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Central prostaglandin D_2 exhibits anxiolytic-like activity via the DP_1 receptor in mice

Hui Zhao [a,c,1], Kousaku Ohinata [a,c,*,1], Masaaki Yoshikawa [a,b,c]

[a] Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Gokasho Uji, Kyoto 611-0011, Japan
[b] Fronteir Research Center, Graduate School of Engineering, Osaka University, Suita, Osaka 565-0871, Japan
[c] CREST, Japan Science and Technology Agency, Kawaguchi, Saitama, Japan

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ABSTRACT

We found that prostaglandin (PG) D_2, the most abundant PG produced in the central nervous system (CNS), exhibited anxiolytic-like activity at a dose of 10–100 pmol/mouse after intracerebroventricular (i.c.v.) administration in the elevated plus-maze test in mice. A DP_1 receptor-selective agonist, BW245C, mimicked the anxiolytic-like activity of PGD_2, while a DP_2 receptor agonist 13,14-dihydro-15-keto-PGD_2 was inactive. The anxiolytic-like activity of PGD_2 was blocked by a DP_1 antagonist, BWA868C, suggesting that PGD_2-induced anxiolytic-like activity was mediated by the DP_1 receptor. Adenosine A_{2A} or $GABA_A$ receptor antagonists, SCH58261 or bicuculline, respectively, also blocked its anxiolytic-like activity. Taken together, centrally administered PGD_2 may induce anxiolytic-like activity via the A_{2A} and $GABA_A$ receptors, downstream of the DP_1 receptor.

1. Introduction

Prostaglandin (PG) D_2, the most abundant PG in the mammalian central nervous system (CNS) [1], possesses a number of functions, such as sleep induction, hypothermia and attenuation of the pain response [2–7]. Recently we have found that central PGD_2 stimulates food intake in mice [8]. PGD_2 is produced from arachidonic acid, via PGH_2, by lipocalin-type PGD synthase (L-PGDS), which is localized in the arachnoid membrane and choroid plexus of the brain [9,24]. There are two receptor subtypes for PGD_2, DP_1 receptor [10,11] and DP_2 receptor [12–14], which was originally termed a chemoattractant receptor-homologous molecule expressed on TH2 (CRTH2). Both subtypes are G-protein coupled receptors present in the CNS. We have reported that orexigenic activity of PGD_2 is mediated by the DP_1 receptor [8].

Recently we also have found that a bioactive tripeptide rubimetide (Met-Arg-Trp), which was isolated from a pepsin–pancreatin digest of spinach ribulose bisphosphate carboxylase/oxygenase (Rubisco), exhibited anxiolytic-like activity by activating a PGD_2 system in mice [15]. We then investigated whether centrally administered PGD_2 alone shows anxiolytic-like activity using the elevated plus-maze test in mice. We also investigated which of two receptor subtypes for PGD_2 mediates the anxiolytic-like activity of PGD_2, and the mechanism underlying its activity downstream of the receptor for PGD_2.

2. Materials and methods

2.1. Animals

Four-week-old male ddY mice were obtained from SLC (Shizuoka, Japan) [15–19]. 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.

2.2. Reagents

PGD_2 was obtained from Nacalai Tesque, Inc. (Kyoto, Japan). BWA868C, a DP_1 receptor antagonist; BW245C, a DP_1 receptor agonist and 13,14-dihydro-15-keto-PGD_2 ($DKPGD_2$), a DP_2 receptor agonist, were purchased from Cayman Chemical Company (Ann Arbor, MI). Bicuculline, a γ-aminobutyric acid type A ($GABA_A$) antagonist, and SCH58261, an adenosine A_{2A} receptor antagonist, were obtained from Tocris Bioscience (Bristol, UK).

* Corresponding author. Tel.: +81 774 38 3733; fax: +81 774 38 3774.
E-mail address: ohinata@kais.kyoto-u.ac.jp (K. Ohinata).
[1] These authors contributed equally to this work.

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2.3. Elevated plus-maze test

The elevated plus-maze test was performed as described previously [15–19]. Four arms (24 cm long × 5 cm wide) were placed 50 cm above the ground. Two opposite arms were delimited by acrylic vertical walls (13 cm high, closed arms), whereas the other two, opposite arms 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 the 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 number of total entries (% of visit). In this system, diazepam (1 mg/kg, i.p.) exhibited significant anxiolytic-like activity. Intracerebroventricular (i.c.v.) administration was performed as described previously [20–22]. 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. PGD_2, a DP_1 agonist BW245C or DP_2 agonist $DKPGD_2$ was dissolved in dimethylsulfoxide (DMSO) and the solution was diluted 20 times 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). Each drug in 4 μl 5% DMSO ACSF was i.c.v. administered 20 min before the test. An antagonist for the DP_1 receptor was i.c.v. co-administered with PGD_2 20 min before the test. The total number of visits to the closed and open arms, and the cumulative time spent in the open and closed arms were measured on a monitor through a video camera system. Data were checked by observers who were unaware of the experimental groups.

