Molecular Mechanisms of Fever and Endogenous Antipyresis

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ABSTRACT: This review summarizes recent studies on endogenous antipyretic mechanisms. Fever is the result of a balance between pyrogenic and cryogenic cytokines and hormones. Although there is considerable evidence that fever evolved as a host defense response, it is important that the rise in body temperature not be too high. Many endogenous cryogens or antipyretics that limit the rise in body temperature have been identified during the last 25 years. These include α-MSH, arginine vasopressin, glucocorticoids, TNF (under certain circumstances), and IL-10. Most recently, evidence has accumulated that cytochrome *P*-450 (*P*-450), part of the alternative pathway for arachidonic acid metabolism, plays an important role in reduction of fever and inflammation. Supporting a role for *P*-450 in endogenous antipyresis and antiinflammation includes evidence that (1) inducers of *P*-450 reduce fever, (2) inhibitors of *P*-450 cause a larger fever, (3) and *P*-450 arachidonic acid metabolites reduce fever.

INTRODUCTION

In the preface to the *Molecular Mechanisms of Fever,*[1] Kluger suggests that "One reason basic researchers have been attracted to this [fever] area is that fever is an example of a *regulated* change in homeostasis" and that "fever represents a *physiology of change.*" Indeed, since the classic work by Liebermeister in the middle of nineteenth century, it has been known that temperature regulation is not disrupted during fever. Rather, an individual upon contracting a disease associated with fever (e.g., infection, inflammation, trauma, and some tumors) regulates body temperature at a higher level than does a healthy individual. Hence, it is believed that the febrile temperature is a result of the upward resetting of the controlling variables, the setpoint of thermoregulation, presumably in the preoptic-anterior hypothalamus. On the other hand, since a provocative paper by DuBois published in 1949,[2] investigators and clinicians have appreciated that the febrile range has an upper limit,[3] in that

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the elevation of body temperature during fever does not exceed safe measures. In other words, the process of resetting the thermoregulatory set point upward during disease is controlled and, apparently, must be carefully guarded from reaching a dangerously high level. In this respect, fever appears to be a tightly controlled and regulated response which, on the one hand, reflects the shift of the set point of thermoregulation and, on the other hand, involves mechanisms acting to prevent fever from reaching dangerous heights. These latter mechanisms have been collectively called *endogenous antipyresis.* The study of the mechanisms underlying the generation of fever has a longer history than the investigation of endogenous antipyresis. Accordingly, there is a great deal more knowledge regarding endogenous pyretic than antipyretic mechanisms.[4] This review summarizes recent studies on endogenous antipyretic mechanisms.

MOLECULAR COMPONENTS OF FEVER AND ANTIPYRESIS

Over the past 25 years, evidence has accumulated that the endogenous febrile and antipyretic systems are operating at the level of neuroendocrino-immunomodulation. In fact, studies into the mechanisms underlying fever and endogenous antipyresis have significantly contributed to our understanding of the interaction between the immune system and central nervous system (CNS). Fever is triggered by the release of *endogenous pyrogens* from a large number of various immune types of cells including macrophage-like cells. Endogenous mediators of fever include cytokines, among which interleukin-1 (IL-1) and IL-6 are considered most important.[5,6] Inhibition of the production of one of these cytokines or neutralization of their activity results either in abrogation (e.g., if IL-6 is attenuated) or in substantial reduction (e.g., if IL-1β is attenuated) of fever during systemic or localized experimental inflammation.[7–9]

The search for *endogenous antipyretics* responsible for limiting the height of the febrile response began with studies of periparturient ewes and newborn lambs demonstrating refractoriness to pyrogenic effects of lipopolysaccharide (LPS).[10] Subsequent studies revealed that arginine vasopressin (AVP),[11,12] α-melanocyte-stimulating hormone (αMSH),[13,14] and glucocorticoids[15,16] can act as endogenous antipyretics opposing the effects of pyrogens. This led to the notion that fever is the result of a balance between pyrogenic cytokines and antipyretic hormones modulating the action of cytokines, presumably at the CNS level.[4] Nevertheless, components of the endogenous antipyresis can be also demonstrated within the cytokine network. Studies from our laboratory and others, using various techniques of neutralization of the cytokines (antibodies, inactivating binding proteins and receptors) as well as gene-deletion techniques (cytokine gene knockout mice), revealed that, besides pyrogenic cytokines, there are also cytokines that can be defined as endogenous antipyretics. They include tumor necrosis factor-α (TNF-α) and IL-10.

