochemical studies of human semen. The biological action of semen on the human uterus. *Proc Soc Exp Biol Med* 28: 268-272, 1930.

176. **Kutchera W, Jones DA, Matsunami N, Groden J, McIntyre TM, Zimmerman GA, White RL, and Prescott SM.** Prostaglandin H synthase 2 is expressed abnormally in human colon cancer: evidence for a transcriptional effect. *Proc Natl Acad Sci U S A* 93: 4816-4820, 1996.
177. **Laine VJ, Grass DS, and Nevalainen TJ.** Protection by group II phospholipase A2 against Staphylococcus aureus. *J Immunol* 162: 7402-7408, 1999.
178. **Lam BK, Penrose JF, Freeman GJ, and Austen KF.** Expression cloning of a cDNA for human leukotriene C4 synthase, an integral membrane protein conjugating reduced glutathione to leukotriene A4. *Proc Natl Acad Sci U S A* 91: 7663-7667, 1994.
179. **Lane NE.** Pain management in osteoarthritis: the role of COX-2 inhibitors. *J Rheumatol Suppl* 49: 20-24, 1997.
180. **Langenbach R, Morham SG, Tiano HF, Loftin CD, Ghanayem BI, Chulada PC, Mahler JF, Lee CA, Goulding EH, Kluckman KD, and et al** Prostaglandin synthase 1 gene disruption in mice reduces arachidonic acid-induced inflammation and indomethacin-induced gastric ulceration. *Cell* 83: 483-492, 1995.
181. **Larsson PK, Claesson HE, and Kennedy BP.** Multiple splice variants of the human calcium-independent phospholipase A2 and their effect on enzyme activity. *J Biol Chem* 273: 207-214, 1998.
182. **Lawrence T, Willoughby DA, and Gilroy DW.** Anti-inflammatory lipid mediators and insights into the resolution of inflammation. *Nat Rev Immunol* 2: 787-795, 2002.
183. **Lazarus M, Kubata BK, Eguchi N, Fujitani Y, Urade Y, and Hayaishi O.** Biochemical characterization of mouse microsomal prostaglandin E synthase-1 and its colocalization with cyclooxygenase-2 in peritoneal macrophages. *Arch Biochem Biophys* 397: 336-341, 2002.
184. **Le Gallois.** Experiences sur le principe de la vie. *D' Hautel, Paris*, 1812.
185. **Learn CA, Mizel SB, and McCall CE.** mRNA and protein stability regulate the differential expression of pro- and anti-inflammatory genes in endotoxin-tolerant THP-1 cells. *J Biol Chem* 275: 12185-12193, 2000.
186. **Ledeboer A, Binnekade R, Breve JJ, Bol JG, Tilders FJ, and Van Dam AM.** Site-specific modulation of LPS-induced fever and interleukin-1 beta expression in rats by interleukin-10. *Am J Physiol Regul Integr Comp Physiol* 282: R1762-1772, 2002.
187. **Leon LR, Conn CA, Glaccum M, and Kluger MJ.** IL-1 type I receptor mediates acute phase response to turpentine, but not lipopolysaccharide, in mice. *Am J Physiol* 271: R1668-1675, 1996.
188. **Leon LR, Kozak W, Rudolph K, and Kluger MJ.** An antipyretic role for interleukin-10 in LPS fever in mice. *Am J Physiol* 276: R81-89, 1999.
189. **Leon LR, White AA, and Kluger MJ.** Role of IL-6 and TNF in thermoregulation and survival during sepsis in mice. *Am J Physiol* 275: R269-277, 1998.
190. **Lewis AB, Freed MD, Heymann MA, Roehl SL, and Kensey RC.** Side effects of therapy with prostaglandin E1 in infants with critical congenital heart disease. *Circulation* 64: 893-898, 1981.
191. **Li RC, Row BW, Gozal E, Kheirandish L, Fan Q, Brittian KR, Guo SZ, Sachleben LR, Jr., and Gozal D.** Cyclooxygenase 2 and intermittent hypoxia-induced spatial deficits in the rat. *Am J Respir Crit Care Med* 168: 469-475, 2003.
192. **Lien E, Means TK, Heine H, Yoshimura A, Kusumoto S, Fukase K, Fenton MJ, Oikawa M, Qureshi N, Monks B, Finberg RW, Ingalls RR, and Golenbock DT.** Toll-like receptor 4 imparts ligand-specific recognition of bacterial lipopolysaccharide. *J Clin Invest* 105: 497-504, 2000.
193. **Lim H, Gupta RA, Ma WG, Paria BC, Moller DE, Morrow JD, DuBois RN, Trzaskos JM, and Dey SK.** Cyclo-oxygenase-2-derived prostacyclin mediates embryo implantation in the mouse via PPARdelta. *Genes Dev* 13: 1561-1574, 1999.
194. **Lim H, Paria BC, Das SK, Dinchuk JE, Langenbach R, Trzaskos JM, and Dey SK.** Multiple female reproductive failures in cyclooxygenase 2-deficient mice. *Cell* 91: 197-208, 1997.
195. **Lin H, Lin TN, Cheung WM, Nian GM, Tseng PH, Chen SF, Chen JJ, Shyue SK, Liou JY, Wu CW, and Wu KK.** Cyclooxygenase-1 and bicistronic cyclooxygenase-1/prostacyclin synthase gene transfer protect against ischemic cerebral infarction. *Circulation* 105: 1962-1969, 2002.

196. **Lin LL, Lin AY, and DeWitt DL.** Interleukin-1 alpha induces the accumulation of cytosolic phospholipase A2 and the release of prostaglandin E2 in human fibroblasts. *J Biol Chem* 267: 23451-23454, 1992.
197. **Lin YM and Jarabak J.** Isolation of two proteins with 9-ketoprostaglandin reductase and NADP-linked 15-hydroxyprostaglandin dehydrogenase activities and studies on their inhibition. *Biochem Biophys Res Commun* 81: 1227-1234, 1978.
198. **Loftin CD, Tiano HF, and Langenbach R.** Phenotypes of the COX-deficient mice indicate physiological and pathophysiological roles for COX-1 and COX-2. *Prostaglandins Other Lipid Mediat* 68-69: 177-185, 2002.
199. **Long NC, Kunkel SL, Vander AJ, and Kluger MJ.** Antiserum against tumor necrosis factor enhances lipopolysaccharide fever in rats. *Am J Physiol* 258: R332-337, 1990.
200. **Long NC, Morimoto A, Nakamori T, and Murakami N.** Systemic injection of TNF-alpha attenuates fever due to IL-1 beta and LPS in rats. *Am J Physiol* 263: R987-991, 1992.
201. **Long WA.** Prostaglandins and control of breathing in newborn piglets. *J Appl Physiol* 64: 409-418, 1988.
202. **Loo RW, Conde-Frieboes K, Reynolds LJ, and Dennis EA.** Activation, inhibition, and regiospecificity of the lysophospholipase activity of the 85-kDa group IV cytosolic phospholipase A2. *J Biol Chem* 272: 19214-19219, 1997.
203. **Lu R, Kanai N, Bao Y, and Schuster VL.** Cloning, in vitro expression, and tissue distribution of a human prostaglandin transporter cDNA(hPGT). *J Clin Invest* 98: 1142-1149, 1996.
