[Frontiers in Bioscience 9, 3046-3057, September 1, 2004]

PROSTAGLANDIN E_2 AS A MEDIATOR OF FEVER: THE ROLE OF PROSTAGLANDIN E (EP) RECEPTORS

Takakazu Oka

Division of Psychosomatic Medicine, Department of Neurology, University of Occupational and Environmental Health, Japan

TABLE OF CONTENTS

1. Abstract
2. Introduction
3. EP receptors in the PGE2-sensitive febrogenic zone
3.1. PGE2 sensitive febrogenic zone
3.2. EP receptor distribution within the OVLT/POA region
3.3. Activated EP receptors during fever response
4. Effects of EP receptor agonists on body temperature
5. Febrile response in EP receptor knockout mice
6. Changes in firing rate responses of OVLT/POA neurons by PGE2
7. Efferent fever-producing pathways from the OVLT/POA region
8. Perspectives
9. Acknowledgements
10. References

1. ABSTRACT

Prostaglandin E_2 (PGE_2) is a principal fever mediator that induces hyperthermia when injected into the organum vasculosum lamina terminalis (OVLT) and the adjacent preoptic area of the hypothalamus (POA). PGE (EP) receptors have four subtypes, i.e. EP_1, EP_2, EP_3, and EP_4. In the rat OVLT/POA region, at least three of these receptors, i.e. EP_1, EP_3, and EP_4 receptors, have distinctively different distribution patterns. In rats, intracerebroventricular injection of EP_1 receptor agonists and EP_3 receptor agonists increased core temperature (Tc) and that of an EP_4 receptor agonist decreased it. IntraPOA injection of an EP_1 receptor agonist increased Tc. IntraPOA injection of an EP_3 receptor agonist, however, induced hyperalgesia but not hyperthermia. Studies using mice with EP receptor gene deletions have indicated that EP_3 receptors play a crucial role in febrile response. Therefore, the involvement of EP_3 receptors at other levels of the nervous system should be considered. Such nuclei include the raphe pallidus nucleus, intermediolateral cell column in the spinal cord, or the nucleus of the solitary tract.

2. INTRODUCTION

After Milton and Wendlandt (1970) reported that injection of prostaglandin (PG) E_1 into the third ventricle elevated body temperature in cats (1), much attention has been focused on PGEs as principal mediators of fever. Since prostaglandin E_2 (PGE_2) is the predominant isoform of PGE in the brain, PGE_2 is currently considered to be an essential mediator of fever (2). Investigators have subsequently sought to identify the sites where PGEs act to produce fever by microinjecting PGE_1, PGE_2, and PGE receptor agonists into the brains of rats (3-6) and other species (7-10). These studies localized the site of PGE_2 febrigenic activity to within the anteroventral tip of the third ventricle, including the organum vasculosum of the lamina terminalis (OVLT) and the adjacent preoptic area (POA).

In the 1990s, the PGE (EP) receptor was cloned, and four subtypes were identified: EP_1, EP_2, EP_3, and EP_4. The EP receptors are all G-protein-coupled receptors, but they have different signal transduction pathways, i.e. elevation of intracellular Ca^{2+} (EP_1) and stimulation of adenylate cyclase (EP_2 and EP_4). The major signaling pathway for the EP_3 receptor is inhibition of adenylate cyclase. However, the EP_3 receptor has several isoforms (three in rats and mice, four in cows, and seven in humans), and these splicing variants of EP_3 receptors are coupled to different signaling pathways (11-13) (table 1).

In this decade, researchers have discovered that each EP receptor has its own distinctive distribution pattern within the OVLT/POA region (14-21) and that each EP receptor plays a different role in the acute phase response, including fever (6, 11, 22-25), sleep (26, 27), activation of hypothalamic-pituitary-adrenal axis (28), and changes in nociception (29-35). Researchers have also proposed several efferent neural pathways from the PGE_2-sensitive febrogenic zone to the interscapular brown adipose tissue (BAT), the major organ for thermogenesis (36-42). This article reviews recent advances in understanding the role that EP receptors play in the febrile response.

3. EP RECEPTORS IN THE PGE_2-SENSITIVE FEBROGENIC ZONE

3.1. PGE_2 sensitive febrogenic zone

Fever is induced by elevation of PGE_2 in the brain. Inflammatory stimuli may cause fever through activation of cyclooxygenase-2 (COX-2) and microsomal PGE synthase in brain endothelial cells (43-45) or in perivascular cells (46, 47). PGE_2 is then released from these cells into the brain and acts on the EP receptor-expressing neurons, resulting in fever (for further details, see (48-50)).

Table 1. EP receptor subtypes and their signal transduction in mice

Subtype	Isoform	Second messenger
EP1		$[Ca^{2+}]$ increase
EP2		cAMP increase
EP3		
	EP3α	cAMP decrease
	EP3β	cAMP decrease
	EP3γ	cAMP decrease, increase
EP4		cAMP increase

Table 2. Distribution of EP_1, EP_3, and EP_4 receptor mRNA's in the OVLT/POA region

Brain region	EP1R mRNA	EP3R mRNA	EP4R mRNA
Meningeal strand	+/++	-	-
Organum vasculosum laminae terminalis	++	+	+++
Median preoptic nucleus	++	+++	++++
Ventromedial preoptic nucleus	++	-/+	++++
Anterodorsal preoptic nucleus	++	-	-
Anteroventral periventricular nucleus	+/++	-	+
Preoptic periventricular nucleus	++	++	+++
Ventrolateral preoptic nucleus	+	+/++	+++
Medial preoptic nucleus	+	-/+	+
Border region between the medial preoptic area and the lateral preoptic area	+	++	++/+++
Coexpression with LPS-induced Fos (%)	50-60	< 10	70-80

Qualitative analysis of hybridization signal for EP_1, EP_3, and EP_4 receptor (EP1R, EP3R, and EP4R, respectively) mRNA's in the preoptic area of non-treated rats. *In situ* hybridization histochemistry was accomplished by using specific 35S-labelled antisense riboprobes complementary to the rat EP_1, EP_3, or EP_4 receptors mRNA. Relative grain densities: ++++, very strong; +++, strong; ++, moderate; +, weak but positive; -, background level. R: receptor. [Modified from (20) with permission from John Wiley & Sons, Inc.]

The sensitive site where PGEs produce hyperthermia is located within the region of the brain that includes the OVLT and the surrounding POA (3-6). Evidence for the pivotal role of PGE_2 on the OVLT/POA region in febrogenesis includes the following findings: [1] PGE_2 levels increase in this region during the febrile response to lipopolysaccharide (LPS), a cell wall component of Gram-negative bacteria (51-53); [2] Microinjection of COX inhibitors which block PGE_2 synthesis into this region attenuates fever (9, 10, 54, 55); [3] A high density of PGE_2 binding has been observed in this region (56); and [4] PGE_2 alters the firing rate activity of thermosensitive neurons within the OVLT/POA region (57-59).

Within this region, the hyperthermic action of PGE is more potent when it is microinjected into the anteroventral region of the third ventricle (AV3V), which includes the OVLT, the median preoptic nucleus (MnPO), and the ventromedial preoptic nucleus (VMPO), compared with other regions within the POA (4). This observation was confirmed by an electrophysiological study demonstrating that OVLT neurons respond to PGE_2 at lower doses than do POA neurons (58). Scammell et al. injected a minute volume of PGE_2 (10 – 100 nl) and observed that the induced hyperthermia was most pronounced when PGE_2 was microinjected into the cell-sparse zone below the diagonal band of Broca, the meningeal strand supporting the optic chiasm and parenchyma immediately above the strand, the MnPO, the VMPO, and the anteroventral periventricular nucleus (AVPV) (5) (figure 1).

