Pyrogen Sensing and Signaling: Old Views and New Concepts

Clark M. Blatteis, Elmir Sehic,[a] and Shuxin Li *Department of Physiology, University of Tennessee, Memphis*

Fever is thought to be caused by endogenous pyrogenic cytokines, which are elaborated and released into the circulation by systemic mononuclear phagocytes that are activated by exogenous inflammatory agents and transported to the preoptic-anterior hypothalamic area (POA) of the brain, where they act. Prostaglandin (PG) E2 is thought to be an essential, proximal mediator in the POA, and induced by these cytokines. It seems unlikely, however, that these factors could directly account for early production of PGE2 following the intravenous administration of bacterial endotoxic lipopolysaccharides (LPS), because PGE2 is generated before the cytokines that induce it are detectable in the blood and the before cyclooxygenase-2, the synthase that they stimulate, is expressed. Hence other, more quickly evoked mediators are presumed to be involved in initiating the febrile response; moreover, their message may be conveyed to the brain by a neural rather than a humoral pathway. This article reviews current conceptions of pyrogen signalling from the periphery to the brain and presents new, developing hypotheses about the mechanism by which LPS initiates fever.

Our knowledge of the mechanisms by which fever-producing agents, or pyrogens, act upon the thermoregulatory regions of the brain to raise body core temperature (T_c) is still incomplete. The conventional view of fever genesis is that it develops in concatenated steps, starting with exposure to the infectious noxa (e.g., bacterial endotoxic lipopolysaccharide [LPS]) and including, in this order, formation of pyrogenic cytokines (e.g., IL-1β) in the periphery, action of these cytokines on targets accessible from blood with generation of stimulatory signals directed to the brain, and certain consequent neurohumoral changes (in particular, elevation of prostaglandin E_2 [PGE_2]) inside the brain, affecting thermoregulatory neurons and resulting in elevation of T_c (i.e., fever). But in fact, the mechanism by which peripheral pyrogens signal the brain to induce fever is still not clear. And although it seems definite that central PGE_2 is an essential mediator of fever, it is not at all certain which cells in the brain synthesize this PGE_2. But it is established that its generation requires the mediation of a slowly inducible enzyme, cyclooxygenase (COX)–2, which is elicited in various cells, including phagocytic and endothelial cells, by inflammatory (i.e., pyrogenic) stimuli. It has been suggested, therefore, that perivascular phagocytic cells, microvascular endothelial cells, or both (collectively termed barrier cells) in the brain may be the direct targets of circulating cytokines, inducing COX-2, consequently producing PGE_2, and hence causing fever. A difficulty with this notion, however, is that fever is initiated after the iv injection of a pathogenic agent such as LPS significantly more quickly than COX-2 is synthesized, and, for that matter, faster than the appearance of cytokines in the circulation.

The purpose of this article is to review the current views and to present new, developing hypotheses about the mechanism by which systemic infectious pyrogens, in particular LPS, may act to produce fever.

Pathogenesis of Fever

When infectious microorganisms invade the body through its natural barriers, an array of systemic reactions promptly develops that mitigates the deleterious effects of the invading pathogens and, ultimately, restores health. These reactions represent the primary host defense response to infection; collectively, they are called the "acute-phase reaction." Fever is the most manifest and familiar among these early responses; indeed, it is the hallmark of infection. Specifically, the term "fever" describes the regulated rise in T_c provoked by the invading organisms, their degradation products (exogenous pyrogens [ExP]; e.g., LPS), or both. Functionally, this is accomplished by increases in metabolic heat production (shivering and nonshivering thermogenesis) and decreases in heat loss (cutaneous vasoconstriction and cessation of sweating or panting, if present), and by heat-seeking behaviors. (For more detail and references on the effector mechanisms of fever, see Blatteis [1].)

It is now generally considered that an array of host-generated factors (endogenous pyrogens [EnP]), rather than the primary ExP, mediate fever as well as most other components of the acute-phase reaction (reviewed in Kluger [2]). These mediators are members of the class of immunoregulatory polypeptides termed "cytokines" that are produced by various cell types, but in the context of fever, are produced primarily by mononuclear

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[a] Present affiliation: Department of Medicine, Veterans Affairs Medical Center, Wilkes-Barre, PA 18711 (ES).

Reprints or correspondence: Dr. Clark M. Blatteis, Dept. of Physiology, University of Tennessee-Memphis, 894 Union Ave., Memphis, TN 38163 (blatteis@physio1.utmem.edu).

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phagocytes activated by the ExP. TNF-α, IL-1β, and IL-6 are considered to be the principal pyrogenic cytokines [3]. Because T_c is regulated centrally, it has been assumed that these cytokines are released into the systemic circulation and transported to the preoptic-anterior hypothalamic area (POA, the brain region where T_c is thought to be regulated; see the article by Boulant in this issue), where they act. Indeed, febrile responses are promptly evoked when EnP are microinjected into this site, and it is now generally accepted that thermosensitive neurons contained in this region modulate the thermoregulatory mechanisms that effect the development of fever [4]. An additional, more proximal mediator, PGE_2, is thought to play an essential (if not the ultimate) role in the POA [5].

Possible Peripheral Pyrogen-to-Brain Signaling Mechanisms

Cytokines. It is controversial, however, how circulating cytokines might reach the POA because as large, hydrophilic peptides, they are unlikely to cross the blood-brain barrier by simple diffusion. Although some researchers have concluded that cytokines may be actively transported across the blood-brain barrier [6], the time and quantity of this passage are too slow and minimal to account for the rapid onset of fever, in particular after they are administered iv. About 16 years ago, we proposed that IL-1β, and presumably other cytokines too, may interact with sensory elements in or near the organum vasculosum laminae terminalis (a circumventricular organ in the medial POA that lacks a blood-brain barrier), thereby evoking secondary signals that transduce the bloodborne pyrogenic messages inwardly to the POA [7]. Since then, various scenarios have been proposed about the events that may consequently be triggered, such as intra–organum vasculosum laminae terminalis or POA production of cytokines, PGE_2, or both. However, the exact nature of the signals for fever production remains uncertain. Because of their strategic location at the interface between blood and brain, cerebromicrovascular endothelial cells have lately been postulated to be the primary targets of circulating ExP, EnP, or both, releasing abluminally either additional cytokines, PGE_2, or both [8, 9]; perivascular microglia and meningeal macrophages may be similarly affected [10, 11]. The mediators that are so released are postulated then to affect by paracrine actions the activities of local thermosensitive neurons.

However, there are challenges to the various hypotheses about bloodborne cytokines communicating signals to the brain. These involve, in particular, the temporal discrepancy between the first detection of cytokines in the blood and the onset of the febrile response after the iv administration of LPS (or ip administration at higher doses) [12]. Indeed, the kinetics of the various synthetic processes involved are slower than the latency of fever onset, there being at a minimum a 30-min delay after the iv injection of LPS before the detection in blood exiting the liver (the major source of pyrogenic cytokines in response to LPS) of TNF-α [13, 14], the first of the cascade of cytokines released by such stimulation. This may be compared with a delay of only 10–15 min until the beginning of fever after the iv injection of 2 μg of LPS/kg into guinea pigs [15] or 5 μg of LPS/kg into rats [16]. Moreover, it was recently shown that TNF-α may not have a role in the initiation of LPS fever in guinea pigs, but only in its maintenance [17].

