[Frontiers in Bioscience 9, 3339-3355, September 1, 2004]

NON-PROSTAGLANDIN EICOSANOIDS IN FEVER AND ANAPYREXIA

Wieslaw Kozak [1] and Vadim Fraifeld [2]

[1] Department of Immunology, Institute of General and Molecular Biology, Faculty of Biology and Earth Sciences, Nicolas Copernicus University, 87-100 Torun, Poland, [2] Department of Microbiology and Immunology, Faculty of Health Sciences, Center for Multidisciplinary Research in Aging, Ben-Gurion University of the Negev, Beer-Sheva 84105, Israel

TABLE OF CONTENTS

1. ABSTRACT

Until recently, studies on the role of the metabolites of arachidonic acid (AA), eicosanoids in fever have primarily focused on prostaglandins, prostaglandin E_2 (PGE_2) in particular, derived from the pathway related to cyclooxygenases (COX). COX exists in two known isoforms; a constitutive COX-1, and COX-2, which is inducible upon the action of pyrogens. Data accumulated in our laboratories suggest a thermoregulatory role for two other pathways of arachidonate metabolism; 5-lipoxygenase (5-LOX) and cytochrome P-450 (epoxygenase). We have demonstrated that leukotrienes (LTs; 5-LOX-derived eicosanoids) and various isomers of epoxyeicosatrienoic acids (EETs; epoxygenase-derived eicosanoids) contribute to the process of endogenous antipyresis or cryogenesis, which limits the height of fever. In support of this are several lines of evidence based on both *in vivo* and *in vitro* experiments. 1) Intracerebroventricular (icv) injections of LTC_4 at nanomolar concentrations cause a dose-dependent decrease of body temperature (T_b) in mice. 2) Lipopolysaccharide (LPS)-induced anapyrexia in mice is preceded and accompanied by elevation in hypothalamic cysteinyl-LT (CysLT) production. 3) The inhibitor of LT synthesis MK-886 suppresses both of these processes. 4) EETs as well as inducers of the epoxygenase attenuate, whereas inhibitors of epoxygenase enhance the LPS-induced fever in rats. 5) One of the isomers of EET, 11,12-EET, in *in vitro* studies inhibited both the generation of PGE_2 and IL-6 in monocytes stimulated with LPS. These results, together with a well-established pyrogenic role of PGE_2, indicate that AA cascade may be regarded as an endogenous system to regulate the temperature response upon disease. COX, 5-LOX, and epoxygenase products may act at the level of hypothalamus as proximal mediators of, respectively, fever (PGE_2) or cryogenesis (CysLTs and EETs), or indirectly by influencing the other endogenous cryogens and pyrogens.

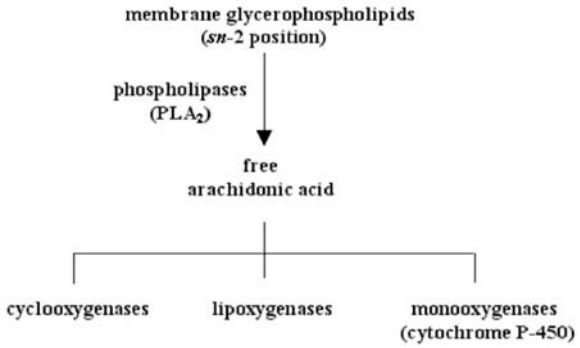

Figure 1. Schematic illustration of pathways of the free arachidonic acid metabolism.

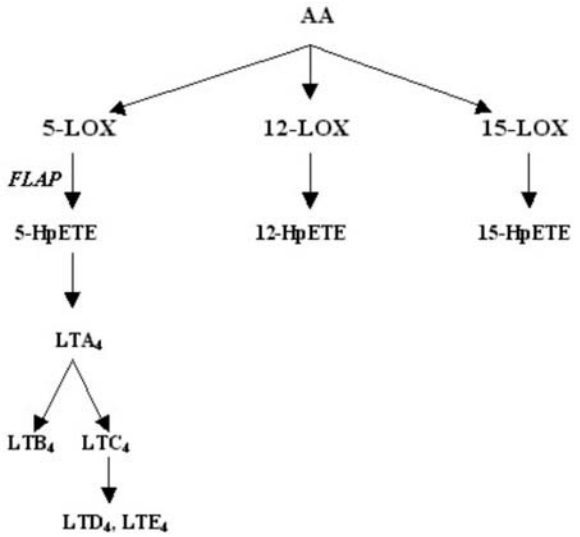

Figure 2. Pathways of arachidonic acid via lipoxygenases.

2. INTRODUCTION

Previous chapters of this volume focused on the role of a cyclooxygenase-derived eicosanoids, prostaglandins, in generation of fever (see, e.g., ref. 1). The other known major groups of enzymes of the arachidonic acid (AA) metabolism are the lipoxygenase and monooxygenase pathways (Figure 1).

Although lipoxygenases and monooxygenases generate potent biological modifiers, they have not been studied extensively in connection to the response to pyrogens. Perhaps because these two pathways, in contrast to the cyclooxygenases (COX) pathway, do not produce factors directly involved in the onset of fever. They appear to play a role, however, in the modulation of fever. They have also been discovered later than prostaglandins. The lipoxygenases convert AA to labile hydroperoxy intermediates that go on to form the leukotrienes (LTs), lipoxins, and hydroxyeicosatetraenoic acids. Monooxygenases, on the other hand, are mixed function oxidase enzymes belonging to the cytochrome P-450 (CYP) superfamily. They metabolize AA to three types of eicosanoid products: (1) midchain conjugated dienols formed by allylic oxidation of AA; (2) C_{16}-C_{20} alcohols formed by omega-terminal hydroxylation of AA; and (3) *cis*-epoxyeicosatrienoic acids formed by olefin epoxidation of AA (also called the epoxygenase reaction). Thus, in addition to prostanoids (prostaglandins, thromboxanes, and prostacyclins), the composition of the eicosanoid family has expanded immensely to include large number of the oxygenated AA metabolites, which possess both pro- and anti-inflammatory properties. In this chapter, data suggesting a role of lipoxygenase and monooxygenase pathways in regulation of body temperature (T_b) will be highlighted.

3. PHYSIOLOGICAL CHARACTERISTICS OF THE LIPOXYGENASE PATHWAY

Mammalian lipoxygenases are categorized as 5-, 12-, or 15-lipoxygenases (5-, 12-, and 15-LOX, respectively), with the number denoting the site of insertion of a single oxygen molecule into arachidonate to form initially a hydroperoxyeicosatetraenoic acid (HpETE; 5-, 12-, and 15-HpETE, respectively (Figure 2). The primary localization of the lipoxygenases is in leukocytes (5-LOX), platelets (12-LOX), and airway cells (15-LOX). Nevertheless, they are also expressed in numerous other tissues and cell types, including brain (2). With respect to the role in fever, 5-LOX has actually been the only pathway studied so far (3 - 14).

3.1. Enzymatic activity of 5-LOX

5-LOX (arachidonate:oxygen 5-oxidoreductase, EC 1.13.11.34) is the key enzyme to transform AA into LTs, potent pro-inflammatory mediators. The term "leukotriene" was coined by Samuelsson and his colleagues to reflect their cells of origin, inflammatory cells, and the recognition that these compounds have a conjugated triene structure (15). In resting cells, 5-LOX appears to be predominantly a cytosolic protein. Phosphorylation of 5-LOX and its translocation to the nuclear membrane are necessary steps in the enzyme activation (16, 17). Cellular stimulation by number of factors such as immune complexes, bacterial proteins, calcium ionophores, formyl peptides, platelet-activating factor (PAF), and other stimuli elicit a sequence of events that result in translocation of cytosolic 5-LOX to the nuclear envelope (18). In particular, priming the cells with cytokines or LPS has been shown to enhance the process of 5-LOX translocation (19). Elevation of intracellular Ca^{2+} and phosphorylation of 5-LOX by MAPK pathway-associated molecules are considered to be important for activation and translocation of 5-LOX. However, the relative role of each of these factors may differ between various cell types (17, 20, 21). In intact inflammatory cells, the presence of the 5-LOX-activating protein (FLAP) is required to make this enzyme fully active (22). FLAP acts as an AA transfer protein that "presents" the substrate to the 5-LOX on the leukocyte nuclear membrane (23). Thus, in contrast to the prostaglandin formation, AA released from the nuclear membrane rather than the outer cell membrane may be the primary substrate for the LT synthesis (24). 5-LOX catalizes two reactions: oxygenation of AA at the fifth carbon generating unstable hydroperoxyl intermediate (5-HpETE), and then a dehydration reaction, forming the unstable epoxide

intermediate, LTA_4. LTA_4 can then be further metabolized to LTB_4 by LTA_4 hydrolase or to LTC_4 by conjugation of glutathione at the sixth carbon by the action of LTC_4 synthase. The peptide moiety of LTC_4 is subjected to extracellular metabolism, forming LTD_4 and LTE_4 (Figure 2). It has been established that LTC_4 and its extracellular metabolites LTD_4 and LTE_4 are the constituents of slow-reacting substance of anaphylaxis, but they are now more properly termed cysteinyl leukotrienes (CysLT). Synthesis of these end products represents the most firmly established functions of 5-LOX. This pathway, although largely restricted to cells of myeloid origin, has also been documented in the brain (25, 26). *In vitro* studies showed LTC_4 synthesis in most regions of the rat brain stimulated by the ionophore A23187, with the highest levels in the hypothalamus and the median eminence (27). Binding studies revealed LTC_4 binding sites throughout the rat brain (28). Chen and Reiss (29) reported a shunting effect between 5-LOX and COX-2 in the mouse brain during viral encephalitis. When 5-LOX activity is inhibited, there was more COX-2 expressed in the brain of mice infected with vesicular stomatitis virus. On the other hand, when COX-2 activity was inhibited, more 5-LOX mRNA was expressed in the mouse brain at day 1 post-infection.

Leukotrienes may convey messages by interacting with specific membrane G-protein-coupled CysLT receptors. CysLT receptors are classified into two major subtypes: $CysLT_1$, which could be blocked by specific antagonists, and $CysLT_2$, which are basically insensitive to $CysLT_1$ antagonists (30). $CysLT_1$ receptors are present in numerous peripheral tissues, whereas the $CysLT_2$ subtype is expressed in brain, heart and coronary vessels, and in adrenal gland (30). The 5-LOX has also been found in the cell nucleus in association with chromatin (16), and nuclear import sequences on the 5-LOX have been identified (31) suggesting involvement of the nuclear 5-LOX in the regulation of gene expression. Indeed, it has been demonstrated that LTs can affect transcription of genes involved in inflammatory responses (24).

3.2. Nonenzymatic activity of 5-LOX

Apart from its well-established catalytic activity related to LT synthesis, the 5-LOX may also influence the cellular functions via nonenzymatic way. This could be associated with the ability of 5-LOX to form complexes with other proteins and to enter the nucleus. In particular, 5-LOX protein contains a Src homology (SH) 3-binding motif, which enables the 5-LOX to interact with proteins containing SH3 domains (32). Among them are Grb2, an adaptor protein, which together with Shc and Sos transduce the signals from tyrosine kinase receptors to Ras/MAPK signaling, and cytoskeletal proteins (32, 33). Two other candidate proteins are related to TGF-beta receptor-1-mediated signaling and to RNA processing (33). These data have been obtained using *in vitro* model systems and a physiological role of 5-LOX-protein complexes remains unclear. Nevertheless, such a possibility should be taken into consideration, especially with respect to a potential modulation of MAPK signaling. During the last years, the role of this pathway in regulation of key enzymes of arachidonic acid cascade and mediating the pharmacological effects of anti-inflammatory drugs have gained growing attention (34-36).