3.2. EP Receptor distribution within the OVLT/POA region

To assess which EP receptor subtypes are expressed within the PGE_2-sensitive febrogenic zone, we mapped the distribution of EP_{1-4} receptor messenger RNA (mRNA) in the OVLT/POA region in rats and found that at least three of these receptors, i.e. the EP_1, EP_3, and EP_4 receptors, have distinctively different distribution patterns (20). In the meningeal strand which supports the optic chiasm, only EP_1 receptor mRNA was found. In the OVLT, EP_1 receptor mRNA was expressed in the medial part, and EP_4 receptor mRNA was expressed in the lateral part. Low levels of EP_3 receptor mRNA were found within the OVLT. In the POA, both EP_3 receptor mRNA and EP_4 receptor mRNA were strongly expressed in the MnPO and in the border region between the medial preoptic area (MPO) and the lateral preoptic area (LPO), forming an inverted Y shape. EP_4 receptor mRNA was also observed within the VMPO and the AVPV, while EP_3 receptor mRNA was not seen in both nuclei. EP_1 receptor mRNA was distributed diffusely throughout the POA (table 2 and figure 2). EP_2 receptor mRNA is reportedly expressed within the MPO (17). These anatomical studies thus indicate that all four EP receptors are distributed within the PGE_2-sensitive febrogenic zone and that they are expressed on neurons (14-21). Other cell types, such as astrocytes (60), microglia (61), or cerebral microvessel endothelial cells (62-64) are also known to express some EP receptor subtypes. Although the involvement of glial cells in the PGE_2-mediated febrile response is proposed (2), it awaits further study if EP receptors on non-neuronal cells contribute to the development of fever.

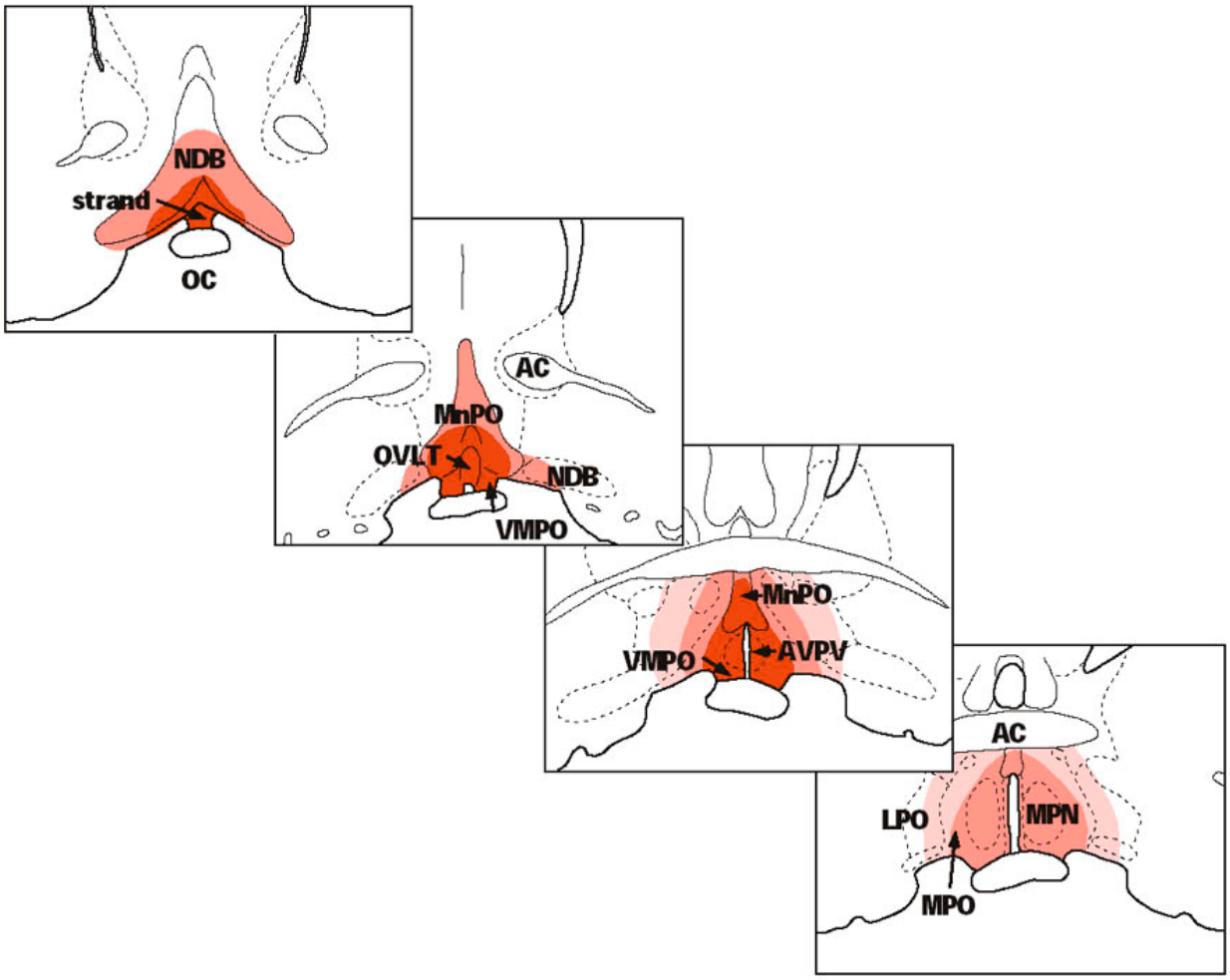

Figure 1. PGE_2-sensitive febrogenic zone as revealed by microinjection of PGE_1, PGE_2 and EP receptor agonists in rats. Coronal sections of the rat brain are illustrated. Red areas represent the region where the microinjection of PGE_2 increases core temperature with darker areas representing greater increases of core temperature. [From (97) with permission from IGAKU-SHOIN].

3.3. Activated EP receptors during fever response

Subsequently, we assessed the coexpression of LPS-induced Fos-like immunoreactivity (Fos-LI) and EP_{1-4} receptor mRNA in nuclei in the rat OVLT/POA region to investigate which EP receptor-expressing neurons are activated during fever (20). Intravenous (i.v.) injection of LPS (5 μg/kg) produced a 1 ^{o}C rise in core temperature (Tc) within 2 hours. At this time point, LPS-induced Fos-LI was observed in the VMPO, in the MnPO, and in the border region between the MPO and the LPO. Within these areas, most Fos-immunoreactive neurons (70-80 %) contained EP_4 receptor mRNA, and about half of them (50-60%) contained EP_1 receptor mRNA. In contrast, despite strong EP_3 receptor mRNA expression within the MnPO, few Fos-immunoreactive neurons (<10%) contained EP_3 receptor mRNA (figure 2).

This high coexpression of Fos-LI and EP_4 receptor mRNA indicates that EP_4 receptor-expressing neurons are activated during LPS-induced fever and suggests some role for EP_4 receptors in the development of fever. However, since the *c-fos* gene is activated by either increased intracellular Ca^{2+} or cAMP, this high coexpression might simply reflect differences in signal transduction pathways between EP isoforms: stimulation of EP_4 receptors increases cAMP, whereas stimulation of the EP_1 receptor increases intracellular Ca^{2+}, and stimulation of EP_3 receptor decreases or increases cAMP depending on its isoform (65, 66). The functional role of activated EP_4 receptor-expressing POA neurons in febrile response should thus be considered in conjunction with physiological studies.

4. EFFECTS OF EP RECEPTOR AGONISTS ON BODY TEMPERATURE

To determine which EP receptor mediates febrile response, we therefore injected EP receptor agonists and antagonists into the rat brain and observed changes in core

