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CHAPTER 1

# The onset of fever: new insights into its mechanism

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**Abstract:** The classical view of fever production is that it is modulated in the ventromedial preoptic area (VMPO) in response to signaling by pyrogenic cytokines elaborated in the periphery by mononuclear phagocytes and the consequent induction of cyclooxygenase (COX)-2-dependent prostaglandin (PG)$E_2$ in the VMPO. This mechanism has, however, been questioned, in particular because the appearance of circulating cytokines lags the onset of the febrile response to intravenously (iv) injected bacterial endotoxic lipopolysaccharide (LPS), an exogenous pyrogen. Moreover, COX-2, in this case, is itself an inducible enzyme, the de novo synthesis of which similarly lags significantly the onset of fever. Issues also exist regarding the accessibility of the POA to blood-borne cytokines. New data adduced over the past 10 years indicate that the peripheral febrigenic message is conveyed to the VMPO via a neural rather than a humoral route, specifically by the vagus to the nucleus tractus solitarius (NST), and that the peripheral trigger is $PGE_2$, not cytokines; vagal afferents express $PGE_2$ receptors ($EP_3$). Thus, the initiation of the febrile responses to both iv and intraperitoneal (ip) LPS is temporally correlated with the appearance of LPS in the liver's Kupffer cells (Kc), its arrival immediately activating the complement (C) cascade and the consequent production of the anaphylatoxin C5a; the latter is the direct stimulus for $PGE_2$ production, catalyzed non-differentially by constitutive COX-1 and -2. From the NST, the signal proceeds to the VMPO via the ventral noradrenergic bundle, causing the intrapreoptic release of norepinephrine (NE) which then evokes two distinct core temperature ($T_c$) rises, viz., one $\alpha_1$-adrenoceptor (AR)-mediated, rapid in onset, and $PGE_2$-independent, and the other $\alpha_2$-AR-mediated, delayed, and COX-2/$PGE_2$-dependent, i.e., the prototypic febrile pattern induced by iv LPS. The release of NE is itself modulated by nitric oxide contemporaneously released in the VMPO.

**Keywords:** pyrogenic agents; preoptic-anterior hypothalamus; vagal afferents; Kupffer cells; prostaglandin $E_2$; complement 5a; norepinephrine; nitric oxide

## Introduction

The conventional view of the mechanism by which infectious fevers are produced holds that infectious noxa (e.g., Gram-negative bacteria and/or their products [bacterial endotoxic lipopolysaccharides, LPS]) that invade the body activate mononuclear phagocytes that then produce and release pyrogenic cytokines. These, in turn, are transported by the bloodstream to the ventromedial preoptic area (VMPO) of the anterior hypothalamus, the "fever-producing center", where they act (for reviews, see Saper, 1998; Roth and De Souza, 2001; Dunn, 2002; Dinarello, 2004). It is generally agreed that evidence of their central action is the depression of

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warm-sensitive neurons in this region, leading, in accordance with Hammel's classical model (1965), to the conservation of heat and, hence, to a rise in body core temperature ($T_c$) (Boulant, 2000). It is, however, uncertain how cytokines, as hydrophilic peptides, could penetrate the brain and, indeed, whether they or other, secondarily elaborated factors mediate the febrile response. To wit, it is generally believed that, rather than acting directly, the cytokines induce the local generation and release of prostaglandin (PG)$E_2$, a lipid mediator that is clearly thermogenic when injected centrally (for reviews, see Blatteis, 1997; Ivanov and Romanovsky, 2004). Its production in this instance has been demonstrated to be dependent on the activation of two enzymes, cyclooxygenase (COX)-2 and microsomal PGE synthase (mPGES)-1, which catalyze its conversion from arachidonic acid (AA) present in the membranes of cells (Ivanov et al., 2002).

Questions have arisen over the past 10 years, however, about the validity of this concept. They are based, in particular, on findings that low-to-moderate doses of LPS injected intravenously (iv) elicit in many species, e.g., guinea pigs (Sehic et al., 1996a, b), significant elevations of $T_c$ and VMPO $PGE_2$ levels within 10 min after administration, whereas the first cytokine to appear in the blood, tumor necrosis factor (TNF)α, is not detectable until, at the earliest, 30 min after injection (Jansky et al., 1995). This would make it unlikely, therefore, that TNFα or other pyrogenic cytokines released subsequently could play a major role in the quick induction of these responses. This is not completely surprising, however, since cytokines are not generally expressed constitutively in mononuclear phagocytes, but are induced de novo in response to these cells' activation by LPS, a synthetic process that requires some time (Conti et al., 2004). By the same token, it has been demonstrated that both the COX-2 and mPGES-1 that catalyze the production of preoptic $PGE_2$ in response to peripheral LPS are also not constitutive, but rather are upregulated after a ca. 60 plus-min delay (Inoue et al., 2002). Hence, it would also seem improbable that $PGE_2$ elaborated by this mechanism could mediate the initiation of the febrile response. A different mechanism must therefore be operating to account for the prompt onset of fever after iv LPS.