3.3. Regulation of 5-LOX expression and activity

There are numerous endogenous factors that regulate expression and activity of 5-LOX. The 5-LOX gene promoter belongs to housekeeping genes and its activity is regulated by several transcription factors, most likely via DNA methylation/demethylation processes (37). It has been shown that the 5-LOX gene promoter contains hormone response element, which indicates its strong susceptibility to hormonal regulation (38). In line with this are reports showing that glucocorticoids stimulate the expression of 5-LOX mRNA *in vitro* (39) and *in vivo* (40). It has been documented that treatment of rats with dexamethasone induced an increase of the brain 5-LOX mRNA accompanied by an increase in 5-LOX protein content (40), whereas melatonin exerts an inhibitory effect (2, 41). Dexamethasone is known to inhibit fever (42, 43) suggesting that induction of 5-LOX may be associated with an antipyretic effect of the steroid. In contrast, agents that raise intracellular cAMP, including PGE_2 and adenosine, down-regulate the activity of 5-LOX (44, 45). Brain PGE_2 is regarded as the proximal mediator of fever (46, 47). Brain adenosine, on the other hand, may be involved in down-regulation of fever (48, 49). These data suggest that if there is a role for 5-LOX in fever, it is probably indirect and complex, and could be related to both enzymatic and nonenzymatic activities.

4. STUDIES ON LEUKOTRIENES AND BODY TEMPERATURE

Studies on putative involvement of LTs in thermoregulatory responses could be divided into two groups with respect to laboratory animal species: 1) studies using laboratory mammals (rats, guinea pigs, cats) not including the mice, and 2) those conducted on mice. Such a division reflects chronology of investigations in the field and, on the other hand, the diversity in working hypotheses on a possible role for LTs, i.e., contribution either to fever or to cryogenesis.

4.1. Studies on laboratory mammals other than mice

In general, studies using laboratory mammals other than mice demonstrate that LTs do not contribute to the generation of fever. Some data suggest, however, that the involvement of LTs in thermoregulatory responses upon disease may vary across species, may depend on a kind of pyrogen, and that LTs may play a role in anapyrexia.

4.1.1. Thermal effects of the central injection of LTs

Early studies designed to examine whether the LTB_4, LTC_4, LTD_4, and LTE_4 can be ascribed to as mediators of the febrile response, brought about negative data. They demonstrated that, in contrast to the central administration of PGE_2, central injection of LTs did not induce the increase of T_b. For example, Brus *et al*. (4) found a hypothermic response to icv injection of LTC_4 and LTD_4 in rats. In contrast, O'Rourke & Rudy (3) and Sri

Kantra *et al.* (10) failed to observe any significant changes in T_b of rats after either icv or intra-hypothalamic injection of LTC_4, LTD_4, and LTE_4. Similar results have been reported by Mashburn *et al.* (5) in guinea pig. Administration of lipoxins, 15-LOX-derived eicosanoids, into the rat brain (10) resulted in a mild hyperthermia (after injection of LXA_4) or no change in T_b (after injection of LXB_4). Collectively, these data were in line with a widely accepted assertion that LTs are unlikely to play a role in fever (see, e.g., 50, 51). It should be stressed however, that the experimental conditions (restrained animals, rectal probe, or arousal state) could influnce the effects of LTs in these studies. In addition, negative results in the experiments with such unstable compounds as LTs, may require parallel testing of their bioactivity using well-established models (for example, the guinea pig tracheal strip bioassay or hypotensive effects of CysLTs). Such an approach was used by O'Rourke & Rudy (3) and by Mashburn *et al.* (5), while Sri Kantra *et al.* (10) did not confirm a high bioactivity of LTs used in their study. The hypothermic effect of icv administered LTs reported by Brus *et al.* (4) should also be taken with caution, since apparently too high doses of LTs were used (e.g., 12 nmole LTC_4 ~7.5 μg). These experiments were designed to search for fever after the administration of LTs into apparently afebrile animal. There have been no studies investigating the effect of the injection of LTs on a time-course of fever induced by, e.g., bacterial pyrogen.

4.1.2. Use of 5-LOX inhibitors in studies on fever

Studies applying various inhibitors of LT synthesis in different models of fever also have generated conflicting results, and fail to support a role for LTs in generation of fever. For example, Bhattacherjee *et al.* (6) demonstrated that the 5-LOX inhibitor BW A797C reduced yeast-induced fever in rats. However, in another study on rats challenged with a baker's yeast, Muller-Peddinghaus *et al.* (9) have reported no effect on fever of Bay X1005, an inhibitor of LTB_4 and LTC_4 synthesis. Similarly, Cornell (7) has demonstrated no effect of another inhibitor of 5-LOX, propyl gallate, on fever in rats treated centrally or peripherally with IL-1. However, lack of the important experimental details (including T_b values; 6, 9) or specific experimental design (24-h fasting before the experiment; 7) does not offer firm conclusions regarding the role for LTs in T_b responses to pyrogens. Hynes *et al*. (8), on the other hand, reported that U-60257, an inhibitor of 5-LOX, suppressed PAF-induced elevation of LTs in cerebrospinal fluid of cats, and enhanced the PAF-induced fever in this species. They also have shown that pyrogens such as LPS and IL-1, in contrast to PAF, did not affect the LT level in cerebrospinal fluid of cats (8). These data, generated in studies using inhibitors that directly affect the molecule of 5-LOX, suggest that LTs may not be required for the induction of fever. Nevertheless, the enhancement of fever in cats treated with PAF and U-60257 (8) suggests that LTs may act as endogenous antipyretics.

4.2. Studies on mice

Mice have long been considered as not quite appropriate animal model for studies on fever (reviewed, for example, by Kluger (52). The reason for this was that mice often responded to pyrogenic stimuli by a decrease in T_b. The diverse T_b responses were also reported for other mammalian species including humans (53 - 58). Depending on the mouse strain, a kind of pyrogen, and ambient temperature, both increase and decrease in T_b (13, 57), as well as polyphasic responses have been observed (for recent review see: 58). From our point of view, this "disadvantage" makes, however, the mice an extremely interesting and suitable model for searching the mechanisms of cryogenesis (endogenous antipyresis). Studies on various murine strains (differing in their patterns of T_b response to LPS) using icv microinjections of LTs through a chronically implanted cannula, determination of CysLT production by an *ex vivo* incubated hypothalamus and expression of neuronal 5-LOX, pretreatment of mice with the inhibitors of LT synthesis have led us to conclusion that LTs, 5-LOX-derived eicosanoids, may contribute to the process of endogenous cryogenesis (antipyresis).

4.2.1. Icv injections of LTC_4 decrease T_b of CD-1 and C57BL/6 mice

PGE_2 administered icv at nanomolar doses generated fever in the CD-1 and C57/BL/6 mice. In contrast, the central administration of LTC_4, a major CysLT in the CNS, caused a dose-dependent decrease in T_b in these two murine strains (data prepared for publication). The magnitude of the drop of T_b induced by nanomolar doses of LTC_4 was similar to that induced by "septic" doses of LPS administered intraperitoneally. We did not, however, observe any significant change in T_b following icv injection of LTB_4, which represents another metabolic branch of 5-LO pathway.

Our studies demonstrated that morning injections of LTC_4 icv (0.3 nmole) caused a decrease in T_b in mice, whereas injection of LTC_4 before the onset of darkness significantly reduced a nighttime rise of T_b (14). These effects of LTC_4 resemble the action of the inhibitors of PGE_2 synthesis on the circadian elevation of T_b (14, 59, 60). These results suggest that LTC_4 may act as an endogenous cryogen.

4.2.2. LPS-induced decrease in T_b of mice is associated with up-regulation of brain 5-LOX and elevation in hypothalamic CysLT production

Presuming a physiological role for LTs in mediating the cryogenesis (antipyresis), one could expect some quantitative relationship between the level of hypothalamic LTs and the T_b response (e.g., to LPS). Indeed, we found that the LPS (1.35 mg/kg ip)-induced drop in T_b in CD-1 mice was accompanied by a significant (~2-3-fold) elevation in total CysLT production by *ex vivo* incubated hypothalamus (12, 13), and this elevation negatively correlated with T_b changes ($R = -0.77$; $P < 0.05$), i.e., the lower values of T_b were associated with higher levels of CysLTs. LPS also resulted in a rapid up-regulation of brain 5-LOX. An increased level of 5-LOX protein was recorded as early as at least 30 min following LPS injection. Notably, more pronounced drop in T_b in response to a high dose of LPS in older mice correlated with the age-related increase in the level of brain 5-LOX

(unpublished data). In the mGSTM5 knockout mice (developed in the laboratory of Dr. Irving Listowsky), expression of neuronal 5-LOX mRNA was down-regulated compared to the wild-type animals. Accordingly, mGSTM5 knockout mice developed more pronounced fever in response to low doses of LPS, and reduced anapyrexia to high doses of LPS (61).

Analysis of temporal changes in T_b and hypothalamic eicosanoid production in 3 murine strains (CD-1, BALB/c, and C57BL/6) revealed that LPS-induced decrease in T_b was consistently associated with an initial increase in CysLTs, which preceded the elevation in PGE_2 (for details see: 13). Collectively, the results coincide well with the hypothesized cryogenic role for the 5-LOX products, in particular CysLT.

4.2.3. Inhibitors of LT synthesis suppress the LPS-induced decrease in T_b in mice

MK-886 is a selective inhibitor of FLAP, a membrane-bound protein required for full enzymatic activity of 5-LO (see section 3.1.). Pretreatment of CD-1 mice with MK-886 abrogated both the decrease in T_b and elevation in hypothalamic CysLT production caused by LPS (1.35 mg/kg ip) (12). This inhibitory effect of MK-886 was dose- and time-dependent. The T_b decrease due to LPS was partially prevented when MK-886 (1 mg/kg ip) was administered 3 h before and completely blocked by MK-886 injected 4 h before LPS. No significant effect was observed if MK-886 was injected 1 h prior to LPS. That is, MK-886 required a relatively long period for exhibiting its inhibitory activity. Perhaps, a temporal factor should be taken into account when the effects of other LT inhibitors are evaluated. For example, esculetin, a potent inhibitor of LTs synthesis (IC_{50} ~4 μM), injected ip 1 h prior to LPS had no effect on the LPS-induced fever in Swiss Webster mice (11).

Data obtained in Swiss Webster mice using nordihydroguaiaretic acid (NDGA), a dual inhibitor of epoxygenase and lipoxygenase (5-, 12, and 15-LOX), could be interpreted in favor of a cryogenic role for 5-LOX products. These mice developed a biphasic T_b response to LPS (2.5 mg/kg ip), i.e., a transient decrease in T_b followed by fever. We found that the low NDGA dose prevented the LPS-induced drop of T_b without influencing the onset or peak of fever, whereas the high dose affected both phases of T_b response (11). In this regard, it should be noted that the lowest IC_{50} of NDGA was found for 5-LOX (~0.2 μM), whereas IC_{50} for cytochrome P-450 was approximately two-order higher (~40 μM) (62). Hence, it is tempting to speculate that the dose-dependent effects of NDGA on T_b response to LPS in mice may be attributed to a differential sensitivity of 5-LOX and CYP to inhibitory action of NDGA.

We also examined whether the effect of MK-886 on T_b response to LPS in CD-1 mice could be associated with factors other than the inhibition of LT synthesis. In particular, those may include an indirect action of MK-886 on PGE_2 synthesis or possible changes in the production of cryogenic cytokine, such as TNFalpha (12). The first possibility seems quite reasonable, in view of an increased availability of AA for processing via COX pathway under inhibition of LT synthesis. We, however, found only a slight potentiation of the LPS-induced stimulation of hypothalamic PGE_2 production in MK-886-pretreated mice. The second possibility could be rejected, since the pretreatment of mice with MK-886 did not alter the LPS-induced elevation in plasma TNFalpha. Hence, the inhibition of 5-LOX activity and LT synthesis was responsible for the "anti-anapyrexic" effect of MK-886, most likely due to its direct central action. The latter was further supported by using the icv route of MK-886 administration (data prepared for publication). We found that 5 μg MK-886 injected icv 2 h prior to LPS (1.35 mg/kg ip) significantly suppressed LPS-induced decrease in T_b of CD-1 mice.