On the basis of data demonstrating that the injection of TNF-α induced elevation of body temperature in, for example, rabbits and humans,[4–6] this cytokine has been regarded as an endogenous pyrogen. Surprisingly, Long *et al.*[17] reported that pretreatment with TNF-α antiserum, instead of preventing fever, enhanced the febrile response to LPS in rats. In subsequent studies in mice, we observed that TNF-α can

cause a drop in body temperature rather than fever.[18] A decrease in temperature induced by injection of TNF-α has also been observed in rats.[19] Furthermore, we observed that TNF-α prevented fever in mice[18] and rats[20] if co-injected with LPS. More interesting, unlike IL-1β and IL-6, inactivation of TNF-α provokes higher LPS-induced fever in mice,[18] the effect previously seen in rats. In support of these data, mice deficient in genes for production of the signaling receptors for TNF-α, p55, and p75 receptors (TNF double receptor-knockout mice) respond with a higher fever to injection of LPS compared to wild-type mice.[21] IL-10 knockout mice also demonstrated a higher fever to LPS than control wild-type mice.[22] Accordingly, injection of a recombinant murine IL-10 into mice does not generate fever. It does prevent, however, the LPS-induced elevation of body temperature in mice.[22] A similar inhibitory effect of IL-10 on fever has also been observed in humans.[23] Thus, one can hypothesize that, in addition to the balance between cytokines and hormones, the height of fever can also be affected and regulated by a balance between pyrogenic and antipyretic cytokines. The cytokine-to-cytokine effects during fever may be accompanied and further supplemented by the interactions of cytokines with a number of cytokine receptor antagonists, inhibitory soluble/shedded cytokine receptors, inhibitory cytokine-binding proteins, and cytokine-chaperon proteins,[5] which are also ascribed to the continuously growing cytokine family. Although this matter needs thorough investigation, the inhibitory effect of IL-1 receptor antagonist on fever in rats has already been demonstrated.[9]

Recently, we and others have reported that endogenous antipyresis may also be operating at the level of arachidonic acid cascade. Prostaglandin (PG) E_2 is considered a key mediator of fever acting downstream of the endogenous pyrogens and ultimately responsible for the upward resetting of the set point of thermoregulation.[24] It seems reasonable, therefore, to postulate that the regulation of the rate of production and/or metabolization of PGE_2 may possibly constitute an additional physiological system, to complement the cytokine–hormones and the cytokine–cytokine interactions in endogenous antipyresis and the regulation of fever. Data presented by Fraifeld *et al.*[25] implicate the involvement of the lipoxygenase pathway in endogenous antipyresis, whereas our data[26–28] and that from Nakashima and coworkers[29] indicate that the cytochrome *P*-450/epoxygenase pathway of the arachidonate metabolism is also part of the endogenous antipyretic system.

ALTERNATIVE PATHWAYS OF ARACHIDONIC ACID METABOLISM

Liberation of arachidonic acid from membrane structural glycerophospholipids is a hallmark for most, if not all, infectious as well as chronic and/or acute inflammatory disorders. Free arachidonic acid can be metabolized by cyclooxygenase and lipoxygenase enzyme systems to generate prostaglandins, thromboxanes, leukotrienes, and hydroperoxyeicosatetraenoic acids. The importance of the cyclooxygenase pathway in generation of fever and in induction of other symptoms of the acute-phase response during disease has been substantially documented.[24] The earliest studies on lipoxygenase, on the other hand, did not support any role for this pathway in fever.[30] However, as mentioned above, data have recently been reported suggesting a role for lipoxygenases in endogenous antipyresis.[25]