**Pyrogen activation of the brain: the role of the vagus**

The first part of the old concept that can probably be rejected is the notion that the cytokines are transported to the brain by the circulation for their action. If they are not detectable in the bloodstream before or coincidently with the beginning of fever, their signaling of the brain is most likely not related to their physical arrival at this site. Indeed, if they had a role at all in initiating fever, the only pathway that would allow the rapid and direct transmission of their message from the periphery to the brain would be neural. Moreover, since the liver contains the body's largest population of mononuclear phagocytes (Kupffer cells, Kc), it is the primary clearinghouse of circulating LPS and therefore also the principal source of LPS-induced cytokines; the pyrogenic signal should therefore originate there. Indeed, the essential role of Kc in fever production was recently verified (Feleder et al., 2003; Li and Blatteis, 2004; Li et al., 2004). Furthermore, a number of studies have now established that the subdiaphragmatic section of both vagal trunks, and its hepatic branches in particular, significantly attenuates the febrile response to LPS (Watkins et al., 1995; Sehic and Blatteis, 1996; Romanovsky et al., 1997a, b; Fleshner et al., 1998; Gaykema et al., 1998; Simons et al., 1998; Wieczorek et al., 2005), albeit that it is still controversial whether this effect is conditional on the route and dose of LPS administered (Goldbach et al., 1997; Hansen et al., 2001). Biotinylated IL-1-receptor antagonist has also been shown to bind to glomus cells in hepatic vagal paraganglia (Goehler et al., 1997), suggesting that this could be the mechanism by which IL-1β activates the vagal afferents. In support, the intraportal vein administration of IL-1β increases the discharge rate of vagal afferents (Niijima, 1996) and the expression of c-*fos* in the nucleus of the solitary tract (NTS, the primary projection area of the vagal nerves) is enhanced after iv and intraperitoneal (ip) IL-1β and iv LPS (Wan et al., 1994). Subdiaphragmatic vagotomy abrogates this effect and electrolytic lesions of the NTS attenuate the febrile response to ip LPS (Wan et al., 1994). However, since Kc do not express cytokines constitutively, they cannot be the factors that rapidly

stimulate these terminals after LPS administration. Consequently, we should look for another factor that is quickly liberated in response to circulating LPS and capable of binding to vagal afferents to mediate the febrile response.

## $PGE_2$, not cytokines, is the peripheral fever trigger

Such a factor could be $PGE_2$. It is produced by Kc activated by LPS and its level rises in venous blood and, to a lesser extent, in arterial blood very quickly after the peripheral administration of both exogenous (e.g., LPS) and endogenous (e.g., IL-1β) pyrogens (Skarnes et al., 1981; Rotondo et al., 1988; Perlik et al., 2005; Li et al., 2006). This raises the possibility that, as was conceived earlier in regard to the cytokines, the $PGE_2$ that acts in the VMPO could enter it from the blood: being lipophilic, it could either cross the blood-brain barrier (BBB) or diffuse to this site through "leaky" ports in the barrier, e.g., the organum vasculosum laminae terminalis (Blatteis and Sehic, 1997). It is, however, controversial whether $PGE_2$ can actually pass from the blood into the brain and, especially, whether $PGE_2$ entering the brain in this manner can raise $T_c$ (Morimoto et al., 1992; Sehic et al., 1996a, b; Abul et al., 1997; Romanovsky et al., 1999). Indeed, although the rise of $T_c$ and preoptic $PGE_2$ levels induced by iv LPS are both abrogated by peripherally injected antipyretics, findings that they are also prevented by the intraVMPO microinjection of COX-2 inhibitors would indicate that it is more likely that the applicable $PGE_2$ is generated inside rather than outside the BBB (Steiner et al., 2001). Hence, again, it would seem more probable that its message should be rapidly conveyed from the liver to the brain neurally rather than humorally. In support, abundant $PGE_2$ receptors of the $EP_3$ subtype occur in nodose ganglion vagal sensory neurons receiving information from the abdominal compartment (Ek et al., 1998).

There is a problem, however, with the notion that $PGE_2$ produced by Kc in quick response to LPS is the candidate mediator of the febrile response, because LPS is actually a weak trigger of AA release, the activation of group IV cytosolic phospholipase $A_2$ ($cPLA_2$) by LPS being very slow (Ambs et al., 1995). Moreover, as indicated earlier, the increased biosynthesis of $PGE_2$ induced by LPS is selectively catalyzed by inducible COX-2 and mPGES-1, and, in conscious rats at least, their mRNAs are not detectable in liver until 30 min after iv LPS (Ivanov et al., 2002). The delay imposed by this synthetic process consequently implies that the prompt elevation of plasma $PGE_2$ levels observed in response to iv LPS cannot be accounted for by its COX-2/mPGES-1-mediated production in Kc. A different factor must therefore be involved.

