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PROSTAGLANDIN E_2 AS A MEDIATOR OF FEVER: SYNTHESIS AND CATABOLISM

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1. ABSTRACT

Prostaglandin (PG) E_2 is a principal downstream mediator of fever. It is synthesized in three steps catalyzed by phospholipase (PL) A_2, cyclooxygenase (COX), and terminal PGE synthase (PGES), where each catalytic activity is represented by multiple enzymes and/or isoenzymes. Inactivation of PGE_2 occurs primarily in the lungs and liver via carrier-mediated cellular uptake and enzymatic oxidation. The two principal carriers are PG transporter (PGT) and multispecific organic anion transporter (MOAT); the two principal PGE_2-inactivating enzymes are 15-hydroxy-PG dehydrogenase (15-PGDH) and carbonyl reductase (CR). Our data [Ivanov A. I. *et al. Am J Physiol Regul Integr Comp Physiol* 283, R1104-R1117 (2002); *ibid.* 284, R698-R706 (2003)] are used to analyze the relationship between transcriptional regulation of PLA_2, COX, PGES, PGT, MOAT, 15-PHDH, and CR, on one hand, and the triphasic febrile response of rats to lipopolysaccharide (LPS), on the other. It is concluded that LPS fever is accompanied by up-regulation of four PGE_2-synthesizing enzymes [secretory (s) PLA_2-IIA, cytosolic (c) PLA_2-alpha, COX-2, and microsomal (m) PGES-1] and down-regulation of all PGE_2 carriers and dehydrogenases studied (PGT, MOAT, 15PGDH, and CR). It is further concluded that different febrile phases employ different mechanisms to mount an increase in the PGE_2 level. *Phase 1* involves transcriptional up-regulation of the couple COX-2 $\rightarrow$ mPGES-1 in the liver and lungs. *Phase 2* entails robust up-regulation of the major inflammatory triad $sPLA_2$-IIA $\rightarrow$ COX-2 $\rightarrow$ mPGES-1 throughout the body. *Phase 3* involves induction of $cPLA_2$-alpha in the hypothalamus and further up-regulation of $sPLA_2$-IIA and mPGES throughout the body. Importantly, *Phase 3* occurs despite a drastic decrease in the expression of COX-1 and -2 in both the brain and periphery, thus suggesting that transcriptional up-regulation of COX-2 is not an obligatory mechanism of PGE_2-dependent inflammatory responses at later stages. Of importance is also that LPS fever is accompanied by transcriptional down-regulation of PGE_2 transporters and dehydrogenases: 15-PGDH in the lungs at *Phase 1*; 15-PGDH and CR in the lungs at *Phase 2*; and PGT, MOAT, 15-PGDH, and CR in the liver and lungs at *Phase 3*. The transcriptional down-regulation of proteins involved in PGE_2 inactivation is a largely unrecognized mechanism of systemic inflammation. By increasing the blood-brain gradient of PGE_2, this mechanism likely facilitates penetration of PGE_2 into the brain. The high magnitude of up-regulation of mPGES and $sPLA_2$-IIA (1,260 and 130 fold, respectively) and that of down-regulation of 15-PGES (30 fold) during LPS fever makes these enzymes attractive targets for anti-inflammatory therapy.

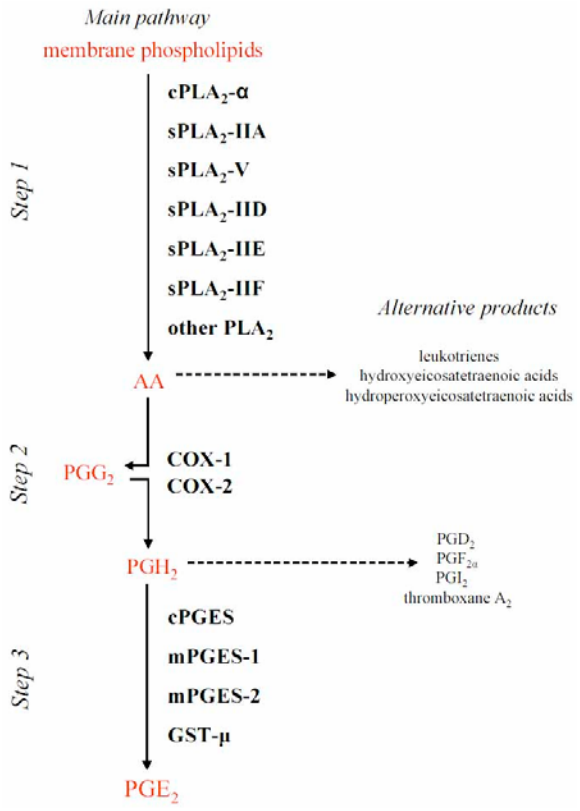

Figure 1. Three steps of the PGE_2-synthesizing cascade. Substrates and products are shown in red (major pathway) or black (selected final products of alternative pathways); enzymes are shown in bold; explanatory signs are shown in italics. For abbreviation, see Section 2. (Modified from Ref. 31; published with permission of the American Physiological Society).

2. INTRODUCTION

Upon a systemic inflammatory challenge [*e.g.*, with bacterial lipopolysaccharide (LPS) or pyrogenic cytokines, such as interleukin (IL)-1beta and tumor necrosis factor (TNF)-alpha], the brain launches a powerful defense response: fever (1). Both clinical and experimental fevers are polyphasic. A single, bolus injection of LPS causes several sequential rises in body temperature, so-called febrile phases, each having its own thermoregulatory mechanism (2). Three phases (*Phases 1-3*) have been identified in the febrile response of the rat (3, 4) and mouse (5, 6), the species commonly used in research on inflammation and sepsis. In addition to having different thermoregulatory mechanisms (2), different febrile phases are accompanied by different sickness symptoms (*e.g.*, hyperalgesia or allodynia at *Phase 1 vs.* hypoalgesia at *Phase 2*; see Refs. 7-9) and are likely to be mediated by different cascades of endogenous mediators triggered by LPS (10). However, a common feature of all phases seems to be their dependence on prostaglandin (PG) E_2 (5, 10-14), which is widely accepted as the principal downstream mediator of LPS fever (15-17). During the febrile response to LPS, PGE_2 concentration is increased in the blood (10, 11), cerebrospinal fluid (18), and hypothalamus (12). Two main mechanisms responsible for such an increase, *viz.*, stimulation of PGE_2 synthesis and inhibition of its catabolism, are discussed in this review. While discussing these mechanisms, we will emphasize two points that have not received the deserved attention in the literature. Specifically, we will show that peripheral, blood-borne PGE_2 is important in triggering fever. We will also show the increases of PGE_2 in body fluids during different febrile phases are achieved by different mechanisms.