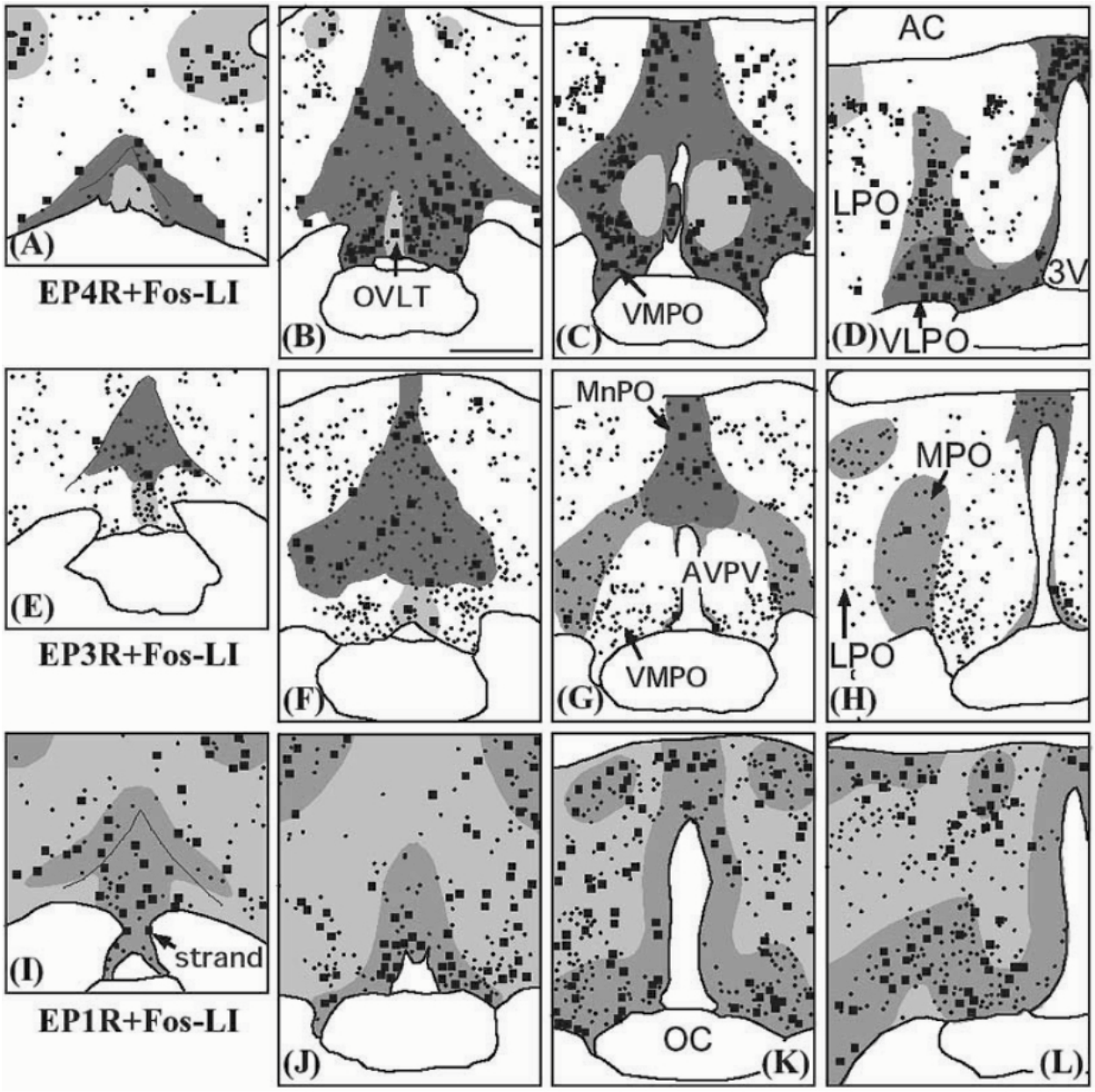

Figure 2. Distribution of activated EP receptor containing neurons within the OVLT-POA region two hours after intravenous administration of lipopolysaccharide. Coronal sections of the rat brain are illustrated. Squares represent Fos-immunoreactive neurons expressing EP_4, EP_3, or EP_1 receptor mRNA. Small dots represent Fos-immunoreactive neurons. Gray areas represent the regions where EP receptor mRNA is predominantly expressed with darker areas representing a greater density of silver grains. (A-D): Fos-like immunoreactivity and its coexpression with EP_4 receptor mRNA (EP4R+Fos-LI). (E-H): Fos-like immunoreactivity and its coexpression with EP_3 receptor mRNA (EP3R+Fos-LI). (I-L): Fos-like immunoreactivity and its coexpression with EP_1 receptor mRNA (EP1R+Fos-LI). Scale bar: 500 μm. [From (20) with permission from John Wiley & Sons, Inc.]

body temperature (Tc). Intracerebroventricular (i.c.v.) injection of PGE_2 induces a monophasic hyperthermia, peaking 30 min after injection. I.c.v. injection of 17-phenyl-ω-trinor PGE_2 (an EP_1 and $EP_{3\alpha}$ receptor agonist) mimicked PGE_2 (i.c.v.)-induced hyperthermia, but injections of butaprost (an EP_2 agonist), M&B28767 (an $EP_{3\alpha}$ agonist), and 11-deoxy-PGE_1 (an EP_4 agonist) did not mimic PGE_2 (i.c.v.)-induced hyperthermia (22). I.c.v. injection of SC19220 (an EP_1 receptor antagonist) blocked PGE_2 (i.c.v.)-induced hyperthermia (22, 67). Microinjection of 17-phenyl-ω-trinor PGE_2 into the OVLT/POA region also increased Tc, whereas microinjections of butaprost and M&B28767 did not increase Tc. The 17-phenyl-ω-trinor PGE_2-induced hyperthermic effect was stronger in the AV3V than the POA. These findings suggest an EP_1 receptor mediation of PGE_2-induced hyperthermia in rats (6).

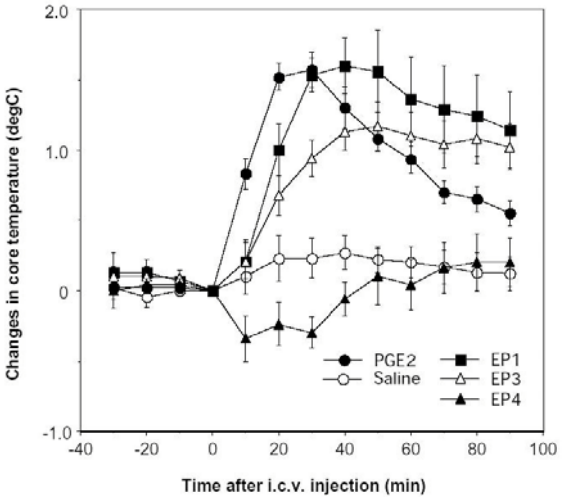

Figure 3. Effects of i.c.v. injection of PGE_2 (0.3 nmol) and ONO EP receptor agonists (20 nmol) on core body temperature in rats. Rats were injected with PGE_2 (closed circle), ONO-DI-004 (an EP_1 agonist, closed square), ONO-AE-248 (an EP_3 agonist, open triangle), ONO-AE1-329 (an EP_4 agonist, closed triangle) and 0.9% saline (open circle) at time zero. Changes in core temperature after i.c.v. injection of vehicle of each EP receptor agonist are not shown in the figure. Each point represents mean ± SEM.

Because of the low selectivity of these agonists to specific receptor subtypes (with the exception of butaprost) (12, 13), a similar pharmacological study was performed using a new generation of more selective EP agonist drugs (24, 68). I.c.v. injection of DI-004 (2 and 20 nmol), an EP_1 receptor agonist, increased Tc in a dose dependent manner with a peak 30 min after injection. ONO-AE1-259-01 (20 nmol), an EP_2 receptor agonist, did not change Tc. ONO-AE-248 (20 nmol), an EP_3 receptor agonist, also increased Tc, but its peak effect was delayed (50 min after injection) compared with PGE_2 (30 min after injection). Given that ONO-AE-248 has more than a 1000-fold higher affinity for EP_3 receptors than for EP_1 receptors (68) and that it inhibits the forskolin-induced increase in cAMP (69), the results with ONO-AE-248 suggest that while EP_3 receptors also may mediate hyperthermia, they probably employ different mechanisms than those associated with EP_1 receptors. In contrast, ONO-AE1-329 (2 nmol), an EP_4 receptor agonist, decreased Tc (figure 3) (24).

To summarize, pharmacological studies in rats suggest that the EP_1 and EP_3 receptors mediate a hyperthermic action and that the EP_4 receptor mediates a hypothermic action. A similar pharmacological study in pigs suggests that the EP_2 receptor is necessary for fever production (70).

5. FEBRILE RESPONSE IN EP RECEPTOR KOCKOUT MICE

Another approach to understanding the mechanisms for the febrile response is to assess responses to pyrogens in mice with EP receptor gene deletions. Ushikubi et al. reported the first such studies, in which they observed changes in rectal temperature after i.v. injections of LPS and interleukin-1β (IL-1β), and after i.c.v. injections of IL-1β and PGE_2 in four EP receptor knockout (KO) mice (11). They found that febrile response was impaired only in EP_3 receptor KO mice. Although this finding suggests a crucial role for EP_3 receptors in evoking fever in mice, several questions remain. [1] Is the EP_3 receptor involved in all phases of LPS-induced fever? Ushikubi et al. conducted their observations during a limited time frame (one hour) following i.v. injection of a single dose of LPS (10 mg/kg). Other investigators have reported that systemic administration of LPS in rats induces monophasic fever, multiphasic fever, or hypothermia, depending on the dose (71), findings which suggests that each phase is mediated by different neural mechanisms (72). [2] What role, if any, does EP_3 receptor have in local inflammation-induced fever? Published studies suggest that local inflammation-induced fever is mediated by afferent neural pathways (73, 74) and cytokines (75, 76) that differ from the neural pathways and cytokines associated with systemic inflammation-induced fever. [3] Most importantly, did Ushikubi et al. properly control for the effects of stress hyperthermia? After they administered LPS (i.v.), IL-1β (i.v.), and PGE_2 (i.c.v.) to their mice, the resultant elevations in Tc all peaked 20 min after injections, a pattern which coincides with the temperature response pattern of psychological stress-induced hyperthermia (77, 78). Thus it is possible that they merely observed stress-induced hyperthermia, and that the impaired rise in Tc that their EP_3 receptor KO mice exhibited in response to LPS and IL-1β might be due to a resistance to stress-induced hyperthermia unique to EP_3 receptor KO mice.

