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Central PGE_2 exhibits anxiolytic-like activity via EP_1 and EP_4 receptors in a manner dependent on serotonin 5-HT_{1A}, dopamine D_1 and $GABA_A$ receptors

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ABSTRACT

We found that centrally administered prostaglandin (PG) E_2 exhibited anxiolytic-like activity in the elevated plus-maze and open field test in mice. Agonists selective for EP_1 and EP_4 receptors, among four receptor subtypes for PGE_2, mimicked the anxiolytic-like activity of PGE_2. The anxiolytic-like activity of PGE_2 was blocked by an EP_1 or EP_4 antagonist, as well as in EP_4 but not EP_1 knockout mice. Central activation of either EP_1 or EP_4 receptors resulted in anxiolytic-like activity. The PGE_2-induced anxiolytic-like activity was inhibited by antagonists for serotonin 5-HT_{1A}, dopamine D_1 and $GABA_A$ receptors. Taken together, PGE_2 exhibits anxiolytic-like activity via EP_1 and EP_4 receptors, with downstream involvement of 5-HT_{1A}, D_1 and $GABA_A$ receptor systems.

1. Introduction

Prostaglandin (PG) E_2 is produced in the mammalian central nervous system (CNS), and has a variety of central actions such as in fever, pain generation, wakefulness and anorexia under both physiological and pathophysiological conditions [1–5]. PGE_2 exerts its actions through four different types of G-protein-coupled seven-transmembrane receptors, known as EP_1, EP_2, EP_3 and EP_4 [6,7]. Recently, functions of these receptor subtypes in the CNS have been revealed using their highly selective ligands or knockout mice. It was reported that EP_4 receptor mediates wakefulness of PGE_2 [2]. We also demonstrated that central activation of EP_4 receptor suppresses food intake and gastrointestinal motility [8]. Various neural responses are evoked by sickness. It was reported that EP_3 receptor mediates the febrile response induced by bacterial endotoxin lipopolysaccharide (LPS), which mimics the condition of systemic sickness [3]. Both EP_1 and EP_3 receptor work critically in activation of the hypothalamic–pituitary–adrenal (HPA) axis after LPS administration [9].

Interestingly, psychological stress not related sickness also enhances PG production in the CNS [10,11]; however, the mechanism underlying the emotional regulation of central PGs is largely unknown. We have recently reported that PGD_2, a structural isomer of PGE_2, has anxiolytic-like activity via DP_1 receptor among two receptor subtypes for PGD_2 [12]. In this study, we focused on the role of PGE_2 in the emotional regulation, behavioral pharmacologically using highly selective ligands for receptor subtypes for PGE_2 [13] as well as knockout mice. We also investigated the neurotransmitters associated with the anxiolytic-like activity induced by central administration of PGE_2.

2. Materials and methods

2.1. Materials

PGE_2 was obtained from Nacalai Tesque Inc. (Kyoto, Japan). The highly selective EP_1 agonist (ONO-DI-004), EP_2 agonist (ONO-AE1-259-01), EP_3 agonist (ONO-AE-248), EP_4 agonist (ONO-AE1-329), EP_1 antagonist (ONO-8713) and EP_4 antagonist (ONO-AE3-208) were provided by Ono Pharmaceutical Co. Ltd. (Osaka, Japan). WAY100135 dihydrochloride, a serotonin 5-HT_{1A} receptor antagonist; R(+)-SCH-23390 hydrochloride, a dopamine D_1 receptor

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antagonist; and (−)-bicuculline, a γ-amino butyric acid $(GABA)_A$ receptor antagonist; were obtained from Tocris Bioscience (Bristol, UK).

2.2. Animals

Four-week-old male ddY mice were obtained from SLC (Shizuoka, Japan). We also used EP_1- and EP_4-knockout (KO) and their wild-type mice as previously reported [9,14]. All animals were housed in a temperature-controlled room (23 °C) on a 12 h light–dark cycle with lights on at 07:00. All animals had free access to food pellets and water. All experiments were approved by the Kyoto University Ethics Committee for Animal Research Use. All animals were euthanized by an overdose of anesthesia drugs after the experiment.

2.3. Intracerebroventricular (i.c.v.) administration

i.c.v. administration to mice weighing 20–24 g was performed as described previously [15–17]. Briefly, a 28-gauge stainless steel needle attached to a 0.05-ml Hamilton syringe was inserted perpendicularly through the skull into the brain. The site of injection was 2 mm from either side of the midline on a line drawn through the anterior base of the ears (the lateral ventricle).