A third pathway of the arachidonate metabolism, producing epoxyeicosatrienoic acids (EETs) and mono-hydroxyeicosatetraenoic acids (mono-HETEs), has been described.[31] The pathway is carried out by the cytochrome *P*-450 (CYP450) monooxygenases, referred to as the epoxygenase pathway (*P*-450). Specific CYP450 enzymes catalyze monoxygenation of arachidonic acid leading to (a) epoxidation and giving rise to four regioisomers, 5,6-, 8,9-, 11,12-, and 14,15-EETs, which in turn are converted by epoxide hydrolases to corresponding dihydroxyeicosatrienoic acids (DHETs); (b) allylic oxidation to produce six regioisomers, 5-, 8-, 9-, 11-, 12-, and 15-HETEs, and (c) ω/ω-1 hydroxylation to result in 19- and 20-HETEs.[32,33] Among many tissues and organs, *P*-450 epoxygenase activity has also been detected in brain structures, including hypothalamus.[34] It has been shown that rat astrocytes[35] and isolated brain slices[36] make EET regioisomers from arachidonic acid. A generation of HETE regioisomers in cerebral tissues has also been documented.[37] *P*-450 arachidonic acid metabolites are currently implicated in a variety of biological functions, including kidney function,[32,33] cerebral blood flow,[36,37] and blood pressure.[38]

Involvement of the cytochrome *P*-450-dependent epoxygenase pathway of arachidonic acid in inflammation and fever has not been thoroughly investigated. It is known, however, that infections and inflammatory stimuli induce changes in the activities and expression of various forms of CYP in humans and experimental animals.[39] These effects could be a part of the homeostatic mechanisms associated with inflammation and infection. They also could be a part of the pathophysiology of fever. The formation of biologically active metabolites of arachidonic acid via *P*-450s indicates that pharmacological modulations of these enzymes may have consequences for the inflammatory disorders. Indeed, we have recently found that administration of the compounds that induce *P*-450 attenuates some histopathological and molecular measures of the LPS-provoked lung inflammation in the rat (unpublished data, manuscript in preparation). Our data have shown that inhibitors of *P*-450 augment fever in rodents, enhance the LPS-induced increase of plasma IL-6,[26] and exacerbate the elevation of PGE_2 in plasma and cerebrospinal fluid.[27] On the other hand, administration of the inducers of *P*-450 reduce fever in mice and rats.[28] Furthermore, intracerebral (i.c.v.) infusion of *P*-450 arachidonic acid metabolites reduce LPS-induced fever in rats.[28] The experimental protocols and data from these experiments are briefly discussed in the following sections of the article.

INDUCERS OF CYTOCHROME *P*-450 DECREASE FEVER IN MICE AND RATS

The rat *P*-450 monooxygenase isoforms known to catalyze the epoxidation of arachidonic acid are CYP1A1, CYP1A2, CYP4A1, CYP2B1, CYP2B4, CYP2C9, CYP2C11, CYP2C23, CYP2E1, CYP2G1, and CYP2J3.[40,41] All of these gene families are markedly inducible as a result of the exposure to xenobiotics. The fibrates (oxyisobutyrates) are the largest structurally related group of inducers investigated, and detailed induction protocols have been described for clofibrate, ciprofibrate, clobuzarit, and bezafibrate.[42] Administration of dehydroepiandrosterone (DHEA), a naturally occurring C_{19} steroid found in mammals, is also effective in induction of

TABLE 1. Effect of *P*-450 modulators on acute-phase responses of mice

Strain	*P*-450 Modulator (route of admin)		Acute-phase response[a] (stimulus)	Effect	Reference
	Inducer	Inhibitor			
Swiss Webster		NDGA (i.m.)	Fever (LPS) Motor act. (LPS)	Increase No effect	26
Swiss Webster		SKF-525A (i.p.)	Fever (LPS) Motor act. (LPS)	Increase No effect	26
Swiss Webster		Clotrimazole (i.m.)	Fever (LPS) Motor act. (LPS)	Increase[b] No effect	26
Swiss Webster		Clotrimazole (i.m.)	Fever (turp.) Motor act. (turp.)	Increase Decrease	Unpublished
Swiss Webster	Clofibrate (s.c.)		Fever (turp.) Motor act. (turp.) Food intake (turp.)	Decrease Increase Increase	28
Swiss Webster	Clofibrate (s.c.)		Fever (LPS) Motor act. (LPS) Food intake (LPS)	Decrease Increase Increase	28
Swiss Webster	Clofibrate (s.c.)		High dose of LPS[c] Morbidity Motor act. (LPS)	 Decrease Increase	Unpublished
Swiss Webster		SKF-525A (i.p.)	Plasma IL-6 bioactivity (LPS)	Increase	26
Swiss Webster		SKF-525A (i.p.)	Plasma TNF bioactivity (LPS)	Decrease	26

