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Review

The differential role of prostaglandin E2 receptors EP3 and EP4 in regulation of fever

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The innate immune system of mammals is able to detect bacteria when they infect local tissue or enter the blood stream, and initiate an immediate immune response. Prostaglandin (PG) E2 is considered as the most important link between the peripheral immune system and the brain. Due to four PGE2 receptors (EP receptors) and their differential expression in various areas of the hypothalamus and brain stem, PGE2 mediates different components of the acute phase reaction. A fever model is discussed in which the preoptic area contains the mechanisms for both hyperthermic and hypothermic responses and EP receptors in the median preoptic area (MnPO) modulate the thermogenic system. The neuron-specific modulation of EP receptors in the MnPO can be critically tested by using Cre-recombinase-mediated DNA recombination in genetically engineered mice. A concept for mice with conditional expression of EP3R and EP4R to investigate the different roles of those receptors in lipopolysaccharide (LPS)-induced fever is presented.

Keywords: EP receptors / Fever / Hypothermia / Median preoptic area / Prostaglandin E2

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1 Introduction

It is critical for mammals to be able to detect bacteria immediately when they infect local tissue and enter the bloodstream, and to mount a vigorous immune response. Fever, sickness behavior (malaise, increased pain sensitivity, changes in wake-sleep cycles, and feeding), and changes in secretion of hormones such as corticosteroids are components of the acute phase reaction, an adaptive suite of responses to systemic inflammatory stimuli.

Sir John Vane has made in 1971 the fundamental discovery that anti-inflammatory compounds such as aspirin act by blocking the formation of prostaglandins (PGs) [1]. Originally, PGs were discovered independently by Goldblatt and von Euler [2, 3] in 1935 as a "vasodilatory substance" in seminal fluid and seminal vesicles from most animals including man and are involved in a large number of biochemical processes, often in extremely low concentrations. PGs are formed from unsaturated fatty acids, primarily arachidonic acid, whereby cyclic endoperoxides constitute an important branching point from which the stable PGs as well as the more unstable thromboxanes and prostacyclin are formed.

PGE2 is now considered as the most important link between the peripheral immune system and the brain. Produced at the boundaries between the bloodstream and brain tissue and small enough to penetrate the blood-brain barrier (BBB) and enter the CNS, PGE2 is the ideal candidate to translate a peripheral immune signal into an acute phase response by modulating neural activity through four specific receptors. In this short review, the differential role of PGE receptors EP3 and EP4 in the regulation of fever will be discussed.

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Abbreviations: AAVs, adenoassociated viruses; **BBB,** blood-brain barrier; **COX-2,** cyclooxygenase type 2; **DMH,** dorsomedial hypothalamus; **IL-1**β, interleukin-1β; **LPS,** lipopolysaccharide; **MnPO,** median preoptic area; **OVLT,** organum vasculosum of the lamina terminalis; **PG,** prostaglandin; **PVH,** paraventricular hypothalamus; **RP,** raphe pallidus nucleus; **VMPO,** ventromedial preoptic nucleus

2 Febrile response and hypothermia

A hallmark of the CNS response to inflammation is that many of its manifestations, including fever and corticotro-

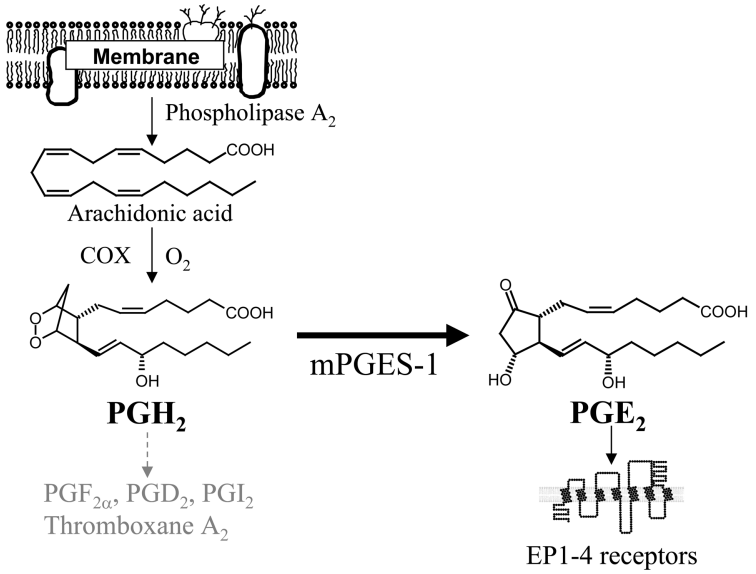

Figure 1. Biosynthesis of PGs. Microsomal PG E synthase-1 (mPGES-1) catalyzes the isomerization of the 9,11-endoperoxide group of PGH2, a common precursor of various prostanoids, to PGE2 having 9-keto and 11-hydroxy groups. PGH2 is produced from arachidonic acid by cyclooxygenases.