204. **Luong C, Miller A, Barnett J, Chow J, Ramesha C, and Browner MF.** Flexibility of the NSAID binding site in the structure of human cyclooxygenase-2. *Nat Struct Biol* 3: 927-933, 1996.
205. **Lyte M.** Regulation of interleukin-1 production in murine macrophages and human monocytes by a normal physiological constituent. *Life Sci* 38: 1163-1170, 1986.
206. **Mabuchi T, Kojima H, Abe T, Takagi K, Sakurai M, Ohmiya Y, Uematsu S, Akira S, Watanabe K, and Ito S.** Membrane-associated prostaglandin E synthase-1 is required for neuropathic pain. *Neuroreport* 15: 1395-1398, 2004.
207. **Mackowiak PA.** Concepts of fever. *Arch Intern Med* 158: 1870-1881, 1998.
208. **Madore E, Harvey N, Parent J, Chapdelaine P, Arosh JA, and Fortier MA.** An aldose reductase with 20 alpha-hydroxysteroid dehydrogenase activity is most likely the enzyme responsible for the production of prostaglandin f2 alpha in the bovine endometrium. *J Biol Chem* 278: 11205-11212, 2003.
209. **Maggi LB, Jr., Sadeghi H, Weigand C, Scarim AL, Heitmeier MR, and Corbett JA.** Anti-inflammatory actions of 15-deoxy-delta 12,14-prostaglandin J2 and troglitazone: evidence for heat shock-dependent and -independent inhibition of cytokine-induced inducible nitric oxide synthase expression. *Diabetes* 49: 346-355, 2000.
210. **Maimone D, Cioni C, Rosa S, Macchia G, Aloisi F, and Annunziata P.** Norepinephrine and vasoactive intestinal peptide induce IL-6 secretion by astrocytes: synergism with IL-1 beta and TNF alpha. *J Neuroimmunol* 47: 73-81, 1993.
211. **Mancini JA, Abramovitz M, Cox ME, Wong E, Charleson S, Perrier H, Wang Z, Prasit P, and Vickers PJ.** 5-lipoxygenase-activating protein is an arachidonate binding protein. *FEBS Lett* 318: 277-281, 1993.
212. **Mancini JA, Blood K, Guay J, Gordon R, Claveau D, Chan CC, and Riendeau D.** Cloning, expression, and up-regulation of inducible rat prostaglandin e synthase during lipopolysaccharide-induced pyresis and adjuvant-induced arthritis. *J Biol Chem* 276: 4469-4475, 2001.
213. **Mathew O.** Apnea, bradycardia, and desaturation. In respiratory control and disorders in the new born. *O Mathew, Ed,* Marcel Dekker, Inc, New York: pp. 277-281, 2003.
214. **Matsumoto H, Naraba H, Murakami M, Kudo I, Yamaki K, Ueno A, and Oh-ishi S.** Concordant induction of prostaglandin E2 synthase with cyclooxygenase-2 leads to preferred production of prostaglandin E2 over thromboxane and prostaglandin D2 in lipopolysaccharide-stimulated rat peritoneal macrophages. *Biochem Biophys Res Commun* 230: 110-114, 1997.
215. **Matsuoka T, Hirata M, Tanaka H, Takahashi Y, Murata T, Kabashima K, Sugimoto Y, Kobayashi T, Ushikubi F, Aze Y, Eguchi N, Urade Y, Yoshida N,**

**Kimura K, Mizoguchi A, Honda Y, Nagai H, and Narumiya S.** Prostaglandin D2 as a mediator of allergic asthma. *Science* 287: 2013-2017, 2000.
216. **McCleskey EW and Gold MS.** Ion channels of nociception. *Annu Rev Physiol* 61: 835-856, 1999.
217. **McVea KL, Turner PD, and Peppler DK.** The role of breastfeeding in sudden infant death syndrome. *J Hum Lact* 16: 13-20, 2000.
218. **Means TK, Golenbock DT, and Fenton MJ.** Structure and function of Toll-like receptor proteins. *Life Sci* 68: 241-258, 2000.
219. **Merlie JP, Fagan D, Mudd J, and Needleman P.** Isolation and characterization of the complementary DNA for sheep seminal vesicle prostaglandin endoperoxide synthase (cyclooxygenase). *J Biol Chem* 263: 3550-3553, 1988.
220. **Mestre JR, Mackrell PJ, Rivadeneira DE, Stapleton PP, Tanabe T, and Daly JM.** Redundancy in the signaling pathways and promoter elements regulating cyclooxygenase-2 gene expression in endotoxin-treated macrophage/monocytic cells. *J Biol Chem* 276: 3977-3982, 2001.
221. **Miggin SM and Kinsella BT.** Expression and tissue distribution of the mRNAs encoding the human thromboxane A2 receptor (TP) alpha and beta isoforms. *Biochim Biophys Acta* 1425: 543-559, 1998.
222. **Miller AJ, Luheshi GN, Rothwell NJ, and Hopkins SJ.** Local cytokine induction by LPS in the rat air pouch and its relationship to the febrile response. *Am J Physiol* 272: R857-861, 1997.
223. **Miller DK, Gillard JW, Vickers PJ, Sadowski S, Leveille C, Mancini JA, Charleson P, Dixon RA, Ford-Hutchinson AW, Fortin R, and et al** Identification and isolation of a membrane protein necessary for leukotriene production. *Nature* 343: 278-281, 1990.
224. **Milton AS and Wendlandt S.** A possible role for prostaglandin E1 as a modulator for temperature regulation in the central nervous system of the cat. *J Physiol* 207: 76P-77P, 1970.
225. **Minami T, Nishihara I, Uda R, Ito S, Hyodo M, and Hayaishi O.** Characterization of EP-receptor subtypes involved in allodynia and hyperalgesia induced by intrathecal administration of prostaglandin E2 to mice. *Br J Pharmacol* 112: 735-740, 1994.
226. **Mitchell JA, Akarasereenont P, Thiemermann C, Flower RJ, and Vane JR.** Selectivity of nonsteroidal antiinflammatory drugs as inhibitors of constitutive and inducible cyclooxygenase. *Proc Natl Acad Sci U S A* 90: 11693-11697, 1993.
227. **Miyamoto T, Ogino N, Yamamoto S, and Hayaishi O.** Purification of prostaglandin endoperoxide synthetase from bovine vesicular gland microsomes. *J Biol Chem* 251: 2629-2636, 1976.
228. **Mnich SJ, Veenhuizen AW, Monahan JB, Sheehan KC, Lynch KR, Isakson PC, and Portanova JP.** Characterization of a monoclonal antibody that neutralizes the activity of prostaglandin E2. *J Immunol* 155: 4437-4444, 1995.
229. **Moldawer LL, Gelin J, Schersten T, and Lundholm KG.** Circulating interleukin 1 and tumor necrosis factor during inflammation. *Am J Physiol* 253: R922-928, 1987.
230. **Moon Y, Glasgow WC, and Eling TE.** Curcumin suppresses interleukin 1beta-mediated microsomal prostaglandin E synthase 1 by altering early growth response gene 1 and other signaling pathways. *J Pharmacol Exp Ther* 315: 788-795, 2005.
231. **Moonen P, Buytenhek M, and Nugteren DH.** Purification of PGH-PGE isomerase from sheep vesicular glands. *Methods Enzymol* 86: 84-91, 1982.