2.4. Elevated plus-maze test

Anxiolytic-like behavior was measured using the elevated plus-maze (EPM) test, which was performed as described previously [12,18–21]. Four arms (25 cm long × 5 cm wide) were placed 50 cm above the ground. Two opposing arms were delimited by acrylic vertical walls (15 cm high, closed arms), and two had unprotected edges (open arms). A mouse was placed in the center of the maze facing an open arm and observed for 5 min to measure the cumulative time and frequency of entries into open and closed arms. Arm entry was defined as the entry of four paws into an arm. Open-arm entry time (time spent in open arms) was expressed as a percentage of the total entry time (% of time), and the number of open-arm entries was expressed as a percentage of the total number of entries (% of visit). PGE_2 or the EP_1–EP_4 agonist was dissolved in dimethylsulfoxide (DMSO) and the solution was diluted 20-fold with artificial cerebrospinal fluid (ACSF: 138.9 mM NaCl, 3.4 mM KCl, 1.3 mM $CaCl_2$, 4.0 mM $NaHCO_3$, 0.6 mM NaH_2PO_4, 5.6 mM glucose, pH 7.4). Four microliters of PGE_2 or an EP agonist solution were co-administered with or without an EP antagonist i.c.v. 20 min before the test. The antagonist of $5\text{-}HT_{1A}$, D_1 or $GABA_A$ receptor (i.p.) or PGE_2 (i.c.v.) was administered 30 or 20 min, respectively, before the test. The total number of visits to open and closed arms, and the cumulative time spent in open and closed arms were measured on a monitor through a video camera system. The data were checked by observers unaware of the experimental groups. The EPM test was started at 11:00 am during the light phase of the light/dark cycle.

2.5. Open field test

The open-field test was performed as previously described with a slight modification [21,22]. The apparatus consisted of a black circular arena of 60 cm in diameter and a gray wall 50 cm tall. Four microliters of PGE_2, EP_1 or EP_4 agonist solution were administered i.c.v. 20 min before the open field test. Each mouse was placed in the center of the circular arena and its movement monitored through the video-tracking system Smart Junior (Panlab, S.L., Barcelona, Spain) for 5 min. The time spent in a circular area 12 cm in diameter in the center of the arena was measured. The open-field test was started at 11:00 am.

2.6. Statistical analysis

Values are expressed as the mean ± S.E.M. Statistical comparisons between groups were performed using a one-way analysis of variance (ANOVA) followed by Fisher's test or the unpaired Student's *t*-test. *P* values less than 0.05 were considered significant.

3. Results

3.1. Centrally administered PGE_2 has anxiolytic-like activity

We investigated the role of PGE_2 in emotional regulation using the elevated plus-maze and open field tests. Centrally administered PGE_2 (10–100 pmol/mouse) dose-dependently increased percentages (%) of time and visits in open arms in the elevated plus-maze test (Fig. 1). Total visits in both open and closed arms, which indicate locomotor activity, did not change significantly after the i.c.v. administration of PGE_2. One hundred pmol/mouse of PGE_2 also increased % time spent in the center (12 cm) and the frequency of rearing in the open field test (Table 1). Thus we demonstrated with two paradigms that centrally administered PGE_2 exhibits anxiolytic-like activity in mice.

3.2. Activation of EP_1/EP_4 receptors exhibits anxiolytic-like activity

To investigate which receptor subtypes for PGE_2 mediate the anxiolytic-like activity of PGE_2, we used highly selective agonists for EP_1–EP_4 receptors. The EP_1 and EP_4 agonists at a dose of 100 pmol/mouse increased % of time and visits in open arms (Fig. 2). The EP_3 agonist seemed to slightly increase visits in open arms; however, statistically significant differences compared to the ACSF-treated control group were not detected. Total visits to the open and closed arms were not affected. The EP_1 or EP_4 agonist (100 pmol/mouse) also increased % time spent in the center and the frequency of rearing in the open field test (Table 2). These

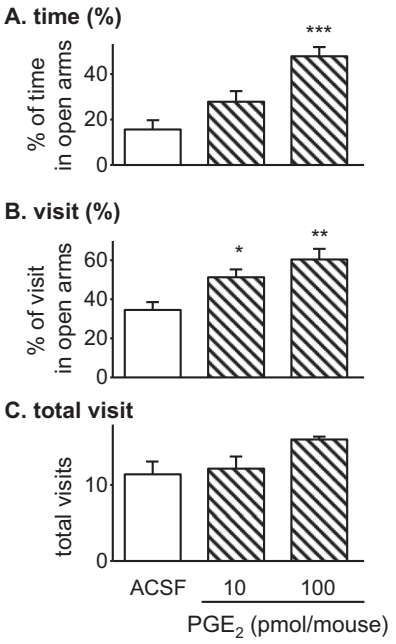

Fig. 1. Anxiolytic-like activity of PGE_2 after central administration in the elevated plus-maze (EPM) test in mice. PGE_2 at a dose of 10–100 pmol/mouse was i.c.v. administered 20 min before the test. The percentages of time (A) and visits (B) spent in the open arms, and total visits (C) to both open and closed arms during the test for 5 min were measured. Each value is expressed as the mean ± S.E.M. (n = 4–6). $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared with the ACSF-treated control group.

Table 1
Anxiolytic-like activity of PGE_2 at a dose of 100 pmol/mouse after i.c.v. administration in the open-field test.

	ACSF	PGE_2
% of time in center circle	0.467 ± 0.214	1.27 ± 0.129*
Frequency of rearing	33.6 ± 6.75	63.8 ± 3.92**

Results are expressed as the mean ± S.E.M. (n = 10).
* $P < 0.05$ compared with the ACSF-treated control group.
** $P < 0.01$ compared with the ACSF-treated control group.