Hence, because the appearance of cytokines in the circulation lags the onset of fever after iv administration of LPS, it is difficult to envision how their levels in blood could provide the trigger for the febrile response under these conditions. But on the other hand, threshold concentrations of pyrogenic cytokines could be reached in the vicinity of their producing cells and activate appropriate local sensors, if they existed, well before these mediators were detectable in the general circulation. Indeed, the rapidity of the febrile response to iv administration of LPS would favor a neural rather than a humoral communication pathway between peripheral EnP and the POA. In support of this hypothesis, it was reported recently that local, abdominal sensory nerve desensitization by the ip administration of low doses of capsaicin inhibits iv LPS-induced fever in rats [18]. Furthermore, LPS injected into a subcutaneous air pouch (mimicking a localized subcutaneous inflammation) evokes rapid-onset fevers without associated, concomitant elevations in circulating cytokine levels [19].

Because circulating LPS is cleared primarily by hepatic macrophages (Kupffer cells [Kc]) [20], it is conceivable that sensory nerves originating in the liver, in particular, may convey the released pyrogenic messages of these macrophages to the brain. In support of this hypothesis, we found that bilateral truncal subdiaphragmatic vagotomy abolished the febrile response of guinea pigs to iv LPS [21]. We therefore suggested that sensory vagal afferent nerves presumably distributed in the vicinity of the primary source of cytokine production: that is, Kc in this case may participate importantly in the transmission of peripheral pyrogenic signals to the brain. Since then, evidence contributed mostly by others [22, 23] (see the article by Romanovsky in this issue) has indeed supported the idea that vagal afferents have a role in LPS and IL-1β fever genesis.

However, cytokines do not exist in a preformed state in macrophages but are transcribed and translated upon LPS stimulation, a process longer than the short latency of the febrile response of guinea pigs to iv LPS at any dose or to ip LPS at doses higher than 16 μg/kg [12]. Therefore it would seem unlikely that Kc-generated cytokines could provide the signals that rapidly initiate the febrile response to an iv bolus of LPS.

PGE_2. An alternative candidate peripheral pyrogenic mediator is PGE_2. It is synthesized by all macrophages, including Kc, in response to LPS; its level rises quickly in plasma after an LPS challenge; and its receptors are widely distributed on sensory neurons, including hepatic and abdominal vagal afferents [24]. It could therefore function as the direct activator of

hepatic vagal afferents. Despite this, however, LPS is actually a weak trigger of arachidonic acid (AA) release; the free AA concentration is rate-limiting in PGE_2 synthesis. Indeed, the activation by LPS of group IV cytosolic phospholipase A_2 ($cPLA_2$, the isoform of the enzyme that initiates the cascade of events leading to the production of PGE_2 from membrane phospholipids by macrophages) is significantly delayed in vitro [25, 26] compared with the prompt elevation of both preoptic and blood PGE_2 in vivo after iv administration of LPS [15, 27]. In fact, LPS and IL-1β both stimulate the increased production of $cPLA_2$ after some hours by inducing posttranscriptional modification and de novo synthesis [25, 26]. Moreover, the increased synthesis of PGE_2 by LPS-stimulated macrophages is entirely caused by the selective expression of COX-2, the transcription and translation of which require at least 1 h (reviewed in Herschman et al. [28], Vane et al. [29], and O'Banion [30]). It would therefore seem improbable that the rapid appearance of PGE_2 in blood and brain, and hence the development of fever after iv administration of LPS, could be accounted for in this manner.

Complement System

It is possible that the intermediary of this process is some other factor that is very quickly elaborated in reaction to the presence of LPS and capable of inducing the rapid release of cytokines or PGE_2 by Kc. Such a factor might be one of the anaphylatoxins. Indeed, the intravascular complement (C) cascade is activated in almost immediate reaction to the presence of LPS via both the classical (the lipid A moiety of LPS) and alternative (the core oligosaccharide) pathways [31], and Kc express the receptors for various C-derived components [32]. C3a induces the production and release of IL-1β by human monocytes in vitro, and C5a enhances the release of both IL-1β and TNF-α by LPS-stimulated monocytes and macrophages [33]. Moreover, Kc very quickly release PGE_2 in response to C3a and to the membrane attack complex (C5b-9) [34], whereas C depletion limits this release [35]. The C system has long been known to be activated by LPS, but apparently it has never before been considered in the context of LPS fever induction. We therefore considered whether the rapid onset of iv LPS-induced fever may be mediated by intravascular C activation by LPS and subsequent Kc stimulation by C fragments, as well as by LPS itself.

To test this hypothesis, we depleted guinea pigs of C by administering cobra venom factor [36] or temporarily eliminated the Kc by pretreating the animals with gadolinium chloride ($GdCl_3$) [37] and subsequently monitored the animals' febrile responses to LPS. The dynamics of T_c increases are different after iv-administered and after ip-administered LPS [12], and analysis of other data suggests that the fever caused by ip-administered LPS may be more vulnerable to the antipyretic effect of vagotomy than that caused by iv LPS [38]. Therefore, we investigated whether the C system may be involved in the febrile responses both to iv and ip LPS. We found [12], unexpectedly, that the magnitude and course of the febrile responses to iv LPS were not demonstrably affected by C reduction, whereas the fevers caused by ip LPS were attenuated in direct correlation ($r = 0.614$) with the amount of C reduction (figure 1).

The reason for this differential susceptibility to depression by iv and ip administered LPS was not evident. We speculate that it may be due to different functional and biochemical properties of peritoneal and hepatic macrophages [39]. Moreover, there is evidence that the activation of macrophages by LPS for synthetic responses may proceed by several pathways. Thus, whereas membrane CD14 and its associated Toll-like receptors 2 and 4 are the predominant LPS coreceptors on macrophages, their activation requires that LPS complexes to LPS-binding protein, which is present in normal plasma but normally scarce in plasma-free peritoneal and other fluids [40]. Indeed, it has been shown that only minimal amounts of LPS-induced cytokines and PGE_2 are released by macrophages in the absence of LPS-binding protein. If, then, LPS-CD14 interactions are not favored in the peritoneal fluid because of the lack of LPS-binding protein, and yet fever develops after administration of ip LPS, it may be surmised that a different signaling mechanism may activate peritoneal macrophages—one that, according to analysis of the present data, may be critically dependent on C. In support, we found that LPS delivered ip at 8 μg/kg caused per se a 40% reduction in serum C activity within 30 min after its administration—that is, in conjunction with the initial increase of T_c that indicates C is activated and consumed soon after a pyrogenic LPS challenge and thus may indeed contribute to the development of the febrile response [12]. In further support, we recently found that congenitally C5-deficient mice fail to develop fever in response to ip-injected LPS, which implies that this C fragment is a factor in the febrile response to LPS [41]. Wild-type controls pretreated with cobra venom factor also did not exhibit fever after ip administration of LPS.