232. **Moore AH, Olschowka JA, and O'Banion MK.** Intraparenchymal administration of interleukin-1beta induces cyclooxygenase-2-mediated expression of membrane- and cytosolic-associated prostaglandin E synthases in mouse brain. *J Neuroimmunol* 148: 32-40, 2004.
233. **Morgenstern R, Guthenberg C, and Depierre JW.** Microsomal glutathione S-transferase. Purification, initial characterization and demonstration that it is not identical to the cytosolic glutathione S-transferases A, B and C. *Eur J Biochem* 128: 243-248, 1982.
234. **Morimoto A, Watanabe T, Morimoto K, Nakamori T, and Murakami N.** Possible involvement of prostaglandins in psychological stress-induced responses in rats. *J Physiol* 443: 421-429, 1991.

235. **Morita I, Schindler M, Regier MK, Otto JC, Hori T, DeWitt DL, and Smith WL.** Different intracellular locations for prostaglandin endoperoxide H synthase-1 and -2. *J Biol Chem* 270: 10902-10908, 1995.
236. **Mosialou E, Piemonte F, Andersson C, Vos RM, van Bladeren PJ, and Morgenstern R.** Microsomal glutathione transferase: lipid-derived substrates and lipid dependence. *Arch Biochem Biophys* 320: 210-216, 1995.
237. **Mukhopadhyay P, Bian L, Yin H, Bhattacherjee P, and Paterson C.** Localization of EP(1) and FP receptors in human ocular tissues by in situ hybridization. *Invest Ophthalmol Vis Sci* 42: 424-428, 2001.
238. **Murakami M and Kudo I.** Secretory phospholipase A2. *Biol Pharm Bull* 27: 1158-1164, 2004.
239. **Murakami M, Matsumoto R, Urade Y, Austen KF, and Arm JP.** c-kit ligand mediates increased expression of cytosolic phospholipase A2, prostaglandin endoperoxide synthase-1, and hematopoietic prostaglandin D2 synthase and increased IgE-dependent prostaglandin D2 generation in immature mouse mast cells. *J Biol Chem* 270: 3239-3246, 1995.
240. **Murakami M, Nakashima K, Kamei D, Masuda S, Ishikawa Y, Ishii T, Ohmiya Y, Watanabe K, and Kudo I.** Cellular prostaglandin E2 production by membrane-bound prostaglandin E synthase-2 via both cyclooxygenases-1 and -2. *J Biol Chem* 278: 37937-37947, 2003.
241. **Murakami M, Nakatani Y, Tanioka T, and Kudo I.** Prostaglandin E synthase. *Prostaglandins Other Lipid Mediat* 68-69: 383-399, 2002.
242. **Murakami M, Naraba H, Tanioka T, Semmyo N, Nakatani Y, Kojima F, Ikeda T, Fueki M, Ueno A, Oh S, and Kudo I.** Regulation of prostaglandin E2 biosynthesis by inducible membrane-associated prostaglandin E2 synthase that acts in concert with cyclooxygenase-2. *J Biol Chem* 275: 32783-32792, 2000.
243. **Murakami M, Shimbara S, Kambe T, Kuwata H, Winstead MV, Tischfield JA, and Kudo I.** The functions of five distinct mammalian phospholipase A2S in regulating arachidonic acid release. Type IIa and type V secretory phospholipase A2S are functionally redundant and act in concert with cytosolic phospholipase A2. *J Biol Chem* 273: 14411-14423, 1998.
244. **Murakami N, Sakata Y, and Watanabe T.** Central action sites of interleukin-1 beta for inducing fever in rabbits. *J Physiol* 428: 299-312, 1990.
245. **Myers LK, Kang AH, Postlethwaite AE, Rosloniec EF, Morham SG, Shlopov BV, Goorha S, and Ballou LR.** The genetic ablation of cyclooxygenase 2 prevents the development of autoimmune arthritis. *Arthritis Rheum* 43: 2687-2693, 2000.
246. **Nakano T, Ohara O, Teraoka H, and Arita H.** Glucocorticoids suppress group II phospholipase A2 production by blocking mRNA synthesis and post-transcriptional expression. *J Biol Chem* 265: 12745-12748, 1990.
247. **Nakayama Y, Omote K, and Namiki A.** Role of prostaglandin receptor EP1 in the spinal dorsal horn in carrageenan-induced inflammatory pain. *Anesthesiology* 97: 1254-1262, 2002.
248. **Naraba H, Yokoyama C, Tago N, Murakami M, Kudo I, Fueki M, Oh-Ishi S, and Tanabe T.** Transcriptional regulation of the membrane-associated prostaglandin E2 synthase gene. Essential role of the transcription factor Egr-1. *J Biol Chem* 277: 28601-28608, 2002.
249. **Narumiya S and FitzGerald GA.** Genetic and pharmacological analysis of prostanoid receptor function. *J Clin Invest* 108: 25-30, 2001.
250. **Needleman P, Moncada S, Bunting S, Vane JR, Hamberg M, and Samuelsson B.** Identification of an enzyme in platelet microsomes which generates thromboxane A2 from prostaglandin endoperoxides. *Nature* 261: 558-560, 1976.
251. **Nemunaitis J, Appelbaum FR, Lilleby K, Buhles WC, Rosenfeld C, Zeigler ZR, Shadduck RK, Singer JW, Meyer W, and Buckner CD.** Phase I study of recombinant interleukin-1 beta in patients undergoing autologous bone marrow transplant for acute myelogenous leukemia. *Blood* 83: 3473-3479, 1994.
252. **Netea MG, Kullberg BJ, and Van der Meer JW.** Circulating cytokines as mediators of fever. *Clin Infect Dis* 31 Suppl 5: S178-184, 2000.
253. **Neutze JM, Starling MB, Elliott RB, and Barratt-Boyes BG.** Palliation of cyanotic congenital heart disease in infancy with E-type prostaglandins. *Circulation* 55: 238-241, 1977.

254. **Newton R, Seybold J, Kuitert LM, Bergmann M, and Barnes PJ.** Repression of cyclooxygenase-2 and prostaglandin E2 release by dexamethasone occurs by transcriptional and post-transcriptional mechanisms involving loss of polyadenylated mRNA. *J Biol Chem* 273: 32312-32321, 1998.
255. **Nicholson DW, Ali A, Vaillancourt JP, Calaycay JR, Mumford RA, Zamboni RJ, and Ford-Hutchinson AW.** Purification to homogeneity and the N-terminal sequence of human leukotriene C4 synthase: a homodimeric glutathione S-transferase composed of 18-kDa subunits. *Proc Natl Acad Sci U S A* 90: 2015-2019, 1993.
256. **Noguchi M, Kimoto A, Kobayashi S, Yoshino T, Miyata K, and Sasamata M.** Effect of celecoxib, a cyclooxygenase-2 inhibitor, on the pathophysiology of adjuvant arthritis in rat. *Eur J Pharmacol* 513: 229-235, 2005.
257. **Ogletree ML.** Overview of physiological and pathophysiological effects of thromboxane A2. *Fed Proc* 46: 133-138, 1987.
258. **Ogorochi T, Ujihara M, and Narumiya S.** Purification and properties of prostaglandin H-E isomerase from the cytosol of human brain: identification as anionic forms of glutathione S-transferase. *J Neurochem* 48: 900-909, 1987.