3. SYNTHESIS OF PROSTAGLANDIN E_2 IN FEVER

3.1. Three steps of the enzymatic cascade

The synthesis of PGE_2 occurs in three steps (19) schematically presented in Figure 1. First, arachidonic acid (AA) is released from membrane phospholipids via action of phospholipase (PL) A_2 (19-21). Second, AA is converted to PGG_2 and then to PGH_2 by cyclooxygenase (COX) (19, 22). Third, PGH_2 is isomerized to PGE_2 by terminal PGE synthase (PGES) (23, 24). Each step of this cascade can be catalyzed by several nonhomologous enzymes and/or multiple isoforms of the same enzyme. Thus, the mammalian PLA_2 family consists of more than a dozen members, both cytosolic (c) and secretory (s) (20, 21). Two COX isoforms, *i.e.*, COX-1 and COX-2, have been firmly identified (22). The $PGH_2 \rightarrow PGE_2$ isomerizing activity is attributed to several PGES enzymes (23, 24), both in cytosol (cPGES) and bound to intracellular membranes, so-called microsomal PGES (mPGES).

An important feature of the PGE_2-synthesizing cascade is a functional coupling between selective isoforms of the enzymes at different steps of the cascade (21, 24-26). Examples of such a coupling are presented below. The functional coupling plays an important role in selective distribution of AA and its metabolites between the PGE_2 pathway *vs.* several alternative eicosanoid pathways. Under basal conditions, the rate of PGE_2 synthesis in various cells is limited by both the release of AA by PLA_2 and the consumption of AA by alternative pathways (19, 27). Along with robust activation all three steps of PGE_2 synthesis, inflammatory stimuli cause a preferential redistribution of AA and its metabolites toward the PGE_2 pathway (28, 29).

3.2. Step 1: phospholipases A_2

The superfamily of the PLA_2 enzymes (all of which hydrolyze the *sn*-2 ester bond of membrane phospholipids; see Refs. 20, 21) is divided into four classes: calcium-dependent $cPLA_2$; $sPLA_2$; calcium-independent $cPLA_2$; and platelet-activating factor (PAF)-acetylhydrolase (21). Since two later classes are unlikely to play a significant role in PGE_2 synthesis, they are not discussed in this review.

3.2.1. Calcium-dependent cytosolic phospholipases A_2

The group of calcium-dependent $cPLA_2$ contains three isoenzymes expressed abundantly in various mammalian tissues: $cPLA_2$-alpha, $cPLA_2$-beta, and $cPLA_2$-gamma, of which the alpha form is the most studied (21).

In resting cells, the inactive form of $cPLA_2$-alpha is present in cytosol. Upon stimulation by inflammatory agents, $cPLA_2$-alpha becomes rapidly (within minutes) phosphorylated and translocates to the Golgi complex, endoplasmic reticulum, and nuclear envelope (20, 21), *i.e.*, into close vicinity of the downstream PGE_2-synthesizing enzymes (see Sections 3.3, 3.4). Translocation to intracellular membranes results in release of fatty acids, predominantly AA. Besides posttranslational activation, LPS has been shown to cause a modest (up to 3 fold) transcriptional up-regulation of $cPLA_2$-alpha in cultured rat liver macrophages (30) and in the rat hypothalamus *in vivo* (31). To what extent this transcriptional up-regulation contributes to the elevated activity of the enzyme remains unclear. Many authors consider $cPLA_2$-alpha a key enzyme for PG production during inflammation (25, 32, 33). Indeed, immune cells generated from $cPLA_2$-alpha knockout mice show a marked reduction in PG synthesis (34). Furthermore, both genetic (35) and pharmacological (36) blockage of $cPLA_2$-alpha attenuates LPS- and zymozan-induced lung injury in rats. The indispensability of this enzyme for PGE_2 synthesis in stimulated immune cells and its rapid activation in inflammation suggest an involvement of $cPLA_2$-alpha in LPS fever. Yet, a first attempt to reveal an involvement of $cPLA_2$-alpha in the febrile response to LPS by using the knockout mouse produced negative results (37). This is rather surprising, because Bosetti and Weerasinghe (38) have shown that the expression of COX-2 is selectively down-regulated in the same knockout. Furthermore, the authors have not found any compensation by $sPLA_2$ or calcium-independent-PLA_2 for the lack of $cPLA_2$-alpha in this model (at least under basal conditions), thus suggesting that $cPLA_2$-alpha is selectively coupled with COX-2, and that its role as a supplier of AA for COX-2 cannot be taken by other PLA_2 enzymes.

3.2.2. Secretory phospholipases A_2

In mammals, the rapidly expanding $sPLA_2$ family now includes ten enzymes known as groups IB, IIA, IIC, IID, IIE, IIF, III, V, X, and XII (21). The cellular localization and the principal mechanism of activation of $sPLA_2$ differ from those of $cPLA_2$. A substantial fraction of $sPLA_2$ (all groups) is secreted into the extracellular space to release AA from the outer leaflet of plasma membrane (20, 21). The major mechanism of $sPLA_2$ activation in stimulated cells is transcriptional up-regulation (20, 21, 39).

One of the better-known members of the $sPLA_2$ family, $sPLA_2$-IIA, was initially described in the synovial fluid of patients with rheumatoid arthritis (40). Not surprisingly, the expressional regulation of this isoform during experimental and clinical inflammation has attracted keen attention. Increased blood levels of $sPLA_2$-IIA and its transcriptional up-regulation have been repeatedly demonstrated in patients with systemic inflammation and in laboratory animals with LPS-induced shock (for review, see Refs 40, 41). In the rat, large, shock-inducing doses of LPS (up to 10 mg/kg) induce up-regulation of $sPLA_2$-IIA in a variety of peripheral organs (42-44) and the brain (43). Importantly, functional coupling with COX-2 has been proposed for $sPLA_2$-IIA and its homolog $sPLA_2$-V (20, 25, 45).

We have found a strong up-regulation of $sPLA_2$-IIA during the polyphasic febrile response to a mild (50 microg/kg) dose of LPS in rats (31). Interestingly, the up-regulation of $sPLA_2$-IIA transcription was readily detectable in both the brain and periphery (liver and lungs), but the magnitude of the up-regulation was much higher in the hypothalamus (133 fold) than in the peripheral, LPS-processing organs (up to 9 fold). In the same study, we failed to detect any effect of LPS on expression of the homologous enzyme $sPLA_2$-V (31). The genes encoding $sPLA_2$-IIA and -V are located on the same chromosome; their transcriptional regulation is thought to be coordinated (21, 46); and their functional redundancy is evident in cell culture experiments (20, 25, 46). However, a dissociation in the expression of $sPLA_2$-IIA and -V was found by Sawada *et al.* (44) in two *in-vivo* models of LPS shock. Based on these findings (31, 44), the roles of $sPLA_2$-IIA and -V in inflammation are likely to be different and possibly tissue- and/or species-specific. Different responses of these two enzymes to several inflammatory stimuli have been also observed in the recent study by Hamaguchi *et al*. (47).

Recently, three novel $sPLA_2$ isoforms were found to be over-expressed in peripheral tissues (isoforms IID, IIE, and IIF) and the brain (isoforms IIE and IIF) at a late stage of LPS shock in mice (47-50). Being highly cationic, the IIA, IID, and IIE isoforms bind to negatively-charged molecules of glypican, a heparan sulfate-containing protein of the cell membrane. Such binding to glypican augments the release of AA from the cell membrane, thus representing an additional mechanism regulating the activity of these $sPLA_2$ isoforms (21, 49, 50). Small cationic proteins annexins (lipocortins)-I and -V compete with $sPLA_2$ of these groups for negatively-charged binding sites on the membrane (51, 52). Because annexins attenuate experimental fevers in rodents (53, 54), the $sPLA_2$-mediated, glypican-binding-dependent augmentation of AA release can be speculated to play a role in the febrile response.