4.2.4. "Paradoxical" T_b response to LPS in 5-LOX$^{-/-}$ (LT-deficient) mice

The lack of LT production in 5-LOX knockout mice offers a "natural model" of a total inhibition of LT synthesis, both in peripheral and central tissues. Presuming a cryogenic action of LTs, one could predict an exacerbated fever, or attenuated anapyrexia in these mice in response to LPS, compared to their wild-type controls. Paradoxically, ip injection of LPS did not cause any significant changes in T_b of 5-LOX$^{-/-}$ mice, whereas the wild-type animals (genetically close to C57BL/6 strain) responded with fever (63). It is reasonable to suggest that the lack of 5-LOX would lead to a re-distribution of AA between alternative metabolic pathways. In particular, in 5-LOX$^{-/-}$ mice more AA may be available for processing via COX. In fact, the hypothalamic PGE_2 production of LPS-treated 5-LOX$^{-/-}$ mice was aproximately 3-fold higher than that of 5-LOX$^{+/+}$ wild-type. It may be speculated that the lack of fever in 5-LOX$^{-/-}$ mice could be attributed to a desensitization of PGE_2 receptors in these mice due to a permanently elevated PGE_2 level throughout their life. "Problems" inherent with using knockout mice for studying pathophysiological aspects of fever have been discussed in details by Kluger *et al.* (64). In addition, independently to LT synthesis, gene deletion for the 5-LOX may affect a proper functioning of signaling pathways specific for fever and thermoregulation.

4.2.5. Leukotrienes may be involved in modulation of normal T_b in mice

Further evidence for the cryogenic role of LTs has been obtained from the studies focusing to the involvement of LTs in modulation of normal photoperiod-related variations of T_b (14). We noticed that the T_b of CD-1 mice tended to decline gradually during the light period, reaching the lowest values between 12:00 to 14:00 (4-6 h before the light was switched off). The decline was generally within ~0.5 °C, and part of the mice exhibited T_b changes comparable to those induced by LPS (see 3.2.3). In this group of mice, the injection of "anti-anapyrexic" dose of MK-886 (1 mg/kg ip) at 09:00 (3 h after light on) significantly reduced the daytime decrease in T_b. The mean values of T_b in saline-treated controls (n = 13) were 37.1 ± 0.1, 35.9 ± 0.1, and 36.2 ± 0.1 °C at 09:00, 12:00, and 15:00, respectively, whereas the corresponding T_b values in MK-886-treated mice (n = 9) were 36.9 ± 0.2, 36.5 ± 0.2*, and 36.8 ± 0.1* °C (*P < 0.05 vs. control).

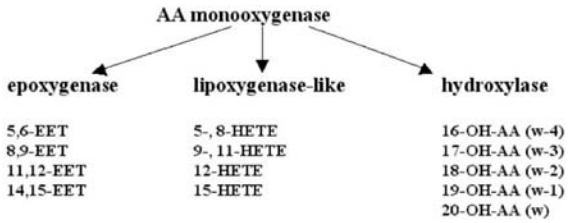

Figure 3. Oxygenation products of arachidonic acid catalyzed by monooxygenases.

These data as well as those previously discussed (see 4.2.1.) support the concept that LTs are involved not only in an anapyrexic response, but also in regulation of the circadian variations of T_b in mice. Below we present data that another pathway of the arachidonic acid metabolism, i.e., monooxygenase pathway, may be involved in an antifebrile mechanism.

5. PHYSIOLOGICAL CHARACTERISTICS OF THE CYP-DEPENDENT MONOOXYGENASE PATHWAYS

The participation of cytochrome P-450 (CYP) in the metabolism of arachidonic acid was first suggested in studies by Cinti & Feinstein (65), who observed that arachidonate-induced platelet aggregation could be blocked by known CYP inhibitors. It was not until early 80s, however, that the role of microsomal CYP in the oxidative metabolism of arachidonic acid was unequivocally demonstrated (66 - 68). These observations were largely ignored for many years due to the focus of researchers on the cyclooxygenase pathway. In recent years, the investigation of the biochemistry and biological significance of arachidonate metabolism via CYP has developed into an area of exciting research. Although the biological implications of this pathway remain to be fully understood, work from several laboratories is beginning to establish biochemical and functional correlations that can be interpreted as suggestive of a physiological and pathological function (see for example: 69 - 76, and references therein).

The microsomal CYP can oxidaze arachidonic acid (Figure 3) by one or more of the following types of reaction (70, 77): 1) lipoxygenase-like *bis*-allylic oxidation to generate six regioisomeric hydroxyeicosatetraenoic acid (HETEs; 5-, 8-, 9-, 11-, 12-, and 15-HETE) demonstrated in skin, liver, kidney, olfactory epithelium, lung, and brain; 2) omega/omega-1 hydroxylation (omega-oxygenase reaction) affording five omega-n arachidonate alcohols (OH-AAs; 16-, 17-, 18-, 19-, and 20-OH-AA) demonstrated in liver, kidney, lung, intestine, olfactory epithelium, and anterior pituitary (this reaction is the most prevalent in renal tissue); and 3) olefin epoxidation (epoxygenase reaction) furnishing four regioisomeric cis-epoxyeicosatrienoic acids (EETs; 5,6-, 8,9-, 11,12-, and 14,15-EET); demonstrated in numerous tissues, including liver, brain, kidney, adrenal, endothelium, and ovary. There is an interesting concept already supported by data that significant portion of generated EETs may be taken up from cytosole and circulation and re-tailored into the *sn*-2 position of the cell membrane glycerophospholipids (78 - 80). This feature differentiates EETs from PGs, which are generated exclusively for the export from cells for a receptor-mediated endocrine and paracrine actions.

As with most CYP-catalyzed reactions, the type of products generated from arachidonic acid during metabolism are highly dependent on the tissue, animal species, sex, age, hormonal status, diet, and exposure to xenobiotics. Fitzpatric & Murphy (81), McGiff (69), Oliw (82), Capdevila *et al*. (70, 77), Imig (73), and Roman (76) have reviewed the biochemical and enzymologic aspects of these processes, and concluded that although the metabolites of arachidonic acid via CYP have vascular, endocrine, renal, and ocular effects, the physiological importance of this pathway remains to be clarified. It has been shown that exposure to an inflammatory agent (e.g., LPS) results in reduction of the activity of epoxygenase by a cytokine- and nitric oxide-dependent mechanism (83).

5.1. Expression of monooxygenases in mammals

CYP comprises a large, but closely related superfamily of distinct gene products with different substrate specificities (84). Members of the CYP superfamily are heme-containing membrane-bound mixed function oxidases which catalyze important steps in the biosynthesis and/or degradation of endogenous compounds such as steroid hormones, cholesterol, and fatty acids, including arachidonic acid (69). In addition, CYP catalyze oxidation of many therapeutic agents and other xenobiotics, which results in their inactivation and facilitation of their excretion from the body. Thus, CYP mediated metabolism is potentially involved in the expression of the pharmacological and/or toxicological effects of drugs and environmental chemicals in tissues, which contain the CYP metabolic system. CYP family is regarded as a part of the bodily detoxification system. The remarkable versatility of this system is due to a large number of isozymes expressed in tissues [e.g., approximately 29 subfamilies have been identified in mammalian tissues (85)]. Some isozymes are only expressed significantly after chemical induction [e.g., specific molecular isoforms of CYP are preferentially induced by exposure to chemical stimuli such as phenobarbital, β-naphtoflavone, theophylline, fibrates, and 3-methylcholantrene], whereas others are expressed constitutively (86, 87). The constitutive enzymes are thought to provide a host defense against foreign compounds (88).

The highest concentrations of CYP are found in the liver, kidney, adrenals, testis, ovary, lung, and skin, but the enzyme is present in most, if not all, tissues of the body, albeit at lower concentration. The hepatic CYP accounts for 4-5% of rat hepatic microsomal protein, of which epoxygenase constitute major component (89). CYP is present in the brain at a concentration of approx. 1% of the liver level (90 - 92). The presence of CYP in the rat medial preoptic and anterior hypothalamus has also been demonstrated (93). It is worth noticing that the expression of CYP in the brain, implicating involvement of the brain tissues in steroidogenesis (generation of neurosteroids) has recently captured attention of neuroendocrinologists (92).

In mammals, expression of monooxygenases and synthesis of EETs has been detected, among other tissues, in the brain, lungs, liver and kidney, and in cells such as astrocytes, hepatocytes, and endothelium (73, 94). Monocytes, the cells playing a key role in inflammation, contain various CYP capable of EET biosynthesis (95, 96) and, in addition, they express specific high affinity receptors for EETs (97). Exhibition of monooxygenases in the brain and immune cells suggest that there is a role of this pathway of arachidonic acid in the neuroimmunomodulation.

5.2. Monooxygenases and neuroimmunomodulation

Immunological stimuli depress the CYP-mediated hepatic metabolism of a variety of drugs. The first demonstration of altered drug metabolism during infectious disease came from observation showing delayed theophylline elimination in patients with influenza (98). Human volunteers injected with small doses of LPS show decreased hepatic CYP-mediated clearance of antipyrine, hexobarbital, and theophylline (99). Clinical infections cause a reduction in total hepatic content of CYP (100). Partly attenuated CYP activity (40-50% inhibition) has also been seen in animals after infection with bacteria and viruses, after administration of vaccines, or after treatment with pyrogenic agents (e.g., LPS, Poly I:C, turpentine, carrageenan) and cytokines (88, 101 - 106). Dexamethasone has been shown to reverse the inhibitory effect of IL-1 and LPS on liver CYP (107).

Expression of CYP is hormonally regulated (69). Growth hormone is believed to be the most important physiological regulator of the expression of CYP (87, 108). Administration of endotoxin causes suppression of the growth hormone secretion that lasts for several hours (109). Therefore, one could hypothesize that alteration in secretion of growth hormone is responsible for the inhibition of CYP by endotoxin. In addition, according to the concept suggested by Ghezzi *et al.* (110), and others (104-106), cytokines and nitric oxide (NO) may mediate the inhibitory effect of inflammatory agents on CYP. As has already been thoroughly discussed in this volume, cytokines and NO are both involved in fever, indicating that suppression of the monooxygenase activity may have a regulatory feedback on the generation of fever. On the other hand, it has been well established that endogenous febrile and antipyretic mechanisms are operating at the level of neuroendocrino-immunomodulation.

EETs were found to stimulate *in vitro* secretion of several brain, pituitary and pancreatic hormones such as somatostatin, luteinizing hormone, growth hormone, adrenocorticotropic hormone, prolactin, insulin, and glucagon (69, 90 - 92). EETs are also potent stimulators of the release of alphaMSH and AVP (69, 111), the hormones known to be involved in endogenous antipyresis (112 - 114). It has also been shown that AVP can stimulate the CYP-related metabolism of arachidonic acid (115 - 118), implying a positive feedback between EETs and AVP. This vast spectrum of interactions of EETs with functioning of immune and brain tissues is grounds for a number of hypotheses regarding a role of the epoxygenase pathway in various aspects of neuroimmunomodulation, including fever.

6. STUDIES ON THE ROLE OF CYP IN FEVER

Studies focusing on the role of CYP in fever may be ascribed into three categories: (i) effect of CYP inhibitors; (ii) effect of CYP inducers; and (iii) effect of CYP metabolites on fever in laboratory mammals. These studies allow for a conclusion that monooxygenase pathway contributes to the endogenous antipyretic mechanism.