This mediator is the anaphylatoxic complement (C) component C5a (for review, see Blatteis et al., 2004a, b). The C cascade is activated on contact by LPS via the alternative pathway, resulting in the very quick production of all its components, including C5a. Kc express its cognate receptor, $C5aR_1$ (Schieferdecker et al., 1997, 2001; Schlaf et al., 2003). The production of $PGE_2$ by Kc is initiated within minutes after the addition of C5a, both *in vitro* and *in vivo*; C depletion inhibits this release (Schieferdecker et al., 2001). $PGE_2$, under these conditions, could be generated by the hydrolysis of membrane-associated phosphoinositide (PI, which has a high arachydonoyl chain content) by PI-specific phospholipase C (PI-PLC); indeed, AA liberation by PI-PLC is 10-fold more rapid (within seconds) than that mediated by $cPLA_2$ (Rhur, 1994). Moreover, PI-PLC is activated by C, but not by LPS or IL-1β, and the subsequent conversion of this AA to $PGE_2$ is unselectively catalyzed by COX-1 and/or COX-2 (Schütze and Krönki, 1994), which are both constitutive in Kc. Hence, the initial peripheral fever trigger could indeed be $PGE_2$ released by Kc stimulated by LPS-activated C5a and binding to $EP_3$ receptors on vagal afferents.

This hypothesis was recently substantiated in a series of studies (for review, see Blatteis et al., 2004a, b; Perlik et al., 2005). Thus, the intraportal vein injection into anesthetized guinea pigs of LPS or cobra venom factor (CVF, another immediate activator of the C cascade that quickly elevates the levels of all the C components, but, unlike LPS, that eventually depletes the C substrate, thereby ultimately causing hypocomplementemia) induced similar increases of $PGE_2$ in the inferior vena cava (ivc, near its confluence with the hepatic veins) within the first 5 min after treatment. The rises in $PGE_2$ due to


Fig. 1. Depiction of the sequence of steps that occur in the periphery following the iv or ip bolus injection of a small-to-moderate dose of LPS into conscious guinea pigs, culminating in the rapid production and release by Kc of $PGE_2$, the endogenous trigger of the febrile response. Pyrogenic cytokines do not play a role in this phase of fever onset.

CVF returned to control levels within 15 min, whereas those due to LPS increased further 30–45-min later, then stabilized. LPS given to the same animals 3 h after CVF (when the pool of C5a was exhausted) also elevated ivc $PGE_2$, but after a 30–45-min delay. CVF *per se* did not affect the basal level of ivc $PGE_2$. It also did not alter basal ivc TNFα, IL-1β, and IL-6 levels nor their responses to LPS; in the latter case, their rise lagged significantly that of $PGE_2$. These results thus confirm that the $PGE_2$ that appears in the ivc immediately after an LPS challenge originates in the liver and is probably due to the postulated activation of C5a by LPS. The secondary elevation of $PGE_2$ 30–45 min after LPS is presumably due to the Toll-like receptor 4-mediated activation of Kc by LPS and the consequent, delayed upregulation of COX-2/mPGES-1. The essential role of Kc as the cellular source of this $PGE_2$ had been shown earlier (Blatteis et al., 2004a) and was reconfirmed in this study (Perlik et al., 2005). The association between the uptake by Kc of iv or ip injected LPS and the febrile response and that between the latter and the rise in plasma $PGE_2$ were also demonstrated in parallel studies in conscious animals (Blatteis et al., 2004a; Li et al., 2006). Especially important in this regard was the finding that $PGE_2$ antiserum administered iv 10 min before iv LPS prevented the febrile response (Li et al., 2006). In sum, these results strongly support the view that $PGE_2$ is generated by C5a-activated Kc in immediate response to LPS and that it, rather than pyrogenic cytokines, triggers the febrile response. In view of the rapid onset of fever following the iv administration of LPS and the likelihood reviewed earlier that the pyrogenic signal is conveyed to the VMPO neurally, it is probable that the released $PGE_2$ acts on hepatic vagal afferents for this transmission, although its binding to these terminals has not yet been specifically demonstrated. Figure 1 summarizes the sequence of events triggered in the periphery by the iv or ip injection of LPS that initiate the febrile response.