Whereas the abovementioned groups IIA, V, IID, IIE, IIF, and V are responsive to inflammatory stimuli, other members of the $sPLA_2$ family are not (20, 21).

3.3. Step 2: cyclooxygenases

Formerly known as PGH synthase, COX catalyzes the double reaction of cyclization and peroxidation of AA producing sequentially PGG_2 and PGH_2 (Figure 1; also see Refs. 19, 22). Two isoforms of this enzyme, COX-1 and COX-2, are well characterized (22). Recently, a variant of COX-1 (named COX-3, or COX-1b) was cloned (55). Although COX-1 and -2 share ~60% of their sequences and catalyze the same reaction, they are differentially regulated and have different physiological functions (22). The reasons for the different functional properties of COX-1 and -2 may include subtle kinetic differences (*viz.*, different thresholds for hydroperoxide activation and different sensitivities to allosteric inhibition by high concentrations of AA; see Refs.

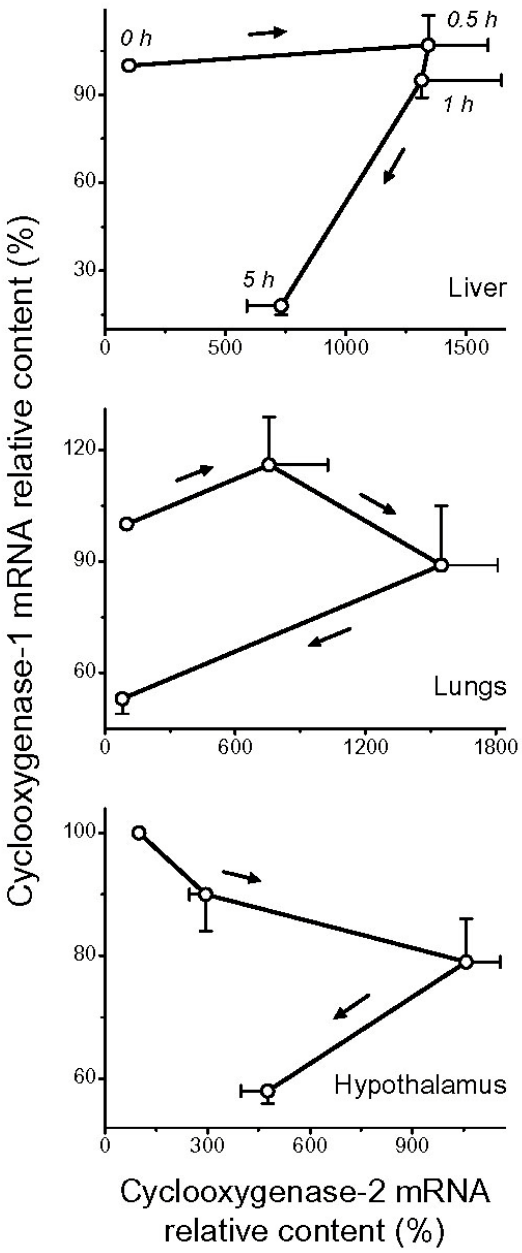

Figure 2. Dynamic changes of transcriptional regulation of COX-1 and COX-2 during the triphasic febrile response to intravenous LPS (50 microg/kg) in the liver, lungs, and hypothalamus. For each organ, point 1 (0 h) corresponds to the basal conditions (relative expression of both enzymes is ~100%); point 2 (0.5 h post LPS) corresponds to the maximal rate of body temperature change at *Phase 1* of the triphasic response to intravenous LPS; next point (1 h) corresponds to the maximal rate of body temperature change at *Phase 2*; and the end point (5 h post-LPS) corresponds to *Phase 3*. In all three organs the levels of both messages dramatically decrease (moves downward for COX-1 and to the left for COX-2) between *Phases 2* and *3*. In saline-treated rats (not shown), all points (0, 0.5, 1, and 5 h) are located close to each other, and their abscissas and ordinates are all equal ~100%. (This original figure uses data reported in Ref. 31.)

56, 57), the difference in coupling with other PGE_2-synthesizing enzymes (covered elsewhere in this review), and a possible difference in the distribution of the two isoforms between the endoplasmic reticulum, perinuclear envelope, and nuclear matrix (58, 59). Although the latter difference is relative, perhaps cell-specific, and may even have methodological causes, COX-1 is often viewed as a mostly extranuclear form, whereas COX-2 is often considered mostly intranuclear (59, 60).

3.3.1. Cyclooxygenase-1

COX-1 is expressed constitutively in most tissues, where it plays a wide range of housekeeping roles (22, 61). Although a low-magnitude (2-to-3-fold) induction of COX-1 by mitogens and growth factors was found in several studies *in vitro* (for review, see Ref. 61) and confirmed in LPS shock (62) and polymicrobial peritonitis (63) *in vivo*, this isoform is generally considered resistant to transcriptional up-regulation (22, 61). A recent study shows that the COX-1 variant COX-1b also does not appear to be induced by acute inflammatory stimulation (64).

Studies involving selective pharmacological inhibition of COX-1 found no role for this isoform in *Phases 2* and/or *3* of LPS fever in rats (65, 66) and guinea pigs (14), but did not decisively answer whether COX-1 is involved in *Phase 1*. Several methodological conditions (non-stressful injection of LPS, near-neutral ambient temperature, *etc.*) are required to study febrile *Phase 1* (4), but those conditions were not satisfied in the studies conducted (65, 66). The published work using COX-1 and COX-2 knockout mice (67) did not shed light on this issue, because LPS was administered in such a way that pronounced stress hyperthermia occurred and completely masked *Phase 1* of the response. We have eliminated this shortcoming by administering LPS intravenously in a non-stressful way (6). Our in-progress study (Steiner A. A, A. Y. Rudaya & A. A. Romanovsky) involving such a non-stressful administration of LPS shows that *Phase 1* is missing in COX-2 knockout mice, whereas it is preserved (and possibly even exaggerated) in COX-1 knockouts. This observation suggests that *Phase 1* is mediated entirely by COX-2, and that COX-1 is uninvolved.

An interesting phenomenon was observed in our recent study (31). In addition to the expected finding that expression of COX-1 is unchanged during *Phases 1* and *2* of triphasic LPS fever in rats, we found that the level of COX-1 mRNA decreased in the liver (7 fold) and lungs (3 fold) and showed a strong tendency for a decrease in the hypothalamus at *Phase 3* (Figure 2). This finding agrees with data of Liu *et al.* (68) and Devaux *et al.* (69) showing down-regulation of the COX-1 gene in the liver, lungs, and heart of rats challenged with shock-inducing doses of LPS. In our study (31), the decrease of COX-1 mRNA to below its basal level occurred concurrently with the decline of COX-2 mRNA from the peak of its response. Indeed, Figure 2 shows that when the ordinate (COX-1 expression) decreases (moves downward) from the 1-h point to the 5-h point, the abscissa (COX-2 expression) also decreases (moves from right to left) in all tissues studied. The simultaneous suppression of both genes suggests a common

mechanism for their transcriptional down-regulation at the later stages of the febrile response. Such a mechanism may involve activation of the peroxisome proliferator-activated receptors, which are considered negative regulators of the inflammatory response (70). A potent endogenous agonist of these receptors, 15-deoxy-delta^{12,14}-PGJ_2 (15d-PGJ_2), was recently shown to suppress LPS-induced COX-2 expression in cultured macrophage-like cells (71) and in rat hypothalamus (72); it was also shown to suppress LPS-induced fever in rats (72). This natural metabolite of PGD_2 is synthesized via a COX-dependent pathway and may, therefore, constitute a negative feedback mechanism for COX expression (70).