259. **Oka K, Oka T, and Hori T.** Prostaglandin E2 may induce hyperthermia through EP1 receptor in the anterior wall of the third ventricle and neighboring preoptic regions. *Brain Res* 767: 92-99, 1997.
260. **Oka S and Arita H.** Inflammatory factors stimulate expression of group II phospholipase A2 in rat cultured astrocytes. Two distinct pathways of the gene expression. *J Biol Chem* 266: 9956-9960, 1991.
261. **Oka T and Hori T.** EP1-receptor mediation of prostaglandin E2-induced hyperthermia in rats. *Am J Physiol* 267: R289-294, 1994.
262. **Oka T, Oka K, Kobayashi T, Sugimoto Y, Ichikawa A, Ushikubi F, Narumiya S, and Saper CB.** Characteristics of thermoregulatory and febrile responses in mice deficient in prostaglandin EP1 and EP3 receptors. *J Physiol* 551: 945-954, 2003.
263. **Oka T, Oka K, Scammell TE, Lee C, Kelly JF, Nantel F, Elmquist JK, and Saper CB.** Relationship of EP(1-4) prostaglandin receptors with rat hypothalamic cell groups involved in lipopolysaccharide fever responses. *J Comp Neurol* 428: 20-32, 2000.
264. **Oldenburg HS, Rogy MA, Lazarus DD, Van Zee KJ, Keeler BP, Chizzonite RA, Lowry SF, and Moldawer LL.** Cachexia and the acute-phase protein response in inflammation are regulated by interleukin-6. *Eur J Immunol* 23: 1889-1894, 1993.
265. **Olsson A, Kayhan G, Lagercrantz H, and Herlenius E.** IL-1 beta depresses respiration and anoxic survival via a prostaglandin-dependent pathway in neonatal rats. *Pediatr Res* 54: 326-331, 2003.
266. **Onodera M, Horiuchi Y, Nakahama K, Muneta T, Mano Y, and Morita I.** Induction of cyclooxygenase-1 in cultured synovial cells isolated from rheumatoid arthritis patients. *Inflamm Res* 53: 217-222, 2004.
267. **Oshima M, Dinchuk JE, Kargman SL, Oshima H, Hancock B, Kwong E, Trzaskos JM, Evans JF, and Taketo MM.** Suppression of intestinal polyposis in Apc delta716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). *Cell* 87: 803-809, 1996.
268. **Oshima M, Oshima H, Matsunaga A, and Taketo MM.** Hyperplastic gastric tumors with spasmolytic polypeptide-expressing metaplasia caused by tumor necrosis factor-alpha-dependent inflammation in cyclooxygenase-2/microsomal prostaglandin E synthase-1 transgenic mice. *Cancer Res* 65: 9147-9151, 2005.
269. **Ouellet M, Falgueyret JP, Hien Ear P, Pen A, Mancini JA, Riendeau D, and Percival MD.** Purification and characterization of recombinant microsomal prostaglandin E synthase-1. *Protein Expr Purif* 26: 489-495, 2002.
270. **Ouellet M, Riendeau D, and Percival MD.** A high level of cyclooxygenase-2 inhibitor selectivity is associated with a reduced interference of platelet cyclooxygenase-1 inactivation by aspirin. *Proc Natl Acad Sci U S A* 98: 14583-14588, 2001.
271. **Palea S, Toson G, Pietra C, Trist DG, Artibani W, Romano O, and Corsi M.** Pharmacological characterization of thromboxane and prostanoid receptors in human isolated urinary bladder. *Br J Pharmacol* 124: 865-872, 1998.
272. **Paton JF, Abdala AP, Koizumi H, Smith JC, and St-John WM.** Respiratory rhythm generation during gasping depends on persistent sodium current. *Nat Neurosci* 9: 311-313, 2006.

273. **Patrignani P, Filabozzi P, and Patrono C.** Selective cumulative inhibition of platelet thromboxane production by low-dose aspirin in healthy subjects. *J Clin Invest* 69: 1366-1372, 1982.
274. **Pecchi E, Dallaporta M, Thirion S, Salvat C, Berenbaum F, Jean A, and Troadec JD.** Involvement of central microsomal prostaglandin E synthase-1 (mPGES-1)in IL-1{beta}- induced anorexia. *Physiol Genomics* in press, 2006.
275. **Penning TD, Talley JJ, Bertenshaw SR, Carter JS, Collins PW, Docter S, Graneto MJ, Lee LF, Malecha JW, Miyashiro JM, Rogers RS, Rogier DJ, Yu SS, AndersonGd, Burton EG, Cogburn JN, Gregory SA, Koboldt CM, Perkins WE, Seibert K, Veenhuizen AW, Zhang YY, and Isakson PC.** Synthesis and biological evaluation of the 1,5-diarylpyrazole class of cyclooxygenase-2 inhibitors: identification of 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benze nesulfonamide (SC-58635, celecoxib). *J Med Chem* 40: 1347-1365, 1997.
276. **Penton-Rol G, Orlando S, Polentarutti N, Bernasconi S, Muzio M, Introna M, and Mantovani A.** Bacterial lipopolysaccharide causes rapid shedding, followed by inhibition of mRNA expression, of the IL-1 type II receptor, with concomitant up-regulation of the type I receptor and induction of incompletely spliced transcripts. *J Immunol* 162: 2931-2938, 1999.
277. **Peri KG, Quiniou C, Hou X, Abran D, Varma DR, Lubell WD, and Chemtob S.** THG113: a novel selective FP antagonist that delays preterm labor. *Semin Perinatol* 26: 389-397, 2002.
278. **Pickard RT, Strifler BA, Kramer RM, and Sharp JD.** Molecular cloning of two new human paralogs of 85-kDa cytosolic phospholipase A2. *J Biol Chem* 274: 8823-8831, 1999.
279. **Picot D, Loll PJ, and Garavito RM.** The X-ray crystal structure of the membrane protein prostaglandin H2 synthase-1. *Nature* 367: 243-249, 1994.
280. **Polyak K, Xia Y, Zweier JL, Kinzler KW, and Vogelstein B.** A model for p53-induced apoptosis. *Nature* 389: 300-305, 1997.
281. **Portanova JP, Zhang Y, Anderson GD, Hauser SD, Masferrer JL, Seibert K, Gregory SA, and Isakson PC.** Selective neutralization of prostaglandin E2 blocks inflammation, hyperalgesia, and interleukin 6 production in vivo. *J Exp Med* 184: 883-891, 1996.
282. **Prandota J.** Possible pathomechanisms of sudden infant death syndrome: key role of chronic hypoxia, infection/inflammation states, cytokine irregularities, and metabolic trauma in genetically predisposed infants. *Am J Ther* 11: 517-546, 2004.
283. **Pucci ML, Bao Y, Chan B, Itoh S, Lu R, Copeland NG, Gilbert DJ, Jenkins NA, and Schuster VL.** Cloning of mouse prostaglandin transporter PGT cDNA: species-specific substrate affinities. *Am J Physiol* 277: R734-741, 1999.
284. **Quraishi O, Mancini JA, and Riendeau D.** Inhibition of inducible prostaglandin E(2) synthase by 15-deoxy-Delta(12,14)-prostaglandin J(2) and polyunsaturated fatty acids. *Biochem Pharmacol* 63: 1183-1189, 2002.
285. **Raza MW and Blackwell CC.** Sudden infant death syndrome, virus infections and cytokines. *FEMS Immunol Med Microbiol* 25: 85-96, 1999.