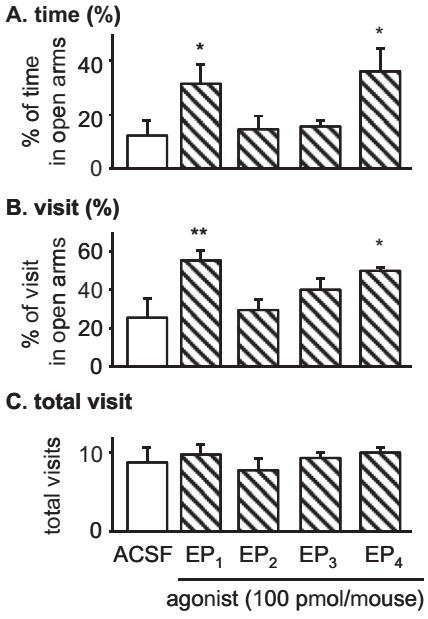

Fig. 2. Anxiolytic-like activity of a highly selective EP_1, EP_2, EP_3 or EP_4 receptor agonist after central administration in the EPM test in mice. ONO-DI-004, ONO-AE1-259-01, ONO-AE-248 or ONO-AE1-329, an agonist for the EP_1, EP_2, EP_3 or EP_4 receptor, respectively, which are receptor subtypes for PGE_2, was i.c.v. administered at a dose of 100 pmol/mouse 20 min before the test. Each value is expressed as the mean ± S.E.M. (n = 4). *$P < 0.05$, **$P < 0.01$, compared with each group.

Table 2
Anxiolytic-like activities of $EP_1$and EP_4 agonists at a dose of 100 pmol/mouse after i.c.v. administration in open-field test.

	ACSF	EP_1 agonist	EP_4 agonist
% of time in center circle	0.683 ± 0.222	1.74 ± 0.189**	2.11 ± 0.367***
Frequency of rearing	31.4 ± 3.02	43.9 ± 3.48*	53.1 ± 5.65**

Results are expressed as the mean ± S.E.M. (n = 8–9).
* $P < 0.05$ compared with the ACSF-treated control group.
** $P < 0.01$ compared with the ACSF-treated control group.
*** $P < 0.001$ compared with the ACSF-treated control group.

results indicate that EP_1 and EP_4 agonists have anxiolytic-like activity.

Next, we investigated whether the anxiolytic-like activity of PGE_2 was mediated by EP_1 and EP_4 receptors. The increase in time and visits in open arms after the i.c.v. administration of the EP_1 or EP_4 agonist (100 pmol/mouse) was inhibited by an EP_1 or EP_4 antagonist (1 nmol/mouse), respectively (Fig. 3). The EP_1 or EP_4 antagonist alone (1 nmol/mouse) was without effect under our experimental condition. PGE_2-induced anxiolytic-like activity was also blocked by the EP_1 or EP_4 antagonist after a central administration (Fig. 4). Thus we found that PGE_2 exhibits anxiolytic-like activity via EP_1/EP_4 receptors.

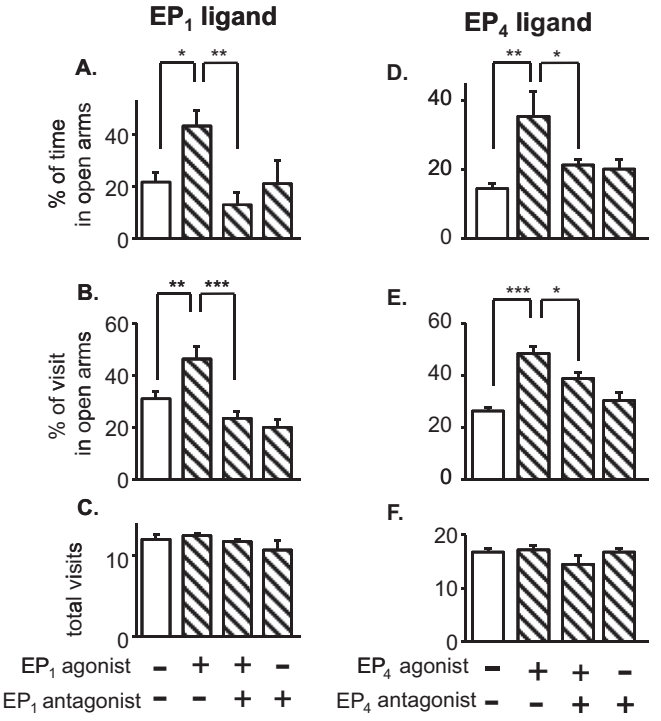

Fig. 3. Effect of an EP_1 or EP_4 antagonist on the anxiolytic-like activity of the EP_1 or EP_4 agonist, respectively. ONO-DI-004 (100 pmol/mouse, i.c.v.), an agonist for the EP_1 receptor, was co-administered with ONO-8713 (1 nmol/mouse), an antagonist for the EP_1 receptor, 20 min before the test (A–C). ONO-AE1-329 (100 pmol/mouse, i.c.v.), an agonist for the EP_4 receptor, was also co-administered ONO-AE3-208 (1 nmol/mouse), an antagonist for the EP_4 receptor, 20 min before the test (D–F). Each value is expressed as the mean ± S.E.M. (n = 4–5). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with each group.