3.3.2. Cyclooxygenase-2

Because COX-2 is by far the most studied enzyme of the PGE_2-synthesizing cascade, we will describe its role in fever briefly and refer the reader to recent reviews published in this special issue of the *Frontiers in Bioscience* (73) and elsewhere (17, 22, 61, 74). In contrast to COX-1, COX-2 transcript is barely detectable in most quiescent cells, but it is robustly up-regulated by inflammatory stimuli (22, 61, 74). The major role of COX-2 in LPS fever has been shown by several recent studies involving pharmacological analyses (14, 65, 66) and using gene knockouts (67). Studies of the LPS-induced COX-2 expression *in vivo* (for review, see Ref. 73) confirm such a role: intravenous LPS drastically up-regulates COX-2 mRNA and immunoreactivity in the brain vasculature and the meninges, while leaving COX-1 expression unchanged (75-77). COX-2 expression in the brain is also stimulated by intravenous IL-1beta and TNF-alpha (78, 79), but not by IL-6 (76). Interestingly, different pyrogens and different doses of the same pyrogen up-regulate expression of COX-2 in different cells in the brain (80, 81). A subpyrogenic dose of LPS (0.1 microg/kg) induces COX-2 expression selectively in brain perivascular macrophages. Higher doses of LPS up-regulate expression of this enzyme in both perivascular macrophages and endotheliocytes. Over-expression of COX-2 in the brain in response to a wide range of doses of IL-1beta is restricted to perivascular macrophages.

Also of interest are the relative contributions of brain-synthesized *vs.* peripherally produced PGE_2 to the febrile response, especially at the onset of the response. The dynamics of the COX-2 mRNA expression in LPS-processing organs (liver and lungs) and the hypothalamus in the dynamics of triphasic LPS fever was studied in our recent work (31). The kinetics of COX-2 up-regulation by LPS *in vivo* was found to be remarkably fast. Already at the beginning of the febrile *Phase 1* (~30 min after LPS injection), the level of transcript was increased both in the periphery and in the brain. Importantly, the *Phase-1*-associated response of the COX-2 gene was much stronger in the peripheral organs (17 fold over-expression in the liver and 5 fold over-expression in the lungs) than in the brain (3 fold over-expression) — in line with the idea of peripherally produced PGE_2 playing a role in fever (10, 11, 82, 83). In all the tissues studied, the induction of COX-2 peaked during *Phase 2* and then rapidly declined simultaneously with downregulation of COX-1 (Figure 2). Although the expression of COX-2 throughout the body (at least, in the liver, lungs and hypothalamus) was drastically decreasing from *Phase 2* to *Phase 3*, (*e.g.*, from 15 fold over-expression to below the basal level in the lungs), expression of other enzymes of the PGE_2-synthesizing cascade (*viz.*, hepatic sPLA$_2$-IIA and mPGES-1, hypothalamic cPLA$_2$-alpha, sPLA$_2$-IIA, and mPGES-1) was skyrocketing (*e.g.*, from 280 to 1,260 fold over the basal level for hepatic mPGES), and body temperature was rising.

Such a discrepancy between the expression of COX-2 and the dynamics of the PGE_2-mediated febrile response suggests that to consider the level of COX-2 mRNA as a measure of PGE_2 concentration (as done in many studies) is an oversimplification. The discrepancy observed in our study (31) agrees well with the lack of correlation between the tissue level of COX-2 protein and the concentration of PGE_2 found by Inoue *et al.* (18) and with the observation by Imai-Matsumura *et al.* (84) that some LPS-treated pregnant rats have the number of COX-2-positive brain cells well within the range observed in their non-pregnant counterparts but still exhibit a drastically blunted PGE_2 response to LPS. These data suggest that the reactions catalyzed by COX (Figure 1, step 2) are not rate-limiting within the PGE_2-synthesizing cascade. Different opinions on this issue can be found in a recent discussion (85).

3.4. Step 3: terminal synthases

The final step of the PGE_2-synthesizing cascade is a nonoxidative rearrangement of the COX product, PGH_2 into PGE_2 (23, 24; Figure 1). Although this isomerization can occur nonenzymatically, living cells produce PGE_2 *via* a catalytic reaction using reduced glutathione as an obligatory cofactor (23, 24). This reaction is attributed to several newly discovered proteins, PGES. Jakobsson *et al.* (86) identified the first mPGES, a 16-kD member of the so-called MAPEG (for membrane-associated proteins involved in eicosanoid and glutathione metabolism) superfamily; this protein is now designated as mPGES-1. A different PGES was found by Tanioka *et al.* (87) in the cytosol of various mammalian cells and is known as cPGES. More recently, Tanikawa *et al.* (88) purified and cloned one more mPGES; this protein belongs to the thioredoxin family and is termed mPGES-2. In addition, mu2 and mu3 representative of the mu class of glutathione-*S*-transferase (GST) have been identified as potential PGES (89).

In-vitro COX-PGES co-expression experiments have shown that cPGES is functionally coupled with COX-1 (which is localized preferentially outside the nucleus, at least according to some authors, see Refs. 59, 60); this couple is responsible for basal synthesis of PGE_2 and for the immediate release of PGE_2 in response to calcium-mobilizing agents (87). In contrast, mPGES-1 strongly prefers the mostly nuclear isoform COX-2 over COX-1; the COX-2-mPGES-1 couple is crucial for the delayed synthesis of PGE_2 in response to proinflammatory stimuli (26, 28). The other microsomal enzyme, mPGES-2, may contribute to both immediately and delayed prostaglandin

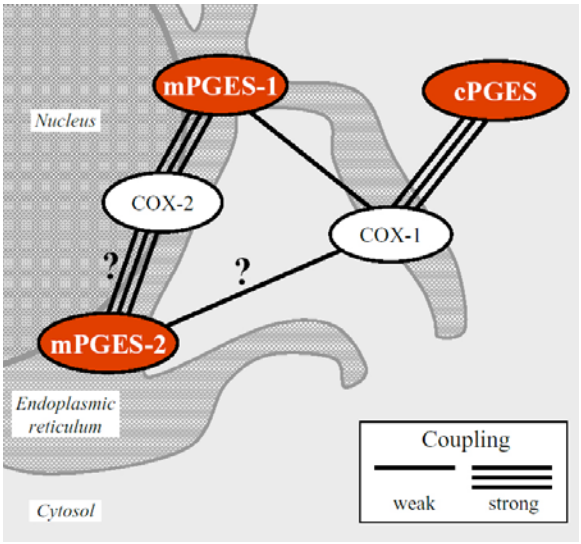

Figure 3. Schematic representation of coupling of different PGES with COX-1 and COX-2 in relationship to the intracellular localization of the enzymes involved (for discussion, see Section 3.4). The question marks refer to the less established relationships.

responses and can couple with both COX isoforms (90); which of the two COX isoforms is preferred by mPGES-2 and to what extent remains unclear. For different PGES, the relationship between their preferential localization and preferential (or even exclusive) coupling with different COX isoforms is schematically presented in Figure 3.