286. **Reddy BS, Hirose Y, Lubet R, Steele V, Kelloff G, Paulson S, Seibert K, and Rao CV.** Chemoprevention of colon cancer by specific cyclooxygenase-2 inhibitor, celecoxib, administered during different stages of carcinogenesis. *Cancer Res* 60: 293-297, 2000.
287. **Regan JW, Bailey TJ, Pepperl DJ, Pierce KL, Bogardus AM, Donello JE, Fairbairn CE, Kedzie KM, Woodward DF, and Gil DW.** Cloning of a novel human prostaglandin receptor with characteristics of the pharmacologically defined EP2 subtype. *Mol Pharmacol* 46: 213-220, 1994.
288. **Rekling JC and Feldman JL.** PreBotzinger complex and pacemaker neurons: hypothesized site and kernel for respiratory rhythm generation. *Annu Rev Physiol* 60: 385-405, 1998.
289. **Reynolds LJ, Hughes LL, Louis AI, Kramer RM, and Dennis EA.** Metal ion and salt effects on the phospholipase A2, lysophospholipase, and transacylase activities of human cytosolic phospholipase A2. *Biochim Biophys Acta* 1167: 272-280, 1993.
290. **Ristimaki A, Garfinkel S, Wessendorf J, Maciag T, and Hla T.** Induction of cyclooxygenase-2 by interleukin-1 alpha. Evidence for post-transcriptional regulation. *J Biol Chem* 269: 11769-11775, 1994.

291. **Rognum TO.** Definition and pathologic features. In Sudden Infant Death syndrome: Problems, Progress and Possibilities. *RW Byard, HF Krouse, eds* London, UK: 4-30, 2001.
292. **Romanovsky AA, Ivanov AI, and Szekely M.** Neural route of pyrogen signaling to the brain. *Clin Infect Dis* 31 Suppl 5: S162-167, 2000.
293. **Ruan KH, Wang LH, Wu KK, and Kulmacz RJ.** Amino-terminal topology of thromboxane synthase in the endoplasmic reticulum. *J Biol Chem* 268: 19483-19490, 1993.
294. **Saag K, van der Heijde D, Fisher C, Samara A, DeTora L, Bolognese J, Sperling R, and Daniels B.** Rofecoxib, a new cyclooxygenase 2 inhibitor, shows sustained efficacy, comparable with other nonsteroidal anti-inflammatory drugs: a 6-week and a 1-year trial in patients with osteoarthritis. Osteoarthritis Studies Group. *Arch Fam Med* 9: 1124-1134, 2000.
295. **Saha S, Engstrom L, Mackerlova L, Jakobsson PJ, and Blomqvist A.** Impaired febrile responses to immune challenge in mice deficient in microsomal prostaglandin E synthase-1. *Am J Physiol Regul Integr Comp Physiol* 288: R1100-1107, 2005.
296. **Sakamoto K, Ezashi T, Miwa K, Okuda-Ashitaka E, Houtani T, Sugimoto T, Ito S, and Hayaishi O.** Molecular cloning and expression of a cDNA of the bovine prostaglandin F2 alpha receptor. *J Biol Chem* 269: 3881-3886, 1994.
297. **Samad TA, Sapirstein A, and Woolf CJ.** Prostanoids and pain: unraveling mechanisms and revealing therapeutic targets. *Trends Mol Med* 8: 390-396, 2002.
298. **Samuelsson B and Hamberg M.** Role of endoperoxides in the biosynthesis and action of prostaglandins. In: *Proceedings of an International Symposium on Prostaglandin Synthetase Inhibitors*, edited by Vane JR. New York: Raven Press, 1974, p. 107-119.
299. **Satoh K, Nagano Y, Shimomura C, Suzuki N, Saeki Y, and Yokota H.** Expression of prostaglandin E synthase mRNA is induced in beta-amyloid treated rat astrocytes. *Neurosci Lett* 283: 221-223, 2000.
300. **Sawada H, Murakami M, Enomoto A, Shimbara S, and Kudo I.** Regulation of type V phospholipase A2 expression and function by proinflammatory stimuli. *Eur J Biochem* 263: 826-835, 1999.
301. **Scheinman RI, Cogswell PC, Lofquist AK, and Baldwin AS, Jr.** Role of transcriptional activation of I kappa B alpha in mediation of immunosuppression by glucocorticoids. *Science* 270: 283-286, 1995.
302. **Schmidt-Krey I, Kanaoka Y, Mills DJ, Irikura D, Haase W, Lam BK, Austen KF, and Kuhlbrandt W.** Human leukotriene C(4) synthase at 4.5 A resolution in projection. *Structure* 12: 2009-2014, 2004.
303. **Schmidt-Krey I, Mitsuoka K, Hirai T, Murata K, Cheng Y, Fujiyoshi Y, Morgenstern R, and Hebert H.** The three-dimensional map of microsomal glutathione transferase 1 at 6 A resolution. *Embo J* 19: 6311-6316, 2000.
304. **Schneider A, Zhang Y, Zhang M, Lu WJ, Rao R, Fan X, Redha R, Davis L, Breyer RM, Harris R, Guan Y, and Breyer MD.** Membrane-associated PGE synthase-1 (mPGES-1) is coexpressed with both COX-1 and COX-2 in the kidney. *Kidney Int* 65: 1205-1213, 2004.
305. **Schuligoi R, Grill M, Heinemann A, Peskar BA, and Amann R.** Sequential induction of prostaglandin E and D synthases in inflammation. *Biochem Biophys Res Commun* 335: 684-689, 2005.
306. **Schuster VL.** Prostaglandin transport. *Prostaglandins Other Lipid Mediat* 68-69: 633-647, 2002.
307. **Schuster VL, Lu R, and Coca-Prados M.** The prostaglandin transporter is widely expressed in ocular tissues. *Surv Ophthalmol* 41 Suppl 2: S41-45, 1997.
308. **Seibert K, Zhang Y, Leahy K, Hauser S, Masferrer J, Perkins W, Lee L, and Isakson P.** Pharmacological and biochemical demonstration of the role of cyclooxygenase 2 in inflammation and pain. *Proc Natl Acad Sci U S A* 91: 12013-12017, 1994.
309. **Sheng H, Shao J, Dixon DA, Williams CS, Prescott SM, DuBois RN, and Beauchamp RD.** Transforming growth factor-beta1 enhances Ha-ras-induced expression of cyclooxygenase-2 in intestinal epithelial cells via stabilization of mRNA. *J Biol Chem* 275: 6628-6635, 2000.

310. **Sheng H, Shao J, Kirkland SC, Isakson P, Coffey RJ, Morrow J, Beauchamp RD, and DuBois RN.** Inhibition of human colon cancer cell growth by selective inhibition of cyclooxygenase-2. *J Clin Invest* 99: 2254-2259, 1997.
311. **Shoemaker AR, Gould KA, Luongo C, Moser AR, and Dove WF.** Studies of neoplasia in the Min mouse. *Biochim Biophys Acta* 1332: F25-48, 1997.
312. **Smith JC, Ellenberger HH, Ballanyi K, Richter DW, and Feldman JL.** Pre-Botzinger complex: a brainstem region that may generate respiratory rhythm in mammals. *Science* 254: 726-729, 1991.