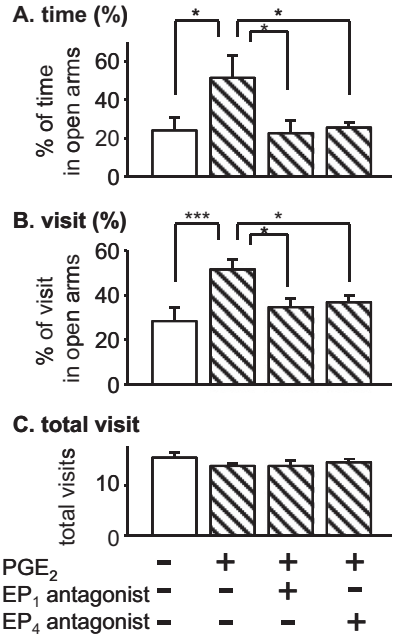

Fig. 4. Involvement of both EP_1 and EP_4 receptors in the anxiolytic-like activity of PGE_2. PGE_2 (100 pmol/mouse, i.c.v.) was co-administered with ONO-8713 (1 nmol/mouse) or ONO-AE3-208 (1 nmol/mouse), an antagonist for the EP_1 or EP_4 receptor, respectively, 20 min before the test. Each value is expressed as the mean ± S.E.M. (n = 4–5). *$P < 0.05$, ***$P < 0.001$, compared with each group.

Although the EP selective drugs used in this study have been shown to be selective across EP receptor subtypes, we sought to confirm the involvement of EP_1/EP_4 receptors in the anxiolytic-like activity by using knockout mice deficient in each EP subtype [9,14]. Genetic deletion of the EP_4 receptor abolished the anxiolytic-like activity of PGE_2 (Fig. 5A). Thus we demonstrated that anxiolytic-like action of PGE_2 is mediated through the EP_4 receptor. Whereas the anxiolytic activity of PGE_2 was spared in EP_1-deficient mice (Fig. 5B), these mice failed to show the anxiolytic-like activity of an EP_1 agonist seen in wild-type mice (Fig. 5C). Thus, activation of EP_1 receptor can reduce the level of anxiety, though this receptor is dispensable for the anxiolytic action of PGE_2.

3.3. The anxiolytic-like activity of PGE_2 is associated with the activation of serotonin 5-HT_{1A}, dopamine D_1 and $GABA_A$ receptors

Next, we investigated mediators associated with the anxiolytic-like activity of PGE_2. Serotonin 5-HT_{1A}, dopamine D_1 and $GABA_A$ receptors are known to be involved in anxiolytic-like activity in animals and humans [21,23–25]; thus, we investigated whether these receptors were involved in the anxiolytic-like activity of PGE_2 using antagonists for them.

The increase in % of time and visits in open arms after the central administration of PGE_2 (100 pmol/mouse, i.c.v.) was blocked by WAY100135 (10 mg/kg, i.p.), a 5-HT_{1A} receptor antagonist (Fig. 6). We previously confirmed that WAY100135 at this dose by itself had no effect [21]. These results suggest that the anxiolytic-like activity of PGE_2 is mediated by the activation of 5-HT_{1A} receptors. The anxiolytic-like activity of PGE_2 was also blocked by SCH23390 (30 μg/kg, i.p.), a dopamine D_1 receptor antagonist (Fig. 6). Bicuculline (5 mg/kg, i.p.) and flumazenil, antagonists for the binding site of GABA and benzodiazepine, respectively, of the $GABA_A$ receptor, also inhibited the anxiolytic-like activity induced by the central administration of PGE_2 (Fig. 6). When administered alone these antagonists, including SCH23390, bicuculline and flumazenil, did not affect any parameters in the elevated plus-maze test under our experimental conditions [21]. Taken together, the anxiolytic-like activity of PGE_2 is mediated by the activation of serotonin 5-HT_{1A}, dopamine D_1 and $GABA_A$ receptors.

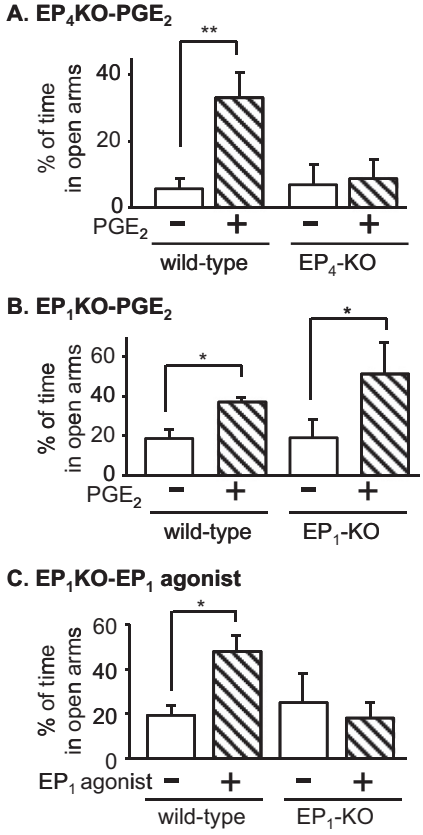

Fig. 5. Anxiolytic-like activity of PGE_2 or an EP_1 agonist in the EP_4- or EP_1-KO mice. The EP_4-KO and wild-type mice were administered PGE_2 (A, 10 pmol/mouse, i.c.v.) 20 min before the test. The EP_1-KO and wild-type mice were also i.c.v. administered PGE_2 (B, 10 pmol/mouse) or ONO-DI-004, an EP_1 agonist (C, 100 pmol/mouse) 20 min before the test. Each value is expressed as the mean ± S.E.M. (A: n = 5–7; B: n = 3–10; C: n = 4–7). $^{*}P < 0.05$, $^{**}P < 0.01$, compared with each group.