To verify the involvement of Kc in the process, we temporarily eliminated these cells by pretreating guinea pigs with $GdCl_3$ and injected fluorescein isothiocyanate–labeled LPS iv or ip into the conscious animals [42]. By use of confocal laser scanning microscopy, we examined their livers for accumulation of the label 15 and 60 min later. Granular fluorescent patches were detectable within presumptive Kc in the liver sinusoids 15 min and in hepatocytes 60 min after its iv administration; only normal autofluorescence was apparent in control animals that received fluorescein salt or LPS alone. Similarly, iv LPS induced an ~1°C fall in the T_c of the $GdCl_3$-pretreated guinea pigs, in contrast to the characteristically biphasic ~1.4°C fevers evoked in the control animals (figure 2). Analysis of these data therefore supported our proposition that LPS injected iv may induce fever within 15 min via a Kc-dependent process. However, no fluorescence was detected at either 15 or 60 min after ip-injected

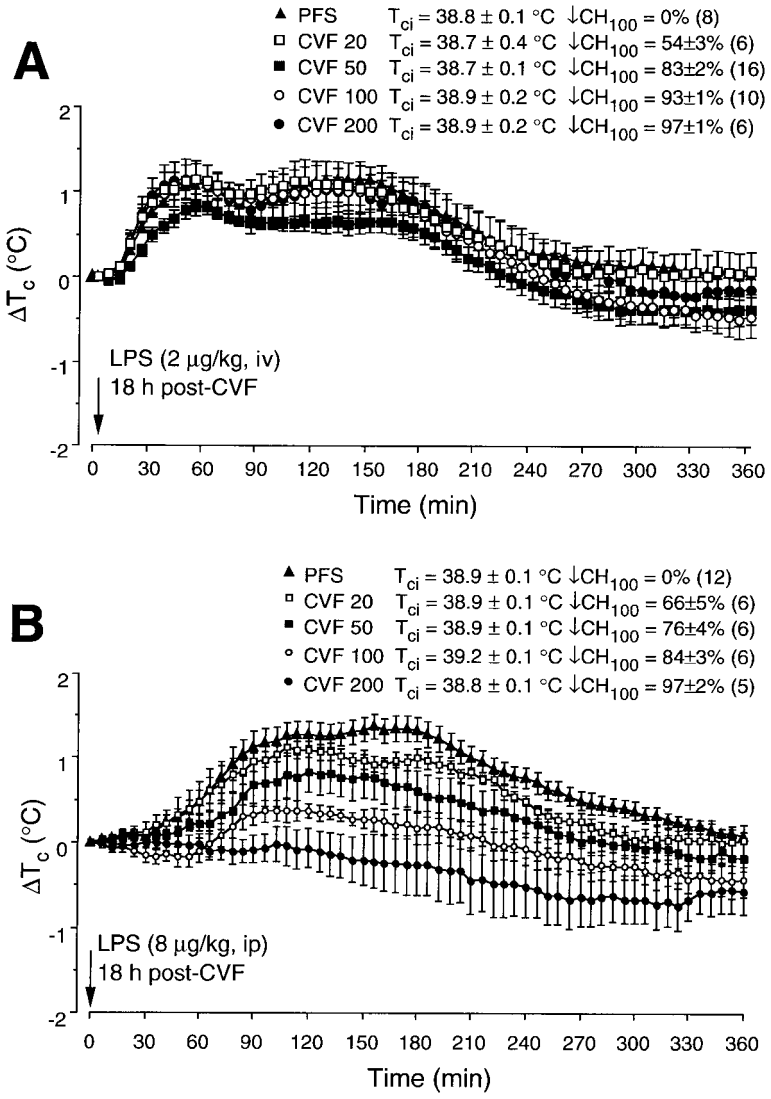

Figure 1. Effects of *(A)* iv administration and *(B)* ip administration of lipopolysaccharide (LPS) on the core temperature (T_c) courses of conscious guinea pigs not pretreated (PFS) or pretreated with cobra venom factor (CVF; 20–200 U per animal iv 18 h before). A 90-min stabilization period preceded the collection of these data. The data are expressed as differences (ΔT_c) relative to their initial levels (T_{ci}, average of the T_c over the last 10 min before the injection of PFS or LPS). The ambient temperature was 24 ± 1°C. The values are means ± SE; ↓, time of injection. *Horizontal lines,* time in min; numbers in parentheses indicate the number of animals. Based on data from [12].

fluorescein isothiocyanate–LPS or fluorescein salt, although prototypic, slow-onset, monophasic fevers developed ~32 min after ip LPS in both the $GdCl_3$-untreated and $GdCl_3$-pretreated animals. It would therefore appear that LPS in pyrogenic doses given ip may induce fever without binding to Kc. We presume—but have not yet verified—that peritoneal and lymph node macrophage uptake of ip LPS forestalled its systemic spread and thereby accounted for these results.

In contrast, we found that the fevers produced by iv or ip administration of muramyl dipeptide (a synthetic gram-positive bacterial cell-wall analog) and polyriboinosinic:polyribocytidylic acid (a synthetic viral double-stranded RNA analog)—that is, factors that reportedly induce fever through a cytokine- and PGE_2-mediated process similar to that of LPS—are not C dependent [43]. The fever caused by iv administration of muramyl dipeptide was also not affected by subdiaphragmatic vagotomy [38] or $GdCl_3$ pretreatment [43], indicating that its genesis may proceed by a different mechanism than that induced by LPS. Indeed, muramyl dipeptide may penetrate the brain directly and stimulate local production of IL-1β, PGE_2, or both [44]. Thus, the mediating role of C appears to be specifically limited to the febrile response to ip administered LPS.

In short, the results to date are consistent with the hypothesis that pyrogenic doses of LPS delivered by the ip route rapidly