313. **Smith WL.** The eicosanoids and their biochemical mechanisms of action. *Biochem J* 259: 315-324, 1989.
314. **Snipes JA, Kis B, Shelness GS, Hewett JA, and Busija DW.** Cloning and characterization of cyclooxygenase-1b (putative cyclooxygenase-3) in rat. *J Pharmacol Exp Ther* 313: 668-676, 2005.
315. **Solomon DH, Schneeweiss S, Glynn RJ, Kiyota Y, Levin R, Mogun H, and Avorn J.** Relationship between selective cyclooxygenase-2 inhibitors and acute myocardial infarction in older adults. *Circulation* 109: 2068-2073, 2004.
316. **Sonoshita M, Takaku K, Sasaki N, Sugimoto Y, Ushikubi F, Narumiya S, Oshima M, and Taketo MM.** Acceleration of intestinal polyposis through prostaglandin receptor EP2 in Apc(Delta 716) knockout mice. *Nat Med* 7: 1048-1051, 2001.
317. **Spik I, Brenuchon C, Angeli V, Staumont D, Fleury S, Capron M, Trottein F, and Dombrowicz D.** Activation of the prostaglandin D2 receptor DP2/CRTH2 increases allergic inflammation in mouse. *J Immunol* 174: 3703-3708, 2005.
318. **Spriggs DR, Deutsch S, and Kufe DW.** Genomic structure, induction, and production of TNF-alpha. *Immunol Ser* 56: 3-34, 1992.
319. **Starling MB, Neutze JM, Elliott RL, and Elliott RB.** Studies on the effects of prostaglandins E1, E2, A1, and A2 on the dustus arteriosus of swine in vivo using cineangiography. *Prostaglandins* 12: 355-367, 1976.
320. **Steiner G and Smolen J.** Autoantibodies in rheumatoid arthritis and their clinical significance. *Arthritis Res* 4 Suppl 2: S1-5, 2002.
321. **Stichtenoth DO and Frolich JC.** COX-2 and the kidneys. *Curr Pharm Des* 6: 1737-1753, 2000.
322. **Stichtenoth DO, Thoren S, Bian H, Peters-Golden M, Jakobsson PJ, and Crofford LJ.** Microsomal prostaglandin E synthase is regulated by proinflammatory cytokines and glucocorticoids in primary rheumatoid synovial cells. *J Immunol* 167: 469-474, 2001.
323. **St-John WM and Paton JF.** Role of pontile mechanisms in the neurogenesis of eupnea. *Respir Physiol Neurobiol* 143: 321-332, 2004.
324. **Sugimoto Y, Namba T, Honda A, Hayashi Y, Negishi M, Ichikawa A, and Narumiya S.** Cloning and expression of a cDNA for mouse prostaglandin E receptor EP3 subtype. *J Biol Chem* 267: 6463-6466, 1992.
325. **Suzuki N, Ishizaki J, Yokota Y, Higashino K, Ono T, Ikeda M, Fujii N, Kawamoto K, and Hanasaki K.** Structures, enzymatic properties, and expression of novel human and mouse secretory phospholipase A(2)s. *J Biol Chem* 275: 5785-5793, 2000.
326. **Tai HH, Ensor CM, Tong M, Zhou H, and Yan F.** Prostaglandin catabolizing enzymes. *Prostaglandins Other Lipid Mediat* 68-69: 483-493, 2002.
327. **Takahashi M, Fukutake M, Yokota S, Ishida K, Wakabayashi K, and Sugimura T.** Suppression of azoxymethane-induced aberrant crypt foci in rat colon by nimesulide, a selective inhibitor of cyclooxygenase 2. *J Cancer Res Clin Oncol* 122: 219-222, 1996.
328. **Takenaka K, Ogawa E, Oyanagi H, Wada H, and Tanaka F.** Carbonyl reductase expression and its clinical significance in non-small-cell lung cancer. *Cancer Epidemiol Biomarkers Prev* 14: 1972-1975, 2005.
329. **Tamai I, Nezu J, Uchino H, Sai Y, Oku A, Shimane M, and Tsuji A.** Molecular identification and characterization of novel members of the human organic anion transporter (OATP) family. *Biochem Biophys Res Commun* 273: 251-260, 2000.
330. **Tanaka Y, Ward SL, and Smith WL.** Immunochemical and kinetic evidence for two different prostaglandin H-prostaglandin E isomerases in sheep vesicular gland microsomes. *J Biol Chem* 262: 1374-1381, 1987.

331. **Tang J, Kriz RW, Wolfman N, Shaffer M, Seehra J, and Jones SS.** A novel cytosolic calcium-independent phospholipase A2 contains eight ankyrin motifs. *J Biol Chem* 272: 8567-8575, 1997.
332. **Tanikawa N, Ohmiya Y, Ohkubo H, Hashimoto K, Kangawa K, Kojima M, Ito S, and Watanabe K.** Identification and characterization of a novel type of membrane-associated prostaglandin E synthase. *Biochem Biophys Res Commun* 291: 884-889, 2002.
333. **Tanioka T, Nakatani Y, Kobayashi T, Tsujimoto M, Oh-ishi S, Murakami M, and Kudo I.** Regulation of cytosolic prostaglandin E(2) synthase by 90-kDa heat shock protein. *Biochem Biophys Res Commun* 303: 1018-1023, 2003.
334. **Tanioka T, Nakatani Y, Semmyo N, Murakami M, and Kudo I.** Molecular identification of cytosolic prostaglandin E2 synthase that is functionally coupled with cyclooxygenase-1 in immediate prostaglandin E2 biosynthesis. *J Biol Chem* 275: 32775-32782, 2000.
335. **Teismann P, Tieu K, Choi DK, Wu DC, Naini A, Hunot S, Vila M, Jackson-Lewis V, and Przedborski S.** Cyclooxygenase-2 is instrumental in Parkinson's disease neurodegeneration. *Proc Natl Acad Sci U S A* 100: 5473-5478, 2003.
336. **Teismann P, Vila M, Choi DK, Tieu K, Wu DC, Jackson-Lewis V, and Przedborski S.** COX-2 and neurodegeneration in Parkinson's disease. *Ann N Y Acad Sci* 991: 272-277, 2003.
337. **Telgkamp P, Cao YQ, Basbaum AI, and Ramirez JM.** Long-term deprivation of substance P in PPT-A mutant mice alters the anoxic response of the isolated respiratory network. *J Neurophysiol* 88: 206-213, 2002.
338. **Terada T, Sugihara Y, Nakamura K, Sato R, Sakuma S, Fujimoto Y, Fujita T, Inazu N, and Maeda M.** Characterization of multiple Chinese hamster carbonyl reductases. *Chem Biol Interact* 130-132: 847-861, 2001.
339. **Thoren S and Jakobsson PJ.** Coordinate up- and down-regulation of glutathione-dependent prostaglandin E synthase and cyclooxygenase-2 in A549 cells. Inhibition by NS-398 and leukotriene C4. *Eur J Biochem* 267: 6428-6434, 2000.
340. **Tietge UJ, Maugeais C, Cain W, Grass D, Glick JM, de Beer FC, and Rader DJ.** Overexpression of secretory phospholipase A(2) causes rapid catabolism and altered tissue uptake of high density lipoprotein cholesteryl ester and apolipoprotein A-I. *J Biol Chem* 275: 10077-10084, 2000.