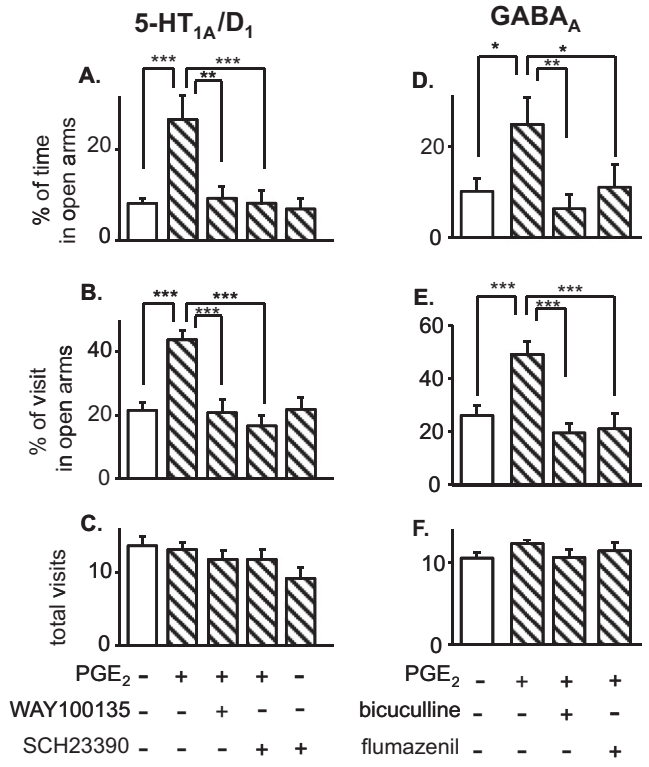

Fig. 6. Effect of an antagonist for the serotonin 5-HT_{1A}, dopamine D_1, $GABA_A$ receptor on the anxiolytic-like activity of PGE_2. WAY100135 (10 mg/kg) or SCH23390 (30 μg/kg), an antagonist for the 5-HT_{1A} or D_1 receptor, respectively, was administered i.p. 30 min before the EPM test (A–C). Bicuculline (5 mg/kg) or flumazenil (1 mg/kg), an antagonist for the GABA- or benzodiazepine-binding site of the $GABA_A$ receptor, respectively, was administered i.p. 30 min before the EPM test (D–F). PGE_2 (100 pmol/mouse, i.c.v.) was administered 20 min before the test. Each value is expressed as the mean ± S.E.M. (A–C: n = 4–6; D–F: n = 5–8). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with each group.

4. Discussion

We found that central administration of PGE_2 exhibits anxiolytic-like activity in mice as measured by the elevated-plus maze and open field test. This action of PGE_2 was abolished in mice treated with an EP_4 antagonist and in EP_4-KO mice. Further, central administration of an EP_4 agonist mimicked the action of PGE_2. These results demonstrate that central administration of PGE_2 has anxiolytic-like activity via the EP_4 receptor. We also found that central activation of EP_1 receptor has anxiolytic-like activity as well. Consistently, an EP_1 antagonist blocked the anxiolytic-like effect of PGE_2, suggesting an involvement of the EP_1 receptor in the anxiolytic-like action of PGE_2. However, genetic deletion of the EP_1 receptor did not interfere with the action of PGE_2. Thus, the EP_4 receptor plays a dominant role in mediating the anxiolytic-like action of PGE_2, and EP_1 deficiency in the long term could be compensated by the remaining EP_4 action.

PGE_2 is a bioactive lipid produced from arachidonic acid, via PGH_2, by cyclooxygenase (COX), and acts on its receptors close to its synthesis [6]. Under normal physiological conditions, brain-cell population expressing either COX isoform constitutively, namely, glial COX-1 and neuronal COX-2, might contribute to PGE_2 production [11,26]. Further investigation will elucidate how PGE_2 is released in response to various stresses including psychological stress not related to sickness. The EP_1 and EP_4 receptors are present in areas such as the paraventricular nucleus of the hypothalamus (PVN) and the amygdala [9,11,27].

Acute infections and other immune changes trigger acute phase reactions including a variety of central responses such as fever, anorexia, HPA axis activation, reduced social interaction and exploration behavior, namely, sickness behavior [28]. Pro-inflammatory cytokines released by activation of the innate immune system are known to induce expression of COX-2 and microsomal PGE synthase (mPGES)-1 in the brain microvessels, and PGE_2 might be released and distributed to the large brain area [11]. In the PVN, an important site in the HPA axis, expression of COX-2 and EP_4 is particularly induced by LPS administration [9,27]. The sickness behavior is thought to be at least in part associated with the cytokines, including interleukin-1β (IL-1β) and tumor necrosis factor (TNF)-α [29,30]. Corticotropin-releasing factor (CRF), which is release from the PVN via PGE_2 synthesis upon treatment with LPS and IL-1β, was shown to increase anxiety behavior after central administration [31]. In contrast, we found that PGE_2 decreases anxiety via EP_1/EP_4 receptors. Thus, we hypothesize that the central PGE_2 system might suppress excessive anxiety-like behavior during sickness. Complement C3a after central administration also induced anxiolytic-like activity, and the anxiolytic-like activity of C3a was blocked by a COX inhibitor and an EP_1/EP_4 antagonist (unpublished data), suggesting that central C3a exhibits anxiolytic-like activity through PGE_2 production followed by activation of EP_1/EP_4 receptors. Further investigation should reveal the roles of the novel anxiolytic pathway through PGE_2–EP_1/EP_4 receptor under physiological and pathophysiological conditions.