6.1. Inducers of CYP attenuate fever in laboratory rodents

The fibrates (oxyisobutyrates) are the largest structurally related group of CYP inducers investigated, and detailed induction protocols have been described for clofibrate, ciprofibrate, clobuzarit, and bezafibrate (119). Bezafibrate administered three times prior to injection of LPS reduced fever in rats in a dose-dependent manner (120). Figure 4 illustrates changes in fever in rats pretreated three times at 24-h intervals with bezafibrate, and injected 24 h later with LPS. To test the effect of bezafibrate administration on CYP activity in the liver, in a separate experiment tissues were collected from corn oil- and bezafibrate-treated rats. Microsomes were prepared and enzymatic activities using three derivatives of resorufin were analyzed according to the method described elsewhere (121). The metabolism of ethoxyresorufin (EROD) is associated with CYP1A1 family. Methoxyresorufin dealkylation (MROD) is carried out by CYP1A2 family, while the dealkylation of pentoxyresorufin (PROD) is attributed to CYP2B1/2. All three CYP families possess monooxygenase activity known to catalyze the epoxidation of arachidonic acid (70). As can be seen in Figure 5, liver samples from the rats treated with bezafibrate showed a significant 3 – 5-fold increase in activities of EROD, MROD, and PROD compared to rats treated with corn oil as control. This indicates that in the rat liver all three CYP families were affected by treatment with bezafibrate.

6.2. Inhibitors of CYP exacerbate fever induced by LPS and interleukin-1beta

Nakashima *et al.* (122) reported that im administration of imidazole inhibitors of CYP, econazole and clotrimazole, augmented fever in the rats injected icv with IL-1beta. We have shown that various structurally dissimilar inhibitors of CYP including SKF-525A, clotrimazole, miconazole, 1-ABT, and 17-ODYA, administered ip (mice and rats) and im or icv (rats) possess the capacity of enhancing fever induced by peripheral injection of LPS (11, 75, 120, 123). Data reported by Nakashima *et al.* (122) indicated that the mechanism of action of the inhibitors of monooxygenases on fever in these species was downstream to the production of IL-1beta, and presumably affecting a generation of other cytokines. In line with this assumption are data showing that injection of the inhibitor of CYP resulted in suppression of the generation of TNFalpha (11, 124), and potentiation of the elevation of plasma IL-6 (11) in laboratory rodents challenged with LPS. Interestingly, indomethacin, a well-known antipyretic and an inhibitor

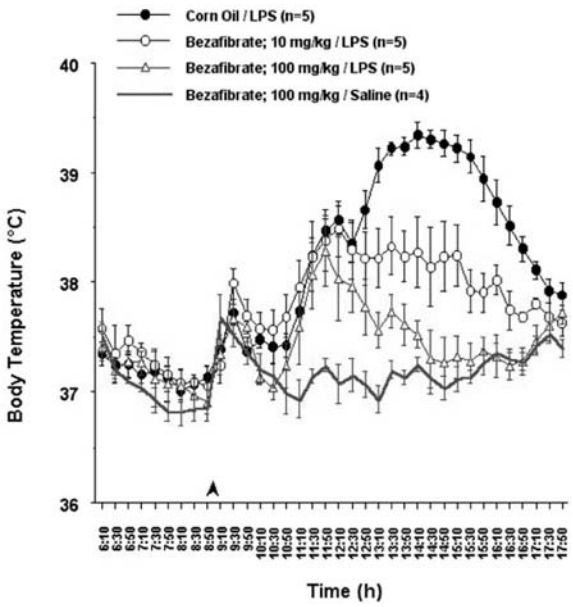

Figure 4. Effect of two doses of bezafibrate, 10 and 100 mg/kg, on lipopolysaccharide (LPS)-induced fever in rats. Bezafibrate, or corn oil as control, were injected intraperitoneally 3 times at 24-h intervals before intraperitoneal LPS (80 μg/kg or saline as control; arrowhead at 0900; 24 h after the 3rd injection of bezafibrate). Values are means ± SE at 20-min averages; n, number of rats in each group (data from Kozak *et al*., 120).

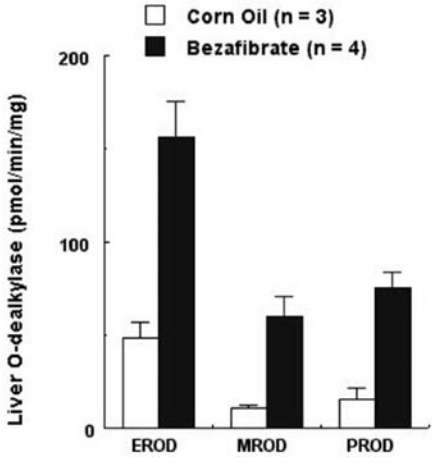

Figure 5. Effect of bezafibrate on ethoxyresorufin (EROD), methoxyresorufin (MROD), and pentoxyresorufin (PROD) *O*-dealkylase activity in microsomes purified from liver. Bezafibrate was injected ip three times at 24-h intervals at a dose of 50 mg/kg each. Tissues for analyses were collected 24 h after the third injection. Values are means ± SE (n indicates sample size; unpublished data).

of cyclooxygenases, exerted the opposite effects than that of CYP inhibitor on the generation of the two cytokines in mice injected with LPS. Namely, indomethacin suppressed generation of IL-6 and exacerbated the levels of TNFalpha (11). These results suggest that higher levels of IL-6 may account for the augmentation of LPS-induced fever in mice and rats treated with CYP inhibitors.

We have found, however, that the potentiation of fever in mice treated with clotrimazole was prevented by indomethacin (11), indicating that prostanoids are also involved in this effect. It is known that generation of IL-6 in cells stimulated with LPS or IL-1beta is prostaglandin-dependent (125 - 127). Subsequent studies in our laboratory revealed that inhibitors of mono-oxygenases (e.g., SKF-525A), exerted profound effects on the levels of prostaglandins measured in body fluids during LPS-induced fever, and *in vitro* in monocyte cultures upon stimulation with LPS. PGE_2 levels measured in the blood and cerebrospinal fluid of rats during fever were several-fold higher in animals treated with SKF-525A and LPS than in those treated with control vehicle and LPS (123). Similar potentiating effects of the two structurally dissimilar CYP inhibitors (SKF-525A and 1-ABT) on the levels of PGE_2 in supernatants from the rat mononuclear cells stimulated with LPS have also been detected (128). Thus, the elevated levels of PGE_2 may account for the enhancing effect of the CYP inhibitors on fever.

The mechanism of this effect of CYP inhibitors on levels of PGE_2 in body fluids and cell-culture supernatants remains to be established. One may hypothesize that inhibition of CYP results in the shunting effect in favor of the cyclooxygenase activity, similar to that observed in mice when lipoxygenases were inhibited under inflammation (29). Another possibility is that treatment with CYP inhibitors affected clearance of PGE_2, since some CYP are thought to be involved in a downstream metabolism of the prostaglandins (69). It is also plausible that SKF-525A and other inhibitors of CYP blocked the synthesis of the modulators of fever, resulting in a higher elevation of core temperature. In favor of the latter hypothesis, we have demonstrated that administration of EETs prevented fever.

6.3. Central EETs inhibit fever induced by LPS and interleukin-1beta

Administration of monooxygenase metabolites into the brain of the febrile rats exerted an opposite effect on fever to that seen after the injection of the inhibitor of CYP, e.g. SKF-525A (Figure 6).

Three out of four studied EET regioisomers injected into the lateral ventricle prevented fever induced by peripheral injection of LPS in rats (120). The effect was dose-dependent at different ranges for each isomer. The most potent antipyretic isomers were 11,12-EET followed by 14,15-EET, and 8,9-EET. Isomer 5,6-EET at the dose examined (1 and 10 μg/rat) did not affect fever. EETs at doses used did not affect normal T_b of rats. In subsequent studies, Nakashima *et al.* (129) reported that 11,12-EET administered into the rat pre-optic anterior hypothalamus (PO/AH) prevented fever induced by IL-1beta infused into the PO/AH. These data indicate that central EETs exert their antipyretic effects acting on the centers of thermoregulation, and that they act downstream to IL-1beta, an endogenous mediator of fever.

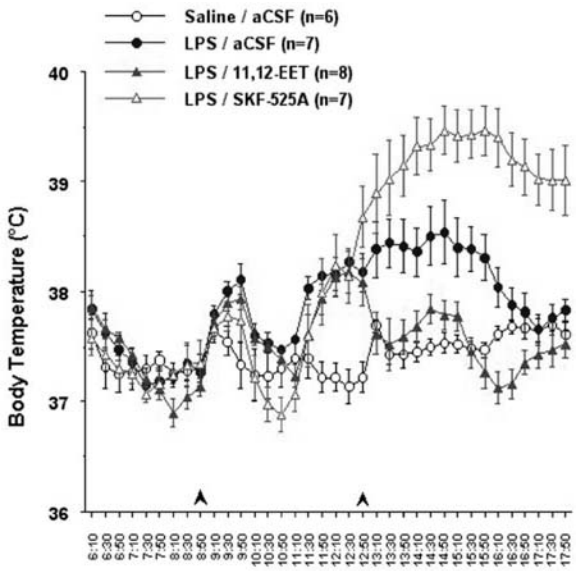

Figure 6. Twenty eight rats were implanted with biotelemetry devices to monitor body temperature, and with brain cannulea to the lateral ventricle. At the day of experiment at 0850 (arrowhead) rats were divided into two groups; 6 rats were injected ip with saline and 22 rats were injected ip with LPS (80 μg/kg). After 4 hrs (arrowhead at 1250) all rats received icv infusion of agents as shown. Saline-treated rats received 5 μl aCSF icv (vehicle control group). LPS-treated rats were divided into 3 groups each receiving: aCSF, SKF-525A (5 μg/rat), and 11,12-EET (100 ng/rat; in control injections this dose of 11,12-EET did not affect normal T_b in rats). Values are means ± SE at 20-min averages; n, number of rats in each group (unpublished data).

Effect of peripheral infusion of EETs on fever has not yet been studied. The major problem with determination of the effects of peripheral administration of EETs is the presence of soluble epoxide hydrolases, enzymes that rapidly hydrolyze EETs into the respective dihydroxyeicosatrienoic acids (see, e.g.: 73, 76). Activity of the epoxide hydrolases in the brain appears to be very low compared to the activity of this enzyme in peripheral tissues (130, 131). This is consistent with a general assumption that expression of the eicosanoids neutralizing enzyme system, including 15-hydroxy-PG dehydrogenase (enzyme that normally neutralizes PGE_2) is reduced in adult brain compared to the peripheral tissues (132, 133). Resistant to the enzymatic metabolization analogs of EETs will be required to assess their effects on fever following peripheral administration.

7. STUDIES INTO THE MECHANISM OF ANTIPYRETIC ACTION OF EETs

Based on *in vivo* data demonstrating that administration of inhibitors of CYP exacerbated the LPS provoked increase levels of PGE_2 in the cerebrospinal fluid and blood, we hypothesized that the mechanism underlying the antipyretic effect of EETs may involve inhibition of the PGE_2 generation by inflammatory cells.

7.1. Regulatory feedback imposed by monoxygenases on the generation of PGE_2

11,12-EET prevented, while CYP inhibitors (SKF-525A and ABT) augmented the release of PGE_2 in monocytes co-cultured with LPS (128). In addition to that, 11, 12-EET added back to the monocytes incubates inhibited the augmenting effect of SKF-525A on LPS-induced release of PGE_2. Enhancement and reduction of PGE_2 synthesis in monocytes, respectively, stimulated with LPS in the presence of SKF-525A and/or 11, 12-EET might be due to an up- and/or down-regulation of COX-2 induction in the cells. As expected, LPS markedly up-regulated the expression of COX-2 protein in the rat monocytes. However, we have not been able to determine any significant effects of SKF-525A and/or 11, 12-EET on the LPS-induced elevation of the enzyme protein (128). We reasoned, therefore, that 11, 12-EET affected PGE_2 synthesis via inhibiting the activity of COX-2.