341. **Tilley SL, Coffman TM, and Koller BH.** Mixed messages: modulation of inflammation and immune responses by prostaglandins and thromboxanes. *J Clin Invest* 108: 15-23, 2001.
342. **Tjoelker LW, Wilder C, Eberhardt C, Stafforini DM, Dietsch G, Schimpf B, Hooper S, Le Trong H, Cousens LS, Zimmerman GA, and et al** Anti-inflammatory properties of a platelet-activating factor acetylhydrolase. *Nature* 374: 549-553, 1995.
343. **Topol EJ and Falk GW.** A coxib a day won't keep the doctor away. *Lancet* 364: 639-640, 2004.
344. **Trebino CE, Eskra JD, Wachtmann TS, Perez JR, Carty TJ, and Audoly LP.** Redirection of eicosanoid metabolism in mPGES-1-deficient macrophages. *J Biol Chem* 280: 16579-16585, 2005.
345. **Trebino CE, Stock JL, Gibbons CP, Naiman BM, Wachtmann TS, Umland JP, Pandher K, Lapointe JM, Saha S, Roach ML, Carter D, Thomas NA, Durtschi BA, McNeish JD, Hambor JE, Jakobsson PJ, Carty TJ, Perez JR, and Audoly LP.** Impaired inflammatory and pain responses in mice lacking an inducible prostaglandin E synthase. *Proc Natl Acad Sci U S A* 100: 9044-9049, 2003.
346. **Tredget EE, Yu YM, Zhong S, Burini R, Okusawa S, Gelfand JA, Dinarello CA, Young VR, and Burke JF.** Role of interleukin 1 and tumor necrosis factor on energy metabolism in rabbits. *Am J Physiol* 255: E760-768, 1988.
347. **Ueda N, Yamashita R, Yamamoto S, and Ishimura K.** Induction of cyclooxygenase-1 in a human megakaryoblastic cell line (CMK) differentiated by phorbol ester. *Biochim Biophys Acta* 1344: 103-110, 1997.
348. **Uematsu S, Matsumoto M, Takeda K, and Akira S.** Lipopolysaccharide-dependent prostaglandin E(2) production is regulated by the glutathione-dependent prostaglandin E(2) synthase gene induced by the Toll-like receptor 4/MyD88/NF-IL6 pathway. *J Immunol* 168: 5811-5816, 2002.

349. **Ueno A, Matsumoto H, Naraba H, Ikeda Y, Ushikubi F, Matsuoka T, Narumiya S, Sugimoto Y, Ichikawa A, and Oh-ishi S.** Major roles of prostanoid receptors IP and EP(3) in endotoxin-induced enhancement of pain perception. *Biochem Pharmacol* 62: 157-160, 2001.
350. **Ujihara M, Tsuchida S, Satoh K, Sato K, and Urade Y.** Biochemical and immunological demonstration of prostaglandin D2, E2, and F2 alpha formation from prostaglandin H2 by various rat glutathione S-transferase isozymes. *Arch Biochem Biophys* 264: 428-437, 1988.
351. **Ujihara M, Urade Y, Eguchi N, Hayashi H, Ikai K, and Hayaishi O.** Prostaglandin D2 formation and characterization of its synthetases in various tissues of adult rats. *Arch Biochem Biophys* 260: 521-531, 1988.
352. **Uozumi N, Kume K, Nagase T, Nakatani N, Ishii S, Tashiro F, Komagata Y, Maki K, Ikuta K, Ouchi Y, Miyazaki J, and Shimizu T.** Role of cytosolic phospholipase A2 in allergic response and parturition. *Nature* 390: 618-622, 1997.
353. **Urade Y and Eguchi N.** Lipocalin-type and hematopoietic prostaglandin D synthases as a novel example of functional convergence. *Prostaglandins Other Lipid Mediat* 68-69: 375-382, 2002.
354. **Ushikubi F, Segi E, Sugimoto Y, Murata T, Matsuoka T, Kobayashi T, Hizaki H, Tuboi K, Katsuyama M, Ichikawa A, Tanaka T, Yoshida N, and Narumiya S.** Impaired febrile response in mice lacking the prostaglandin E receptor subtype EP3. *Nature* 395: 281-284, 1998.
355. **Wada M, Yokoyama C, Hatae T, Shimonishi M, Nakamura M, Imai Y, Ullrich V, and Tanabe T.** Purification and characterization of recombinant human prostacyclin synthase. *J Biochem (Tokyo)* 135: 455-463, 2004.
356. **Valentin E, Ghomashchi F, Gelb MH, Lazdunski M, and Lambeau G.** On the diversity of secreted phospholipases A(2). Cloning, tissue distribution, and functional expression of two novel mouse group II enzymes. *J Biol Chem* 274: 31195-31202, 1999.
357. **van Dorp DA, Beerthuis RK, Nugteren DH, and Vonkeman H.** The biosynthesis of prostaglandins. *Biochim Biophys Acta*: 204-207, 1964.
358. **Vane JR.** Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nat New Biol* 231: 232-235, 1971.
359. **Wanebo HJ.** Tumor necrosis factors. *Semin Surg Oncol* 5: 402-413, 1989.
360. **Warner TD, Giuliano F, Vojnovic I, Bukasa A, Mitchell JA, and Vane JR.** Nonsteroid drug selectivities for cyclo-oxygenase-1 rather than cyclo-oxygenase-2 are associated with human gastrointestinal toxicity: a full in vitro analysis. *Proc Natl Acad Sci U S A* 96: 7563-7568, 1999.
361. **Watanabe K.** Prostaglandin F synthase. *Prostaglandins Other Lipid Mediat* 68-69: 401-407, 2002.
362. **Watanabe K, Iguchi Y, Iguchi S, Arai Y, Hayaishi O, and Roberts LJ, 2nd.** Stereospecific conversion of prostaglandin D2 to (5Z,13E)-(15S)-9 alpha-11 beta,15-trihydroxyprosta-5,13-dien-1-oic acid (9 alpha,11 beta-prostaglandin F2) and of prostaglandin H2 to prostaglandin F2 alpha by bovine lung prostaglandin F synthase. *Proc Natl Acad Sci U S A* 83: 1583-1587, 1986.
363. **Watanabe K, Kurihara K, and Suzuki T.** Purification and characterization of membrane-bound prostaglandin E synthase from bovine heart. *Biochim Biophys Acta* 1439: 406-414, 1999.
364. **Watanabe K, Kurihara K, Tokunaga Y, and Hayaishi O.** Two types of microsomal prostaglandin E synthase: glutathione-dependent and -independent prostaglandin E synthases. *Biochem Biophys Res Commun* 235: 148-152, 1997.
365. **Vaughn LK, Veale WL, and Cooper KE.** Antipyresis: its effect on mortality rate of bacterially infected rabbits. *Brain Res Bull* 5: 69-73, 1980.
366. **Webb AC, Collins KL, Auron PE, Eddy RL, Nakai H, Byers MG, Haley LL, Henry WM, and Shows TB.** Interleukin-1 gene (IL1) assigned to long arm of human chromosome 2. *Lymphokine Res* 5: 77-85, 1986.
367. **Vege A, Rognum TO, Scott H, Aasen AO, and Saugstad OD.** SIDS cases have increased levels of interleukin-6 in cerebrospinal fluid. *Acta Paediatr* 84: 193-196, 1995.