We also investigated the mechanism underlying the anxiolytic-like activity of PGE_2, downstream of EP_1/EP_4 receptor. The anxiolytic-like activity of PGE_2 was completely blocked by antagonists for 5-HT_{1A}, D_1 and $GABA_A$ receptors; however, each antagonist by itself has no effect under our experimental condition. These results suggest that the PGE_2-induced anxiolytic-like activity is mediated by activation of their receptors. By pharmacological experiments using agonists and antagonists, we previously determined that the order of activation was 5-HT_{1A}, D_1 and $GABA_A$ receptors [21]. Thus, we also hypothesized that the anxiolytic-like activity of PGE_2 was mediated as follows: EP_1/EP_4 receptor activation → serotonin release → 5-HT_{1A} receptor → dopamine release → D_1 receptor → GABA release → $GABA_A$ receptor → anxiolytic-like activity; however, it cannot be ruled out that 5-HT_{1A}, D_1 and $GABA_A$ systems might be activated in parallel after central administration of PGE_2.

The serotonin–5-HT_{1A} system is involved in the anxiolytic activity [32–35]. Serotonin neurons project to broad area such as the PVN and central nucleus of the amygdala (CeA), in which the EP_1/EP_4 receptors are present [9,11,27,36]. In the dorsal raphe nucleus (DR), the most prominent serotonin source, EP_4 receptor was also reported to exist [27]. PGE_2 administration into the DR suppressed food intake, and LPS-induced anorexia was blocked by injection of a COX-2 inhibitor NS-398 into the DR [36], implying that PGE_2 released in response to LPS acts on serotonergic neurons. 5-HT_{1A} receptors are located not only in the serotonergic cell bodies of the DR but also in non-serotonergic neurons in various brain regions, including the hippocampus, prefrontal cortex and ventral tegmental area (VTA) [37]. The mesocorticolimbic pathway with dopaminergic projections from the VTA to frontal cortex and/or nucleus accumbens is implicated in emotional and cognitive regulation, and the nigrostriatal pathway from the substantia nigra to the striatum is involved in motor function [38]. Systemic administration of a 5-HT_{1A} agonist facilitated dopamine release in the frontal cortex or nucleus accumbens [37]. The anxiolytic-like activity of PGE_2 was blocked by bicuculline, an antagonist of the GABA binding site of the $GABA_A$ receptor, suggesting that PGE_2 might show an anxiolytic-like effect after activating the GABA site, probably by stimulating presynaptic GABA release. Further investigation should reveal the functional or structural relevance of 5-HT_{1A}, D_1 and $GABA_A$ receptors in anxiolytic-like activity of PGE_2.

PGE_2 is produced from arachidonic acid by COX followed by PGE synthase. We also found that another COX product, PGD_2, also has anxiolytic-like activity via the DP_1 receptor among two receptor subtypes for PGD_2 [12]. This anxiolytic-like activity was mediated by activating the adenosine A_{2A} and $GABA_A$ receptors, which is consistent with the pathway of sleep induction. The anxiolytic-like activity of PGE_2 was not blocked by an A_{2A} receptor antagonist (data not shown), whereas the PGD_2-induced anxiolytic-like activity was not inhibited by a 5-HT_{1A} receptor antagonist [12], indicating that PGE_2 and PGD_2 exhibit anxiolytic-like activities via independent pathways in the CNS; however, the $GABA_A$ receptor might be commonly involved downstream of all other neurotransmitters.

Among a number of bioactive peptides isolated from the enzymatic digests of food proteins, several short peptides exhibited anxiolytic-like activity. Soymorphin-5 and rubiscolin-6, which are μ and δ opioid peptides derived from soy β-conglycinin and spinach Rubisco, respectively, have anxiolytic-like activities after oral administration [18,19]. Rubiscolin-6 exhibits anxiolytic activity by activating σ_1 receptor, downstream of δ opioid receptor [19]; however, PGE_2-induced anxiolytic-like activity was not blocked by antagonists for μ and δ opioid receptors, naloxone and naltrindole and BMY14802, respectively (data not shown), suggesting that PGE_2 shows anxiolytic-like activity independently of these opioid systems. It was also reported that rubimetide, a tripeptide derived from Rubisco, a tripeptide exhibits anxiolytic-like activities via activation of the PGD_2 system [20]. It is worthwhile testing the anxiolytic-like activities of PG-releasing peptides derived from dietary proteins.

In conclusion, we found that centrally administered PGE_2 exhibited anxiolytic-like activity in mice. We also demonstrated that the anxiolytic-like activity of PGE_2 is mediated by the EP_1/EP_4 receptors using selective ligands. Since genetic deletion of the EP_4 rather than the EP_1 receptor abolished the anxiolytic effect of PGE_2, the EP_4 receptor must play a dominant role in this PGE_2 action. The PGE_2-induced anxiolytic-like activity was mediated via serotonin 5-HT_{1A}, dopamine D_1 and $GABA_A$ receptors. Taken together, activation of central EP_4 receptors for PGE_2 exhibits anxiolytic-like activity, dependent on the 5-HT_{1A}, D_1 and $GABA_A$ receptors system.