3.4.1. Microsomal prostaglandin E synthase-1

Because of its strong preferential coupling with COX-2, mPGES-1 is probably the most important PGES in inflammation-associated PGE_2 synthesis. In rats, high (120-400 microg/kg) doses of LPS increase mPGES-1 mRNA and protein levels in the brain and many peripheral organs, including the lungs and spleen (26, 91). Yamagata *et al.* (92) reported the induction of mPGES-1 transcript by LPS in many areas of the rat brain. The message was localized in brain vasculature, and the protein was abundant in the perinuclear envelope of endothelial cells, where mPGES-1 was co-localized with COX-2 (92). Ek *et al.* (93) also found co-induction of mPGES-1 and COX-2 by IL-1beta in brain vascular cells, presumably endotheliocytes and perivascular macrophages. Engblom *et al.* (94) demonstrated a crucial role of mPGES-1 in LPS fever by using recently developed (95, 96) knockout mice.

In our recent study of LPS-induced fever in rats, we found strong transcriptional up-regulation of the mPGES-1 gene in peripheral LPS-processing organs and in the brain (31). The dynamics of this up-regulation was tissue-specific. In the liver and lungs, the level of mPGES-1 transcript was already increased at *Phase 1*; in the hypothalamus, it was not induced until *Phase 2*. Remarkable features of the mPGES-1 response were its magnitude and duration. Indeed, the expression of this gene was up-regulated more than 1,200 fold in the liver and more than 30 fold in the lungs and hypothalamus and persisted for several hours after a single LPS injection. Even at the times when COX-2 expression had returned to its baseline, mPGES-1 remained over-expressed. The most terminal position in the PGE_2-synthesizing cascade, the highest magnitude of up-regulation among all PGE_2-synthesizing enzymes studied, and the long duration of its upregulation makes mPGES an attractive target for antipyretic/anti-inflammatory therapy (31). The abovementioned fact that mPGES is strongly up-regulated when expression of COX-2 declines adds to its attractiveness as a pharmacological target.

3.4.2. Cytosolic prostaglandin E synthase

According to Tanioka *et al.* (87), LPS, IL-1beta, and TNF-alpha have no effect on the level of cPGES mRNA in cultured human and murine gliocytes, epitheliocytes, and fibroblasts. Neither do these pyrogens affect cPGES expression in rat tissues *in vivo*, with the exception of the brain, where a several-fold increase in the level of cPGES transcript was found at a late stage of LPS shock. When using a lower dose of LPS, we did not see any changes in the expression of cPGES in the liver, lungs, or hypothalamus during LPS fever in rats (31). As shown by these data and suggested by the exclusive coupling of cPGES with COX-1 (Figure 3), this PGES is likely uninvolved in inflammatory PGE_2 synthesis.

3.4.3. Other prostaglandin E synthases

Not much is known about involvement of mPGES-2 and GST-mu in inflammation and fever. Murakami *et al.* (90) have shown that mPGES-2 expression in epithelial and fibroblastic cells is insensitive to IL-1beta and TNF-alpha and just marginally increased in mice liver during endotoxemia (90). As for the mu class of GST, one of them, *i.e.*, GST-mu subunit 6 (Yb3), is abundant in the hypothalamus (97) — the brain's febrigenic "center". Studies involving selective inhibitors and knockout animals are needed to determine involvement of different PGES in fever.

4. CATABOLISM OF PROSTAGLANDIN E_2 IN FEVER

PGE_2 is a rapidly acting, short-lived mediator. Its physiological effects (*e.g.*, fever following an intrabrain administration) typically have a latent period of a few minutes and duration of tens of minutes (for examples of PGE fever curves, see Refs. 98-100). The half-life of exogenous PGE_2 in the blood plasma is less than 60 s (101). Rapid inactivation of blood-borne PG in the lungs, kidney, and liver (102, 103) is a mechanism for changing the blood level of these short-lived molecules. It is noteworthy that PGE_2 is not catabolized in the brain of adult mammals (104). To be inactivated, brain PGE_2 has to escape the brain tissue (supposedly via the choroid plexus) and enter the blood (105). A high brain-to-blood PGE_2 gradient has been shown to facilitate the escape of PGE_2 from the brain, whereas a low gradient counteracts such an escape (106). Hence, by changing the blood concentration of PGE_2, the processes of transport and catabolism of PGE_2 in peripheral organs can affect the level of PGE_2 not only in peripheral tissues, but also in the brain. It is tempting, therefore, to speculate that catabolism of PGE_2 in peripheral tissues can be used by the body to regulate the febrile and other

physiological responses driven by both peripheral and central PGE_2. Since little or no PGE-catabolizing activity can be found in the blood or associated with the cell surface, a two-step model of metabolic clearance of PGE_2 implies its carrier-mediated uptake across the plasma membrane followed by its intracellular enzymatic oxidation (107-109).

4.1. Carrier-mediated cellular uptake

Newly synthesized molecules of PG readily leave the cell due to a passive diffusion of the protonated PG species, which prevail in a mildly acidic cytosolic environment (108). In the extracellular space (pH 7.4) they became anionic, and this charge greatly inhibits the ability of PG to cross the cell membrane. Indeed, several studies reported that the ability of PG to permeate in erythrocytes, kidney epithelial cells, and other cultured cells is poor (for review, see Refs. 108, 109). Almost three decades ago, Bito *et al.* (106, 107) provided compelling evidence for the existence of carrier-mediated PG transport across the plasma membrane. In fact, this transport is the rate-limiting step of PGE_2 inactivation, at least in the lungs (110). The two most specific transmembrane carriers of PGE_2 are PG transporter (PGT; a.k.a. matrin; Ref. 111) and multispecific organic anion transporter (MOAT; Ref. 112). Other putative PG transporters have been recently found in the organic anion transporter and organic cation transporter protein families (113).

PGT is the first discovered and the best characterized transporter. It is widely expressed in various peripheral tissues and in the brain of several mammalian species and humans (for review, see Refs. 108, 109). Interestingly, in the polarized kidney and prostate epithelial cells, PGT is localized at the apical membrane, where it facilitates PG transport in the apical-to-basolateral direction (108, 114). If PGT has similar polarized distribution in choroid plexus epithelial cells, it is perfectly positioned to facilitate efflux of PGE_2 from cerebrospinal liquid into the blood.