To address these questions, we extended the experiments of Ushikubi et al. by observing changes in Tc using telemetry in wild type (WT) mice, EP_1 receptor KO mice, and EP_3 receptor KO mice (78) (figure 4). [1] We investigated for at least 10 hours following injection the effect of intraperitoneal (i.p.) LPS on Tc over a range of LPS dosages (1 μg/kg - 1 mg/kg). In WT mice, i.p. injection of LPS at 10 μg/kg induced the early (within 60 min) and the middle (2 – 3 hrs) phases of fever. At 100 μg/kg, LPS induced the late phase (5 – 10 hrs) of fever in addition to early and middle phases. At 1 mg/kg, LPS decreased Tc, possibly due to endotoxin shock. In EP_1 receptor KO mice, LPS at 10 μg/kg reduced the early phase, but not the middle phase of fever . LPS at 100 μg/kg suppressed fever completely. LPS at 1 mg/kg exacerbated hypothermia. In EP_3 receptor KO mice, LPS lowered Tc in a dose- and time-dependent manner. Diurnal changes in Tc remained normal in both EP_1 and EP_3 receptor KO mice. [2] We also investigated the effect of the subcutaneous (s.c.) injection of turpentine on Tc over a 48 hr observation period. S.c. injection of turpentine induced fever in EP_1 receptor KO mice and WT mice, but it did not induce fever in EP_3 receptor KO mice. [3] Psychological stress, including cage-exchange stress and buddy-removal stress, induced a monophasic hyperthermia peaking at 20 min in both EP_1 receptor KO mice and EP_3 receptor KO mice. These patterns of hyperthermia were identical to those observed in WT mice. Therefore, inhibition of stress-induced hyperthermia is not a mechanism responsible for impairment of the febrile response in EP_3 receptor KO mice.

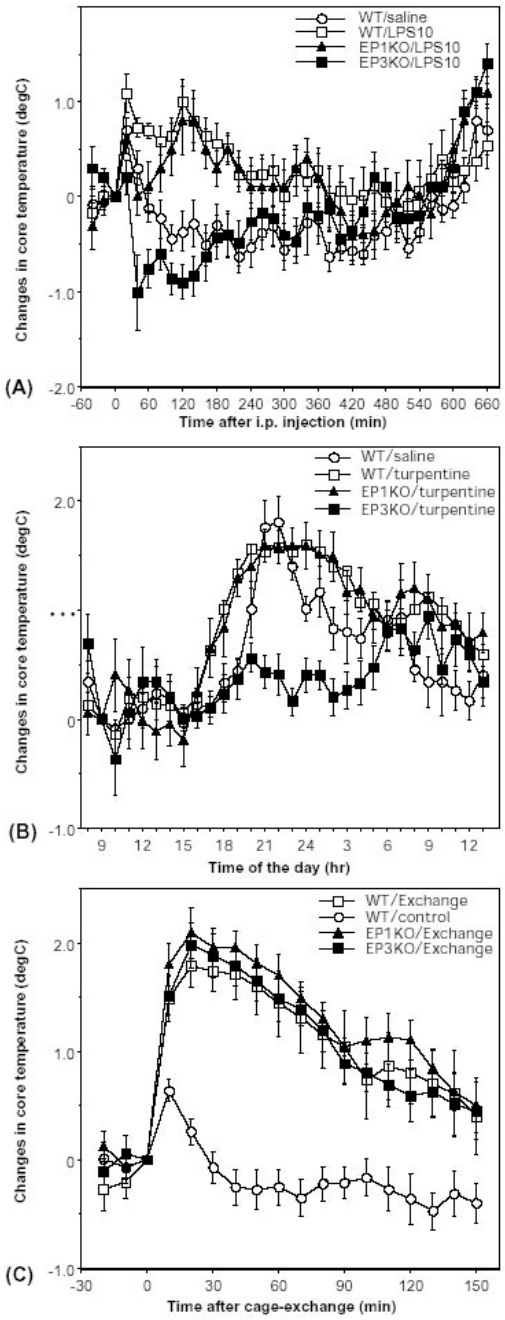

Figure 4. Effects of i.p. injection of LPS (A), s.c. injection of turpentine (B), and psychological stress (C) on core body temperature in WT mice (open square), EP_1 receptor KO mice (closed triangle), and EP_3 receptor KO mice (closed square). In A, mice were injected with LPS at 10 μg/kg or 0.9% saline (open circle) at time zero (9 a.m.). In B, mice were injected with turpentine or 0.9% saline (open circle) at 9 a.m. In C, cages of two mice were exchanged, or mice were removed and replaced in the same cages (open circle) as a control at time zero. Each point represents mean ± SEM. Bar shows dark period. Changes in core temperature after i.p. or s.c. injection of vehicle in EP_1 receptor KO mice and EP_3 receptor KO mice are not shown in the figure. [Modified from (78) with permission from The Physiological Society.]

Although pharmacological studies in rats suggest that the EP_1 and EP_3 receptors mediate a hyperthermic action and that the EP_4 receptor mediates a hypothermic action, our study suggests that in mice, EP_3 receptors play a crucial role in fever responses to both systemic inflammation and local inflammation. EP_1 receptors may be partially involved in LPS-induced fever. Neither EP_1 nor EP_3 receptors are involved in circadian rises in Tc or psychological stress-induced hyperthermia. In addition, EP_3 receptor KO mice tended to show a hypothermic response after systemic injection of LPS (11, 78), s.c. injection of turpentine (78) and i.c.v. injection of PGE_2 (25), suggesting that certain EP receptors have a hypothermic action in mice.

6. CHANGES IN FIRING RATE RESPONSES OF OVLT/POA NEURONS BY PGE_2

Electrophysiological studies have shown that i.v. injection of IL-1β, a pyrogenic cytokine, changes the firing rate of neurons within the OVLT/POA region, i.e. it inhibits 50% of neurons in the AV3V and the MnPO, and it also excites 30% of them (table 3) (79). According to Hammel's model, fever is induced by inhibiting firing rate of warm-sensitive (W) neurons and exciting that of temperature-insensitive (I) neurons in the hypothalamus (80). In accordance with this, direct bath application of PGE_2 predominantly inhibited the activity of W neurons in the OVLT (58) and in the VMPO (59) and excited that of I neurons in the VMPO (59) in rat tissue slices. In the POA, however, PGE_2 excited a considerable population of W neurons (57, 58). In any case, since PGE_2-induced changes in the firing rate of the thermosensitive neurons were still observed even in a Ca^{2+} free and high Mg^{2+} solution which blocks synaptic activity, post-synaptic responsiveness of OVLT and POA neurons to PGE_2 is suggested (58).

So far, however, researchers have not definitively identified which EP receptors are involved in inhibition or excitation of thermosensitive or temperature-insensitive neuronal activities. Available evidence indicates that AH6809, an EP_1 and/or D type PG receptor antagonist (12), blocks the PGE_2-induced inhibition of both OVLT and POA neurons (58).