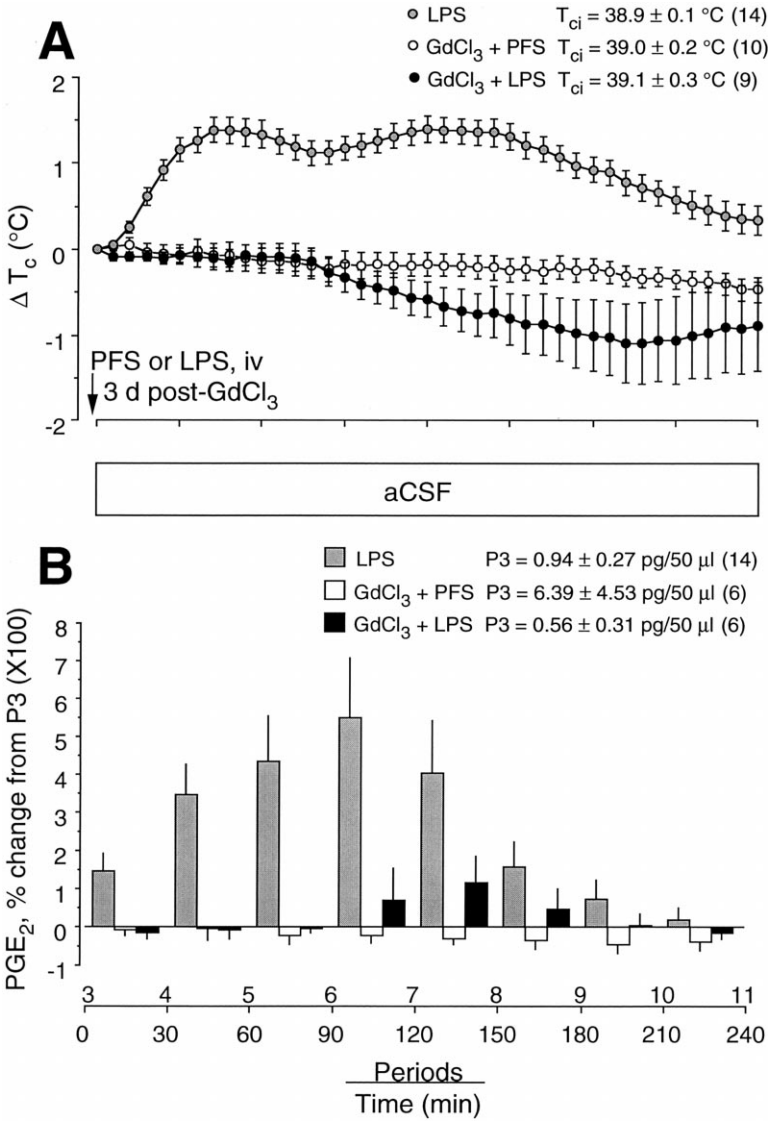

Figure 2. Effects of iv administration of PFS or lipopolysaccharide (LPS) on the T_c courses *(top panel)* and interstitial fluid levels of PGE_2 in the preoptic-anterior hypothalamic area *(bottom panel)* of conscious guinea pigs not pretreated (PFS) or pretreated with gadolinium chloride ($GdCl_3$; 7.5 mg/kg iv 3 d before). The values are means ± SE. *Horizontal bar,* Duration of intrapreoptic microdialysis; ↓, time of injection; aCSF, artificial cerebrospinal fluid, the intracerebral microdialysis perfusate; P3, the last of 3 consecutive 30-min PGE_2 collections during the stabilization period; T_{ci}, average of the T_c over the last 10 min before the injection of PFS or LPS. Numbers in parentheses indicate the number of animals. Based on data from [42].

activate the C cascade and that at least one of the components generated, C5, contributes critically to the induction of the febrile response. We speculate that it causes the quick release by macrophages of a mediator or mediators capable of stimulating local neural sensory terminals that convey the pyrogenic message to the POA. The exact nature of the mediator or mediators thus released, however, remains to be elucidated. Because of the time constraints, it is probably not IL-1β at the beginning, even though its receptors exist within abdominal vagal paraganglia [45]. Alternatively, it has been suggested that it could be PGE_2. Indeed, under these conditions, PGE_2 could be generated by the hydrolysis of membrane-associated phosphoinositide (PI, which has a high arachidonoyl chain content) by PI-specific phospholipase C (PI-PLC). AA production by PI-PLC is 10 times more rapid (within seconds) than AA production mediated by $cPLA_2$. PI-PLC is activated by C, but not by LPS or IL-1β. Moreover, the subsequent conversion of this AA to PGE_2 is catalyzed in resident peritoneal macrophages by constitutive COX-1 (unpublished data). It could thus bind to its receptors (EP_3 R) [46] in the immediate vicinity of its

source of production. Indeed, EP_3 R are expressed on sensory afferent nerves, where they mediate activation, and EP_3 R mRNA was also recently found to be induced in the nodose ganglion by systemic LPS and IL-1β [24]. Hence, PGE_2 released by peritoneal macrophages stimulated by LPS-activated C fragments could be the initial peripheral fever trigger. A problem with this hypothesis, however, is that in this case, COX-1 knockouts should be unable to develop fever in response to LPS—yet they do. Hence, it is alternatively possible that other (i.e., nonphagocytic) cell types responsive to C5 may be induced to secrete as-yet-unknown mediators.

PGE_2 and Fever

Much evidence has accumulated indicating that PGE_2 is the proximal mediator of fever (reviewed by Blatteis et al. [5] and Coceani [47]). Thus, it is a potent hyperthermic agent, thought to act on thermoregulatory neurons in the POA; its level increases and decreases in this brain region in conjunction with the febrile course; and COX inhibitors (e.g., indomethacin) inhibit pyrogen fever in parallel with the reversal of PGE_2 synthesis. The level of PGE_2 in the blood increases promptly after the entry of microorganisms or after systemic administration of ExP or EnP [27], but the general view is that the PGE_2 detected in brain is not derived from the blood but is produced directly in the brain [48, 49] (although some contradictory data exist [50–52]). As reviewed above, there are many explanations of how bloodborne pyrogens may signal the brain and promote PGE_2 synthesis, but they are all still tentative. Therefore, the cell source and nature of the triggering mechanism that induces PGE_2 in the brain in response to systemic pyrogens, as well as its precise mode of action, remain unclear. Although the consensus implicates the barrier cells (i.e., cerebral microvascular endothelium, perivascular microglia, and meningeal macrophages) as direct targets of circulating LPS or IL-1β, the data must be interpreted with caution because of the temporal discrepancy between the latency of onset of the febrile response to iv LPS and the appearance of any cytokine in the circulation.

On the basis of our own work [21] and that of others (reviewed in Blatteis et al. [53]), we have postulated that the pyrogenic message of peripheral LPS is conveyed very rapidly via vagal afferents to the nucleus tractus solitarius, where it passes to the A1/A2 noradrenergic cell groups, which transmit it to the anteroventral third ventricle (AV3V)/POA region via the ventral noradrenergic bundle. In support of this hypothesis, we have provided evidence that the norepinephrine released in this site [54] stimulates the local release of PGE_2 [55, 56], thus presumably triggering the febrile response.

PGE_2 is formed by the cleavage of membrane phospholipids by $cPLA_2$, yielding AA. The released AA, in turn, is converted into the prostaglandin endoperoxides PGG_2 and PGH_2 by a 2-step reaction: cyclization and oxygenation by COX forms PGG_2, and hydroperoxidation by hydroperoxidase yields PGH_2. These 2 enzymes coexist in a single protein, prostaglandin H synthase. PGH_2 is then quickly isomerized to PGE_2 by PGE_2 isomerase. It is now well recognized that there are 2 isoforms of COX that differ in their tissue distribution and that are activated by distinct mechanisms. COX exists as a constitutive COX-1, localized to the endoplasmic reticulum, and an inducible COX-2, found more extensively in the nuclear envelope [57, 58]; they are encoded by separate genes [59], but the enzymes share 60%–70% homology [60, 61]. COX-1 is constitutively expressed in most cells; it is not affected by inflammatory mediators, and its basal activity is not altered by anti-inflammatory glucocorticoids.

COX-2, on the other hand, is up-regulated by proinflammatory mediators in, among other cell types, stimulated, but not unstimulated, macrophages and endothelial cells; it is selectively down-regulated by anti-inflammatory glucocorticoids (reviewed by Vane et al. [29] and Herschman [62]). COX-2, however, is also expressed constitutively in the brain [10, 11, 63–65]. It is located primarily in neuronal cell bodies and in dendritic spines (i.e., at sites where intercellular communication occurs) [11, 66, 67]; but the data differ on whether it is also up-regulated by pyrogenic stimuli [10, 68–71].