Little is known about regulation of transmembrane transport of PGE_2 in fever and systemic inflammation. One study showed that PGE_2 uptake was inhibited in the rabbit uvea by topical application of LPS (115), whereas another study failed to detect any effect of IL-1beta on PGT expression in human endothelial cells *in vitro* (116). Recently we found that expression of the two major transmembrane carriers of PGE_2 (*viz.*, PGT and MOAT) is down-regulated during the triphasic febrile response to a mild dose of systemic LPS in rats (117). Expression of both transporters was inhibited at the transcriptional level in the liver and lungs but not in hypothalamus, and this effect was evident only during febrile *Phase 3*. The level of PGT mRNA in the hypothalamus showed a tendency to increase during *Phases 2* and *3*. More recently, Matsumura *et al*. (118) presented *in-situ* hybridization data showing that PGT mRNA was weakly expressed in the brain of intact rats, but remarkably induced in the arachnoidal membrane and in blood vessels of the subarachnoidal space at late stages (5-48 h) of the response to systemic LPS administration. However, PGT was expressed only in a fraction of COX-2-positive endothelial cells. These data confirm that PGT is not essential for the release of PGE_2 from cells. Rather PGT is involved in the uptake of PGE_2 from the cerebrospinal fluid and in its excretion into the blood. This mechanism may limit the febrile response.

4.2. Intracellular oxidation

Intracellular catabolism of PGE_2 consists of several subsequent reactions, of which the first reaction, enzymatic oxidation of the 15-hydroxyl group, is crucial because it leads to the great reduction of biological activities (119). This reaction is catalyzed by 15-hydroxy-PG dehydrogenase (15-PGDH; formerly known as 15-PGDH type I) and carbonyl reductase (CR; formerly, 15-PGDH type II) (see Refs. 120, 121). CR also possesses the 9-keto-reductase activity and thus inactivates PGE_2 by converting it to PGF_{2alpha} (122), a much less potent inductor of fever and other inflammatory symptoms (16, 123). Both enzymes are widely distributed in peripheral tissues but are weakly expressed in mammalian brain (121, 124). This is not surprising, because the brain of adult mammals is virtually devoid of PG-oxidizing activity (104, 124). The physiological importance of 15-PGDH is supported by the fact that its genetic deletion (125) or transcriptional down-regulation *(e.g.*, as a result of thermal injury; see Ref. 126) leads to increases in the blood and/or tissue levels of PGE_2.

Surprisingly, there are only a few reports of the expressional regulation of 15-PGDH and CR by inflammatory agents. Both IL-1beta and TNF-alpha were reported to decrease the expression of 15-PGDH and inhibit PGE_2 catabolism in cultured human trophoblasts (127, 128), whereas shock-inducing doses of LPS were shown to inhibit the expression of pulmonary 15-PGDH and catabolism of PGE_1 and PGE_2 *in vivo* (129-131). Our study of 15-PGDH and CR expression during a triphasic LPS fever in rats demonstrated a rapid (starting during *Phase 1*) and dramatic (up to 26 fold) decrease in 15-PGDH mRNA level in the liver and lungs; we also found a similar, although less pronounced, down-regulation of CR expression in the liver and lungs (117). Since the half-life of the 15-PGDH protein is on the order of tens of minutes (132), its dramatic transcriptional down-regulation should rapidly decrease the level of the active enzyme, and thus contribute to the increased PGE_2 level during LPS fever.

The transcriptional downregulation of PGE_2 transporters and inactivating enzymes found in our recent study (117) provides new explanations for the experimental phenomenon observed by Davidson *et al.* (133). These authors reported that intravenous administration of LPS, IL-1beta, or TNF-alpha in rabbits readily facilitates PGE_2 entry from the peripheral circulation into the brain. The authors hypothesized that this phenomenon reflects a disruption of the blood-brain barrier (BBB) in systemic inflammation. Based on our findings (117), we suggest that an accelerated influx of circulating PGE_2 into the brain during fever may occur even if the integrity of the barrier is uncompromised. The simultaneous, drastic transcriptional down-regulation of four major PGE_2-inactivating enzymes in the liver and lungs is likely to increase the blood

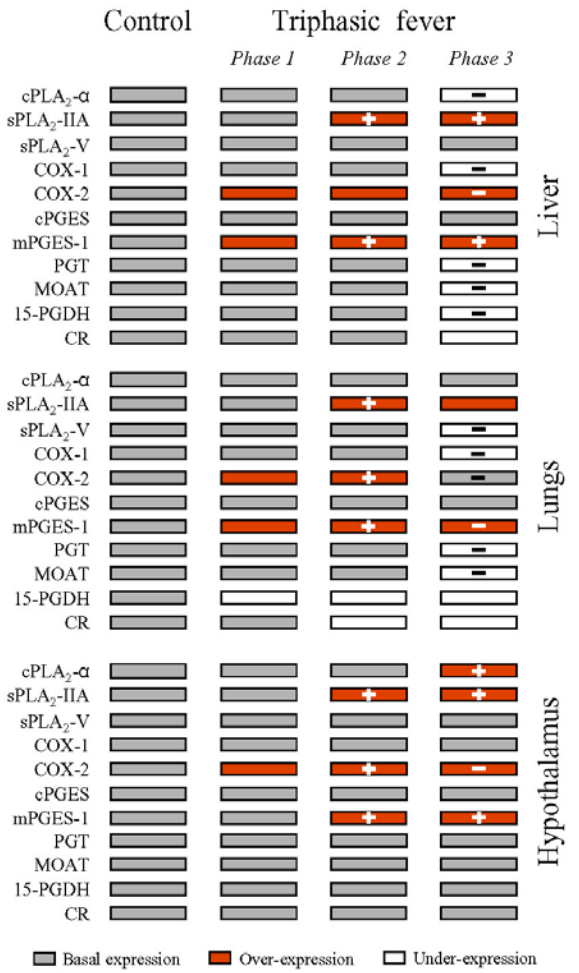

Figure 4. Schematic summary of our recent studies (31, 117). Up-regulation of a gene involved in PGE_2 metabolism in LPS-treated rats at a given febrile phase (compared to the corresponding saline-treated rats) is shown in red; down-regulation is shown in white; gray represents no statistically significant changes. +, statistically significant up-regulation of a gene at the given febrile phase compared to the preceding phase; -, statistically significant down-regulation of a gene at the given febrile phase compared to the preceding phase. Note that some genes (COX-2 in the liver and hypothalamus and mPGES in the lungs) are up-regulated at *Phase 3* as compared to their expression in saline-treated rats, but down-regulated as compared to their expression at *Phase 2*. (Modified from Ref. 31; published with permission of the American Physiological Society.)

concentration of PGE_2 and, therefore, the blood-to-brain PGE_2 gradient. Yet, the expression of PGE_2 carriers in the hypothalamus *per se* remains unaltered (or even increased, see Ref. 118) during fever. In the presence of normally expressed or over-expressed PGE_2 carriers, the increased blood-brain PGE_2 gradient is likely to facilitate transport of circulating PGE_2 into the brain.