7. EFFERENT FEVER-PRODUCING PATHWAYS FROM THE OVLT/POA REGION

As described above, the action of PGE_2 on neurons in the OVLT/POA region is thought to be crucial for fever induction. However, the neuroanatomical chain of functionally connected neurons from the PGE_2-sensitive febrogenic zone to the effectors that contribute to fever induction has not yet been fully demonstrated. The interscapular BAT is the major organ for non-shivering thermogenesis during fever and the tail artery contributes to fever development by inhibiting heat-loss via vasoconstriction in rats. Therefore, to define the central circuitry responsible for the febrile response, pseudorabies virus (PRV) was injected into the interscapular BAT, and the distribution of infected neurons was traced in rats (37, 38, 42) and hamsters (81). In the rat forebrain, infected neurons were found in the OVLT, the ventral MnPO, the

Table 3. Effects of bath-applied PGE_2 on the activity of OVLT and POA neurons in comparison with those of systemically administered IL-1β

(1) Effects of bath-applied PGE_2 on the activity of OVLT and preoptic neurons.				
(1-1) OVLT (Ref. 58)				
	All neurons (%)	**W neurons (%)**	**I neurons (%)**	**C neurons (%)**
Excitation	14/51 (27)	7/30 (23)	7/20 (35)	0/1 (0)
No effect	9/51 (18)	2/30 (7)	7/20 (35)	0/1 (0)
Inhibition	28/51 (55)	21/30 (70)	6/20 (30)	1/1 (100)
Total	51	30/51 (59)	20/51 (39)	1/51 (2)
(1-2) MPO (Ref. 58)				
Excitation	17/58 (29)	11/28 (39)	6/29 (21)	0/1 (0)
No effect	26/58 (45)	8/28 (29)	17/29 (58)	1/1 (100)
Inhibition	15/58 (26)	9/28 (32)	6/29 (21)	0/1 (0)
Total	58	28/58 (48)	29/58 (50)	1/58 (2)
(1-3) POA (Ref. 57)				
Excitation	48/89 (54)	34/55 (62)	13/31 (42)	1/3 (33)
No effect	36/89 (40)	18/55 (33)	16/31 (52)	2/3 (67)
Inhibition	5/89 (6)	3/55 (5)	2/31 (6)	0/3 (0)
Total	89	55/89 (62)	31/89 (35)	3/89 (3)
(2) Effects of intravenous IL-1β on the activity of AV3V and MnPO neurons. (Ref. 79)				
(2-1) AV3V	**All neurons (%)**		**(2-2) MnPO**	**All neurons (%)**
Excitation	6/19 (32)		Excitation	7/21 (33)
No effect	3/19 (16)		No effect	4/21 (19)
Inhibition	10/19 (52)		Inhibition	10/21 (48)
Total	19		Total	21

medial preoptic nucleus, the VMPO, and the VLPO, suggesting polysynaptic regulation of BAT thermogenesis (38). This region coincides with the region where labeled neurons were found following injection of PRV into the wall of the ventral tail artery (82) and with the PGE_2-sensitive febrogenic zone where EP_1, EP_3, and EP_4 receptors are distributed.

40 % of virally infected POA neurons expressed EP_3 receptor-LI (38). About 90% of EP_3 receptor-immunoreactive POA neurons exhibited signals for GAD67 mRNA. EP_3 receptor-expressing GABAergic neurons send direct projections to the rostral raphe pallidus nucleus (RPa) (36). The RPa contains the sympathetic premotor neurons responsible for BAT thermogenesis (83) and vasoconstriction of the tail artery (84, 85), both of which contribute to the febrile response. IntraPOA PGE_2 microinjection-induced BAT thermogenesis was blocked by the microinjection of muscimol, a $GABA_A$ receptor agonist, into the RPa (36). In addition, microinjection of bicuculline, a $GABA_A$ receptor antagonist, into the RPa increased sympathetic nerve activity (SNA) to the BAT in rats (83). Therefore, it is reasonable to hypothesize that EP_3 receptor-expressing GABAergic POA neurons tonically inhibit RPa neurons and that a PGE_2-triggered mechanism in the POA effectively relieves the tonic inhibition of the RPa neurons, leading to the excitation of SNA to the BAT and the tail artery, thereby producing fever (36, 81).

IntraPOA PGE_2 microinjection-induced hyperthermia was also attenuated by microinjection of kynurenate, an ionotropic excitatory amino acid (EAA) receptor antagonist, into the RPa (39) or the DMH (40). These findings suggest that a PGE_2-induced disinhibition of the RPa neurons is not the sole mechanism for producing fever and that activation of EAA receptors in the DMH or the RPa is also necessary for the BAT thermogenesis evoked by microinjection of PGE_2 into the POA. Involvement of other hypothalamic nuclei, including the paraventricular hypothalamic nucleus (87-89) and the posterior hypothalamus (90), has also been reported. Furthermore, PRV-infected nuclei include the lateral hypothalamic, perifornical and retrochiasmatic nuclei, ventrolateral periaqueductal gray matter, the lateral parabrachial nucleus, and the nucleus of the solitary tract (NTS) (37, 38, 42). So far, however, it remains unknown whether EP_3-receptor expressing or other EP receptors-expressing POA neurons send excitatory output to the RPa or the DMH, and it also remains unknown how specific EP receptor-expressing neurons affect neurons within other PRV-infected nuclei.

8. PERSPECTIVES

The EP_3 receptor is undoubtably crucial for the febrile response in mice. On the other hand, i.c.v. or intraPOA injection of EP_1 receptor agonists produces more potent hyperthermia than EP_3 receptor agonists in rats (6, 22, 24). IntraPOA injection of PGE_2 induces hyperalgesia and hyperthermia, whereas injection of M&B28767, an EP_3 receptor agonist, induces hyperalgesia but has little effect on Tc in rats (6, 33). Furthermore, lesions in the PGE_2-sensitive febrogenic zone did not attenuate PGE_1 or PGE_2-induced hyperthermia in squirrel monkeys (91) or rats (92). These findings raise a question whether the OVLT/POA region is really the only site where PGE_2 acts on EP_3 receptors (or other EP receptors) to produce hyperthermia.

EP_3 receptor mRNA or immunoreactivity has

been demonstrated at other levels of the nervous system, e.g. in the RPa, the intermediolateral cell column (IML) in the spinal cord, and vagal afferents in the NTS (18, 19 93), and in peripheral cells such as macrophages (94, 95). It may be possible that EP_3 receptors in these regions or cell types also contribute to febrile response. For example, two-thirds of infected RPa neurons that polysynaptically project to the interscapular BAT expressed EP_3 receptor-LI (38). Bath application of PGE_2 and M&B28767 depolarized the membrane of dorsal raphe neurons (96). These findings suggest that RPa neurons themselves might possess PG sensitivity that is able to modulate BAT thermogenesis under febrile conditions. Therefore, future studies should include investigation of the role of EP_3 receptors in the RPa, IML, or NTS in thermoregulatory and febrile responses.

9. ACKNOWLEDGEMENTS

I thank Drs. Tetsuro Hori, Clifford B Saper, Shuh Narumiya, Kae Oka, Masako Hosoi, Michie Abe, Thomas E Scammell, Charlotte Lee, Joseph F Kelly, Francois Nantel, Joel K Elmquist, Takuya Kobayashi, Yukihiko Sugimoto, Atsushi Ichikawa, and Fumitaka Ushikubi, for their collaboration with me.

10. REFERENCES

1. Milton, A. S. and S. Wendlandt: A possible role for prostaglandin E1 as a modulator for temperature regulation in the central nervous system of the cat. *J Physiol (Lond)* 207, 76P-77P (1970)

2. Blatteis, C. M. and E. Sehic: Prostaglandin E2: A putative fever mediator. In: Fever basic mechanisms and management. Ed: P. A. Mackowiak, Lippincott-Raven, Philadelphia, 117-145 (1997)

3. Williams, J. W., T. A. Rudy, T. L. Yaksh and C. T. Viswanathan: An extensive exploration of the rat brain for sites mediating prostaglandin-induced hyperthermia. *Brain Res* 120, 251-262 (1977)

4. Stitt, J. T: Differential sensitivity in the sites of fever production by prostaglandin E1 within the hypothalamus of the rat. *J Physiol (Lond)* 432, 99-110 (1991)

5. Scammell, T. E., J. K. Elmquist, J. D. Griffin and C. B. Saper: Ventromedial preoptic prostaglandin E2 activates fever-producing autonomic pathways. *J Neurosci* 16, 6246-6254 (1996)

6. Oka, K., T. Oka and T. Hori: Prostaglandin E2 may induce hyperthermia through EP1 receptor in the anterior wall of the third ventricle and neighboring preoptic regions. *Brain Res* 767, 92-99 (1997)

7. Feldberg, W. and P. N. Saxena: Further studies on prostaglandin E 1 fever in cats. *J Physiol (Lond)* 219, 739-745 (1971)