Therefore it may be anticipated that COX-2 should have a major role in the brain in fever production. Indeed, it is now well documented that ExP (e.g., LPS) and EnP (e.g., IL-1β) activate COX-2 in vivo. For example, in recent studies, COX-2–like immunoreactivity [72] and COX-2 mRNA [10, 11, 73, 74] were found to be expressed in rat cerebral endothelial cells of capillaries and venules ~1.5 h after ip administration of LPS and in perivascular microglia and meningeal macrophages ~2.5 h after iv administration of LPS and IL-1β. In contrast, COX-1 expression was not affected anywhere in the brain by the peripheral administration of pyrogens. Moreover, treatment with specific inhibitors of COX-2 (NS-398, dimethyl furanone, celecoxib, and nimesulide, etc.) administered orally after iv administration of LPS [75], or administered ip before administration of ip LPS [73, 76, 77] or IL-1β [78], suppressed the febrile response but did not affect basal T_c. These antipyretic effects were not different from those produced by conventional nonsteroidal anti-inflammatory drugs, which inhibit both COX-1 and COX-2. Finally, most recently, we found that COX-2 gene–deleted heterozygous ($COX\text{-}2^{+/-}$) and homozygous ($COX\text{-}2^{-/-}$) mice were unable to develop a febrile response to ip and intracerebroventricularly (icv) administered LPS or IL-1β, whereas their COX-1–deficient analogs produced fevers not different from those in their wild-type counterparts (figure 3) [69, 78]. Guinea pigs pretreated with nimesulide similarly failed to increase their T_c in response to LPS administered iv and icv [79]. It is noteworthy in this context that compared with their wild-type counterparts, $COX\text{-}1^{-/-}$ and $COX\text{-}2^{-/-}$ cells overexpress the alternate functional COX isozyme, as well as both basal and IL-1β–induced $cPLA_2$, and consequently exhibit elevated PGE_2 biosynthesis [80]. Taken together, therefore, this infor-

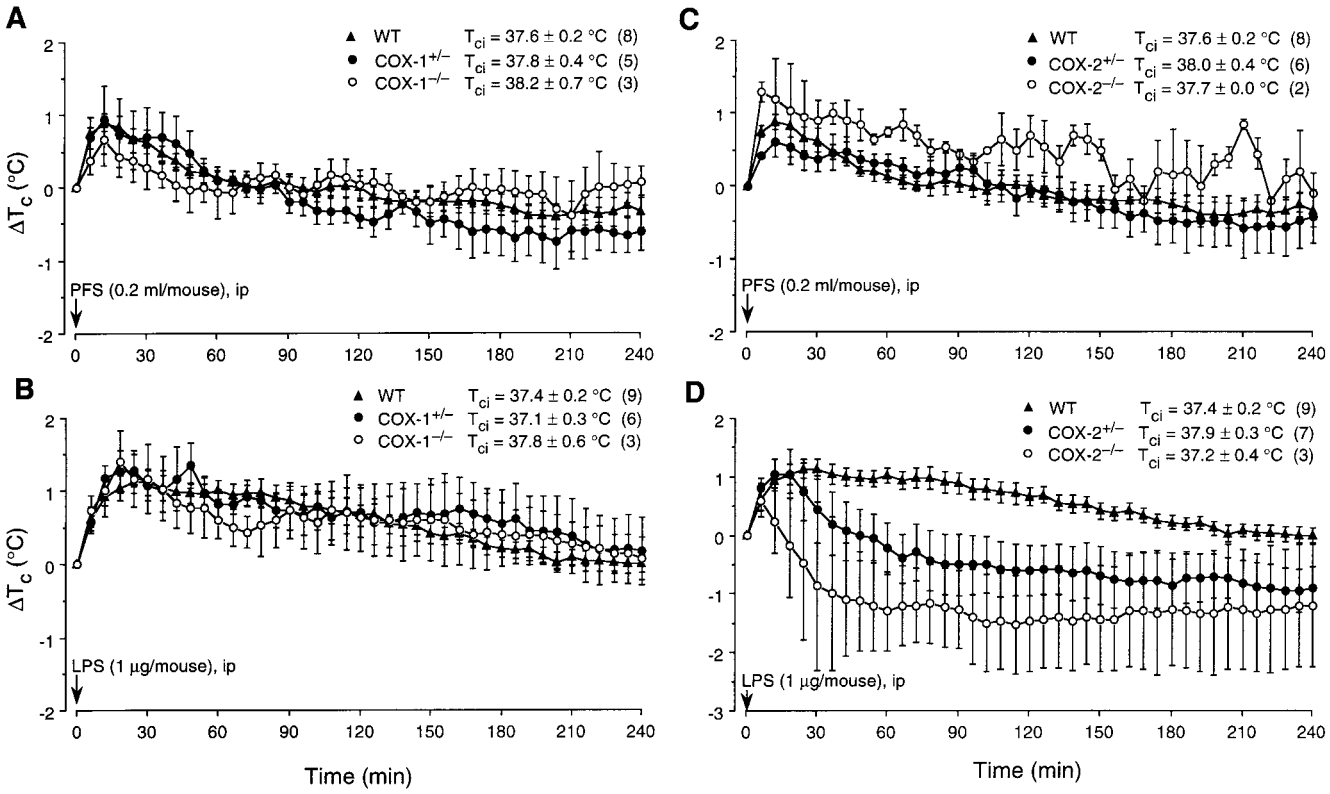

Figure 3. Body core temperature (T_c) of conscious wild-type (WT) and cyclooxygenase (COX)–1 and COX-2 gene-mutated mice injected ip with PFS *(A* and *C)* or *Escherichia coli* lipopolysaccharide (LPS) *(B* and *D)*. A 3-h stabilization period preceded the collection of these data. The values are means ± SE; ↓, time of injection; COX-2$^{+/-}$, COX-2 gene–deleted heterozygous mice; COX-2$^{-/-}$, COX-2 gene–deleted homozygous mice. Numbers in parentheses indicate the number of animals. Based on data from [69].

mation would seem to provide compelling support for the critical importance of COX-2 in fever genesis.

The temporal incongruence between COX-2 induction and fever onset would imply, however, that the prompt, pyrogen-induced elevation of POA PGE_2 cannot be accounted for by the inducible form of this enzyme, but rather by its constitutive form, which occurs only in brain neurons. Hence, the responses of the COX-2 gene knockout mice would suggest that the enzyme implicated in LPS-induced fever may be, first, "constitutive" neuronal COX-2, then "inducible" COX-2 in, presumably, the barrier cells (i.e., phagocytic and endothelial cells), although possibly also in neurons [64, 68, 81]. According to this interpretation, PGE_2 production after the addition of ExP or EnP could occur in the AV3V/POA region at 2 distinct times: an initial release within 30 min and a second, more protracted one lasting 2–4 h, the first increase in PGE_2 levels being COX-2 synthesis independent and the second mediated by de novo synthesis of COX-2.