Our observation (117) that the febrile response to mild doses of LPS is accompanied by drastic (up to 26 fold) transcriptional down-regulation of PGE_2 transporters (PGT and MOAT) and its catabolizing enzymes (15-PGDH and CR) in the lungs and liver, but not the brain, may find clinical applications. We speculate that this down-regulation increases the blood-to-brain gradient of PGE_2 (decreases the brain-to-blood gradient) and is, therefore, likely to both facilitate penetration of PGE_2 into the central nervous system and prevent its elimination from the brain. This largely unrecognized mechanism may constitute a novel target for antipyretic and anti-inflammatory therapy. What adds to the attractiveness of this target is that tissue expression of PGE_2 transporters and dehydrogenases is suppressed most dramatically at the time when COX-2 expression is declined.

5. PHYSIOLOGICAL IMPLICATIONS

5.1. A major role of transcriptional regulation in prostaglandin E_2 metabolism

Transcriptional up-regulation is a major mechanism of activation for several enzymes involved in PG metabolism as suggested by the existence of binding sites for a variety of proinflammatory transcription factors (*e.g.*, AP-2, NF-kappaB, and cAMP regulatory binding protein) in the 5′-flanking region of several enzymes involved, including $cPLA_2$-alpha, $sPLA_2$-IIA and -V (20, 21, 39), and COX-2 (22). As reviewed in this paper, a large amount of data has been accumulated showing that LPS, IL-1beta and TNF-alpha, induce massive release of PGE_2 *via* transcriptional up-regulation of PLA_2, COX-2, and/or mPGES-1 *in vitro* and *in vivo*. On the other hand, transcriptional down-regulation is likely to be a major mechanism of inactivation of PGE_2 transporters and catabolizing enzymes. Genomic sequences of human PGT (134), mouse 15-PGDH (135), and human CR (120) all contain sequences for Sp1 transcription factor binding. LPS and TNF-alpha decrease DNA-binding activity of Sp1 and inhibit Sp1-mediated gene expression (136, 137). Hence, inactivation of constitutive transcription factor Sp1 by LPS or LPS-induced cytokines may be a common mechanism for transcriptional down-regulation of PGT, 15-PGDH, and CR in fever.

5.2. Differential regulation at three phases of lipopolysaccharide fever

Studies with pharmacological inhibition of PG synthesis (10, 13, 14, 66) and experiments in PGE receptor knockout mice (5) strongly suggest that PGE_2 is involved in all three febrile phases. However, they leave open the most intriguing question about pathogenesis of fever: how can the same mediator, PGE_2, bring about three different events, three febrile phases? Our recent studies (31, 117) show that each phase (and each underlying burst of PGE_2 concentration) is characterized by a distinct pattern of the transcriptional regulation of enzymes involved in PG synthesis and catabolism (Figure 4).

5.2.1. Febrile *Phase 1*

At *Phase 1*, the most remarkable event was a strong transcriptional up-regulation of the functional couple COX-2 → mPGES-1 in the peripheral LPS-processing organs (31). This event was accompanied by an up-

regulation of COX-2 transcription in the hypothalamus (31) and by down-regulation of 15-PGDH in the lungs (117). It can be suggested that the major mechanism of *Phase 1* is the synthesis of PGE_2 in the periphery. The proposed mediation of *Phase 1* by peripherally synthesized PGE_2 deserves special attention for two reasons.

First, *Phase 1*, which is characterized by a short duration (~1 h) and small magnitude (a few tenths of a degree) and which is very sensitive to experimental conditions (4) is considered by many unimportant. Yet, it possesses several remarkable features (see Ref. 138). This phase is by definition the earliest thermoregulatory event occurring in response to LPS, which makes it an important end-point measure for studying febrigenic signaling to the brain. Further, *Phase 1* is the only febrile phase characterized by the precise type of body temperature regulation (a narrow dead band) and not "contaminated" by poikilothermia (2); this makes it the only phase that truly satisfies the current definition of fever as an increase in the set point (139). *Phase 1* also has a unique biological significance: it is the only phase coupled with the so-called early sickness symptoms (*i.e.*, those symptoms that alarm the body about the forthcoming inflammation/infection and prepare it for an active response), such as hyperalgesia/allodynia and motor agitation (7-9). Finally, *Phase 1* is the only phase of polyphasic LPS fever that was proposed by Morimoto *et al.* (10) and is currently thought (9, 83, 140) to be triggered by afferent nerve fibers, specifically hepatic (141) vagal fibers. Indeed, hepatic fibers bear PG receptors of the EP3 type (142) [*i.e.*, one of the two types mediating *Phase 1* of LPS fever (5, 143, 144)], and activation of vagal afferents by cytokines is at least partially PG-mediated (142, 145).

The second reason for special attention to the mechanisms of *Phase 1* is that involvement of peripherally synthesized PG in fever goes against the prevailing views of the exclusive role of the centrally synthesized PG (15, 17). Interestingly, the important role of peripheral PGE_2 in fever was proposed in the late seventies by Dascombe and Milton (146) and re-stated in the eighties by at least three groups (11, 147, 148). However, several attempts to induce fever by intravenous or intracarotid PGE_2 or PGE_1 failed, as did attempts to detect PGE_2 in the cerebrospinal fluid or preoptic microdialysate following PGE_2 infusion into the carotid artery (for review, see Ref. 82). As a result, the idea of blood-derived PGE_2 playing an important role in fever has been either ignored [see, *e.g.*, Breder and Saper (149)] or plainly rejected [see, *e.g.*, by Blatteis and Sehic (15) or Zhang and Rivest (17)] by many authors, including some of the early proponents (150). Our two studies [*viz.*, one showing a marked pyrogenic activity of PGE_2 administered intravenously as a complex with albumin (82) and the other demonstrating the expression of PGE_2-synthesizing enzymes in the liver and lungs — but not in the brain — during *Phase 1* of LPS fever (31)] and studies by others [showing that immune activation of vagal fibers is PG-dependent (142, 145)] have shifted the balance back in favor of the role of peripheral PGE_2 (for reviews, see 9, 83, 140). Perhaps a decisive argument in this long-standing dispute, *i.e.*, demonstration of an attenuation of the febrile response by selective elimination of peripheral PGE_2, has been recently obtained by our group (Steiner A. A, A. I. Ivanov, A. Y. Rudaya, A. Dragic & A. A. Romanovsky, in progress). We have shown that intravenous anti-PGE_2 antibodies (that do not cross the BBB) strongly attenuate *Phase 1* of LPS-induced fever in rats.

5.2.2. Febrile *Phases 2* and *3*

Coming back to the transcriptional patterns of proteins involved in PGE_2 metabolism at different febrile phases (Figure 4), our studies (31, 117) showed that *Phase 2* of LPS fever in rats was characterized by a robust transcriptional up-regulation of the entire cascade, $sPLA_2$-IIA $\rightarrow$ COX-2 $\rightarrow$ mPGES-1, both in the periphery and in the brain. Importantly, up-regulation of these PGE_2-synthesizing enzymes was accompanied by transcriptional down-regulation of the major PGE_2-catabolizing enzymes (15-PGDH and CR) in the lungs. We speculate that intrabrain synthesis of PGE_2, peripheral synthesis of PGE_2, and inhibition of PGE_2 catabolism all contribute to this phase.