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References

[1] Hayaishi, O. (1991) Molecular mechanisms of sleep-wake regulation: roles of prostaglandins D_2 and E_2. FASEB J. 5 (11), 2575–2581.
[2] Huang, Z.L., Sato, Y., Mochizuki, T., Okada, T., Qu, W.M., Yamatodani, A., Urade, Y. and Hayaishi, O. (2003) Prostaglandin E_2 activates the histaminergic system via the EP_4 receptor to induce wakefulness in rats. J. Neurosci. 23 (14), 5975–5983.
[3] Ushikubi, F., Segi, E., Sugimoto, Y., Murata, T., Matsuoka, T., Kobayashi, T., Hizaki, H., Tuboi, K., Katsuyama, M., Ichikawa, A., Tanaka, T., Yoshida, N. and

Narumiya, S.M. (2007) Impaired febrile response in mice lacking the prostaglandin E receptor subtype EP3. Nature 395 (6699), 281–284.
[4] Horton, E.W. (1964) Actions of prostaglandins E1, E2 and E3 on the central nervous system. Br. J. Pharmacol. 22, 189–192.
[5] Levine, A.S. and Morley, J.E. (1981) The effect of prostaglandins (PGE2 and PGF2 alpha) on food intake in rats. Pharmacol. Biochem. Behav. 15 (5), 735–738.
[6] Narumiya, S., Sugimoto, Y. and Ushikubi, F. (1999) Prostanoid receptors: structures, properties, and functions. Physiol. Rev. 79 (4), 1193–1226.
[7] Narumiya, S. and FitzGerald, G.A. (2001) Genetic and pharmacological analysis of prostanoid receptor function. J. Clin. Invest. 108 (1), 25–30.
[8] Ohinata, K., Suetsugu, K., Fujiwara, Y. and Yoshikawa, M. (2006) Activation of prostaglandin E receptor EP_4 subtype suppresses food intake in mice. Prostaglandins Other Lipid Mediat. 81 (1–2), 31–36.
[9] Matsuoka, Y., Furuyashiki, T., Bito, H., Ushikubi, F., Tanaka, Y., Kobayashi, T., Muro, S., Satoh, N., Kayahara, T., Higashi, M., Mizoguchi, A., Shichi, H., Fukuda, Y., Nakao, K. and Narumiya, S. (2003) Impaired adrenocorticotropic hormone response to bacterial endotoxin in mice deficient in prostaglandin E receptor EP1 and EP3 subtypes. Proc. Natl. Acad. Sci. USA 100 (7), 4132–4137.
[10] García-Bueno, B., Madrigal, J.L., Pérez-Nievas, B.G. and Leza, J.C. (2008) Stress mediators regulate brain prostaglandin synthesis and peroxisome proliferator-activated receptor-gamma activation after stress in rats. Endocrinology 149 (4), 1969–1978.
[11] Matsuoka, Y., Furuyashiki, T., Yamada, K., Nagai, T., Bito, H., Tanaka, Y., Kitaoka, S., Ushikubi, F., Nabeshima, T. and Narumiya, S. (2005) Prostaglandin E receptor EP1 controls impulsive behavior under stress. Proc. Natl. Acad. Sci. USA 102 (44), 16066–16071.
[12] Zhao, H., Ohinata, K. and Yoshikawa, M. (2009) Central prostaglandin D_2 exhibits anxiolytic-like activity via the DP_1 receptor in mice. Prostaglandins Other Lipid Mediat. 88 (3–4), 68–72.
[13] Suzawa, T., Miyaura, C., Inada, M., Maruyama, T., Sugimoto, Y., Ushikubi, F., Ichikawa, A., Narumiya, S. and Suda, T. (2000) 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 (4), 1554–1559.
[14] Segi, E., Sugimoto, Y., Yamasaki, A., Aze, Y., Oida, H., Nishimura, T., Murata, T., Matsuoka, T., Ushikubi, F., Hirose, M., Tanaka, T., Yoshida, N., Narumiya, S. and Ichikawa, A. (1998) Patent ductus arteriosus and neonatal death in prostaglandin receptor EP4-deficient mice. Biochem. Biophys. Res. Commun. 246 (1), 7–12.
[15] Yang, S., Kawamura, Y. and Yoshikawa, M. (2003) Effect of rubiscolin, a delta opioid peptide derived from Rubisco, on memory consolidation. Peptides 24 (2), 325–328.
[16] Ohinata, K., Sonoda, S., Shimano, T. and Yoshikawa, M. (2007) Enterostatin (APGPR) enhances memory consolidation in mice. Peptides 28 (3), 719–721.
[17] Ohinata, K., Sonoda, S., Inoue, N., Yamauchi, R., Wada, K. and Yoshikawa, M. (2007) β-Lactotensin, a neurotensin agonist peptide derived from bovine blactoglobulin, enhances memory consolidation in mice. Peptides 28 (7).
[18] Ohinata, K., Agui, S. and Yoshikawa, M. (2007) Soymorphins, novel μ opioid peptides derived from soy β-conglycinin β-subunit, have anxiolytic activities. Biosci. Biotechnol. Biochem. 71 (10), 2618–2621.
[19] Hirata, H., Sonoda, S., Agui, S., Yoshida, M., Ohinata, K. and Yoshikawa, M. (2007) Rubiscolin-6, a delta opioid peptide derived from spinach Rubisco, has anxiolytic effect via activating $sigma_1$ and dopamine D_1 receptors. Peptides 28 (10), 1998–2003.