It is of interest in this regard that lipid body–like structures have very recently been found in cultured, quiescent neurons [82]. These organelles, which contain COX-2, are rapidly inducible in leukocytes at sites of inflammation [83]; as a ready source of AA, they are postulated to be a means of quickly up-regulating PGE_2 production [84, 85]. They are not seen in quiescent, nonneural cells or in COX-1–labeled cells [85]. The initiating afferent signal could be provided by norepinephrine or simply by synaptic excitation [66, 67, 86]. Hence, we hypothesize that the regulation of AV3V/POA PGE_2 synthesis for LPS-induced fever production may be determined by 2 successive mechanisms: an early one associated with the first febrile rise, involving norepinephrine-induced activation of the preexisting COX-2 isozyme in neurons, and a later one associated with the second febrile rise, requiring de novo COX-2 synthesis by macrophages, endothelial cells, or both. Importantly, it should be noted that the secondary increased production of PGE_2 is not associated with elevated preoptic norepinephrine levels; these decrease to control levels correlatively with the decline of the first febrile rise [54].

Summary

Peripheral LPS appears to give rise to fever by a sequence of actions that begin in the periphery and may involve C consumption and as yet indeterminate mediator or mediators rap-

idly released by macrophages, other C-responsive cells, or both. These may signal the brain initially via vagal afferent inputs that eventually reach the AV3V/POA via noradrenergic pathways. Here, the released norepinephrine may rapidly activate constitutive (neuronal) COX-2, thereby prompting the quick generation and release of PGE_2 and consequently the first of the 2 febrile increases in T_c prototypically produced by iv administered LPS. The second febrile increase may be due to PGE_2 synthesized via the catalysis of inducible (barrier cells, most likely endothelial cells) COX-2, activated by IL-1β that is meanwhile produced in the brain.

Analysis of the available data also indicates that the signals that initiate fever may differ depending on the nature of the ExP (LPS, muramyl dipeptide, polyriboinosinic:polyribocytidylic acid, and by extension the original microorganisms from which these compounds are derived), its dose and route of administration (iv, ip, etc.), the time lapsed since its administration, and the species (and even the strain) under study. Many other data have also shown that the time of day, the ambient temperature, the age and sex of the challenged host, its nutritional and hydrational status, and many other factors influence febrile responsiveness. Indeed, the cytokine that may be the ultimate, predominant EnP that sustains the late-phase febrile response may be different and dependent or not on a PGE_2-mediated mechanism. Thus the processes that underlie fever initiation and maintenance are multiple, different, and complex. Consequently, generalization from particular findings should be made only with great caution. Much more study is needed to resolve the many questions that remain.

References

1. Blatteis CM. The pyrogenic action of cytokines. In: Rothwell NJ, Dantzer RD, eds. Interleukin-1 and the brain. Oxford: Pergamon, **1992**:93–114.
2. Kluger MJ. Fever: role of pyrogens and cryogens. Physiol Rev **1991**; 71: 93–127.
3. Dinarello CA. Cytokines as endogenous pyrogens. In: Mackowiak PA, ed. Fever: basic mechanisms and management, 2d ed. Philadelphia: Lippincott-Raven, **1997**:87–116.
4. Boulant JA. Hypothalamic neurons regulating body temperature. In: Fregly MJ, Blatteis CM, eds. APS handbook of physiology, section 4: environmental physiology. New York: Oxford University Press, **1996**:105–26.
5. Blatteis CM. Prostaglandin E_2: a putative fever mediator. In: Mackowiak PA, ed. Fever: basic mechanisms and management, 2d ed. Philadelphia: Lippincott-Raven, **1997**:117–45.
6. Banks WA, Kastin AJ, Broadwell RD. Passage of cytokines across the blood-brain barrier. Neuroimmunomodulation **1995**; 2:241–8.
7. Blatteis CM. Role of the OVLT in the febrile response to circulating pyrogens. Prog Brain Res **1992**; 91:409–12.
8. Tilders FJH, DeRijk RH, van Dam A-M, Vincent VAM, Schotanus K, Persoons JHA. Activation of the hypothalamus-pituitary-adrenal axis by bacterial endotoxins: routes and intermediate signals. Psychoneuroendocrinology **1994**; 19:209–32.
9. Matsumura K, Cao C, Nakadate K, Morii H, Watanabe Y. Role of brain endothelial cyclooxygenase-2 in the transmission of immune signals to the central nervous system. In: Oomura Y, Hori T, eds. Brain biodefense. Karger: Basel, **1998**:99–109.
10. Elmquist JK, Breder CD, Sherin JE, et al. Intravenous lipopolysaccharide induces cyclooxygenase-2–like immunoreactivity in rat brain perivascular microglia and meningeal macrophages. J Comp Neurol **1997**; 381:119–28.
11. Quan N, Whiteside M, Herkenham M. Cyclooxygenase-2 mRNA expression in rat brain after peripheral injection of lipopolysaccharide. Brain Res **1998**; 802:189–97.
12. Li S, Sehic E, Wang Y, Ungar AL, Blatteis CM. Relation between complement and the febrile response of guinea pigs to systemic endotoxin. Am J Physiol **1999**; 277:R1635–45.
13. Givalois L, Dornand J, Mekaouche M, et al. Temporal cascade of plasma level surges in ACTH, corticosterone, and cytokines in endotoxin-challenged rats. Am J Physiol **1994**; 266:R164–70.
14. Jansky LS, Vybiral S, Pospisilova D, Roth J. Production of systemic and hypothalamic cytokines during the early phase of endotoxin fever. Neuroendocrinology **1995**; 62:55–61.
15. Sehic E, Székely M, Ungar AL, Oladehin A, Blatteis CM. Hypothalamic prostaglandin E_2 during lipopolysaccharide-induced fever in guinea pigs. Brain Res Bull **1996**; 39:391–9.
16. Elmquist JK, Scammell TE, Jacobson CD, Saper CB. Distribution of Fos-like immunoreactivity in the rat brain following intravenous lipopolysaccharide administration. J Comp Neurol **1996**; 371:85–103.
17. Roth J, Markin D, Störr B, Zeisberger E. Neutralization of pyrogen-induced tumor necrosis factor by its type 1 soluble receptor in guinea pigs: effects on fever and interleukin-6 release. J Physiol **1998**; 509:267–75.
18. Székely M, Balasko M, Romanovsky AA. Peripheral neural inputs: their role in fever development. Ann N Y Acad Sci **1997**; 813:427–34.
19. Miller AJ, Luheshi GN, Rothwell NJ, Hopkins SJ. Local cytokine induction by LPS in the rat air pouch and its relationship to the febrile response. Am J Physiol **1997**; 272:R857–61.
20. Ruiter DJ, van der Meulen J, Brouwer A, et al. Uptake by liver cells of endotoxin following its intravenous injection. Lab Invest **1981**; 45:38–45.
21. Sehic E, Blatteis CM. Blockade of lipopolysaccharide-induced fever by subdiaphragmatic vagotomy in guinea pigs. Brain Res **1996**; 726:160–6.
22. Maier SF, Goehler LE, Fleshner M, Watkins LR. The role of the vagus nerve in cytokine-to-brain communication. Ann N Y Acad Sci **1998**; 840:289–300.
23. Watkins LR, Maier SF. Vagal afferent nerve mediates immune signals to the brain. In: Oomura Y, Hori T, eds. Brain biodefence. Karger: Basel, **1998**: 143–51.
24. Ek M, Kurosawa M, Lundeberg T, Ericsson A. Activation of vagal afferents after intravenous injection of interleukin-1β: role of endogenous prostaglandins. J Neurosci **1998**; 18:9471–9.
25. Dennis EA, Rhee DG, Billah MM, Hannun YF. Role of phospholipases in generating lipid second messengers in signal transduction. FASEB J **1991**; 5:2068–77.
26. Angel J, Berenbaum F, LeDenmat C, Nevalainen T, Masliah J, Fournier C. Interleukin-1–induced prostaglandin E_2 biosynthesis in human synovial cells involves the activation of cytosolic phospholipase A_2 and cyclooxygenase-2. Eur J Biochem **1994**; 226:125–31.
27. Rotondo D, Abul HT, Milton AS, Davidson J. Pyrogenic immunomodulators increase the level of prostaglandin E_2 in the blood simultaneously with the onset of fever. Eur J Pharmacol **1988**; 154:145–52.
28. Herschman HR, Reddy ST, Xie W. Function and regulation of prostaglandin synthase-2. Adv ExP Med Biol **1997**; 407:61–6.
29. Vane JR, Bakhle YS, Boting RM. Cyclooxygenases 1 and 2. Annu Rev Pharmacol Toxicol **1998**; 38:97–120.
30. O'Banion MK. Cyclooxygenase 2: molecular biology, pharmacology, and neurobiology. Crit Rev Neurobiol **1999**; 13:45–82.
31. Vukajlovich SW. Interactions of LPS with serum complement. In: Ryan JL, Morrison JM, eds. Bacterial endotoxic lipopolysaccharides, Vol 2. Boca Raton, FL: CRC Press, **1992**; 213–35.
32. Hinglais N, Kazatchkine MD, Mandet C, Appay M-D, Bariety J. Human liver Kupffer cells express CR1, CR3, and CR4 complement receptor antigens. An immunohistochemical study. Lab Invest **1989**; 61:509–14.
33. Cavaillon J-M, Haeffner-Cavaillon N. Signals involved in interleukin-1 syn-