## Contribution of preoptic $PGE_2$ to fever production

It is well established that the pyrogenic signal of peripheral LPS activates specific neural systems in the VMPO region. The enhanced expression of c-*fos* in the NTS and its blockade by subdiaphragmatic vagotomy were already mentioned. In addition, the central noradrenergic system is activated, causing the release of norepinephrine (NE), an effect that is also blocked by vagotomy (Fleshner et al., 1995; Ishizuka et al., 1997); the increase in the activity of the noradrenergic system produced by proinflammatory stimuli is well documented (for review, see Dunn, 2001). NE microinjected into the VMPO of conscious guinea pigs raises their $T_c$ (Zeisberger, 1987; Quan and Blatteis, 1989), and electrical stimulation of their ascending noradrenergic system in the NTS produces the same effect (Szelenyi et al., 1976, 1977) whereas chemical sympathectomy prevents this response (Zeisberger, 1987). NE also stimulates the release of $PGE_2$ in brain tissue *in vitro* (Hori et al., 1987), and the microdialysis of NE into the VMPO of conscious guinea pigs augments the local production of $PGE_2$ (Sehic et al., 1996a, b; Feleder et al., unpublished observation); in turn, the released $PGE_2$ inhibits the further presynaptic release of NE (Bergstrom et al., 1973; for review, see Hedqvist, 1977). Hence, NE locally released in the VMPO consequent to the vagally transmitted hepatic $PGE_2$ signal could induce the production of preoptic $PGE_2$ and thereby account for its presence and traditional pyrogenic function there. Indeed, the release of NE in the VMPO following the systemic administration of LPS or IL-1β has been demonstrated in several species (Linthorst et al., 1995; Wieczorek et al., 2005; Feleder et al., unpublished observation; for review, see Dunn, 2001). It has further been reported that pretreatment with the non-selective COX inhibitor aspirin prevents the $T_c$ rise produced by $PGE_2$ microinjected into a lateral ventricle (Navarro et al., 1988).

If NE were the direct stimulus for the increased production of $PGE_2$ in the VMPO in response to

peripheral LPS-stimulated, vagally transmitted signals, the induction of $PGE_2$ should begin promptly and, according to current dogma, be mediated by COX-2/mPGES-1. Indeed, the LPS-induced rise in preoptic $PGE_2$ occurs promptly following the peripheral injection of LPS (Sehic et al., 1996a, b; Feleder et al., unpublished observation; for review, see Blatteis, 1997). However, the increase in preoptic NE that attends the course of the febrile response to LPS is associated with the early rather than the late phase of fever (Linthorst et al., 1995; Feleder et al., unpublished observation). Hence, the temporal discrepancy between the occurrence of NE and $PGE_2$ in the VMPO would seem to argue against their functional interaction. There is another challenge: as mentioned earlier, although, in brain, both COX-2 and mPGES-1 are expressed constitutively, but rather minimally, in endothelial cells and, to a lesser extent, in neuronal cell bodies, dentritic spines, and glia, their role in fever production is attributed to their upregulation in endothelial and/or glial cells, but not in neurons (Breder and Saper, 1996; Matsumura et al., 1997; Cao et al., 1998; Quan et al., 1998). Moreover, their mRNAs become detectable in the VMPO between 0.5 and 4 h after the iv or ip administration of LPS or a cytokine (Inoue et al., 2002; Ivanov et al., 2002). The prompt elevation of preoptic $PGE_2$ could therefore not be due to its COX-2/mPGES-1-mediated production. Consequently, its production should be mediated either via COX-1 or via a COX-independent pathway.