The most prominent events occurring at *Phase 3* were: synchronized down-regulation of PGE_2 transmembrane carriers (PGT and MOAT) and oxidizing enzymes (15-PGDH and CR) in both peripheral organs studied and further up-regulation of $sPLA_2$-IIA and mPGES-1 expression in the liver and hypothalamus. This phase also involved trascriptional up-regulation of $cPLA_2$-alpha. We speculate that both peripheral and central bursts of PGE_2 synthesis contribute to febrigenesis at *Phase 3*. We further conjecture that blood-borne PGE_2 more readily enters the brain at this phase due to the decreased carrier-mediated uptake and catabolism of PGE_2 in peripheral organs resulting in an increased blood-to-brain gradient for this mediator.

6. INSTEAD OF CONCLUSIONS

Although antipyretic remedies have been in use for millenia (151) and their mechanism of action (inhibition of PG synthesis) has been known for more than three decades (152), the research on PGE_2 metabolism in fever is now as vigorous as ever. A variety of new genetic, pharmacological, and molecular biological tools to study PGE_2 synthesis and catabolism have been developed within the last few years. As pointed out in Section 3, several enzymes and isoenzymes of the PGE_2 synthesizing cascade were discovered only recently. Even while this review was in preparation, several new studies were reported (*e.g.*, 37, 38, 94, 118). Clearly, not all conclusions that can be made today based on the data available will be immutable. Instead of making formal conclusions, we would like to identify some important observations and outline perspectives.

First, it has become clear that functional coupling between PLA_2 and COX and between COX and PGES (Figure 3) plays a key role in distributing AA and PGH_2 between the many eicosanoid pathways and between

different enzymes or isoenzymes of the same pathway. Because different enzymes or isoenzymes competing for the same substrate have different tissue distribution, intracellular localization, and mechanisms that regulate their activity, selective distribution of AA and PGH_2 between different synthetic pathways via coupling is likely to be functionally important. Although further research is needed to illustrate this statement with specific examples, we speculate that such selective distribution may explain a variety of physiological phenomena. For example, peripheral administration of the same dose of LPS (4, 7, 153), PAF (154), and possibly some pyrogenic cytokines causes fever at thermoneutrality (for current definitions, see Ref. 155) but hypothermia at subneutral ambient temperatures. The activation of $cPLA_2$ by LPS, PAF, or cytokines results in an accumulation of AA, which is converted by COX-2, lipoxygenase, and terminal synthases into several eicosanoids. Whereas some of these eicosanoids (*e.g.*, PGE_2 and PGF_{2alpha}) are highly pyrogenic (16), others [*e.g.*, PGD_2 (156), its metabolite 15d-PGJ_2 (72), and leukotrienes C4 and D4 (157)] can cause hypothermia. We hypothesized that at different ambient temperatures pyrogens may be preferentially delivered with blood flow to different target cells, in which proximal enzymes of eicosanoid synthesis are coupled with different terminal synthases (154).

Second, the idea that the febrile response is not a single, elementary, "homogeneous" act, but rather a complex, "heterogenous" cascade of multiple processes has received strong support over the last three decades (see, *e.g.*, Refs. 2, 3, 7-9, 158). It has also become clear that different febrile phases are mediated differently (10, 159). It is of no surprise, therefore, that our recent studies (31, 117) showed that different febrile phases are accompanied by different changes in the metabolism of the major downstream mediator of fever, PGE_2 (Figure 4). An important corollary of this finding is that different enzymes involved in PGE_2 metabolism are likely to be more effective targets at different stages of an inflammatory process and for acute *vs.* chronic inflammation. For example, inhibitors of COX-2 that do not cross the BBB should be highly effective at early stages of systemic inflammation (*Phase 1* of experimental fever); the same inhibitors are likely to be ineffective during the late stages (*Phase 3* of LPS fever), when PGE_2 synthesis is activated both outside and inside the BBB, and when a high blood-to-brain gradient of PGE_2 is maintained by down-regulation of PGE_2 transporters and catabolizing enzymes.

Third, the fact that both COX-1 and COX-2 enzymes are naturally down-regulated during later stages of fever (Figure 2) should be emphasized. This fact explains the reported lack of correlation between the tissue level of COX-2 (whether protein or mRNA) and either the concentration of PGE_2 or the intensity of a PGE_2-mediated response (height of fever) (18, 31, 84). This fact also allows us to speculate that certain housekeeping functions of COX-1 may suffer during systemic inflammation even when no COX-2 inhibitors are used. Last but not least, this fact suggests that other enzymes of PGE_2 synthesis (those that are up-regulated at the time when COX-2 is down-regulated) may be better candidate targets for anti-inflammatory drugs.

Finally, our recent studies (31, 117) suggest several new antipyretic/anti-inflammatory targets. The most promising one is likely to be mPGES-1. Indeed, the over-expression of this enzyme during fever has the highest magnitude (1,260 fold), the longest duration (throughout all three febrile phases), and the widest tissue distribution (all organs studied, *viz.*, the liver, lungs, and hypothalamus). In addition, because of the distal position of mPGES-1 in the PGE_2-synthesizing cascade (Figure 1), selective pharmacological blockade of this enzyme would affect only PGE_2 (a pro-inflammatory, "bad" PG) and would not decrease the production of anti-inflammatory, "good" PG such as the pair $PGD_2 \rightarrow$ 15d-PGJ_2. Another promising target is $sPLA_2$-IIA. Although its position in the PGE_2 synthesizing cascade is proximal, the transcriptional up-regulation of this enzyme during fever has a very high magnitude (130 fold) and duration (the longest febrile *Phases 2* and *3*). Finally, the transcriptional down-regulation of proteins involved in PGE_2 inactivation is a largely unrecognized mechanism of systemic inflammation. By increasing the blood-brain gradient of PGE_2, this mechanism likely facilitates penetration of PGE_2 into the brain and prevents its elimination from the brain. The high magnitude of down-regulation of the principal PGE_2-catabolizing enzyme 15-PGES (30 fold) during fever makes this protein another attractive target for anti-inflammatory therapy.

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Abbreviations: In addition to the commonly accepted (*e.g.*, mRNA), the following abbreviations are used in this paper (all introduced at first mentioning). AA, arachidonic acid, BBB, blood-brain barrier, c, cytosolic, as in $cPLA_2$ or cPGES, CR, carbonyl reductase, COX, cyclooxygenase(s), GST, glutathione-*S*-transferase(s), IL, interleukin, LPS, lipopolysaccharide, m, microsomal, as in mPGES, MOAT, multispecific organic anion transporter, PAF, platelet-activating factor, PG, prostaglandin(s), 15d-PGJ2, 15-deoxy-delta^{12,14}-PGJ_2, 15-PGDH, 15-hydroxy-PG dehydrogenase, PGES, PGE synthase(s), PGT, PG transporter, PL, phospholipase(s), s, secretory, as in $sPLA_2$, TNF, tumor necrosis factor

Key Words: Body temperature, Thermoregulation, Fever, Febrile phases, Lipopolysaccharide, Prostaglandins, PGE_2, Phospholipases, PLA_2, Cyclooxygenases, COX-1, COX-2, Terminal synthases, PGES, Prostaglandin transporter, PGT, MOAT, Prostaglandin dehydrogenases, 15PGDH, Carbonyl reductase, CR, Expression, Transcriptional regulation, Review

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