thesis and release by lipopolysaccharide-stimulated monocytes/macrophages. Cytokine **1990**; 2:313–329.

34. Püschel GP, Hespeling U, Opperman M, Dieter P. Increase in prostanoid formation in rat liver macrophages (Kupffer cells) by human anaphylatoxin C3a. Hepatology **1993**; 18:1516–21.
35. Fink MP, Rotschild HR, Deniz YF, Cohn SM. Complement depletion by *Naja naja* cobra venom factor limits prostaglandin release and improves visceral perfusion in porcine endotoxin shock. J Trauma **1989**; 29:1076–85.
36. Cochrane CG, Müller-Eberhard HJ, Aikin BS. Depletion of plasma complement in vivo by a protein from cobra venom: its effect on various immunological reactions. J Immunol **1970**; 105:55–69.
37. Koudstaal J, Dijkhuis FWJ, Hardonk JM. Selective depletion of Kupffer cells after intravenous injection of gadolinium chloride. In: Wisse E, Knock DL, McCuskey RS, eds. Cells of the hepatic sinusoid, Vol 3. Leiden: Kupffer Cell Foundation, **1991**:87–91.
38. Goldbach JM, Roth J, Zeisberger E. Fever suppression by subdiaphragmatic vagotomy in guinea pigs depends on the route of pyrogen administration. Am J Physiol **1997**; 272:R675–81.
39. Fox ES, Thomas P, Broitman SA. Comparative studies of endotoxin uptake by isolated rat Kupffer and peritoneal cells. Infect Immun **1987**; 55:2962–6.
40. Fenton MJ, Golenbock DT. LPS-binding proteins and receptors. J Leukoc Biol **1998**; 64:25–32.
41. Li S, Holers VM, Boackle SA, Blatteis CM. Complement fragment 5 (C5) modulates the febrile response of mice to intraperitoneally (ip) administered endotoxin. FASEB J **2000**; 14:A6.
42. Sehic E, Li S, Ungar AL, Blatteis CM. Complement reduction impairs the febrile response of guinea pigs to endotoxin. Am J Physiol **1998**; 274: R1594–603.
43. Li S, Sehic E, Ungar AL, Blatteis CM. Complement does not mediate the febrile response of guinea pigs to muramyl dipeptide and polyriboinosinic-polyribocytidylic acid. J Therm Biol **2000**; 25:51–8.
44. Krueger JM, Johannsen L, Toth LA, Opp M, Kapas L. CNS mechanisms for sleep responses in microbial challenge. In: Frederickson RCA, McGaugh JL, Felten DL, eds. Peripheral signaling of the brain: neuroimmune and cognitive functions. Toronto: Hogrefe and Huber, **1991**: 179–92.
45. Goehler LE, Gaykema RPA, Nguyen KT, et al. Interleukin-1β in immune cells of the abdominal vagus nerve: a link between the immune and nervous systems? J Neurosci **1999**; 19:2799–806.
46. Ushikubi F, Segi E, Sugimoto Y, et al. Impaired febrile response in mice lacking the prostaglandin E receptor subtype EP_3. Nature **1998**; 395:281–4.
47. Coceani F. Prostaglandins and fever: facts and controversies. In: Mackowiak PA, ed. Fever: basic mechanisms and management. Philadelphia: Raven, **1991**:59–70.
48. Coceani F, Lees J, Bishai I. Further evidence implicating prostaglandin E_2 in the genesis of pyrogen fever. Am J Physiol **1988**; 254:R463–9.
49. Morimoto A, Morimoto K, Watanabe T, Sakata Y, Murakami N. Does an increase in prostaglandin E_2 in the blood circulation contribute to a febrile response in rabbits? Brain Res Bull **1992**; 29:189–92.
50. Abul HT, Davidson J, Milton AS, Rotondo D. Prostaglandin E_2 enters the brain following stimulation of the acute phase immune response. Ann N Y Acad Sci **1997**; 813:287–95.
51. Eguchi N, Hayashi H, Urade Y, Ito S, Hayaishi O. Central action of prostaglandin E_2 and its methylester in the induction of hyperthermia after their systemic administration in urethane-anesthetized rats. J Pharmacol ExP Ther **1988**; 247:671–9.
52. Romanovsky AA, Ivanov AI, Karman EK. Blood-borne, albumin-bound prostaglandin E_2 may be involved in fever. Am J Physiol **1999**; 276:R1840–4.
53. Blatteis CM, Sehic E, Li S. Afferent pathways of pyrogen signaling. Ann N Y Acad Sci **1998**; 856:95–107.
54. Linthorst ACE, Flachskamm C, Holsboer F, Reul JMHM. Intraperitoneal administration of bacterial endotoxin enhances noradrenergic neurotransmission in the rat preoptic area: relationship with body temperature and hypothalamic-pituitary-adrenocortical axis activity. Eur J Neurosci **1995**; 7:2418–30.
55. Shido O, Romanovsky AA, Ungar AL, Blatteis CM. Role of intrapreoptic norepinephrine in endotoxin-induced fever in guinea pigs. Am J Physiol **1993**; 265:R1369–75.
56. Sehic E, Ungar AL, Blatteis CM. Interaction between norepinephrine and prostaglandin E_2 in the preoptic area of guinea pigs. Am J Physiol **1996**; 271:R528–36.
57. Morita I, Schindler M, Regier MK, et al. Different intracellular locations for prostaglandin endoperoxide H synthase-1 and -2. J Biol Chem **1995**; 270:10902–8.
58. Spencer AG, Woods JW, Arakawa T, Singer JT, Smith WL. Subcellular localization of prostaglandin endoperoxide H synthases-1 and -2 by immunoelectron microscopy. J Biol Chem **1998**; 273:9886–93.
59. O'Banion MK, Winn VD, Young DA. cDNA cloning and functional activity of a glucocorticoid-regulated inflammatory cyclooxygenase. Proc Natl Acad Sci U S A **1992**; 89:4888–92.