8. Stitt, J. T: Prosaglandin E1 fever induced in rabbits. *J Physiol* 232, 163-179 (1973)

9. Vaughn, L. K., W. L. Veale and K. E. Cooper: Sensitivity of hypothalamic sites to salicylate and prostaglandin. *Can J Physiol Pharmacol* 57, 118-123 (1979)

10. Morimoto, A., N. Murakami, T. Nakamori, and T. Watanabe: Multiple control of fever production in the central nervous system of rabbits. *J Physiol (Lond)* 397, 269-280 (1988)

11. Ushikubi, F., E. Segi, Y. Sugimoto, T. Murata, T. Matsuoka, T. Kobayashi, H. Hizaki, K. Tuboi, M. Katsuyama, A. Ichikawa, T. Tanaka, N. Yoshida and S. Narumiya: Impaired febrile response in mice lacking the prostaglandin E receptor subtype EP3. *Nature* 395, 281-284 (1998)

12. Boie, Y., R. Stocco, N. Sawyer, D. M. Slipetz, M. D. Ungrin, F. Neuschafer-Rube, G. P. Puschel, K. M. Metters, and M. Abramovitz: Molecular cloning and characterization of the four rat prostaglandin E2 prostanoid receptor subtypes. *Eur J Pharmacol* 340, 227-241 (1997)

13. Kiriyama, M., F. Ushikubi, T. Kobayashi, M. Hirata, Y. Sugimoto and S. Narumiya: Ligand binding specificities of the eight types and subtypes of the mouse prostanoid receptors expressed in Chinese hamster ovary cells. *Br J Pharmacol* 122, 217-224 (1997)

14. Sugimoto, Y., R. Shigemoto, T. Namba, M. Negishi, N. Mizuno, S. Narumiya and A. Ichikawa: Distribution of the messenger RNA for the prostaglandin E receptor subtype EP3 in the mouse nervous system. *Neuroscience* 62, 919-928 (1994)

15. Rage, F., B. J. Lee, Y. J. Ma, and S. R. Ojeda: Estradiol enhances prostaglandin E2 receptor gene expression in luteinizing hormone-releasing hormone (LHRH) neurons and facilitates the LHRH response to PGE2 by activating a glia-to-neuron signaling pathway. *J Neurosci* 17, 9145-9156 (1997)

16. Nakamura, K., T. Kaneko, Y. Yamashita, H. Hasegawa, H. Katoh, A. Ichikawa and M. Negishi: Immunocytochemical localization of prostaglandin EP3 receptor in the rat hypothalamus. *Neurosci Lett* 260, 117-120 (1999)

17. Zhang, J. and S. Rivest: Distribution, regulation and colocalization of the genes encoding the EP2- and EP4-PGE2 receptors in the rat brain and neuronal responses to systemic inflammation. *Eur J Neurosci* 11, 2651-2668 (1999)

18. Nakamura, K., T. Kaneko, Y. Yamashita, H. Hasegawa, H. Katoh and M. Negishi: Immunohistochemical localization of prostaglandin EP3 receptor in the rat nervous system. *J Comp Neurol* 421, 543-569 (2000)

19. Ek, M., C. Arias, P. Sawchenko and A. Ericsson-Dahlstrand: Distribution of the EP3 prostaglandin E(2)

receptor subtype in the rat brain: relationship to sites of interleukin-1-induced cellular responsiveness. *J Comp Neurol* 428, 5-20 (2000)

20. Oka, T., K. Oka, T. E. Scammell, C. Lee, J. F. Kelly, F. Nantel, J. K. Elmquist, and C. B. Saper: Relationship of EP(1-4) prostaglandin receptors with rat hypothalamic cell groups involved in lipopolysaccharide fever responses. *J Comp Neurol* 428, 20-32 (2000)

21. Zhang, J. and S. Rivest: A functional analysis of EP4 receptor-expressing neurons in mediating the action of prostaglandin E2 within specific nuclei of the brain in response to circulating interleukin-1beta. *J Neurochem* 74, 2134-2145 (2000)

22. Oka, T., and T. Hori: EP1-receptor mediation of prostaglandin E2-induced hyperthermia in rats. *Am J Physiol* 267, R289-294 (1994)

23. Oka, K., T. Oka, and T. Hori: PGE2 receptor subtype EP1 antagonist may inhibit central interleukin- 1beta-induced fever in rats. *Am J Physiol* 275, R1762-1765 (1998)

24. Oka, T., K. Oka, and C. B. Saper: Contrasting effects of E type prostaglandin (EP) receptor agonists on core body temperature in rats. *Brain Res* 968, 256-262 (2003)

25. Engblom, D., S. Saha, L. Engstrom, M. Westman, L. P. Audoly, P. J. Jakobsson and A. Blomqvist: Microsomal prostaglandin E synthase-1 is the central switch during immune-induced pyresis. *Nat Neurosci* 6, 1137-1138 (2003)

26. Yoshida, Y., H. Matsumura, T. Nakajima, M. Mandai, T. Urakami, K. Kuroda and H. Yoneda: Prostaglandin E (EP) receptor subtypes and sleep: promotion by EP4 and inhibition by EP1/EP2. *Neuroreport* 11, 2127-2131 (2000)

27. Huang, Z. L., Y. Sato, T. Mochizuki, T. Okada, W. M. Qu, A. Yamatodani, Y. Urade and O. Hayaishi: Prostaglandin E2 activates the histaminergic system via the EP4 receptor to induce wakefulness in rats. *J Neurosci* 23, 5975-5983 (2003)

28. Matsuoka, Y., T. Furuyashiki, H. Bito, F. Ushikubi, Y. Tanaka, T. Kobayashi, S. Muro, N. Satoh, T. Kayahara, M. Higashi, A. Mizoguchi, H. Shichi, Y. Fukuda, K. Nakao and S. Narumiya: Impaired adrenocorticotropic hormone response to bacterial endotoxin in mice deficient in prostaglandin E receptor EP1 and EP3 subtypes. *Proc Natl Acad Sci USA* 100, 4132-4137 (2003)

29. Oka, T., S. Aou and T. Hori: Intracerebroventricular injection of prostaglandin E2 induces thermal hyperalgesia in rats: the possible involvement of EP3 receptors. *Brain Res* 663, 287-292 (1994)

30. Oka, T., K. Oka, M. Hosoi and T. Hori: Inhibition of peripheral interleukin-1 beta-induced hyperalgesia by the intracerebroventricular administration of diclofenac and alpha-melanocyte-stimulating hormone. *Brain Res* 736, 237-242 (1996)

31. Oka, T., T. Hori, M. Hosoi, K. Oka, M. Abe and C. Kubo: Biphasic modulation in the trigeminal nociceptive neuronal responses by the intracerebroventricular prostaglandin E2 may be mediated through different EP receptors subtypes in rats. *Brain Res* 771, 278-284 (1997)

32. Oka, T., M. Hosoi, K. Oka and T. Hori: Biphasic alteration in the trigeminal nociceptive neuronal responses after intracerebroventricular injection of prostaglandin E2 in rats. *Brain Res* 749, 354-357 (1997)

33. Hosoi, M., T. Oka and T. Hori: Prostaglandin E receptor EP3 subtype is involved in thermal hyperalgesia through its actions in the preoptic hypothalamus and the diagonal band of Broca in rats. *Pain* 71, 303-311 (1997)

34. Hosoi, M., T. Oka, M. Abe, T. Hori, H. Yamamoto, K. Mine and C Kubo: Prostaglandin E(2) has antinociceptive effect through EP(1) receptor in the ventromedial hypothalamus in rats. *Pain* 83, 221-227 (1999)

35. Abe, M., T. Oka, T. Hori and S. Takahashi: Prostanoids in the preoptic hypothalamus mediate systemic lipopolysaccharide-induced hyperalgesia in rats. *Brain Res* 916, 41-49 (2001)

36. Nakamura, K., K. Matsumura, T. Kaneko, S. Kobayashi, H. Katoh and M. Negishi: The rostral raphe pallidus nucleus mediates pyrogenic transmission from the preoptic area. *J Neurosci* 22, 4600-4610 (2002)

37. Oldfield, B. J., M. E. Giles, A. Watson, C. Anderson, L. M. Colvill and M. J. McKinley: The neurochemical characterisation of hypothalamic pathways projecting polysynaptically to brown adipose tissue in the rat. *Neuroscience* 110, 515-526 (2002)