phin activation, can be prevented by blocking the production of PGs [1, 4]. Both lipopolysaccharide (LPS) and cytokines activate intracellular signaling cascades in endothelial cells in small blood vessels and in vascular-associated macrophages, termed perivascular cells, in the brain (Fig. 1) [5–7]. This cascade results in phospholipase A2 degrading phospholipids into arachidonic acid and increased expression of cyclooxygenase type 2 (COX-2) [8, 9]. COX-2 converts arachidonic acids to PGH2, from which specific PG synthases synthesize various PGs [10, 11]. In addition, macrophages and dendritic cells of the abdominal cavity including Kupffer cells in the liver can produce PGE2 in response to LPS [12, 13] and sensory neurons of the vagus nerves express receptors for PGE2 that can activate afferent nerve fibers [14] (for review see [15, 16]).

In the normal brain, COX-2 is present in certain neurons and meningeal cells but little is found in brain vascular cells [17–21]. Following administration of LPS or interleukin-1β (IL-1β), there is little change in neuronal expression of COX-2, but intense induction of COX-2 is seen in vascular cells of the brain parenchyma and subarachnoidal space [17, 22–24].

A variety of enzymes have been characterized as PG E synthase [25, 26] but it was until 1999 when a membrane-bound PG E synthase (mPGES-1) was identified [27] and its coordinate up-regulation with COX-2 was shown [12, 28]. In the normal brain, mPGES-1 is very little expressed in endothelial cells, but highly abundant in this type of cells in response to systemic administration of LPS or IL-1β and the distribution is very similar to that of COX-2 [29–31].

A key role of both COX-2 and mPGES-1 in producing fever has been demonstrated in mouse strains lacking these key enzymes [32–34]. Animals lacking either COX-2 or mPGES-1 fail to produce a fever response when stimulated with LPS.

Because PGE2 is a lipid mediator, it can cross the BBB in the circumventricular organs, which lack a BBB, or penetrating venules around the margins of the brain, and as a result it can act in the adjacent brain on neurons expressing E type PG receptors (EPRs) to differentially activate various pathways of the CNS in response to systemic immune challenge. EP receptors are G-protein-coupled receptors and classified into four subtypes: EP1, EP2, EP3, and EP4. These receptors can be grouped into three categories on the basis of their signal transduction: the EP1 receptor increases intracellular Ca^{2+}. The receptors, which mediate increases in intracellular cyclic adenosine monophosphate (cAMP), consist of the EP2 and EP4 receptors. The EP3 receptor expressed in the brain is an inhibitory receptor that mediates decreases in intracellular cAMP, although other splicing variants of the EP3R have different signaling pathways.

E-type PGs have been considered as principal mediator of fever since Milton and Wendlandt [35] reported in 1970 that injection of PG E1 into the third ventricle of cats elevated body temperature. Conversely, injecting a COX inhibitor into the preoptic area attenuated fever dramatically [36], indicating that both the production of PGE2 and its neuronal targets for fever production are located at the anterior tip of the third ventricle, a region containing the median preoptic area (MnPO), organum vasculosum of the lamina terminalis (OVLT), and ventromedial preoptic nucleus (VMPO).

One major clue to identifying the key circuitry involved in the CNS responses to systemic immune challenge is the location of EP receptors in the brain. Oka *et al.* [37] mapped

the distribution of EP receptors by using *in situ* hybridization for their mRNA. These studies and those by others demonstrated that EP3 receptors are highly localized to the MnPO appearing at rostral levels as an inverted Y-shaped structure, capping the vascular organ of the OVLT. The localization of intense expression in the MnPO provides an important clue to the genesis of fever responses as the EP3 receptor is probably the most critical for producing fever. EP4 receptors are found at highest levels in the VMPO and the lateral OVLT, but are also found at lower levels in the MnPO.