In a series of experiments designed to clarify the mechanism that could underlie the production of $PGE_2$ in the VMPO in response to local NE and, at the same time, to identify the adrenoceptor (AR) subtype(s) that could be involved in this effect, NE and specific $\alpha_1$- and $\alpha_2$-AR agonists and antagonists were microdialyzed into the VMPO of conscious guinea pigs pretreated intraVMPO with selective COX-1 and COX-2 inhibitors (Feleder et al., 2004). The results unexpectedly revealed that NE mediates not one, but two successive $T_c$ rises, each associated with a different mechanism. Thus, the intraVMPO microdialysis of the selective $\alpha_1$-AR agonist cirazoline induced a prompt $T_c$ rise without, remarkably, affecting basal preoptic $PGE_2$ levels, whereas the selective $\alpha_2$-AR agonist clonidine caused a significantly delayed $T_c$ rise that followed an initial $T_c$ fall; both these responses were accompanied by parallel changes in the levels of VMPO $PGE_2$. The elevation of $PGE_2$, however, was not associated under these conditions with a demonstrable upregulation of COX-2 (Feleder et al., unpublished observation), implying, therefore, that it was mediated by the activation of this enzyme, not its de novo synthesis. The thermal effects of both agonists were validated by their blockade by their respective, selective antagonists, prazosin and yohimbine. Furthermore, both the increases in $T_c$ and preoptic $PGE_2$ levels caused by clonidine were prevented by the intraVMPO microdialysis of a selective COX-2 inhibitor, MK-0663; they were unaffected by that of the selective COX-1 inhibitor SC-560, although it unexpectedly suppressed the initial decreases of both these variables caused by this $\alpha_2$-AR agonist. The intraVMPO microdialysis of NE reproduced the early and the late $T_c$ and preoptic $PGE_2$ level changes induced by clonidine, but not the early $T_c$ rise evoked by cirazoline; both the clonidine-mediated effects were inhibited by yohimbine and MK-0663, but not by prazosin. Cirazoline and clonidine microdialyzed together replicated the late $T_c$ rises but not the early $T_c$ falls elicited by clonidine and NE, and also not the early $T_c$ rises caused by cirazoline; on the other hand, their co-microdialysis induced both the VMPO $PGE_2$ falls and rises associated with clonidine and NE. In further support of the non-involvement of $PGE_2$ in the cirazoline-induced $T_c$ rise and, in contrast, the involvement of COX-2-dependent $PGE_2$ in that caused by clonidine, conscious, wild-type, and COX-$1^{-/-}$ mice respond similarly to the intracerebroventricular (icv) microinjection of these two $\alpha$-AR agonists as the correspondingly treated guinea pigs; and, most relevant to this context, the late clonidine-induced $T_c$ rise is absent in COX-$2^{-/-}$ mice (Blatteis et al., 2004a).

The finding that the specific $\alpha_1$-AR agonist cirazoline evoked very quickly a rise in $T_c$ without the intermediation of $PGE_2$ was indeed remarkable and unexpected. It suggested that the $\alpha_1$-AR activated by NE are located on postsynaptic warm-sensitive or thermoinsensitive neurons in the VMPO and that NE directly reduces or augments,

respectively, the activities of these neurons; according to the classical model of Hammel (1965); both responses promote heat conservation. Since these neurons, moreover, are thought to inhibit synaptically connected cold-sensitive neurons, these are concomitantly facilitated, stimulating heat production; i.e., in combination, these effector mechanisms raise $T_c$. Support for the direct, $PGE_2$-independent involvement of $\alpha_1$-AR is also provided by preliminary data from single-unit extracellular recordings of the discharge rates of thermally characterized, individual neurons in horizontal slices of rat hypothalami. In the experiments to date, cirazoline inhibited three of four warm-sensitive neurons and excited two of three thermoinsensitive neurons (Boulant and Blatteis, unpublished observation). The specific $\alpha_1$-AR subtype involved in this hyperthermic effect remains to be identified.

The findings that, when the microdialysis of clonidine was continued for another 3 h, the initial hypothermic response was followed by a protracted $T_c$ rise and an associated increase in the animals' levels of preoptic $PGE_2$ were both also novel. Since both these responses were also inhibited by yohimbine pretreatment, they were therefore also $\alpha_2$-AR-mediated; and since, moreover, they were prevented by the prior microdialysis of the selective COX-2 inhibitor MK-0663, this late hyperthermic response was specifically mediated by COX-2-dependent $PGE_2$. The brain cell type expressing COX-2 in response to NE in this context remains to be determined. But, since the increase of COX-2 following the peripheral administration of LPS has been observed, as already mentioned, in glial and cerebromicrovascular endothelial cells, but only irregularly in neurons, we conjecture that the $PGE_2$ collected in the microdialysate effluents from the VMPO interstitial space in these experiments was generated by astrocytic processes contacting noradrenergic synaptic regions rather than by postsynaptic (warm-sensitive) neurons. The subsequent effect of the thus released $PGE_2$ on the electrical activities of VMPO warm-sensitive and thermoinsensitive neurons is presumptively similar to that indicated earlier for $\alpha_1$-AR-mediated responses, viz., a reduction and an increase in their discharge rates, respectively. The $PGE_2$-sensitive receptor involved in these neuronal effects is probably the $EP_3$ subtype; it has been linked to the development of fever and is present in the VMPO (Ushikubi et al., 1998; Ek et al., 2000; for review, see Oka, 2004). The receptor implicated in the inhibition of presynaptic NE release by $PGE_2$ has also been previously identified as the $EP_3$ subtype (Schlicker and Gothert, 1998). These results thus demonstrated that the induction of $PGE_2$ by NE in the VMPO is modulated by $\alpha_2$-AR and indeed catalyzed by COX-2. The identity of the $\alpha_2$-AR subtype involved in this effect remains to be elucidated.