368. **Weinander R, Ekstrom L, Andersson C, Raza H, Bergman T, and Morgenstern R.** Structural and functional aspects of rat microsomal glutathione transferase. The

roles of cysteine 49, arginine 107, lysine 67, histidine, and tyrosine residues. *J Biol Chem* 272: 8871-8877, 1997.

369. **Welsch DJ, Creely DP, Hauser SD, Mathis KJ, Krivi GG, and Isakson PC.** Molecular cloning and expression of human leukotriene-C4 synthase. *Proc Natl Acad Sci U S A* 91: 9745-9749, 1994.
370. **Wenninger JM, Pan LG, Klum L, Leekley T, Bastastic J, Hodges MR, Feroah T, Davis S, and Forster HV.** Small reduction of neurokinin-1 receptor-expressing neurons in the pre-Botzinger complex area induces abnormal breathing periods in awake goats. *J Appl Physiol* 97: 1620-1628, 2004.
371. **Wenninger JM, Pan LG, Klum L, Leekley T, Bastastic J, Hodges MR, Feroah TR, Davis S, and Forster HV.** Large lesions in the pre-Botzinger complex area eliminate eupneic respiratory rhythm in awake goats. *J Appl Physiol* 97: 1629-1636, 2004.
372. **Wermuth B, Mader-Heinemann G, and Ernst E.** Cloning and expression of carbonyl reductase from rat testis. *Eur J Biochem* 228: 473-479, 1995.
373. **Westman M, Korotkova M, af Klint E, Stark A, Audoly LP, Klareskog L, Ulfgren AK, and Jakobsson PJ.** Expression of microsomal prostaglandin E synthase 1 in rheumatoid arthritis synovium. *Arthritis Rheum* 50: 1774-1780, 2004.
374. **Williams CS, Mann M, and DuBois RN.** The role of cyclooxygenases in inflammation, cancer, and development. *Oncogene* 18: 7908-7916, 1999.
375. **Williams JW, Rudy TA, Yaksh TL, and Viswanathan CT.** An extensive exploration of the rat brain for sites mediating prostaglandin-induced hyperthermia. *Brain Res* 120: 251-262, 1977.
376. **Willinger M, James LS, and Catz C.** Defining the sudden infant death syndrome (SIDS): deliberations of an expert panel convened by the National Institute of Child Health and Human Development. *Pediatr Pathol* 11: 677-684, 1991.
377. **Wintergalen N, Thole HH, Galla HJ, and Schlegel W.** Prostaglandin-E2 9-reductase from corpus luteum of pseudopregnant rabbit is a member of the aldo-keto reductase superfamily featuring 20 alpha-hydroxysteroid dehydrogenase activity. *Eur J Biochem* 234: 264-270, 1995.
378. **Virta M, Hurme M, and Helminen M.** Increased plasma levels of pro- and anti-inflammatory cytokines in patients with febrile seizures. *Epilepsia* 43: 920-923, 2002.
379. **von Euler US.** On the specific vaso-dilating and plain muscle stimulating substances from accessory genital glands in man and certain animals (prostaglandin and vesiglandin). *J Physiol* 88: 213-234, 1936.
380. **Wooley PH, Luthra HS, Stuart JM, and David CS.** Type II collagen-induced arthritis in mice. I. Major histocompatibility complex (I region) linkage and antibody correlates. *J Exp Med* 154: 688-700, 1981.
381. **Woolf CJ and Salter MW.** Neuronal plasticity: increasing the gain in pain. *Science* 288: 1765-1769, 2000.
382. **Wu WX, Ma XH, Yoshizato T, Shinozuka N, and Nathanielsz PW.** Increase in prostaglandin H synthase 2, but not prostaglandin F2alpha synthase mRNA in intrauterine tissues during betamethasone-induced premature labor and spontaneous term labor in sheep. *J Soc Gynecol Investig* 8: 69-76, 2001.
383. **Xie WL, Chipman JG, Robertson DL, Erikson RL, and Simmons DL.** Expression of a mitogen-responsive gene encoding prostaglandin synthase is regulated by mRNA splicing. *Proc Natl Acad Sci U S A* 88: 2692-2696, 1991.
384. **Xin L and Blatteis CM.** Hypothalamic neuronal responses to interleukin-6 in tissue slices: effects of indomethacin and naloxone. *Brain Res Bull* 29: 27-35, 1992.
385. **Xu XM, Tang JL, Chen X, Wang LH, and Wu KK.** Involvement of two Sp1 elements in basal endothelial prostaglandin H synthase-1 promoter activity. *J Biol Chem* 272: 6943-6950, 1997.
386. **Yamada T, Komoto J, Watanabe K, Ohmiya Y, and Takusagawa F.** Crystal structure and possible catalytic mechanism of microsomal prostaglandin E synthase type 2 (mPGES-2). *J Mol Biol* 348: 1163-1176, 2005.
387. **Yamagata K, Matsumura K, Inoue W, Shiraki T, Suzuki K, Yasuda S, Sugiura H, Cao C, Watanabe Y, and Kobayashi S.** Coexpression of microsomal-type prostaglandin E synthase with cyclooxygenase-2 in brain endothelial cells of rats during endotoxin-induced fever. *J Neurosci* 21: 2669-2677, 2001.

388. **Yan F and Polk DB.** Aminosalicylic acid inhibits IkappaB kinase alpha phosphorylation of IkappaBalpha in mouse intestinal epithelial cells. *J Biol Chem* 274: 36631-36636, 1999.
389. **Yokoyama C, Takai T, and Tanabe T.** Primary structure of sheep prostaglandin endoperoxide synthase deduced from cDNA sequence. *FEBS Lett* 231: 347-351, 1988.
390. **Yokoyama C and Tanabe T.** Cloning of human gene encoding prostaglandin endoperoxide synthase and primary structure of the enzyme. *Biochem Biophys Res Commun* 165: 888-894, 1989.
391. **Yoshida K, Taga T, Saito M, Suematsu S, Kumanogoh A, Tanaka T, Fujiwara H, Hirata M, Yamagami T, Nakahata T, Hirabayashi T, Yoneda Y, Tanaka K, Wang WZ, Mori C, Shiota K, Yoshida N, and Kishimoto T.** Targeted disruption of gp130, a common signal transducer for the interleukin 6 family of cytokines, leads to myocardial and hematological disorders. *Proc Natl Acad Sci U S A* 93: 407-411, 1996.
392. **Yoshimatsu K, Altorki NK, Golijanin D, Zhang F, Jakobsson PJ, Dannenberg AJ, and Subbaramaiah K.** Inducible prostaglandin E synthase is overexpressed in non-small cell lung cancer. *Clin Cancer Res* 7: 2669-2674, 2001.
393. **Yoshimatsu K, Golijanin D, Paty PB, Soslow RA, Jakobsson PJ, DeLellis RA, Subbaramaiah K, and Dannenberg AJ.** Inducible microsomal prostaglandin E synthase is overexpressed in colorectal adenomas and cancer. *Clin Cancer Res* 7: 3971-3976, 2001.
394. Zheng H, Fletcher D, Kozak W, Jiang M, Hofmann KJ, Conn CA, Soszynski D, Grabiec C, Trumbauer ME, Shaw A, and et al Resistance to fever induction and impaired acute-phase response in interleukin-1 beta-deficient mice. Immunity 3: 9-19, 1995.