ABBREVIATIONS: LPS, lipopolysaccharide; turp, turpentine; NDGA, nordihydroguaiaretic acid; i.m., intramuscular; i.p., intraperitoneal; s.c., subcutaneous.
[a]Compared to LPS or turpentine-injected animals treated with vehicle (rather than modulator of *P*-450).
[b]Blocked by indomethacin.
[c]10mg/kg.

TABLE 2. Effect of *P*-450 modulators on fever in the rat

Strain	*P*-450 Modulator (route of admin)		Acute-phase response[a] (stimulus)	Effect	Reference
	Inducer	Inhibitor			
Sprague-Dawley		SKF-52A (i.m.)	Fever (LPS)	Increase	27
Sprague-Dawley		SKF-525A (icv)	Fever (LPS)	Increase	27
Sprague-Dawley		Miconazole (i.m.)	Fever (LPS)	Increase	28
Sprague-Dawley		Clotrimazole (i.m.)	Fever (LPS)	Increase	27
Sprague-Dawley		Econazole (i.m.)	Fever (LPS)	Increase	Unpublished
Sprague-Dawley		1-ABT (i.p.)	Fever (LPS)	Increase	Unpublished
Sprague-Dawley		17-ODYA (i.m.)	Fever (LPS)	Increase	28
Sprague-Dawley	Clofibrate (i.m., i.p.)		Fever (LPS)	Decrease	Unpublished
Sprague-Dawley	Bezafibrate (i.m., i.p.)		Fever (LPS)	Decrease	28
Sprague-Dawley	DHEA (i.p.)		Fever (LPS)	Decrease	28
Sprague-Dawley		SKF-525a (i.c.v.) SKF-525A (i.p.)	CSF PGE_2 (LPS) Plasma PGE_2 (LPS)	Increase	27
Wister		Econazole, clotrimazole	Fever (IL-1)	Increase	29
Sprague-Dawley	8,9-EET; 11,12-EET; 14,15-EET; 12(R)-HETE[b]		Fever (LPS)	Decrease	28

ABBREVIATIONS: LPS, lipopolysaccharide; i.m., intramuscular; i.p., intraperitoneal; i.c.v., intracerebroventricular; CSF., cerebrospinal fluid; 1-ABT, 1-aminobenzotriazole; 17-ODYA, 17-octadecynoic acid; DHEA, dehydroepiandrosterone.

[a]Compared to LPS injected rats treated with vehicle (rather than modulator of *P*-450).

[b]Epoxyeicosatrienoic and hydroxyeicosatetraenoic acids administered i.c.v.

the *P*-450 epoxygenases.[42] To test the effect of *P*-450 inducers on fever in mice and rats, clofibrate, bezafibrate, and DHEA (dehydroisoandrosterone 3-sulfate, all Sigma) were dissolved in a warm sterile corn oil (Sigma) at a stock concentration of 50 mg/ml. Stock solutions were rewarmed (38°C), sonicated, and diluted with warm corn oil to a desired concentration. Inducers of cytochrome *P*-450 were administered at doses 10 and 100 mg/kg (bezafibrate and DHEA), and 0.5 and 5 mg/kg (clofibrate), three times at 24-h intervals before the induction of fever. The routes of administration are indicated in TABLES 1 and 2. Sterile corn oil was used as a control injection.