2.4. Statistical analysis

All values are expressed as the means ± S.E.M. Analysis of variance (ANOVA) followed by Fisher's test was used to assess differences among groups. *P*-values less than 0.05 were considered significant.

3. Results

3.1. PGD_2 exhibits anxiolytic-like activity in the elevated plus-maze test

We investigated whether PGD_2 exhibits anxiolytic-like activity after central administration in the elevated plus-maze test in mice. I.c.v. administered PGD_2 at a dose of 10–100 pmol/mouse significantly increased the percentages of the time and visits to the open arms for 5 min in a dose-dependent manner (Fig. 1A and B). PGD_2 did not change the total visits to open and closed arms (Fig. 1C), suggesting that PGD_2 had no effect on locomotor activity. Thus, we found that centrally administered PGD_2 has anxiolytic-like activity in mice.

3.2. PGD_2-induced anxiolytic-like activity is mediated through the DP_1 receptor

To elucidate which of two receptor subtypes for PGD_2 was involved in anxiolytic-like activity of PGD_2, we used selective agonists for DP_1 and DP_2 receptors, BW245C and $DKPGD_2$, respectively. I.c.v. administered BW245C (0.1–1 pmol/mouse) showed a significant increase in % of time spent in the open arms (Fig. 2A). In contrast, $DKPGD_2$ (1–10 pmol/mouse) was inactive (Fig. 2B). Both agonists had no effect on total motor activity (data not shown).

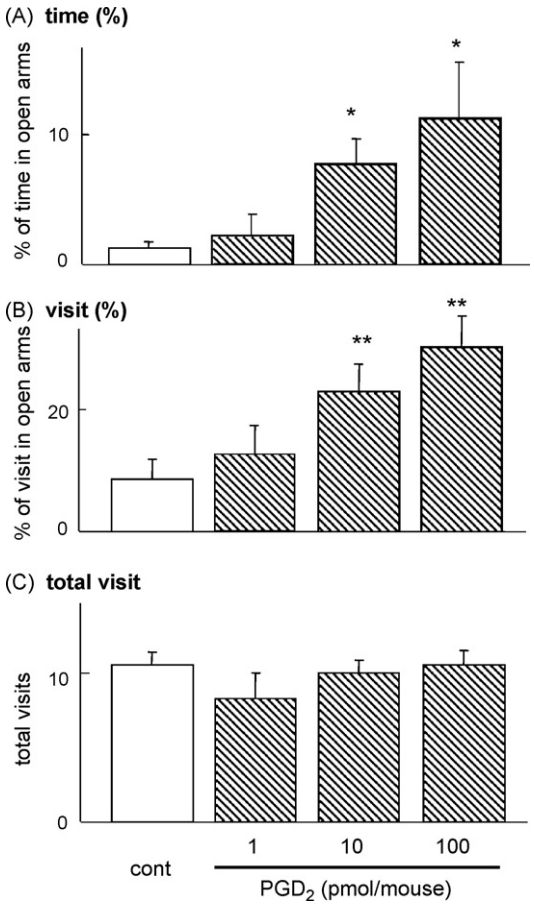

Fig. 1. Anxiolytic-like activity of PGD_2 in the elevated plus-maze test in mice. PGD_2 at a dose of 1–100 pmol/mouse was i.c.v. administered 20 min before the tests. Each value is expressed as the mean ± S.E.M. (n = 5–6). $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ compared with the ACSF-treated control group.

Thus, a DP_1 but not DP_2 receptor agonist exhibited anxiolytic-like activity after central administration. These results suggest that the DP_1 receptor is associated with anxiolytic-like activity of PGD_2.

In order to demonstrate the involvement of the DP_1 receptor in PGD_2-induced anxiolytic-like activity, a DP_1 receptor antagonist, BWA868C was used. PGD_2 (100 pmol/mouse, i.c.v.)-induced increases in % of time and visits to open arms were completely blocked by co-administration with BWA868C (1.6 nmol/mouse, i.c.v.), as shown in Fig. 3A and B. BWA868C alone did not have any effect on anxiety-related behavior (Fig. 3). Thus we demonstrated that the anxiolytic-like activity of PGD_2 after central administration is evidently mediated by the DP_1 receptor.

3.3. Anxiolytic-like activity of PGD_2 is mediated through the $GABA_A$ receptor downstream of the DP_1 receptor

We also tested whether anxiolytic-like activity of PGD_2 was involved in activation of the $GABA_A$ receptor, which is a well-known anxiolytic pathway in the CNS [23], downstream of the DP_1 receptor. I.p. pretreatment of bicuculline, a $GABA_A$ receptor antagonist (5 mg/kg) significantly blocked increases in % of time spent in open arms after i.c.v. administration of PGD_2 (100 pmol/mouse), as shown in Fig. 4A. Bicuculline itself did not have any effect on anxiety-related behavior (Fig. 4A). These results suggest that activation of the $GABA_A$ receptor involves anxiolytic-like behavior induced by PGD_2, downstream of the DP_1 receptor.