Taken together, the preceding data would suggest, therefore, that the NE released in the VMPO in response to the vagally conveyed pyrogenic message of Kc-generated $PGE_2$ could mediate the febrile response of guinea pigs in the following two, successive ways: (1) it could induce the first of the characteristic two $T_c$ rises evoked in conscious guinea pigs by iv LPS by rapidly activating $\alpha_1$-AR without the intermediation of $PGE_2$, and (2) it could cause the second of the two $T_c$ rises and also the delayed, single rise produced by ip LPS (due to the late arrival of LPS in the liver [Li and Blatteis, 2004]) by also stimulating at the same time $\alpha_2$-AR, consequently activating (after the delay imposed by the de novo synthesis of the enzymes) the production and release of COX-2/mPGES-1-dependent $PGE_2$ in the POA. This hypothesis was tested (Feleder et al., unpublished observation) by measuring over short intervals the febrile response and the levels of NE and $PGE_2$ in the interstitial fluid of the VMPO of conscious guinea pigs pretreated with intraVMPO prazosin or yohimbine (or their vehicles) to the iv or Ip injection of *Salmonella enteritidis* LPS, following the same protocol as in the preceding study. In guinea pigs, pyrogenic doses of iv injected LPS rapidly and characteristically evoke two successive $T_c$ rises associated with concurrent elevations in preoptic $PGE_2$ levels (Sehic et al., 1996a, b); however, the second rise is attenuated significantly more than the first by COX-2 blockade (Steiner et al., 2001). Ip injected LPS, on the other hand, induces coincident monophasic $T_c$ and $PGE_2$ responses after some delay (Inoue et al., 2002). The iv injection of a low-to-moderate dose of LPS (2 μg/kg) promptly induced the appearance of NE in the VMPO interstitial fluid; its level culminated in 30 min, then gradually

returned toward its control value over the following 2 h. $T_c$ and preoptic $PGE_2$ levels also both increased promptly, first rapidly, then more slowly, and in correspondence with each other; the first $T_c$ peak was reached in ~60 min and the second in ~150 min. Pretreatment with the $\alpha_1$-AR antagonist prazosin significantly slowed the rate of rise of the early phase of fever and eliminated the first $T_c$ peak; but it did not affect the onset latency of the febrile response nor the magnitude and time of the second peak of fever. It also did not affect the initial, LPS-induced elevation of preoptic $PGE_2$; but, importantly, this increase was not sustained: $PGE_2$ levels returned from their first highs at 30 min to their control values at 60 min. They then resumed their rise, culminating not differently than their untreated counterparts and coincidentally with the second peak of fever (Feleder et al., unpublished observations). These results thus confirmed that the onset of fever evoked in conscious guinea pigs by iv LPS is associated with the intraVMPO release of NE and accompanied by coincident increases in $T_c$ and preoptic $PGE_2$ levels, in conformity with previous findings (Linthorst et al., 1995; Sehic et al., 1996a, b; Wieczorek et al., 2005). They further demonstrated that the initial $T_c$ rise is indeed mediated by the NE-induced activation of $\alpha_1$-AR and intimated that, its very onset apparently excepted, the first phase of fever was maintained by the direct noradrenergic activation of the relevant neurons in the VMPO, without the intermediation of $PGE_2$. Pretreatment with the $\alpha_2$-AR antagonist yohimbine, by contrast, did not change the onset and rate of rise of the early febrile response, the first $T_c$ peak reaching the same value and occurring at the same time as that of the untreated controls; but it completely abrogated the second peak, consequently significantly reducing the overall magnitude of the febrile response by comparison with that of untreated controls. The fever, moreover, abated more slowly in this group than in its controls. Remarkably, this treatment completely suppressed the LPS-induced rise in preoptic $PGE_2$ levels (Feleder et al., unpublished observations). These findings thus indicated that the LPS fever of these $\alpha_2$-AR-blocked guinea pigs is initiated, maintained, and even extended in the total absence of corresponding increases in VMPO $PGE_2$ levels. Hence, by deduction, it is mediated entirely by $PGE_2$-independent, $\alpha_1$-AR activation. Neither the microdialysis of prazosin nor of yohimbine *per se* affected the animals' $T_c$ and preoptic $PGE_2$ levels (Feleder et al., unpublished observations). In sum, it would appear that the early phase of LPS fever is indeed mediated independently of $PGE_2$ by $\alpha_1$-AR stimulation whereas the late phase is dependent on $PGE_2$ induced consequent to $\alpha_2$-AR stimulation, as hypothesized. But then what accounts for the initial rise in preoptic $PGE_2$ of all the animals, including the yohimbine-pretreated ones?