In order to clarify which of the EP receptors might be involved in fever responses, Oka *et al.* [38] have injected highly specific EP receptor agonists intracerebroventricular (i.c.v.) into awake rats and recorded body temperature telemetrically. In these experiments, it was found that the EP3 agonist (ONO-AE-248) caused a 1.0–1.5°C fever that reached its maximum after 40 min after the injection. This response was substantially delayed from PGE2, which causes a brisk fever response in about 20 min. Conversely, the EP4 agonist (ONO-AE1–329) caused a 0.5°C hypothermia. These experiments demonstrated that the EP3 receptors are capable of producing hyperthermic responses, whereas the EP4 receptor is more likely to produce hypothermic responses.

A study of EP receptor knockout mice showed that only mice with EP3 [39], but not EP1, EP2, or EP4 receptor gene deletion lacked a hyperthermic response to i.c.v. PGE2, suggesting a crucial role for EP3 receptors in producing fever in mice. However, in those experiments, body temperature was measured by restraining animals and using rectal probes and only for 1 h after LPS or PGE2 administration. As restraint causes stress that alters the body temperature, Saper and colleagues studied the fever response of unrestrained EP3 receptor knockout mice for up to 12 h after intraperitoneal LPS administration at a variety of doses [40]. They found that different doses of intraperitoneally injected LPS produced different temporal evolutions of fever responses in C57BL/6 wild-type mice. A 1 μg/kg dose caused a brief, early hyperthermia (2–4 h after LPS injection), which was eliminated in EP3 receptor knockout animals. At 10 μg/kg, the early hyperthermia was greater in wild-type mice, whereas the EP3 receptor null mice showed only a brief hypothermia from 1 to 2 h after LPS was administered. At 100 μg/kg, wild-type mice showed little early fever, but a 1°C hyperthermia from 4 to 9 h after LPS administration. EP3R knockout animals had a profound hypothermia of 2°C that lasted for 9 h. At 1 mg/kg of LPS administration, the wild-type mice had a mild hypothermia of 0.5°C from 1 to 3 h after the LPS injection, and then a more profound hypothermia of 4°C from 4 to 8 h. EP3 receptor knockout animals had an even more profound hypothermia, with body temperature dropping more than 6°C from 4 to 8 h after the LPS injection.

It appears that the EP3 receptor is necessary not only to produce fever responses but also to prevent profound hypothermic responses, particularly at higher doses of LPS. Thus, the fever curve at different time points and after different doses of LPS appears to reflect the interaction of both hyperthermic and hypothermic processes. EP3 receptors are providing a strong hyperthermic drive, and without these, the main effect of LPS was hypothermia, which appears from the agonist studies [38] to be due to EP4 receptors. The EP4 receptor animals were excluded from this study, mainly because these animals rarely survive very long after birth, because the ductus arteriosus remains patent, as it requires EP4 receptor stimulation to close [41, 42].

Studies by Nakamura and colleagues [43–45] have examined the neuronal pathways by which the EP3 receptor may cause fever. Sawchenko and colleagues [46] confirmed that EP3 receptors are highly expressed by neurons in the MnPO, and demonstrated descending projections to the raphe pallidus nucleus (RP) in the brain stem. The RP has been shown in recent years to be a key site for integrating sympathetic responses important in producing increased body temperature, including both increased thermogenesis through activation of brown adipose tissue and reduced passive heat loss through the skin by tail artery vasoconstriction [47–50].

The model shown in Fig. 2 [51] also accounts for additional observations, such as the attenuation of fever response by lesion of the paraventricular hypothalamus (PVH) or gamma-amino butyric acid (GABA)-ergic inhibition of the dorsomedial hypothalamus (DMH), and that fever is attenuated by blocking excitatory amino acid neurotransmission in the RP, indicating that the RP receives both an excitatory amino acid input and a reduced GABA input during fever responses [52–54]. In this model, the preoptic area contains the mechanisms for both hyperthermic and hypothermic responses. Hyperthermia is generated by a system of neurons including the PVH, DMH, and the RP, which activate thermogenic brown adipose tissue and heat conservation (vasoconstriction) responses. This thermogenic system is normally restrained by inhibitory neurons in the MnPO. Some MnPO neurons project to the RP; there are also projections to neurons in the PVH and the DMH, which in turn provide excitatory inputs to the RP. During fever, hyperthermic neurons in the MnPO are inhibited by EP3 receptors, thus disinhibiting, *i.e.*, releasing, thermogenesis. The same neurons can contain EP4 receptors, which excite them and thereby limit thermogenesis and in the absence of EP3 receptor action produce hypothermia.