38. Yoshida, K., K Nakamura, K. Matsumura, K. Kanosue, M. Konig, H. J. Thiel, Z. Boldogkoi, I. Toth, J. Roth, R. Gerstberger and T. Hubschle: Neurons of the rat preoptic area and the raphe pallidus nucleus innervating the brown adipose tissue express the prostaglandin E receptor subtype EP3. *Eur J Neurosci* 18, 1848-1860 (2003)

39. Madden, C. J. and S. F. Morrison: Excitatory amino acid receptor activation in the raphe pallidus area mediates prostaglandin-evoked thermogenesis. *Neuroscience* 122, 5-15 (2003)

40. Madden, C. J., and S. F. Morrison: Excitatory amino acid receptors in the dorsomedial hypothalamus mediate prostaglandin-evoked thermogenesis in brown adipose tissue. *Am J Physiol Regul Integr Comp Physiol* 286, R320-325 (2004)

41. Zaretskaia, M. V., D. V. Zaretsky and J. A. DiMicco: Role of the dorsomedial hypothalamus in thermogenesis and tachycardia caused by microinjection of prostaglandin E2 into the preoptic area in anesthetized rats. *Neurosci Lett*

340, 1-4 (2003)

42. Cano, G., A. M. Passerin, J. C. Schiltz, J. P. Card, S. F. Morrison and A. F. Sved: Anatomical substrates for the central control of sympathetic outflow to interscapular adipose tissue during cold exposure. *J Comp Neurol* 460, 303-326 (2003)

43. Ek, M., D. Engblom, S. Saha, A. Blomqvist, P. J. Jakobsson and A. Ericsson-Dahlstrand: Inflammatory response: pathway across the blood-brain barrier. *Nature* 410, 430-431 (2001)

44. Yamagata, K., K. Matsumura, W. Inoue, T. Shiraki, K. Suzuki, S. Yasuda, H. Sugiura, C. Cao, Y. Watanabe and S. Kobayashi: Coexpression of microsomal-type prostaglandin E synthase with cyclooxygenase-2 in brain endothelial cells of rats during endotoxin-induced fever. *J Neurosci* 21, 2669-2677 (2001)

45. Engblom, D., M. Ek, I. M. Andersson, S. Saha, M. Dahlstrom, P. J. Jakobsson, A. Ericsson-Dahlstrand and A. Blomqvist: Induction of microsomal prostaglandin E synthase in the rat brain endothelium and parenchyma in adjuvant-induced arthritis. *J Comp Neurol* 452, 205-214 (2002)

46. Elmquist, J. K., C. D. Breder, J. E. Sherin, T. E. Scammell, W. F. Hickey, D. Dewitt and C. B. Saper: Intravenous lipopolysaccharide induces cyclooxygenase 2-like immunoreactivity in rat brain perivascular microglia and meningeal macrophages. *J Comp Neurol* 381, 119-129 (1997)

47. Schiltz, J. C. and P. E. Sawchenko: Distinct brain vascular cell types manifest inducible cyclooxygenase expression as a function of the strength and nature of immune insults. *J Neurosci* 22, 5606-5618 (2002)

48. Schiltz, J. C. and P. E. Sawchenko: Signaling the brain in systemic inflammation: the role of perivascular cells. *Front Biosci* 8, s1321-1329 (2003)

49. Matsumura, K: Signaling the brain in systemic inflammation: The role of endothelial cells. *Front Biosci*, in press (2004)

50. Ivanov, A. I. and A. A. Romanovsky: Prostaglandin E2 as a mediator of fever: synthesis and catabolism. *Front Biosci* 9, 1977-1993 (2004)

51. Sirko, S., I. Bishai and F. Coceani: Prostaglandin formation in the hypothalamus in vivo: effect of pyrogens. *Am J Physiol* 256, R616-624 (1989)

52. Van Dam, A. M., M. Brouns, A. H. W. Man, and F. Berkenbosch: Immunocytochemical detection of prostaglandin E2 in microvasculature and in neurons of rat brain after administration of bacterial endotoxin. *Brain Res* 613, 331-336 (1993)

53. Sehic, E., M. Szekely, A. L. Ungar, A. Oladehin and C. M. Blatteis: Hypothalamic prostaglandin E2 during lipopolysaccharide-induced fever in guinea pigs. *Brain Res Bull* 39, 391-399 (1996)

54. Cranston, W. I. and M. D. Rawlins: Effects of intracerebral micro-injection of sodium salicylate on temperature regulation in the rabbit. *J Physiol (Lond)* 222, 257-266 (1972)

55. Scammell, T. E., J. D. Griffin, J. K. Elmquist and C. B. Saper: Microinjection of a cyclooxygenase inhibitor into the anteroventral preoptic region attenuates LPS fever. *Am J Physiol* 274, R783-789 (1998)

56. Matsumura, K., Y. Watanabe, K. Imai-Matsumura, M. Connolly, Y. Koyama, H. Onoe and Y. Watanabe: Mapping of prostaglandin E2 binding sites in rat brain using quantitative autoradiography. *Brain Res* 581, 292-298 (1992)

57. Morimoto, A., N. Murakami and T. Watanabe: Effect of prostaglandin E2 on thermoresponsive neurones in the preoptic and ventromedial hypothalamic regions of rats. *J Physiol (Lond) 1988* 405, 713-725 (1988)

58. Matsuda, T., T. Hori and T. Nakashima: Thermal and PGE2 sensitivity of the organum vasculosum lamina terminalis region and preoptic area in rat brain slices. *J Physiol (Lond)* 454, 197-212 (1992)

59. Ranels, H. J. and J. D. Griffin: The effects of prostaglandin E2 on the firing rate activity of thermosensitive and temperature insensitive neurons in the ventromedial preoptic area of the rat hypothalamus. *Brain Res* 964, 42-50 (2003)

60. Fiebich, B. L., S. Schleicher, O. Spleiss, M. Czygan and Hull M: Mechanisms of prostaglandin E2-induced interleukin-6 release in astrocytes: possible involvement of EP4-like receptors, p38 mitogen-activated protein kinase and protein kinase C. *J Neurochem* 79, 950-958 (2001)

61. Patrizio, M., M. Colucci and G. Levi: Protein kinase C activation reduces microglial cyclic AMP response to prostaglandin E2 by interfering with EP2 receptors. *J Neurochem* 74, 400-405 (2000)

62. Li, D. Y., D. Abran, K. G. Peri, D. R. Varma and S. Chemtob: Inhibition of prostaglandin synthesis in newborn pigs increases cerebral microvessel prostaglandin F2 alpha and prostaglandin E2 receptors, their second messengers and vasoconstrictor response to adult levels. *J Pharmacol Exp Ther* 278, 370-377 (1996)

63. Chemtob, S., D. Y. Li, D, Abran, K. G. Peri and D. R. Varma: Regulation of cerebrovascular prostaglandin E2 (PGE2) and PGF2 alpha receptors and their functions during development. *Semin Perinatol* 20, 164-172 (1996)

64. Gobeil, F. Jr., I. Dumont, A. M. Marrache, A. Vazquez-Tello, S. G. Bernier, D. Abran, X. Hou, M. H. Beauchamp, C. Quiniou, A. Bouayad, S. Choufani, M. Bhattacharya, S.