The first possibility is that COX-1 could mediate the initial, LPS-induced $PGE_2$ rise. But this possibility was refuted by findings that the intraVMPO microdialysis of the selective COX-1 inhibitor SC-560 had absolutely no effect on the onset, height, and course of $T_c$ and the associated changes in preoptic $PGE_2$ levels induced in conscious guinea pigs by iv LPS (Feleder et al., unpublished observation). Acetaminophen, a putatively selective inhibitor of COX-1 variant retaining intron 1 (for review, see Botting and Ayoub, 2005), also did not affect the $T_c$ and intraVMPO $PGE_2$ responses to iv LPS (Feleder et al., unpublished observation). Administration of the selective COX-2 inhibitor MK-0663, by contrast, did not alter the early $T_c$ and preoptic $PGE_2$ rises, but prevented both their late rises (Feleder et al., unpublished observation). These results, therefore, documented again that the late, but not the early phase of LPS fever is mediated by COX-2-dependent $PGE_2$, and that COX-1 evidently plays no role in the febrile response (except, presumptively in part, in the initial induction of $PGE_2$ by C5a-stimulated Kc, as mentioned earlier).

Since the intraVMPO microdialysis of selective COX-1, -2, and -3 inhibitors do not prevent the prompt, initial increase in $PGE_2$ levels observed in the POA/VMPO following the peripheral administration of LPS, it may be surmised that this rise is induced by a COX-independent pathway, i.e., presumptively by the non-enzymatic isoprostane pathway of free radical-catalyzed peroxidation of AA (for review, see Basu and Helmersson, 2005). Indirect support for the involvement of free radicals in the observed, initial $PGE_2$ elevation comes from the finding that the intraVMPO

microdialysis of the antioxidant catechin throughout the febrile course had the same effect as pretreatment with the $\alpha_2$-AR antagonist yohimbine, i.e., suppression of both the LPS-induced early and late rises of preoptic $PGE_2$ and of the second peak of fever; the latency of fever onset and its first peak were not affected, the fever continuing at its early phase high level until it abated normally (Feleder et al., unpublished observation). The free radicals in this case could be generated by the auto-oxidation of NE and/or nitric oxide (NO). The latter is also released locally in the VMPO after LPS administration (see below); however, no direct measurement of biomarkers of oxidative stress was made in these studies. It is not clear at this writing whether catechin also inhibited the expression of COX-2 and, hence, the production of COX-2-dependent $PGE_2$ (Wang et al., 2004). But it has been reported previously that fever is prevented when the production of free radicals is blocked. The effect was ascribed then not to the inhibition by antioxidants of isoprostane production, but rather to their reduction of the thiol groups attached to *N*-methyl-D-aspartate receptors, thereby depressing glutamate-mediated neuronal excitability and, hence, limiting fever (Riedel, 1997; Riedel and Maulik, 1999; Riedel et al., 2003); the activation by peripheral pyrogens of glutaminergic pathways both in the NTS and the OVLT has been reported (Mascarucci et al., 1998; Huang et al., 2001, 2004). It was therefore suggested that NO and oxygen radicals, but not $PGE_2$, modulate fever (Riedel et al., 2003). Indeed, since the initial $T_c$ rise provoked by the peripheral administration of LPS is evidently mediated by $PGE_2$-independent $\alpha_1$-AR activation in the VMPO and since, moreover, the febrile rise can evidently be sustained by this mechanism alone until its normal abatement, it raises the heretic possibility that, in contrast to $PGE_2$ generated in the liver, $PGE_2$ in the VMPO, however it may occur there, may not be material to the febrile response!

Finally, reports that the gaseous transmitter NO stimulates the biosynthesis of $PGE_2$ by increasing the activities of both isoforms of COX prompted us to investigate whether NO could also have a pyretic function in the central mediation of the febrile response. Indeed, the various isoforms of NO synthase (endothelial, neural, and inducible NOS), the enzyme that converts L-arginine into citrulline and NO, occur in the hypothalamus (for review, see Mollace et al., 2005), and circulating LPS and cytokines stimulate the release of NO in the VMPO (for review, see Schmid et al., 1998; Simon, 1998; Gerstberger, 1999). Furthermore, as reviewed above, NE induces the production of COX-2/mPGES-1-dependent $PGE_2$ in the VMPO via an $\alpha_2$-AR-mediated mechanism, and others have shown that NE activates NOS (Canteros et al., 1996). Hence, the existence of a pyrogenic NE-NO-$PGE_2$ cascade in the POA in response to iv LPS seems plausible. But the testing of this hypothesis revealed that, quite to the contrary, NO donors microdialyzed into the POA of conscious