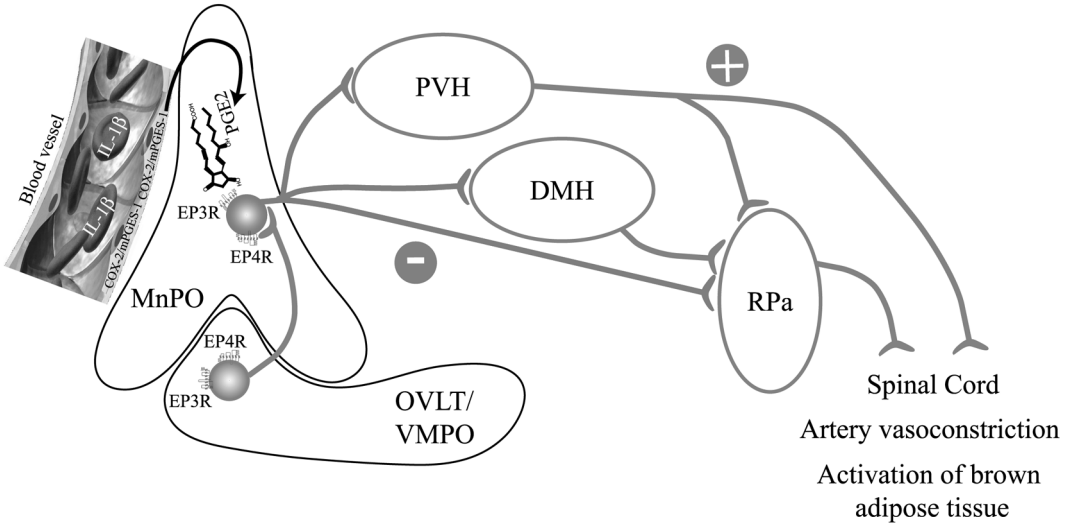

Figure 2. A model for neuronal interactions with EP receptors in the fever response after systemic immune challenge. PGE2 is transported or diffuses across the BBB in small blood vessels around the margins of the brain. Action of PGE2 on EP3 and EP4 receptors surrounding the anteroventral tip of the third ventricle in the preoptic area, including the MnPO, OVLT, and VMPO, appears to be critical for producing fever. EP3 receptors generate hyperthermia, and EP4 responses generate hypothermia. The body temperature of the animal represents the net effect of these counterpoised forces, which may vary at different time points after immune stimuli start signaling the CNS [51].

3 Future studies

Transgenic animals with constitutive gene disruptions of the EP receptors are very valuable to understand the role played by the specific EP receptors for the mechanisms by which an immune stimulus, such as LPS or IL-1β, can cause a set of acute phase responses organized by the brain. However, it is difficult to use EP4 receptor knockout animals because of the poor viability of the animals. Genetic disruption of the mouse EP4 receptor results in perinatal lethality associated with persistent patent ductus arteriosus. Moreover, constitutive knockout animals cannot be used to further localize individual brain areas or neurons involved in the acute phase responses.

The neuron-specific modulation of EP receptors can be critically tested by using Cre-recombinase-mediated DNA recombination in genetically engineered mice [55]. Cre recombinase catalyzes deletion of DNA sequences flanked by 34 bp LOX sites. By transgenically directing Cre to discrete populations of neurons, and by crossing these transgenes with mice bearing LOX-modified alleles, it is possible to modulate these genes in a neuron-specific fashion [56]. Similarly, adenoassociated viruses (AAVs) expressing Cre can be stereotaxically injected into specific nuclei in the brains of mice bearing LOX-modified alleles to modulate these gene expression in pattern dictated by the site of AAV injection [57]. AAV vectors are viral capsids, in which all viral genes were replaced by engineered genes. Thus, AAVs cannot replicate and transgene expression is limited to the area in which the virus is injected. Recombinant AAV vectors are an ideal method to introduce Cre into the brain with almost no inflammatory response and a very popular tool for genetic rescue experiments in mice [57–60].

A mouse line with an EP4R gene amenable to conditional deletion using Cre recombinase was recently created by inserting LOX sites into introns flanking the second exon of the EP4R gene [61]. Moreover, our lab is currently producing mice with conditional expression of EP3 receptors. The conditional knockout mice for the specific EP receptor will be very useful to study the effect of focal gene manipulation in the preoptic area.

4 Conclusion

Due to the variety of four EP receptors and their differential expression in various areas of the hypothalamus and brain stem, PGE2 mediates different components of the acute phase reaction. In the preoptic area, EP3 receptors generate hyperthermia, and EP4 responses generate hypothermia. The body temperature of the animal represents the net effect of these opposing forces, which may vary at different time points after the infection.

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