[20] Zhao, H., Ohinata, K. and Yoshikawa, M. (2008) Rubimetide (Met-Arg-Trp) derived from Rubisco exhibits anxiolytic-like activity via the DP_1 receptor in male ddY mice. Peptides 29 (4), 629–632.
[21] Kanegawa, N., Suzuki, C. and Ohinata, K. (2010) Dipeptide Tyr-Leu (YL) exhibits anxiolytic-like activity after oral administration via activating serotonin 5-HT_{1A}, dopamine D_1 and $GABA_A$ receptors in mice. FEBS Lett. 584 (3), 599–604.
[22] Asakawa, A., Toyoshima, M., Inoue, K. and Koizumi, A. (2007) Ins2Akita mice exhibit hyperphagia and anxiety behavior via the melanocortin system. Int. J. Mol. Med. 19 (4), 649–652.
[23] Leonardo, E.D. and Hen, R. (2008) Anxiety as a developmental disorder. Neuropsychopharmacology 33 (1), 134–140.
[24] Short, J.L., Ledent, C., Drago, J. and Lawrence, A.J. (2006) Receptor crosstalk: characterization of mice deficient in dopamine D_1 and adenosine A_{2A} receptors. Neuropsychopharmacology 31 (3), 525–534.
[25] Jacob, T.C., Moss, S.J. and Jurd, R. (2008) $GABA_A$ receptor trafficking and its role in the dynamic modulation of neuronal inhibition. Nat. Rev. Neurosci. 9 (5), 331–343.
[26] Li, S., Wang, Y., Matsumura, K., Ballou, L.R., Morham, S.G. and Blatteis, C.M. (1999) The febrile response to lipopolysaccharide is blocked in cyclooxygenase-2(−/−), but not in cyclooxygenase-1(−/−) mice. Brain Res. 825 (1–2), 86–94.
[27] Zhang, J. and Rivest, S. (1999) Distribution, regulation and colocalization of the genes encoding the EP2- and EP4-PGE_2 receptors in the rat brain and neuronal responses to systemic inflammation. Eur. J. NeuroSci. 11 (8), 2651–2668.
[28] Pecchi, E., Dallaporta, M., Jean, A., Thirion, S. and Troadec, J.D. (2009) Prostaglandins and sickness behavior: old story, new insights. Physiol. Behav. 97 (3–4), 279–292.
[29] Swiergiel, A.H. and Dunn, A.J. (2007) Effects of interleukin-1beta and lipopolysaccharide on behavior of mice in the elevated plus-maze and open field tests. Pharmacol. Biochem. Behav. 86 (4), 651–659.
[30] Yamada, K., Iida, R., Miyamoto, Y., Saito, K., Sekikawa, K., Seishima, M. and Nabeshima, T. (2000) Neurobehavioral alterations in mice with a targeted deletion of the tumor necrosis factor-alpha gene: implications for emotional behavior. J. Neuroimmunol. 111 (1–2), 131–138.
[31] Momose, K., Inui, A., Asakawa, A., Ueno, N., Nakajima, M., Fujimiya, M. and Kasuga, M. (1999) Intracerebroventricularly administered corticotropin-releasing factor inhibits food intake and produces anxiety-like behaviour at very low doses in mice. Diabetes Obes. Metab. 1 (5), 281–284.
[32] Abe, M., Tabata, R., Saito, K., Matsuda, T., Baba, A. and Egawa, M. (1996) Novel benzodioxan derivative, 5-[3-[((2S)-1,4-benzodioxan-2-ylmethyl) amino]propoxy]-1,3-benzodioxole HCl (MKC-242), with anxiolytic-like and antidepressant-like effects in animal models. J. Pharmacol. Exp. Ther. 278 (2), 898–905.
[33] Matsuda, T., Somboonthum, P., Suzuki, M., Asano, S. and Baba, A. (1995) Antidepressant-like effect by postsynaptic 5-HT1A receptor activation in mice. Eur. J. Pharmacol. 280 (2), 235–238.
[34] Ramboz, S., Oosting, R., Amara, D.A., Kung, H.F., Blier, P., Mendelsohn, M., Mann, J.J., Brunner, D. and Hen, R. (1998) Serotonin receptor 1A knockout: an animal model of anxiety-related disorder. Proc. Natl. Acad. Sci. USA 95 (24), 14476–14481.
[35] Parks, C.L., Robinson, P.S., Sibille, E., Shenk, T. and Toth, M. (1998) Increased anxiety of mice lacking the serotonin1A receptor. Proc. Natl. Acad. Sci. USA 95 (18), 10734–10739.
[36] Asarian, L. and Langhans, W. (2010) A new look on brain mechanisms of acute illness anorexia. Physiol. Behav. 100 (5), 464–471.
[37] Sakaue, M., Somboonthum, P., Nishihara, B., Koyama, Y., Hashimoto, H., Baba, A. and Matsuda, T. (2000) Postsynaptic 5-hydroxytryptamine1A receptor activation increases in vivo dopamine release in rat prefrontal cortex. Br. J. Pharmacol. 129 (5), 1028–1034.
[38] Salamone, J.D., Correa, M., Farrar, A. and Mingote, S.M. (2007) Effort-related functions of nucleus accumbens dopamine and associated forebrain circuits. Psychopharmacology (Berlin) 191 (3), 461–482.