Pre-incubation of the murine COX-2 preparation for 5 minutes with three concentrations of 11,12-EET (1, 5, and 10 μM) led to the inhibition of the activity of the enzyme in a concentration-dependent manner, with an IC_{50} of 6.43 ± 1.58 μM. This inhibitory effect of 11,12-EET was also time-dependent, i.e., the longer pre-incubation time of COX-2 with 11,12-EET the greater inhibition of the activity of COX-2 (128). The parallel experiments using the same enzyme preparation and assay have shown that the potency of inhibition of COX-2 by 11,12-EET was approximately 58-fold greater than that of acetaminophen (IC_{50} = 372 μM; ref. 134), and ca. 120-fold greater than that of sodium salicylate (IC_{50} = 763 μM; ref. 135). Ongoing experiments revealed that 8,9-EET is 10—fold more potent inhibitor of COX-2 (IC_{50} = 0.64 μM) than that of 11,12-EET, and it approximates that of ibuprofen (IC_{50} = 0.60 μM; unpublished data). Despite of these subtle differences, it appeared that EETs surpassed the most commonly used nonspecific anti-inflammatory drugs in their ability to inhibit COX-2 activity.

We conclude based on data presented, that monooxygenase pathway imposes a negative regulatory feedback on the prostaglandin synthesis machinery.

7.2. Monooxygenases and generation of cytokines by inflammatory cells

As mentioned earlier, injection of the inhibitor of CYP resulted in suppression of the generation of TNFalpha and exacerbation of the elevation of plasma IL-6 in laboratory rodents challenged with LPS. Injection of indomethacin, an inhibitor of cyclooxygenases exerted the opposite effects than that of CYP inhibitor on plasma levels of the cytokines: it exacerbated levels of TNFalpha and suppressed generation of IL-6. These results suggest that prostanoids and epoxyeicosanoids may play an opposite regulatory role on the synthesis of IL-6 and TNFalpha in the inflammatory cells stimulated with LPS. To test this hypothesis, we compared the levels of IL-6 and TNFalpha in supernatants of the rat macrophages stimulated with LPS and co-incubated with or without

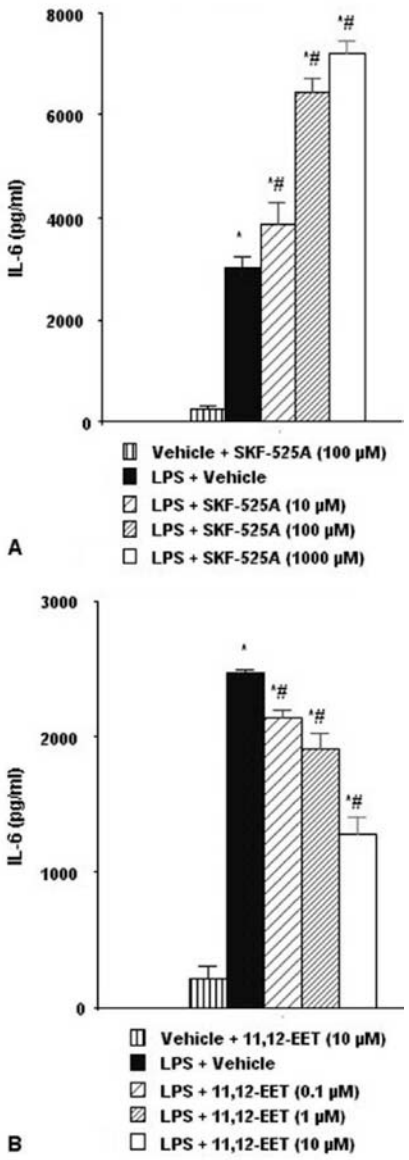

Figure 7. Effect of SKF-525A (panel A) and 11,12-EET (panel B) on IL-6 levels in supernatants of rat monocytes stimulated with LPS. Cells were prepared as described in Kozak *et al*. (128). Ten μl of LPS solution was added to a final concentration of 100 ng/well/2x10^5 cells (saline, vehicle for LPS, was added to the control cell incubates). Immediately after the LPS, 10 μl of SKF-525A or 11,12-EET was added to concentration as indicated. RPMI vehicle was added to control incubates. Supernatants for analyses were collected after 8 hours. ELISA-based immunoassay (Quantikine M Rat IL-6 kit from R&D Systems; Cat #R6000) was used to determine IL-6 in supernatants. The asterisk indicates significant difference from the control incubates (vehicle/SKF525A as well as vehicle/11, 12-EET). # Depicts significant difference of all LPS/SKF-525A and LPS/11, 12-EET groups from respective LPS/vehicle groups. Values are means ± SEM (n = three repetitions, each consisted of four incubation wells per group).

11,12-EET, as well as with and without SKF-525A. A part of this experiment, revealing the levels of IL-6 is shown in Figure 7.

As can be seen, SKF-525A at a concentration of 100 μM doubled while 11,12-EET at a concentration of 10 μM reduced approximately by half the increase of IL-6 in supernatants of the cells stimulated with LPS for 8 h. TNFalpha levels assessed in the same supernatants were also affected by SKF-525A and 11,12-EET. These effects, however, were opposite to that seen for IL-6. Namely, an inhibitor of CYP significantly reduced, while 11,12-EET augmented the levels of TNFα (data not shown). We postulate, therefore, that EETs and PGE_2, metabolites of AA via epoxygenase and COX-2, respectively, are involved in regulation of the release of IL-6 and TNFα from inflammatory cells in opposite fashion. This conclusion is depicted in Figure 8.

Inhibitory action of 11,12-EET, and presumably other EETs, on generation of PGE_2 and IL-6, two major endogenous mediators of fever, may certainly be translated onto the role of these metabolites play during fever. They impose a negative feedback on the febrile response. Hence, we conclude, that epoxygenase pathway of the AA cascade contributes to the endogenous antipyresis mechanism, the process that keeps fever from reaching dangerous heights during infectious and inflammatory disorders.

8. CONCLUDING REMARKS

Fever is a fundamental component of the acute phase response to infection, and has an extremely important diagnostic value. Fever is also thought to evolve as an adaptive response. Nevertheless, unusually high and persistent fever may be harmful and may contribute to morbidity. However, upon normal infections fever almost never reaches such excessive measures. But what keeps fever from reaching dangerous heights?

Since data published in 1949 by DuBois (136), it has been appreciated that fever has an upper limit, and that increase of T_b during infection in most patients is maintained within safe limits. In other words, that the magnitude of fever (fever ceiling) is tightly regulated. As a consequence, the concept of endogenous antipyresis has emerged (137, 138) and number of various factors, including hormones, neurotransmitters, and cytokines has been identified as endogenous antipyretics. These studies are thoroughly reviewed by other authors contributing to this volume (see: 139 - 143). Based on data discussed in this chapter, we conclude that metabolism of AA via lipoxygenases and epoxygenases also operates to limit the magnitude of fever. We hypothesize that arachidonic acid metabolism may yield both pro- and anti-febrile eicosanoids through a cascade inherently requiring tight regulation. Regulation of the arachidonate pathway may help to control both the magnitude and duration of fever. However, mechanisms and endogenous factors involved in this regulation have yet to be identified.

Biological relevance as well as clinical

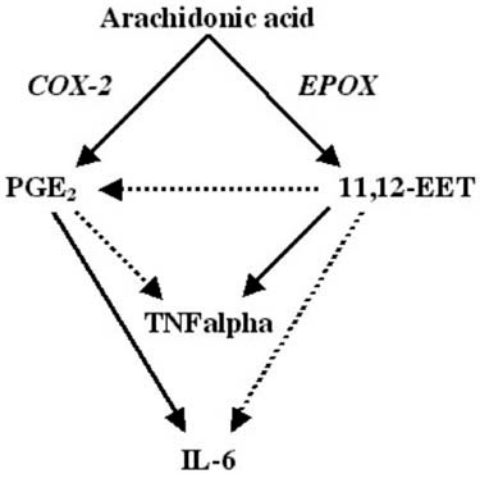

Figure 8. Schematic representation of the action of prostaglandin E_2 (PGE_2) and 11,12-epoxyeicosatrienoic acid (11,12-EET) on the release of IL-6 and TNFalpha from monocytes stimulated with LPS. Solid arrows represent stimulation, whereas dashed arrows depict inhibitory effects. Abbreviations: COX-2, cyclooxygenase-2; EPOX, epoxygenase; IL-6, interleukin-6; TNFalpha, tumor necrosis factor-alpha.

significance of these findings also needs further investigation. There are, however, clinical indications that substantiate the above hypothesis. For example, phenobarbital, a CYP inducer, is one of the most effective and widely used drugs for treatment of febrile convulsions in children (144). Anti-seizure effect of phenobarbital is ascribed predominantly to the enhancement of GABA A receptor-mediated currents. However, phenobarbital-induced induction and activation of CYP may be also responsible for this effect. In accordance, it has been found that metabolites of AA via CYP act as neurotransmitters, and they interact with GABA A receptors (92). There are also reports indicating that aspirin, while inhibiting a cyclooxygenase pathway, stimulates CYP- and 5-LOX-dependent metabolism of arachidonic acid (see, e.g., 75, and references therein). Thus, NSAIDs, while inhibiting cyclooxygenases may also unmask or potentiate an antipyretic pathway via CYP and 5-LOX.

Clinical reports frequently describe compromised and neutropenic patients with high and persistent fevers, which are often resistant to a regular antipyretic regimen. Furthermore, treatment with antifungal agents, known to inhibit CYP, as well as with other drugs that work, in part, via inhibition of CYP, is often accompanied by fever and other untoward effects such as chills, headache, rash, nausea, renal dysfunction, respiratory distress, and body fluid electrolyte imbalance (145 - 151). We postulate that exacerbated and unusually high fevers frequently seen in such patients and in infants and children (152) may result from a deficiency in the endogenous antipyretic mechanisms. Conversely, lack of a history of fever in cancer patients [*clinical oncologists repeatedly report that cancer patients stress in their history that they did not suffer from a febrile illness before the onset of cancer*; see review by Kleef *et al*., (153)] may indicate that there is a higher expression of endogenous antipyresis in these patients. Consistent with this notion are laboratory and clinical studies indicating a potent tumor-promoting activity of LTs (see, e.g., 154). High expression of the endogenous antipyresis has also been proposed to explain lack of fever in females at term of pregnancy (112, 113) and an impaired febrile response in the elderly (57, 155). Clearly, the mechanisms of *endogenous antipyresis* deserve the best of our scientific attention to understand these important clinical observations.

9. ACKNOWLEDGEMENTS

This research was partially supported by Grant No 98-00434 from the United States - Israel Binational Science Foundation (BSF; Jerusalem, Israel), and by National Institute of Health (NIH/NIAID) Grant No AI27556 (USA).