60. Yokoyama L, Tanabe T. Cloning of the human gene encoding prostaglandin endoperoxide synthase and primary structure of the enzyme. Biochem Biophys Res Commun **1989**; 165:888–94.
61. Hla T, Neilson K. Human cyclooxygenase-2 cDNA. Proc Natl Acad Sci U S A **1992**; 89:7384–8.
62. Herschman HR. Prostaglandin synthase 2. Biochim Biophys Acta **1996**; 1299: 125–40.
63. Breder CD, Smith WL, Raz A, et al. Distribution and characterization of cyclooxygenase immunoreactivity in the ovine brain. J Comp Neurol **1992**; 322:409–38.
64. Breder CD, DeWitt D, Kraig RP. Characterization of inducible cyclooxygenase in rat brain. J Comp Neurol **1995**; 355:296–315.
65. Peri KG, Hardy P, Li DY, Varma DR, Chemtob S. Prostaglandin G/H synthase-2 is a major contributor of brain prostaglandins in the newborn. J Biol Chem **1995**; 270:24615–20.
66. Kaufmann WE, Worley PF, Pegg J, Bremer M, Isakson P. COX-2, a synaptically induced enzyme, is expressed by excitatory neurons at postsynaptic sites in rat cerebral cortex. Proc Natl Acad Sci U S A **1996**; 93: 2317–21.
67. Yamagata K, Andreasson KI, Kaufmann WE, Barnes CA, Worley PF. Expression of a mitogen-inducible cyclooxygenase in brain neurons: regulation by synaptic activity and glucocorticoids. Neuron **1993**; 11:371–86.
68. Breder CD. Cyclooxygenase systems in the mammalian brain. Ann N Y Acad Sci **1997**; 813: 296–301.
69. Li S, Wang Y, Matsumura K, Ballou LR, Morham SG, Blatteis CM. The febrile response to lipopolysaccharide is blocked in COX-$2^{-/-}$ but not in COX-$1^{-/-}$ mice. Brain Res **1999**; 825:86–94.
70. Cao C, Matsumura K, Yamagata K, Watanabe Y. Cyclooxygenase-2 is induced in brain blood vessels during fever evoked by peripheral or central administration of tumor necrosis factor. Mol Brain Res **1998**; 56:45–56.
71. van Dam A-M, Brouns M, Man-A-Hing W, Berkenbosh F. Immunocytochemical detection of prostaglandin E_2 in microvasculature and in neurons of rat brain after administration of bacterial endotoxin. Brain Res **1993**; 613:331–6.
72. Matsumura K, Cao C, Ozaki M, Morii H, Nakadate K, Watanabe Y. Brain endothelial cells express cyclooxygenase-2 during lipopolysaccharide-induced fever. Light and electron microscopic immunocytochemical studies. J Neurosci **1998**; 18:6279–87.
73. Cao C, Matsumura K, Yamagata K, Watanabe Y. Involvement of cyclooxygenase-2 in LPS-induced fever and regulation of its mRNA by LPS in the rat brain. Am J Physiol **1997**; 272:R1712–25.
74. Matsumura K, Cao C, Watanabe Y. Possible role of cyclooxygenase-2 in the brain vasculature in febrile response. Ann N Y Acad Sci **1997**; 813:302–9.
75. Futaki N, Takahashi S, Yokoyama A, Arai I, Higuchi S, Ottomo S. NS-398, a new anti-inflammatory agent, selectively inhibits prostaglandin G/H synthase/cyclooxygenase (COX-2) activity in vitro. Prostaglandins **1994**; 47:55–7.

76. Riendeau DV, Percival MD, Boyce S, et al. Biochemical and pharmacological profile of a tetrasubstituted furanone as a highly selective COX-2 inhibitor. Br J Pharmacol **1997**; 121:105–17.
77. Taniguchi Y, Yokoyama K, Inui K, Deguchi Y, Furukawa K, Noda K. Inhibition of brain cyclooxygenase-2 activity and the antipyretic action of nimesulide. Eur J Pharmacol **1997**; 330:221–9.
78. Li S, Ballou LR, Morham SG, Blatteis CM. The febrile response of mice to interleukin (IL)-1β is cyclooxygenase-2 (COX-2)-dependent. FASEB J **2000**; 14:A86.
79. Steiner AA, Blatteis CM. COX-2 is an essential modulator of LPS fever in guinea pigs. FASEB J **2000**; 14:A86.
80. Kirtikara K, Morham SG, Raghow R, et al. Compensatory prostaglandin E_2 biosynthesis in cyclooxygenase-1 or -2 null cell. J ExP Med **1998**; 187: 517–23.
81. Lacroix S, Rivest S. Effect of acute systemic inflammatory response and cytokines on the transcription of the genes encoding cyclooxygenase enzymes (COX-1 and COX-2) in the rat brain. J Neurochem **1998**; 70:452–66.
82. Thore CR, Beasley TC, Busija DW. In vitro and in vivo localization of prostaglandin H synthase in fetal sheep neurons. Neurosci Lett **1998**; 242: 29–32.
83. Dvorak AM, Weller PF, Harvey VS, Morgan ES, Dvorak HF. Ultrastructural localization of prostaglandin endoperoxide synthase (cyclooxygenase) to isolated, purified fractions of guinea pig peritoneal macrophage and line 10 hepatocarcinoma cell lipid bodies. Int Arch Allergy Immunol **1993**; 101:136–42.
84. Weller PF, Dvorak AM. Lipid bodies: intracellular sites for eicosanoid formation. J Allergy Clin Immunol **1994**; 94:1151–6.
85. Bozza PT, Payne JL, Morham SG, et al. Leukocyte lipid body formation and eicosanoid generation: cyclooxygenase-independent inhibition by aspirin. Proc Natl Acad Sci U S A **1996**; 93:11091–6.
86. Adams J, Collaco-Maracs Y, de Belleroche J. Cyclooxygenase-2 induction in cerebral cortex: an intracellular response to synaptic excitation. J Neurochem **1996**; 66:6–13.