Specific pathogen-free young adult male Swiss Webster mice (Taconic) and Sprague-Dawley rats (Charles River) were housed individually in plastic cages in temperature-controlled rooms at 25 ± 1°C for rats and 30 ± 1°C for mice, with a 12:12-h light–dark cycle (lights on at 0600). Body temperature and motor activity of each animal were monitored with intra-abdominal implanted temperature-sensitive telemetry transmitters (Dataquest III System, MiniMitter).[18,20,26,27]

Fever in animals was induced by an i.p. injection of a saline-diluted LPS (*E. coli* endotoxin 0111:B4; Sigma, L2630) at doses of 50 and 80 μg/kg for rats and 100 μg/kg and 2.5 mg/kg for mice. In separate experiments, fever in mice was induced by a subcutaneous (s.c.) injection of turpentine (10 μl/mouse). LPS and turpentine were injected 24 h after the third administration of the *P*-450 inducer. Saline was used as control injections.

Pretreatment of mice (TABLE 1) and rats (TABLE 2) with inducers of the cytochrome *P*-450 resulted in reduction of fever. The effect was dose-dependent; the higher the dose of *P*-450 inducer, the larger the inhibition of fever. In mice, fever was reduced in both models, that is, as a result of the i.p. injection of LPS to model a systemic inflammation and as a result of the s.c. administration of turpentine to model a localized inflammation. Furthermore, pretreatment of mice with clofibrate (5 mg/kg) reduced the other LPS-induced components of the acute-phase response, such as decreased motor activity and food intake (TABLE 1).

INHIBITORS OF CYTOCHROME *P*-450 CAUSE LARGER FEVER IN MICE AND RATS

The facts that the CYP monooxygenases exist in many isoforms and that enzyme inducers and inhibitors are frequently nonspecific complicate the task of elucidating the pathophysiological role of these enzymes. As a result, in studies testing the effects of the inhibition of the enzyme on fever, we used two types of the monooxygenase inhibitors. One approach used a "suicide substrate," an acetylenic derivative 17-octadecynoic acid (17-ODYA) designed to resemble the substrate and at the same time to inactivate the enzyme. 17-ODYA inhibits epoxygenation and ω-hydroxylation of arachidonic acid and other fatty acids.[41] Inactivation is irreversible, and activity is restored on *de novo* synthesis of the enzyme.[41] In the other experiments, we used imidazole antimycotics (clotrimazole, econazole, miconazole), nordihydroguaiaretic acid, and aminobenzotriazole, which are the "mechanism-based" reversible inhibitors of a number of *P*-450 monooxygenase isoforms.[43]

Proadifen (SKF-525A) is a water-soluble inhibitor of a broad spectrum of the *P*-450-dependent oxygenate reactions.

To test the effects of inhibitors of *P*-450 on fever in mice and rats, doses of the inhibitors were injected twice: one injection 24 h before LPS and/or turpentine, and then at the time of the LPS or turpentine injection. Data presented in TABLES 1 and 2 indicate that, in contrast to the effects of *P*-450 inducers on fever in mice and rats, treatment with inhibitors of *P*-450 resulted in augmentation of fever. Amplifying effects of SKF-525A on fever in rats (TABLE 2) have been observed when the drug was administered either peripherally (i.p. or i.m.) or centrally (i.c.v. into the lateral cerebral ventricle). Exacerbation of fever due to pretreatment with *P*-450 inhibitors was dose-dependent. Clotrimazole, an inhibitor of *P*-450, amplified a turpentine-induced lethargy in mice (TABLE 1). In a supplementary experiment, we observed that the exacerbation of fever in mice treated with a *P*-450 inhibitor (clotrimazole; TABLE 1) was abolished by indomethacin,[26] an inhibitor of cyclooxygenases. These data suggest that the amplification of fever using inhibitor(s) of *P*-450 was associated with a shift into a higher rate of the synthesis of PGE_2, tempting a hypothesis that the exacerbation of fever is associated with the higher levels of PGE_2. This hypothesis was tested on rats.[27]