10. REFERENCES

1. Ivanov A. I. and A. A. Romanovsky: Prostaglandin E_2 as a mediator of fever: synthesis and catabolism. *Front Biosci* 9, 1977-1993 (2004)

2. Manev H., T. Uz, K. Sugaya and T. Qu: Putative role of neuronal 5-lipoxygenase in an aging brain. *FASEB J* 14, 1464-1469 (2000)

3. O'Rourke S. T. and T. A. Rudy: Intracerebroventricular and preoptic injection of leukotriene C_4, D_4, and E_4 in the rat: lack of febrile effect. *Brain Res* 295, 283-288 (1984)

4. Brus R., T. Krzeminski, Z. Juraszczyk, A. Kurcok, W. Felinska and W. Kozik: Central effects of leukotriene in rats and mice. *Biomed Biochim Acta* 45, 1153-1158 (1986)

5. Mashburn Jr. T. A., Q. J. Llanos, R. A. Ahokas and C. M. Blatteis: Thermal and acute-phase protein responses of guinea pigs to intrapreoptical injections of leukotrienes. *Brain Res* 376, 285-291 (1986)

6. Bhattacherjee P., N. K. Boughton-Smith, R. L. Follenfant, L. G. Garland, G. A. Higgs, H. F. Hodson, P. J. Islip, W. P. Jackson, S. Moncada, A. N. Payne, R. W. Randall, C. H. Reynolds, J. A. Salmon, J. E. Tateson and B. J. R. Whittle: The effect of the novel series of selective inhibitors of arachidonate 5-lipoxygenase on anaphylactic and inflammatory responses. *Ann NY Acad Sci* 542, 307-320 (1988)

7. Cornell R. P: Central interleukin 1-elicited hyperinsulinemia is mediated by prostaglandins but not autonomics. *Am J Physiol* 276, R52-R58 (1989)

8. Hynes N., I. Bishai, J. Lees and F. Coceani: Leukotrienes in brain: natural occurrence and induced changes. *Brain Res* 553, 4-13 (1991)

9. Muller-Peddinghaus K., R. C. Kohlsdorfer, P. Theisen-

Popp, R. Fruchtmann, E. Perzborn, B. Beckermann, K. Buhner, H. J. Ahr and K. H. Mohrs: BAY X1005, a new inhibitor of leukotriene synthesis: *in vivo* inflammation pharmacology and pharmacokinetics. *J Pharmacol Exp Ther* 267, 51-57 (1993)

10. Sri Kantra S., H. Matsumura, E. Kubo, K. Kawase, R. Takahata, C. N. Serhan and O. Hayaishi: Effects of prostaglandin D_2, lipoxins and leukotrienes on sleep and brain temperature of rats. *Prostaglandins Leukot Essent Fatty Acids* 51, 87-93 (1994)

11. Kozak W., I. Archuleta, K. P. Mayfield, A. Kozak, K. Rudolph and M. J. Kluger: Inhibitors of alternative pathways of arachidonate metabolism differentially affect fever in mice. *Am J Physiol* 275, R1031-R1040 (1998)

12. Paul L., V. Fraifeld and J. Kaplanski: Evidence supporting involvement of leukotrienes in LPS-induced hypothermia in mice. *Am J Physiol* 276, R52-R58 (1999)

13. Fraifeld V., L. Paul and J. Kaplanski. The relationship between hypothalamic prostaglandin E_2 or leukotrienes and the body temperature response to lipopolysaccharide in different murine strains. *J Therm Biol* 25, 17-20 (2000)

14. Fraifeld V., L. Paul, J. Kaplanski, W. Kozak and M. J. Kluger: Evidence for the involvement of eicosanoids in the regulation of normal body temperature. *J Therm Biol* 26, 295-297 (2001)

15. Samuelsson B: Leukotrienes: mediators of immediate hypersensitivity reactions and inflammation. *Science* 220, 568-575 (1983)

16. Bigby T. D: The yin and the yang of 5-lipoxygenase pathway activation. *Mol Pharmacol* 62, 200-202 (2002)

17. Werz O., E. Burkert, L. Fischer, D. Szellas, D. Dishart, B. Samuelsson, O. Radmark and D. Steinhilber: Extracellular signal-regulated kinases phosphorylate 5-lipoxygenase and stimulate product formation in leukocytes. *FASEB J* 16, 1441-1443 (2002)

18. Woods J. W., J. F. Evans, D. Ethier, S. Scott, P. J. Vickers, L. Hearn, J. A. Heiben, S. Charleson and I. I. Singer: 5-Lipoxygenase and 5-lipoxygenase-activating protein are localized in the nuclear envelope of activated human lekocytes. *J Exp Med* 178, 35-1946 (1993)

19. Qu T., T. Uz and H. Manev: Inflammatory 5-LOX mRNA and protein are increased in brain of aging rats. *Neurobiol Aging* 21, 647-652 (2000)

20. Burkert E., D. Szellas, O. Radmark, Steinhilber and O. Werz: Cell type-dependent activation of 5-lipoxygenase by arachidonic acid. *J Leukoc Biol* 73, 191-200 (2003)

21. Fischer L., D. Szellas, O. Radmark, D. Steinhilber and O. Werz: Phosphorylation- and stimulus-dependent inhibition of cellular 5-lipoxygenase activity by nonredox-type inhibitors. *FASEB J* 17, 949-951 (2003)

22. Dixon R. A., R. E. Diehl, E. Opas, E. Rands, P. J. Vickers, J. F. Evans, J. W. Gillard and D. K. Miller: Requirement of a 5-lipoxygenase-activating protein for leukotriene synthesis. *Nature* 343, 282-284 (1990)

23. Mancini J. A., M. Abramovitz, M. E. Cox, E. Wong, S. Charleson, H. Perrier, Z. Wang, P. Prasit and P. J. Vickers: 5-lipoxygenase-activating protein is an arachidonate binding protein. *FEBS Lett* 318, 277-281 (1993)

24. Peters-Golden M: Cell biology of the 5-lipoxygenase pathway. *Am J Resp Crit Care Med* 157, S227-S232 (1998)

25. Lindgren J. A., T. Hokfelt, S. E. Dahlen, C. Patrono and B. Samuelsson: Leukotrienes in the rat central nervous system. *Proc Natl Acad Sci USA* 81, 6212-6215 (1984)

26. Lammers C. H., P. Schweitzer, P. Facchinetti, J. M. Arrang, S. G. Madamba, G. R. Siggins and D. Piomelli: Arachidonate 5-lipoxygenase and its activating protein: prominent hippocampal expression and role in somatostatin signaling. *J Neurochem* 66, 147-152 (1996)

27. Miyamoto T., J. A. Lindgren, T. Hokfelt and B. Samuelsson: Regional distribution of leukotriene and mono-hydroxyeicosatetraenoic acid production in the rat brain. Highest leukotriene C4 formation in the hypothalamus. *FEBS Lett* 216, 123-127 (1987)

28. Schalling M., A. Neil, L. Terenius, J. A. Lindgren, T. Miyamoto, T. Hokfelt and B. Samuelsson: Leukotriene C_4 binding sites in the rat central nervous system. *Eur J Pharmacol* 122, 251-257 (1986)

29. Chen N. and C. S. Reiss: Distinct roles of eicosanoids in the immune response to viral encephalitis: or why you should take NSAIDs. *Viral Immunol.* 15, 133-146 (2002)

30. Hui Y. and C. D. Funk: Cysteinyl leukotriene receptors. *Biochem Pharmacol* 64, 1549-1557 (2002)

31. Jones S. M., M. Luo, M. Peters-Golden and T. G. Brock: Identification of two novel nuclear import sequences on the 5-lipoxygenase protein. *J Biol Chem* 278, 10257-10263 (2003)

32. Lepley R. A. and F. A. Fitzpatrick: 5-lipoxygenase contains a functional Src homology 3-binding motif that interacts with the Src homology 3 domain of Grb2 and cytoskeletal proteins. *J Biol Chem* 269, 24163-24168 (1994)

33. Provost P., B. Samuelsson and O. Radmark: Interaction of 5-lipoxygenase with cellular proteins. *Proc Natl Acad Sci USA* 96, 1881-1885 (1999)

34. Tegeder I., J. Pfeilschifter and G. Geisslinger: Cyclooxygenase-independent action of cyclooxygenase inhibitors. *FASEB J* 15, 2057-2072 (2001)

35. Branger J., B. van den Blink, S. Weijer, J. Madwed, C. L. Bos, A. Gupta, C. L. Yong, S. H. Polmar, D. P. Olszyna,

C. E. Hack, S. J. van Deventer, M. P. Peppelenbosch and T. Van Der Poll: Anti-inflammatory effects of p28 mitogen activated protein kinase inhibitor during human endotoxemia. *J Immunol* 168, 4070-4077 (2002)

36. Fessler M. B., K. C. Malcolm, M. W. Duncan and G. S. Worthen: A genomic and proteomic analysis of activation of the human neutrophil by lipopolysaccharide and its mediation by p38 mitogen-activated protein kinase. *J Biol Chem* 277, 31291-31302 (2002)

37. Uhl J., N. Klan, M. Rose, K.-D. Entian, O. Werz and D. Steinhilber: The 5-lipoxygenase promoter is regulated by DNA methylation. *J Biol Chem* 277, 4374-4379 (2002)

38. Hoshiko S., O. Radmar and B. Samuelsson: Characterization of the human 5-lipoxygenase gene promoter. *Proc Natl Acad Sci USA* 87, 9073-9077 (1990)

39. Riddick C. A., W. L. Ring, J. R. Baker, C. R. Hodulik and T. D. Bigby: Dexamethasone increases expression of 5-lipoxygenase and its activating protein in human monocytes and THP-1 cells. *Eur J Biochem* 246, 112-118 (1997)

40. Uz T., Y. Dwivedi, P. D. Savani, F. Impagnatiello, G. Pandey and H. Manev. Glucocorticoids stimulate inflammatory 5-lipoxygenase gene expression and protein translocation in the brain. *J Neurochem* 73, 693-699 (1999)

41. Steinhilber D., M. Brungs, O. Werz, I. Wiesenberg, C. Danielsson, J. P. Kahlen, S. Nayeri, M. Schrader and C. Carlberg: The nuclear receptor for melatonin represses 5-lipoxygenase gene expression in human B lymphocytes. *J Biol Chem* 270, 7037-7040 (1995)

42. Abul H. J., J. Davidson, A. S. Milton and D. Rotondo: Dexamethasone pre-treatment is antipyretic toward polyinosinic: polycytidylic acid, lipopolysaccharide and interleukin-1/endogenous pyrogen. *Naunyn Schmiedebergs Arch Pharmacol* 335, 305-309 (1987)

43. Kozak W., J. J. Klir, C. A. Conn and M. J. Kluger: Attenuation of lipopolysaccharide fever in rats by protein kinase C inhibitors. *Am J Physiol* 273, R873-R879 (1997)

44. Ham E. A., D. D. Soderman, M. E. Zanetti, H. W. Dougherty, E. McCauley and F. A. Kuehl Jr.: Inhibition by prostaglandins of leukotriene B_4 release from activated neutrophils. *Proc Natl Acad Sci USA* 80, 4349-4353 (1983)

45. Flamand F., M. E. Surette, S. Picard, S. Bourgoin and P. Borgeat: Cyclic AMP-mediated inhibition of 5-lipoxygenase translocation and leukotriene biosynthesis in human neutrophils. *Mol Pharmacol* 62, 250-256 (2002)

46. Milton A. S. and S. Wendlandt: A possible role for prostaglandin E_1 as a modulator for temperature regulation in the central nervous system of the cat. *J Physiol (London)* 207, 76P-77P (1970)

47. Blatteis C. M. and E. Sehic: Prostaglandin E_2: a putative fever mediator. In: *Fever: Basic Mechanism and Management, 2nd ed*. (Ed. Mackowiak PA), Lippincott-Raven, Philadelphia, PA, pp. 117-148 (1997)

48. Steiner A., A., J. Antunes-Rodrigues and L. G. S. Branco: Role of preoptic second messenger systems (cAMP and cGMP) in the febrile response. *Brain Res* 944(1-2), 135-145 (2002)

49. Gourine A. V., E. V. Melenchuk, D. M. Poputnikov, V. N. Gourine and K. M. Spyer: Involvement of purinergic signalling in central mechanisms of body temperature regulation in rats. *Br J Pharmacol* 135, 2047-2055 (2002)

50. Milton A. S: Thermoregulatory actions of eicosanoids in the central nervous system with particular regard to the pathogenesis of fever. *Ann NY Acad Sci* 559, 392-410 (1989)

51. Funk C. D: Prostaglandins and leukotrienes: Advances in eicosanoid biology. *Science* 294, 1871-1875 (2002)

52. Kluger M. J: Fever: role of pyrogens and cryogens. *Physiol Rev* 71, 93-127 (1991)

53. Székely M. and Z. Szelényi: Endotoxin fever in the rat. *Acta Physiol Acad Sci Hung* 53, 265-277 (1979)

54. Clemmer T. P., C. J. Fisher Jr., R. C. Bone, G. J. Slotman, C. A. Metz and F. O. Thomas: Hypothermia in the sepsis syndrome and clinical outcome. The Methylprednisolone Severe Sepsis Study Group. *Crit Care Med* 20:1395-401 (1992)

55. Romanovsky A. A., O. Shido, S. Sakurada, N. Sugimoto and T. Nagasaka: Endotoxin shock: thermoregulatory mechanisms. *Am J Physiol Regul Integr Comp Physiol* 270, R693-R703 (1996)

56. Dogan M. D., H. Ataoglu and E. S. Akarsu: Characterization of the hypothermic component of LPS-induced dual thermoregulatory response in rats. *Pharmacol Biochem Behav* 72: 143-50 (2002)