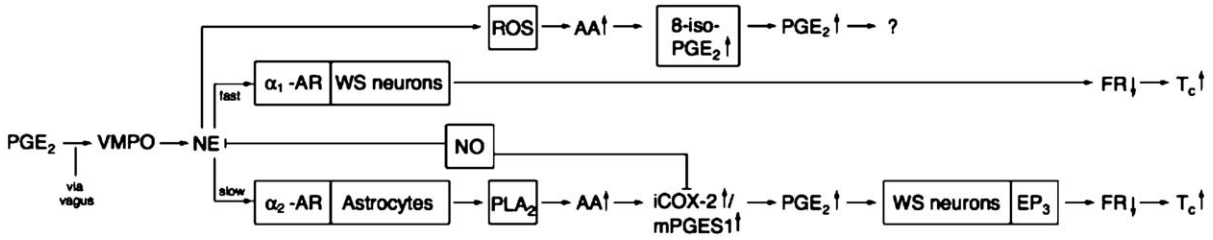


Fig. 2. Schematic illustration of the dual mechanisms, one fast, $\alpha_1$-AR-mediated and $PGE_2$-indenpendent, the other slow, $\alpha_2$-AR-mediated and COX-2/mPGES-1-derived $PGE_2$-dependent, activated by NE liberated in the VMPO. Both mechanisms sequentially lead to the depression of the firing rates (FR) of local warm-sensitive (WS) neurons, thereby causing $T_c$ to rise. NO inhibits the release of NE and may also inhibit the upregulation of COX-2. Reactive oxygen species (ROS) due to the auto-oxidation of NE or derived from NO may also induce isoprostanes (8-iso-$PGE_2$) that are quickly converted to $PGE_2$; it, however, does not appear to contribute to the initial $T_c$ rise. Circulating pyrogenic cytokines and cerebromicrovascular endothelial cells-generated $PGE_2$ probably contribute to sustaining the course of the late phase of fever.

guinea pigs inhibit rather than promote the febrile response to iv LPS and that they do so by inhibiting the LPS-induced release of NE in the VMPO and consequently preventing the $\alpha_2$-AR-mediated activation of COX-2-dependent $PGE_2$ synthesis (Feleder et al., unpublished observation). NO scavengers microdialyzed into the POA have exactly the opposite effects (Feleder et al., unpublished observation). Indeed, previous data regarding a potential role of NO in fever have been conflicting, some indicating pyretic and others antipyretic effects (Riedel, 1997; Gerstberger, 1999; Kozak and Kozak, 2003). The present results would indicate, therefore, that NO, presumptively released in the VMPO coincidentally with or very shortly after NE, serves as a local negative-regulatory factor, i.e., it is a central, endogenous antipyretic mediator of LPS fever. Figure 2 illustrates the sequence of events occurring in the VMPO following the arrival of the peripheral $PGE_2$-induced, vagally transmitted, pyrogenic signal.

## Conclusions

LPS-induced fever arises as the result of a complex, phased sequence of interactions among soluble factors and cells that is initiated in the periphery and then transmitted neurally to the VMPO, which modulates the febrile response. Thus, novel evidence accumulated over the past 10 years suggests that the febrigenic process is initiated by the arrival of LPS in the liver and its uptake by Kc, causing virtually immediately the activation of the C cascade and, hence, the generation of the anaphylatoxin C5a. It, in turn, stimulates the Kc very rapidly to release constitutive COX-1- and COX-2-dependent $PGE_2$. The released $PGE_2$ activates local sensory vagal terminals that project to the NTS. From the NTS, the input of $PGE_2$ is transmitted to the VMPO via the ventral noradrenergic bundle. NE consequently secreted in the VMPO activates both local $\alpha_1$- and $\alpha_2$-AR. The stimulation of the first rapidly evokes an initial rise in $T_c$ which is associated with a decrease in the firing rates of preoptic warm-sensitive neurons, inhibiting heat loss and stimulating heat production, but is not accompanied by any change in the levels of preoptic $PGE_2$. Stimulation of the second causes, after a significant delay, a second, more prolonged $T_c$ rise that is associated with a concurrent increase in preoptic COX-2/mPGES-1-dependent $PGE_2$ levels. Hence, two distinctly produced $PGE_2$s would appear to mediate the febrile response: one, generated in the liver, is the immediate distal trigger of the febrile response, and the other, produced in the VMPO, is its subsequent proximal modulator. The late phase is probably also supported by meanwhile produced circulating cytokines and $PGE_2$ as well as $PGE_2$ generated by cerebral endothelial and/or other cells, all acting presumably as originally proposed for their roles.

In summary, the key, new findings regarding the initiation of LPS fever are: (1) peripheral $PGE_2$ rather than pyrogenic cytokines initiates the febrile response, (2) NE propagates the pyrogenic signal forward within the VMPO, (3) NO modulates its release in the VMPO and, hence, the intensity of the febrile response, and (4) COX-2/mPGES-1-dependent $PGE_2$ mediates the late, but not the early phase of fever; the latter appears to be independent of COX-derived $PGE_2$.

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