PROADIFEN (SKF-525A), AN INHIBITOR OF *P*-450, EXACERBATES PGE_2 LEVELS IN RATS

Two experiments were performed using 44 rats to study the effect of SKF-525A on PGE_2 levels in plasma and cerebrospinal fluid (CSF) during LPS fever.[27] All rats were implanted intra-abdominally with biotelemitters to monitor temperature. In the first experiment, 20 rats were pretreated i.p. with SKF-525A (15 mg/kg) or saline as control and 30 min later were injected with LPS (i.p.; 50 μg/kg) or control saline (four groups, five rats/group). Heparinized blood (cardiac puncture) was taken 3 h after the LPS injection. In the second experiment, 24 rats were also implanted with lateral ventricle cannulae and given artificial cerebrospinal fluid (aCSF, control) or SKF-525A i.c.v. (5 μg/rat) 10 min before LPS (i.p.; 50 μg/kg) or control saline (four groups; six rats/group). Three hours later a 150- to 200-μl CSF sample (cisternal puncture) was collected from each rat.

In the first experiment (SKF-525A given i.p.), the plasma PGE_2 levels in control-injected rats, (i.e., saline/saline and SKF-525A/saline) were, respectively, 14.4 ± 2.5 pg/ml and 33.2 ± 8.6 pg/ml (difference not significant; $p > 0.05$). Injection of LPS triggered a significant elevation of plasma PGE_2 within 3 h to 169.6 ± 37.5 pg/ml. Administration of SKF-525A (15 mg/kg; i.p.) induced an even larger elevation of PGE_2 within that time in LPS-injected rats to 343.4 ± 45.4 pg/ml ($p < 0.05$ between saline/LPS and SKF-525A/LPS groups).

In the second experiment, the concentration of PGE_2 in CSF changed dramatically when SKF-525A was administered i.c.v. into the LPS-injected rats. Injection of LPS (i.p.) induced significant elevation of CSF PGE_2 from 4.5 ± 1.4 pg/ml (aCSF/saline group) to 30.9 ± 5.2 pg/ml ($p < 0.05$ between saline- and LPS-injected groups) in rats infused i.c.v. with aCSF. Administration of SKF-525A (5 μg/rat; i.c.v.)

amplified the effect of LPS about threefold to a CSF PGE_2 of 92.5 ± 21.1 pg/ml in LPS-injected rats ($p < 0.05$ between aCSF/LPS and SKF-525A/LPS groups).

The augmenting effect of the inhibitor of *P*-450 on LPS-induced elevation of PGE_2 reflected the levels of IL-6 and TNF-α.[26] Generation of these two cytokines is regulated in part by PGE_2; PGE_2 is required for IL-6 synthesis, and it inhibits generation of TNF-α. Accordingly, SKF-525A significantly enhanced the LPS-induced levels of IL-6, whereas it suppressed the increase of TNF-α.[26] These alterations to *in vivo* generation of PGE_2, IL-6, and TNF-α paralleled the effects of SKF-525A on fever in mice and rats.

P-450 ARACHIDONIC ACID METABOLITES REDUCE LPS FEVER IN RATS[28]

Data showing the reduction and/or amplification of fever following treatment with, respectively, inducers and/or inhibitors of *P*-450 monooxygenases, implicate the involvement of this enzyme system in fever, particularly in the regulation of the height of fever. Several mechanisms involving *P*-450 can be considered. One possibility is that the modulation of the activity of *P*-450 affects arachidonate metabolism, in that inhibition of *P*-450 results in more arachidonic acid to be metabolized via cyclooxygenases and, hence, more PGE_2 can be produced per unit of time, which translates into a higher fever. Another hypothesis is that *P*-450 itself is engaged in the inactivating metabolism of PGE_2, and that inhibition of the *P*-450 results in reduction of the rate of neutralization of PGE_2. Data discussed above, showing that proadifen (SKF-525A), an inhibitor of cytochrome *P*-450, enhances the LPS-induced elevation of PGE_2, support this hypothesis. However, a complementary hypothesis is that some *P*-450 arachidonic acid metabolites, and possibly *P*-450 eicosanoid metabolites, can act as endogenous antipyretics. Thus, induction of the *P*-450 monooxygenase enzyme system might accelerate the rate of the generation of fever-preventing *P*-450 arachidonate metabolites, resulting in a lower fever. Data from the studies using clofibrate, bezafibrate, and DHEA presented in this report support this hypothesis. To test whether or not the *P*-450 monooxygenase (epoxygenase) products are antipyretic, we administered various regioisomers of epoxyeicosatrienoic and monohydroxyeicosatetraenoic acids into the rat brain and estimated the changes of fever following the peripheral (i.p.) injection of LPS. This route of EET or HETE isomer administration was applied since (i) the preoptic-anterior hypothalamus is considered the center for thermoregulation as well as regulation of fever,[3,4] (ii) expression of *P*-450 has been demonstrated in the hypothalamus,[34] and (iii) infusion of SKF-525A, an inhibitor of *P*-450, into the brain exacerbated the LPS-induced fever in rats.[27]