Molotchnikoff, A. Ribeiro-Da-Silva, D. R. Varma, G. Bkaily and S. Chemtob: Regulation of eNOS expression in brain endothelial cells by perinuclear EP(3) receptors. *Circ Res* 90, 682-689 (2002)

65. Ushikubi, F., M. Hirata and S. Narumiya: Molecular biology of prostanoid receptors; an overview. *J Lipid Mediat Cell Signal* 12, 343-359 (1995)

66. Sheng, M. and M. E. Greenberg: The regulation and function of c-fos and other immediate early genes in the nervous system. *Neuron* 4, 477-485 (1990)

67. Cranston, W. I., G. W. Duff, R. F. Hellon, D. Mitchell and Y. Townsend: Evidence that brain prostaglandin synthesis is not essential in fever. *J Physiol* 259, 239-249 (1976)

68. Suzawa, T., C. Miyaura, M. Inada, T. Maruyama, Y. Sugimoto, F. Ushikubi, A. Ichikawa, S. Narumiya, and T. Suda: The role of prostaglandin E receptor subtypes (EP1, EP2, EP3, and EP4) in bone resorption: an analysis using specific agonists for the respective EPs. *Endocrinology* 141, 1554-1559 (2000)

69. Zacharowski, K., A. Olbrich, J. Piper, G. Hafner, K. Kondo and C Thiemermann: Selective activation of the prostanoid EP(3) receptor reduces myocardial infarct size in rodents. *Arterioscler Thromb Vasc Biol* 19, 2141-2147 (1999)

70. Parrott, R. F. and S. V. Vellucci: Effects of centrally administered prostaglandin EP receptor agonists on febrile and adrenocortical responses in the prepubertal pig. *Brain Res Bull* 41, 97-103 (1996)

71. Romanovsky, A. A., C. T. Simons and V. A. Kulchitsky: "Biphasic" fevers often consist of more than two phases. *Am J Physiol* 275, R323-331 (1998)

72. Szekely, M., M. Balasko, V. A. Kulchitsky, C. T. Simons, A. I. Ivanov and A. A. Romanovsky: Multiple neural mechanisms of fever. *Auton Neurosci* 85, 78-82 (2000)

73. Goldbach, J. M., J. Roth and E. Zeisberger: Fever suppression by subdiaphragmatic vagotomy in guinea pigs depends on the route of pyrogen administration. *Am J Physiol* 272, R675-681 (1997)

74. Gourine, A. V., K. Rudolph, A. S. Korsak, J. Kubatko, J. Tesfaigzi, W. Kozak and M. J. Kluger: Role of capsaicin-sensitive afferents in fever and cytokine responses during systemic and local inflammation in rats. *Neuroimmunomodulation* 9, 13-22 (2001)

75. Leon, L. R., C. A. Conn, M. Glaccum and M. J. Kluger: IL-1 type I receptor mediates acute phase response to turpentine, but not lipopolysaccharide, in mice. *Am J Physiol* 271, R1668-1675 (1996)

76. Leon, L. R., W. Kozak, J. Peschon and M. J. Kluger: Exacerbated febrile responses to LPS, but not turpentine, in TNF double receptor-knockout mice. *Am J Physiol* 272, R563-569 (1997)

77. Oka, T., K. Oka and T. Hori: Mechanisms and mediators of psychological stress-induced rise in core temperature. *Psychosom Med* 63, 476-486 (2001)

78. Oka, T., K. Oka, T. Kobayashi, Y. Sugimoto, A. Ichikawa, F. Ushikubi, S. Narumiya and C. B. Saper: Characteristics of thermoregulatory and febrile responses in mice deficient in prostaglandin EP1 and EP3 receptors. *J Physiol* 551, 945-954 (2003)

79. Ota, K., T. Katafuchi, A. Takaki and T Hori: AV3V neurons that send axons to hypothalamic nuclei respond to the systemic injection of IL-1beta. *Am J Physiol* 272, R532-540 (1997)

80. Hammel, H. T: Neurons and temperature regulation. In: Physiological controls and regulations. Eds: W. S. Yamamoto and J. R. Brobeck, WB Saunders, Philadelphia, 71-97 (1965)

81. Bamshad, M., C. K. Song and T. J. Bartness: CNS origins of the sympathetic nervous system outflow to brown adipose tissue. *Am J Physiol* 276, R1569-1578 (1999)

82. Smith, J. E., A. S. Jansen, M. P. Gilbey and A. D. Loewy: CNS cell groups projecting to sympathetic outflow of tail artery: neural circuits involved in heat loss in the rat. *Brain Res* 786, 153-164 (1998)

83. Morrison, S. F., A. F. Sved and A. M. Passerin: GABA-mediated inhibition of raphe pallidus neurons regulates sympathetic outflow to brown adipose tissue. *Am J Physiol* 276, R290-297 (1999)

84. Rathner, J. A. and R. M. McAllen: Differential control of sympathetic drive to the rat tail artery and kidney by medullary premotor cell groups. *Brain Res* 834, 196-199 (1999)

85. Blessing, W. W. and E. Nalivaiko: Raphe magnus/pallidus neurons regulate tail but not mesenteric arterial blood flow in rats. *Neuroscience* 105, 923-929 (2001)

86. Morrison, S. F: Raphe pallidus neurons mediate prostaglandin E2-evoked increases in brown adipose tissue thermogenesis. *Neuroscience* 121, 17-24 (2003)

87. Horn, T., M. F. Wilkinson, R. Landgraf and Q. J. Pittman: Reduced febrile responses to pyrogens after lesions of the hypothalamic paraventricular nucleus. *Am J Physiol* 267, R323-328 (1994)

88. Caldeira, J. C., C. R. Franci and I. R. Pela: Bilateral lesion of hypothalamic paraventricular nucleus abolishes fever induced by endotoxin and bradykinin in rats. *Ann N Y*

Acad Sci 856, 294-297 (1998)

89. Lu, J., Y. H. Zhang, T. C. Chou, S. E. Gaus, J. K. Elmquist, P. Shiromani and C. B. Saper: Contrasting effects of ibotenate lesions of the paraventricular nucleus and subparaventricular zone on sleep-wake cycle and temperature regulation. *J Neurosci* 21, 4864-4874 (2001)

90. Monda, M., S. Amaro, A. Sullo and B. De Luca: Injection of muscimol in the posterior hypothalamus reduces the PGE1- hyperthermia in the rat. *Brain Res Bull* 37, 575-580 (1995)

91. Lipton, J. M. and G. P. Trzcinka: Persistence of febrile response to pyrogens after PO/AH lesions in squirrel monkeys. *Am J Physiol* 231, 1638-1648 (1976)

92. Matsumura, K., C. Cao, Y. Watanabe and Y. Watanabe: Prostaglandin system in the brain: sites of biosynthesis and sites of action under normal and hyperthermic states. *Prog Brain Res* 115, 275-295 (1998)

93. Ek, M., M. Kurosawa, T. Lundeberg and A. Ericsson: Activation of vagal afferents after intravenous injection of interleukin-1beta: role of endogenous prostaglandins. *J Neurosci* 18, 9471-9479 (1998)

94. Hubbard, N. E., S. Lee, D. Lim and K. L. Erickson: Differential mRNA expression of prostaglandin receptor subtypes in macrophage activation. *Prostaglandins Leukot Essent Fatty Acids* 65, 287-294 (2001)

95. Ma, W. and J. C. Eisenach: Four PGE2 EP receptors are up-regulated in injured nerve following partial sciatic nerve ligation. *Exp Neurol* 183, 581-592 (2003)

96. Momiyama, T., N. Todo, Y. Sugimoto, A. Ichikawa and S. Narumiya: Membrane depolarization by activation of prostaglandin E receptor EP3 subtype of putative serotonergic neurons in the dorsal raphe nucleus of the rat. *Naunyn Schmiedebergs Arch Pharmacol* 353, 377-381 (1996)

97. Oka, T: Functional roles of hypothalamic PGE2 and its receptors in brain-immune system interactions. *Adv Neurol Sci* 45, 901-908 (2001)

Abbreviations: In addition to the commonly accepted (e.g., GABA or cAMP), the abbreviations used in this paper are 3V third ventricle, AC anterior commissure, AVPV anteroventral periventricular nucleus, AV3V anteroventral region of the third ventricle, BAT brown adipose tissue, C cold-sensitive, COX cyclooxygenase, DMH dorsomedial hypothalamic nucleus, EAA excitatory amino acid, I temperature-insensitive, IL interleukin, IML intermediolateral cell column, LI like immunoreactivity, LPO lateral preoptic area, LPS lipopolysaccharide, MnPO median preoptic nucleus, MPN medial preoptic nucleus, MPO medial preoptic area , NDB nucleus of the diagonal band of Broca, NTS nucleus of the solitary tract, OC optic chiasm, OVLT organum vasculosum of the lamina terminalis, PG prostaglandin, POA preoptic area of the hypothalamus, PRV pseudorabies virus, RPa raphe pallidus nucleus, Tc core body temperature, VLPO ventrolateral preoptic nucleus, VMPO ventromedial preoptic nucleus, W warm-sensitive

Key Words: Prostaglandin E_2, EP receptors, Fever, Psychological stress-induced hyperthermia, Review

Send correspondence to: Dr Takakazu Oka, Division of Psychosomatic Medicine, Department of Neurology, University of Occupational and Environmental Health, Japan, Iseigaoka 1-1, Yahatanishi-ku, Kitakyushu, 807-8555, Japan, Tel: 81-93-603-1611, Fax: 81-93-693-9842, E-mail: toka@med.uoeh-u.ac.jp