57. Fraifeld V. and J. Kaplanski: LPS-induced fever: species and age differences. *Prog Brain Res* 115, 141-157 (1998)

58. Romanovsky A: Signaling the brain in the early sickness syndrome: are sensory nerves involved? *Frontiers Biosci* 9: 494-504 (2004)

59. Satinoff E: Salicylate: action on normal body temperature in rats. *Science* 176, 532-533 (1972)

60. Scales W. E. and M. J. Kluger: Effect of antipyretic drugs on circadian rhythm in body temperature of rats. *Am J Physiol* 253, R306-R313 (1987)

61. Fraifeld V., L. Paul, J. Kaplanski, H. Asraf, O. Sagi, M. Wolfson, W. Kozak, M. J. Kluger, T. Tchaikovskaya and I.

Listowsky: 5-lipoxygenase and cytochrome P-450 pathways of arachidonate metabolism contribute to endogenous cryogenesis. *Neural Plasticity* 10, 197-198 (2003)

62. Agarwal R., Z. Y. Wang, D. P. Bik and H. Mukhtar: Nordihydroguaiaretic acid, an inhibitor of lipoxygenases, also inhibits cytochrome P450-mediated monooxygenase activity in rat epidermal and hepatic microsomes. *Drug Metab Dispos* 19, 620-624 (1991)

63. Paul L., V. Fraifeld and J. Kaplanski. Lack of febrile response to LPS in 5-lipoxygenase-deficient mice. *Arch Pharmacol* 358 (Suppl. 2), R724 (Abstract) (1998)

64. Kluger M. J., W. Kozak, L. R. Leon, D. Soszynski, and C. Conn: Fever and antipyresis. *Prog Brain Res* 115, 465-475 (1998)

65. Cinti D. L. and M. B. Feinstein: Platelet cytochrome P450: a possible role in arachidonate-induced aggregation. *Biochim Biophys Res Commun* 73, 171-179 (1976)

66. Capdevila J. H., N. Chacos, J. Werringloer, R. A. Prough and R. W. Estabrook: Liver microsomal cytochrome P450 and the oxidative metabolism of arachidonic acid. *Proc Natl Acad Sci USA* 78, 5362-5366 (1981)

67. Morrison A. R. and N. Pascoe: Metabolism of arachidonic acid through NADPH-dependent oxygenase of renal cortex. *Proc Natl Acad Sci USA* 78, 7375-7378 (1981)

68. Oliw E. H., F. P. Guengerich and J.A. Oates: Oxygenation of arachidonic acid by hepatic monooxzygenases. *J Biol Chem* 257, 3771-3781 (1982)

69. McGiff J. C: Cytochrome P450 metabolism of arachidonic acid. *Annu Rev Pharmacol Toxicol* 31, 339-369 (1991)

70. Capdevila J. H., D. Zeldin, K. Makita, A. Karara and J. R. Falck: Cytochrome P450 and the metabolism of arachidonic acid and oxygenated eicosanoids. In: *Cytochrome P450: Structure, Mechanism, and Biochemiostry*. (Ed. Ortiz de Montellano PR), Plenum Press, NY, pp. 443-471 (1995)

71. Node K., Y. Huo, X. Ruan, B. Yang, M. Spiecker, K. Ley, D. C. Zeldin and J. K. Liao: Anti-inflammatory properties of cytochrome P450 epoxygenase-derived eicosanoids. *Science* 285, 1276-1279 (1999)

72. Quilley J. and J. C. McGiff: Is EDHF an epoxyeicosatrienoic acid? *Trends Pharmacol Sci* 21, 121-124 (2000)

73. Imig J. D: Epoxygenase metabolites: Epithelial and vascular actions. *Mol Biotech* 16, 233-251 (2000)

74. Campbell, W. B: New role for epoxyeicosatrienoic acids as anti-inflammatory mediators. *Trends Pharmacol Sci* 21, 125-127 (2000)

75. Kozak W., M. J. Kluger, J. Tesfaigzi, A. Kozak, K. P. Mayfield, M. Wachulec and K. Dokladny: Molecular mechanisms of fever and endogenous antipyresis. *Ann NY Acad Sci* 917, 121-134 (2000)

76. Roman R. J: Cytochrome P450 metabolites of arachidonic acid in the control of cardiovascular function. *Physiol Rev* 82, 131-185 (2002)

77. Capdevila J. H., J. R. Falck and R. C. Harris: Cytochrome P450 and arachidonic acid bioactivation: molecular and functional properties of the arachidonate monooxygenases. *J Lipid Res* 41, 163-181 (2000)

78. Karara A., E. Dishman, I. Blair, J. R. Falck and J. H. Capdevila: Endogenous epoxyeicosatrienoyl-phospholipids. A novel class of cellular glycerolipids containing epoxidized arachidonate moieties. *J Biol Chem* 269, 7561-7569 (1991)

79. Yoder E. J., M. VanRollins and S. A. Moore: Epoxyeicosatrienoic acid (EET) metabolism by cerebral endothelium (Abstract). *FASEB J* 9, A374 (1995)

80. VanRollins M., T. L. Kaduce, X. Fang, H. R. Knapp and A. A. Spector: Arachidonic acid diols produced by cytochrome P450 monooxygenases are incorporated into phospholipids of vascular endothelial cells. *J Biol Chem* 271, 14001-14009 (1996)

81. Fitzpatric F. A. and R. C. Murphy: Cytochrome P450 metabolism of arachidonic acid: formation and biological actions of "epoxygenase"-derived eicosanoids. *Pharmacol Rev* 40, 229-241 (1989)

82. Oliw E. H: Oxygenation of polyunsaturated fatty acids by cytochrome P450 monooxygenases. *Prog Lipid Res* 33, 329-354 (1994)

83. Morgan E. T: Regulation of cytochromes P450 by inflammatory mediators: Why and How. *Drug Metab Disp* 29, 207-212 (2001)

84. Nebert D. W., D. R. Nelson, M. J. Coon, R. W. Estabrook, R. Feyereisen, Y. Fujii-Kuriyama, F. J. Gonzalez, F. P. Guengerich, I. C. Gunsalus, E. F. Johnson, J. C. Loper, R. Sato, M. R. Waterman and D. J. Waxman: The cytochrome P450 superfamily: update on new sequences, gene mapping, and recommended nomenclature. *DNA Cell Biol* 10, 1-14 (1991)

85. Nelson D. R., L. Koymans, T. Kamataki, J. J. Stegeman, R. Feyereisen, D. J. Waxman, M. R. Waterman, O. Gotoh, M. J. Coon, R. W. Estarbrook, I. C. Gunsalus and D. W. Nebert: Cytochrome P450 superfamily: update on new sequences, gene mapping, accession numbers and nomenclature. *Pharmacogenetics* 6, 1-42 (1996)

86. Lu A. Y. H. and S. B. West: Multiplicity of mammalian

microsomal cytochromes P450. *Pharmacol Rev* 31, 277-295 (1980)

87. Morgan E. T. and J. A. Gustafsson: Sex-specific isozymes of cytochrome P450. *Steroids* 49, 213-245 (1987)

88. Morgan E. T: Suppresion of constitutive cytochrome P450 gene expression in livers of rats undergoing an acute phase response to endotoxin. *Mol Pharmacol* 36, 699-707 (1989)

89. Karara A., E. Dishman, J. R. Falck and J.H. Capdevila: Endogenous epoxyeicosatrienoic acids. *J Biol Chem* 264, 19822-19827 (1989)

90. Warner M., C. Kohler, T. Hansson and J. A. Gustafsson: Regional distribution of cytochrome P450 in the brain: spectral quantitation and contribution of CYP b,e, and c,d. *J Neurochem* 50, 1057-1065 (1988)

91. Warner M., M. Stromstedt, A. Wyss and J. A. Gustafsson: Regulation of cytochrome P450 in the central nervous system. *J Steroid Biochem Mol Biol* 47, 191-194 (1993)

92. Warner M. and J. A. Gustafsson: Cytochrome P450 in the brain: neuroendocrine functions. *Front Neuroendocrin* 16, 224-236 (1995)

93. Hagihara K., S. Shisaka, Y. Lee, J. Kato, O. Hatano, A. Takakusu, Y. Emi, T. Omura and M. Tohyama: Presence of sex difference of cytochrome P450 in the rat preoptic area and hypothalamus with reference to coexistence with oxytocin. *Brain Res* 515, 69-78 (1990)

94. Capdevila J. H., R. C. Harris and J. R. Falck: Microsomal cytochrome P450 and eicosanoid metabolism. *Cell Mol Life Sci* 59, 780-789 (2002)

95. Baron J. M., G. Zwadlo-Klarwasser, F. Jugert, W. Hamann, A. Rubben, H. Mukhtar and H. F. Merk: Cytochrome P450 1B1: a major CYP isoenzyme in human blood monocytes and macrophage subsets. *Biochem Pharmacol* 56, 1105-1110 (1998)

96. Carcillo J. A., R. A. Parise, A. Adedoyin, R. Frye, R. A. Branch and M. Romkes: CYP2D6 mRNA expression in circulating peripheral blood mononuclear cells correlates with *in vivo* debrisoquine hydroxylase activity in extensive metabolizers. *Res Commun Mol Pathol Pharmacol* 91, 149-159 (1996)

97. Wong P. Y., P. S. Lai and J. R. Falck: Mechanism of signal transduction of 14 R, 15 (S)-epoxyeicosatrienoic acid (14,15-EET) binding in guinea pig monocytes. *Prostaglandins Other Lipid Mediat* 62, 321-333 (2000)

98. Chang K. C., B. A. Lauer, T. D. Bell and H. Chai: Altered theophylline pharmacokinetics during acute respiratory viral illness. *Lancet* 1, 1132-1133 (1978)

99. Shedlofsky S. I., B. C. Israel, C. J. McClain, D. B. Hill and R. A. Blouin: Endotoxin administration to humans inhibits hepatic cytochrome P450-mediated drug metabolism. *J Clin Invest* 94, 2209-2214 (1994)

100. Renton K. W: Alteration of drug biotransformation and elimination during infection and inflammation. *Pharmacol Ther* 92, 147-63 (2001)

101. Mannering G. J. and L. B. Deloria: The pharmacology and toxicology of interferons: an overview. *Annu Rev Pharmacol Toxicol* 26, 455-515 (1986)

102. Sakai H., T. Okamoto and Y. Kikkawa: Suppression of hepatic drug metabolism by the interferon inducer, polyriboinosinic:polyribocitidylic acid. *J Pharmacol Exp Therap* 263, 381-386 (1992)

103. Abdel-Razzak Z., P. Loyer, A. Fautrel, J. C. Gautier, L. Corcos, B. Turlin, P. Beaune and A. Guillouzo: Cytokines down-regulate expression of major cytochrome P450 enzymes in adult human hepatocytes in primary culture. *Mol Pharmacol* 44, 707-715 (1993)

104. Khatsenko O. G., S. S. Gross, A. B. Rifkind and J. R. Vane: Nitric oxide is a mediator of the decrease in cytochrome P450-dependent metabolism caused by immunostimulants. *Proc Natl Acad Sci USA* 90, 11147-11151 (1993)

105. Morgan E. T., K. B. Thomas, R. Swanson, T. Vales, J. Hwang and K. Wright: Selective suppression of cytochrome P450 gene expression by interleukins 1 and 6 in rat liver. *Biochim Biophys Acta* 1219, 475-483 (1994)

106. Ansher S. S. and W. Thompson: Modulation of hepatic mRNA levels after administration of lipopolysaccharide and diphtheria and tetanus toxoids and pertussis vaccine adsorbed (DTP vaccine) to mice. *Hepatology* 20, 984-991 (1994)

107. Bertini R., M. Bianchi, A. Erroi, P. Villa and P. Ghezzi: Dexamethasone modulation of *in vivo* effects of endotoxin, tumor necrosis factor, and interleukin-1 on liver cytochrome P450, plasma fibrinogen, and serum iron. *J Leukoc Biol* 46, 254-262 (1989)