For the cerebral injections (into the lateral ventricle; i.c.v.), rats were implanted stereotaxically with a 5-mm long, 22-gauge stainless-steel, thin-walled cannula (Plastic Products) into the lateral ventricle, as described elsewhere.[27,28] Ethanol solutions of 12(R)-HETE, 5,6-EET, 8,9-EET, 11,12-EET, and 14,15-EET were purchased from Cayman Chemical. Before i.c.v. infusion, ethanol was evaporated under nitrogen in a cold room (about 10°C), and the specimens were reconstituted with

aCSF to a desired concentration. In this study, fever in rats was induced by an i.p. injection of LPS at a dose of 80 μg/kg.

Four of five examined isomers appeared to reduce fever when infused into the lateral ventricle a few minutes before the i.p. administration of LPS.[28] The most potent antipyretic isomers (dosage-wise) in our experiments were 11,12-EET followed by 14,15-EET. They significantly and dose-dependently reduced fever if administered i.c.v. in the range of nanograms per rat. All other tested epoxygenase metabolites except the 5,6-EET isomer, significantly reduced fever when infused i.c.v. in a dose range of micrograms per rat (TABLE 2). The effects observed for these isomers, that is, 8,9-EET, 14,15-EET, and 12(R)HETE, were dose-dependent.

CONCLUDING REMARKS

FIGURE 1 summarizes data presented in this report and illustrates hypotheses regarding the interactions within the arachidonate cascade in the modulation of fever. On the basis of the results just described, on our data earlier published,[26–28] and that from Nakashima *et al.*[29] and Fraifeld *et al.*,[25] we conclude that besides interactions among the cytokines and hormones, the process of endogenous antipyresis also operates at the level of the arachidonate cascade, reflecting the interactions among the enzymes, substrates, and metabolites of the cascade.

It is well documented that the metabolism of arachidonic acid via cyclooxygenases and, in turn, production of prostaglandins, particularly PGE_2, is an essential pathway for the generation of fever.[24] Data presented in this report suggest that when arachidonic acid is metabolized via the *P*-450 pathway, the net result can be antipyresis. This latter conclusion is based in part on the study in which we used pharmacological agents to modulate (induce and/or inhibit) *P*-450 monooxygenases. It must be stressed, however, that the selectivity and specificity of such an approach is limited due to the magnitude and complexity of the cytochrome *P*-450 gene superfamily.[41,44] One cannot rule out, therefore, that the drugs used in our study altered not only the expression of the arachidonate epoxygenases, but also might affect other *P*-450 isoforms, which could result in influencing the LPS fever in rats. More direct data were obtained when we applied the exogenous *P*-450 arachidonic acid metabolites to test their effects on fever. These results clearly support the hypothesis that metabolization of the arachidonic acid via *P*-450 can indeed give rise to the antipyretic eicosanoids.

The multiple mechanisms underlying the antipyretic action of epoxyeicosanoids may involve an "endogenously induced" inhibition of the activity of cyclooxygenases. In support of this notion, it has been found that some tested isomers of EET are potent inhibitors of platelet[45] and vascular smooth muscle[46] cyclooxygenases. In the study by Fang *et al.*,[46] 14,15-EET exerted a potent inhibitory effect on the production of PGE_2, whereas 5,6-EET isomer was ineffective in the inhibition of cyclooxygenases, which is in accordance with our findings that 5,6-EET was not effective in the attenuation of fever in rats. Interestingly, the potency of some EETs examined, that is, 8,9-EET, and 14,15-EET, surpassed that of ibuprofen and aspirin, well-known antiinflammatory agents, in the inhibition of the activity of isolated cyclooxygenase enzyme preparation.[45] It is believed that aspirin inhibits fever and attenuates inflammation by blocking cyclooxygenase activity and, in consequence,

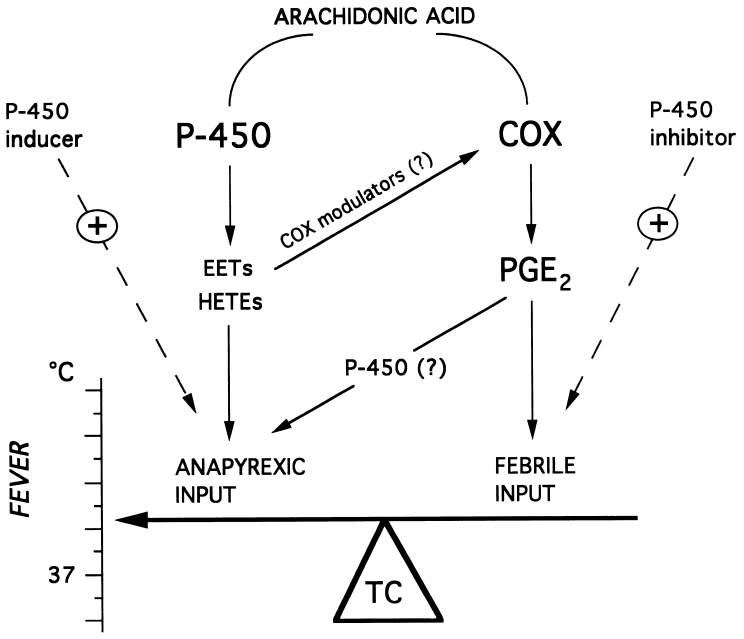

FIGURE 1. Schematic illustrating the summary and interpretation of the data presented in this report. We have observed that inducers of *P*-450 reduced, whereas inhibitors exacerbated, fever in the rat. This observation indicates that treatment with inducers of *P*-450 amplified an antipyretic input (anapyrexic input), whereas treatment with inhibitors of *P*-450 enhanced a febrile input into the thermoregulatory center (TC). The underlying mechanisms of the *P*-450-related antipyresis may involve generation of the antipyretic metabolites from arachidonic acid. These metabolites may either act directly on TC or attenuate the activity of cyclooxygenases (COX) to suppress the production of PGE_2 and, in turn, diminish a pro-febrile signaling into TC. We have shown that an inhibitor of *P*-450 enhanced the LPS-induced PGE_2 level in plasma and cerebrospinal fluid.[27] This finding suggests either a rerouting of the substrate (i.e., arachidonic acid) into the COX pathway as a result of the inhibition of *P*-450 or *P*-450 participation in the metabolization of PGE_2 during inflammation and fever.

the generation of PGE_2.[47] It has recently been found, however, that aspirin is a potent inducer of *P*-450, and administration of a single dose of aspirin produces a significant increase in the activity of CYP2E1 and CYP4A1, the CYP isoforms involved in the metabolism of arachidonic acid.[48,49] Therefore, the possibility arises that aspirin attenuates inflammation and inhibits fever by another mechanism as well, that is, by inducing a cytochrome *P*-450, which metabolizes arachidonic acid. This may result in (1) lowering the concentration of the substrate (i.e., arachidonic acid) for cyclooxygenases and (2) in a generation of the arachidonic acid metabolites, which can act within a feedback loop to attenuate the activity of cyclooxygenases. This hypothesis is consistent with data reported recently by Node *et al.*,[50] showing that several cytochrome *P*-450 epoxygenase-derived metabolites exert antiinflammatory properties.

ACKNOWLEDGMENTS

Research supported by the National Institute of Allergy and Infectious Disease Grant AI-27556 and funds from Lovelace Respiratory Research Institute. The study was conducted in facilities fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International.

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