108. MacGeoch C., E. T. Morgan and J. A. Gustafsson: Hypothalamo-pituitary regulation of cytochrome P450 15b apoprotein levels in rat liver. *Endocrinology* 117, 2085-2092 (1985)

109. Kasting N. W. and J. B. Martin: Altered release of growth hormone and thyrotropin induced by endotoxin in the rat. *Am J Physiol* 243, E332-E337 (1982)

110. Ghezzi P., B. Saccardo, P. Villa, V. Rossi, M. Bianchi and C. A. Dinarello: Role of interleukin-1 in the depression of liver drug metabolism by endotoxin. *Infect Immun* 54, 837-840 (1986)

111. Negro-Vilar A., G. Snyder, J. Falck, S. Manna, N. Chacos and J. Capdevilla: Involvement of eicosanoids in

release of oxitocin and vasopressin from the neural lobe of the rat pituitary. *Endocrinology* 116, 2663-2668 (1985)

112. Cooper K. E: The neurobiology of fever: thoughts on recent developments. *Ann Rev Neurosci* 10, 297-324 (1987)

113. Kasting N. W: Criteria for establishing a physiologic role for brain peptides. A case in point: the role of vasopressin in thermoregulation during fever and antipyresis. *Brain Res Rev* 14, 143-153, (1989)

114. Lipton J. M. and A. Catania: Anti-inflammatory actions of the neuroimmunomodulator alphaMSH. *Immunology Today* 18, 140-145 (1997)

115. Schwartzman M., N. R. Ferreri, M. A. Carroll, E. Songu-Mize and J. C. McGiff: Renal cytochrome P450-related arachidonate metabolite inhibits ($Na^+ + K^+$)ATPase. *Nature* 314, 620-622 (1985)

116. Davis K. L., M. W. Dunn and M. L. Schwartzman: Hormonal stimulation of 12(R)-HETE, a cytochrome P450 arachidonic acid metabolite in the rabbit cornea. *Curr Eye Res* 9, 661-667 (1990)

117. Yoshida S., A. Hirai, Y. Tamura and S. Yoshida: Possible involvement of arachidonic acid metabolism of cytochrome P450 monooxygenase pathway in vasopressin-stimulated glycogenolysis in isolated rat hepatocytes. *Arch Biochem Biophys* 280, 346-351 (1990)

118. Vazquez B., A. Rios and B. Escalante: Arachidonic acid metabolism modulates vasopressin-induced renal vasoconstriction. *Life Sci* 56, 1455-1466 (1995)

119. Gibson G. G. and B. G. Lake: Induction protocols for the cytochrome P450 IVA subfamily in animals and primary hepatocyte cultures. *Methods Enzymol* 206, 353-364 (1991)

120. Kozak W., M. J. Kluger, A. Kozak, M. Wachulec and K. Dokladny: Role of cytochrome P450 in endogenous antipyresis. *Am J Physiol* 279, R455-R460 (2000)

121. Wardlaw S. A., K. J. Nikula, D. A. Kracko, G. L. Finch, J. R. Thornton-Manning and A. R. Dahl: Effect of cigarette smoke on CYP1A1, CYP1A2 and CYP2B1/2 of nasal mucosae in F344 rats. *Carcinogenesis* 19, 655-662 (1998)

122. Nakashima T., Y. Harada, S. Miyata and T. Kiyohara: Inhibitors of cytochrome P450 augment fever induced by interleukin-1beta. *Am J Physiol* 271, R1274-R1279 (1996)

123. Kozak W., K. P. Mayfield, A. Kozak and M. J. Kluger: Proadifen (SKF-525A), an inhibitor of cytochrome P450, augments LPS-induced fever and exacerbates prostaglandin E_2 levels in the rat. *J Therm Biol* 25, 45-50 (2000)

124. Fantuzzi G., L. Cantoni, M. Sironi and P. Ghezzi: Inhibitors of cytochrome P450 suppress tumor necrosis factor production. *Cell Immunol* 150, 417-424 (1993)

125. Spangelo B. L., W. D. Jarvis, A. M. Judd and A. M. MacLoed: Induction of interleukin-6 release by interleukin-1 in rat anterior pituitary cells *in vitro*: evidence for an eicosanoid-dependent mechanism. *Endocrinology* 129, 2886-2894 (1991)

126. Hinson R. M., J. A. Williams and E. Shacter: Elevated interleukin 6 is induced by prostaglandins E_2 in a murine model of inflammation: possible role of cyclooxygenase-2. *Proc Natl Acad Sci USA* 93, 4885-4890 (1996)

127. Ohishi S., L. Utsunomija, T. Yamamoto, Y. Komuro and Y. Hara: Effects of prostaglandins and cyclic AMP on cytokine production in rat leukocytes. *Eur J Pharmacol* 300, 255-259 (1996)

128. Kozak W., D. M. Aronoff, O. Boutaud and A. Kozak: 11,12-epoxyeicosatrienoic acid attenuates synthesis of prostaglandin E_2 in rat monocytes stimulated with lipopolysaccharide. *J Exp Biol Med* 228, 786-794 (2003)

129. Nakashima T., Y. Yoshida, S. Miyata and T. Kiyohara: Hypothalamic 11,12-epoxyeicosatrienoic acid attenuates fever induced by central interleukin-1beta in the rat. *Neurosci Lett* 310, 141-144 (2001)

130. Johansson C., A. Stark, M. Sandberg, B. Ek, L. Rask and J. Meijer: Tissue specific basal expression of soluble murine epoxide hydrolase and effects of clofibrate on the mRNA levels in extrahepatic tissues and liver. *Arch Toxicol* 70, 61-3 (1995)

131. Schilter B., M. R. Andersen, C. Acharya and C. J. Omiecinski: Activation of cytochrome CYP gene expression in the rat brain by phenobarbital-like inducers. *J Pharmacol Exp Ther* 294, 916-22 (2000)

132. Krunic N., S. L. Adamson, C. Ackerey, R. T. Okita and F. Coceani: Perinatal changes in choroidal 15-hydroxyprostagandin dehydrogenase: implications for prostaglandin removal from brain. *Brain Res Dev Brain Res* 121, 145-155 (2000)

133. Ivanov A. I., A. C. Scheck and A. A. Romanovsky: Expression of genes controlling transport and catabolism of prostaglandin E_2 in lipopolysaccharide fever. *Am J Physiol* 284, R698-R706 (2003)

134. Boutaud O., D. M. Aronoff, J. H. Richardson, L. J. Marnett and J. A. Oates: Determinants of the cellular specificity of acetaminophen as an inhibitor of prostaglandin H(2) synthases. *Proc Nat Acad Sci USA* 99, 7130-7135 (2002)

135. Aronoff D. M., O. Boutaud, L. J. Marnett and J. A. Oates: Inhibition of prostaglandin H_2 synthases by salicylate is dependent on the oxidative state of the enzyme. *J Pharmacol Exp Ther* 304, 589-595 (2003)

136. DuBois E. F: Why are fever temperatures over 106°F

rare? *Am J Med Sci* 217, 361-368 (1949)

137. Veale W. L., N. W. Kasting and K. E. Cooper: Arginine vasopressin and endogenous antipyresis: evidence and significance. *Fed Proc* 40, 2750-2759 (1981)

138. Zeisberger E., G. Merker, S. Blahser and M. Krannig: Role of vasopressin in fever regulation. In: *Homeostasis and Thermal Stress.* (Ed: Cooper KE, Lomax P, Schonbaum E, and Veale WL), Karger-Basel, pp. 62-65 (1986)

139. Leon R. L: Hypothermia in systemic inflammation: role of cytokines. *Front Biosci* 9, 1877-1888 (2004)

140. Gourine A. V., N. Dale, V. N. Gourine and M. Spyer: Fever in systemic inflammation: roles of purines. *Front Biosci* 9, 1011-1022 (2004)

141. Roth J., E. Zeisberger, S. Vybíral and L. Jansky: Endogenous antipyretics: neuropeptides and glucocorticoids. *Front Biosci* 9, 816-826 (2004)

142. Szelényi Z., M. Székely, Z. Hummel, M. Balaskó, A. A. Romanovsky and E. Pétervári: Cholecystokinin: possible mediator of fever and hypothermia. *Front Biosci* 9, 301-308, (2004)

143. Steiner A. A. and L. G. S. Branco: Fever and anapyrexia in systemic inflammation: intracellular signaling by cyclic nucleotides. *Front Biosci* 8. s1398-s1408 (2003)

144. Rosman N. P: Evaluation and management of febrile seizures. *Curr Opin Pediatr* 1, 318-323 (1989)

145. Annalaro C., A. Oriana, E. Tagliaferri, V. Bertolli, A. Della Volpe, D. Soligo, A. Ibatici, E. Pozzoli and G. L. Lambertenghi Deliliers: Efficacy of different prophylactic antifungal regimens in bone marrow transplantation. *Haematologica* 80, 512-517 (1995)

146. Barton T. D. and M. G. Schuster: The cause of fever following resolution of neutropenia in patients with acute leukemia. *Clin Infect Dis* 22, 1064-1068 (1996)

147. Carpentier A. F., L. Bernard, M. Poisson and J. Y. Delattre: Central nervous system infections in patients with malignant disease. *Rev Neurolog (Paris) 152*, 587-601 (1996)

148. Gonzalez C. E., D. Shetty, L. L. Lewis, B. U. Mueller, P. A. Pizzo and T. J. Walsh: Cryptococcosis in human immunodeficiency virus-infected children. *Ped Infect Dis J* 15, 796-800 (1996)

149. Martino P. and C. Girmenia: Diagnosis and treatment of invasive fungal infections in cancer patients. *Supp Care Cancer* 1, 240-244 (1993)

150. Rabinovici R., D. Szewczyk, P. Ovadia, J. R. Greenspan, and J. J. Sivalingam: Candida pericarditis: clinical profile and treatment. *Ann Thoracic Surg* 63, 1200-1204 (1997)

151. Viscoli C., E. Castagnola, M. T. Van Lint, C. Moroni, A. Garaventa, M. R. Rossi, R. Fanci, F. Menichetti, D. Caselli, M. Giachcino and M. Congiu: Fluconazole versus amphotericin B as empirical antifungal therapy of unexplained fever in granulocytopenic patients: a pragmatic, multicentre, prospective and randomised clinical trial. *Eur J Cancer* 32A, 814-820 (1996)

152. Crocetti M., N. Moghbeli and J. Serwint: Fever phobia revisited: have parental misconceptions about fever changed in 20 years? *Pediatrics* 107, 1241-1246 (2001)

153. Kleef R., W. B. Jones, W. Knogler and W. Stenzinger: Fever, cancer incidence and spontaneous remissions. *Neuroimmunomodulation* 9, 55-64 (2001)

154. Steele, V. E., C. A. Holmes, E. T. Hawk, L. Kopelovich, R. A. Lubet, J. A. Crowell, C. C. Sigman and G. J. Kelloff: Potential use of lipoxygenase inhibitors for cancerchemoprevention. *Expert Opin Investig Drugs* 9, 2121-38 (2000)

155. Fraifeld V., L. Abramovich and J. Kaplanski: Delayed febrile response in old rats is not associated with an inability of hypothalamus to produce prostaglandin E_2. *Mech Ageing Dev* 79, 137-140 (1995)

Key Words: Body temperature, Thermoregulation, Fever, Endogenous antipyresis, Anapyrexia, Eicosanoids, Lipoxygenases, Leukotrienes, Monooxygenases, Epoxygenases, Epoxyeicosatrienoic acids, Cytokines, Feedback mechanism, Cyclooxygenase inhibition, Mice, Rats, Macrophages, Hypothalamus, Review

Send correspondence to: Wieslaw Kozak, PhD, Department of Immunology, Institute of General and Molecular Biology, Faculty of Biology and Earth Science, Nicolas Copernicus University, 87-100 Torun, Poland, Tel: 48-56-611-4948, Fax: 48-56-4772, E-mail: wkozak@biol.uni.torun.pl