



Neuropharmacology and Analgesia

Prostaglandin D₂ sustains the pyrogenic effect of prostaglandin E₂

Wei Gao^{1,2}, Achim Schmidtke^{*,1}, Ruirui Lu, Christian Brenneis, Carlo Angioni, Ronald Schmidt, Gerd Geisslinger

pharmazentrum frankfurt/ZAFES, Institut für Klinische Pharmakologie, Klinikum der Goethe-Universität, Theodor-Stern-Kai 7, 60590 Frankfurt am Main, Germany

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ABSTRACT

Prostaglandin D₂ (PGD₂) is involved in a variety of physiological and pathophysiological processes, but its role in fever is poorly understood. Here we investigated the effects of central PGD₂ administration on body temperature and prostaglandin levels in the cerebrospinal fluid (CSF) of rats. Administration of PGD₂ into the cisterna magna (i.c.m.) evoked a delayed fever response that was paralleled by increased levels of prostaglandin E₂ (PGE₂) in the CSF. The elevated PGE₂ levels were not caused by an increased expression of cyclooxygenase 2 or microsomal prostaglandin E synthase-1 in the hypothalamus. Interestingly, i.c.m. pretreatment of animals with PGD₂ considerably sustained the pyrogenic effects of i.c.m. administered PGE₂. These data indicate that PGD₂ might control the availability of PGE₂ in the CSF and suggest that centrally produced PGD₂ may play a role in the maintenance of fever.

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1. Introduction

Fever is a state of elevated body temperature and a common symptom associated with systemic infectious diseases and inflammation. Considerable evidence indicates that prostaglandin E₂ (PGE₂) is the final central mediator of fever responsible for the elevation of the set point in the hypothalamic thermoregulatory center (Blatteis, 2006; Romanovsky et al., 2005). 'Fever-relevant' PGE₂ is produced from arachidonic acid by cyclooxygenase-2 (COX-2) and microsomal prostaglandin E synthase-1 (mPGES-1), which are induced in endothelial cells of the hypothalamus and in peripheral organs in response to bacterial pyrogens (Engblom et al., 2003; Schiltz and Sawchenko, 2002; Steiner et al., 2006). PGE₂ evokes fever by stimulating the E type prostaglandin (EP) receptor subtype EP₃ in the thermoregulatory center of the hypothalamus (Ushikubi et al., 1998). Accordingly, central injections of PGE₂ evoke fever in animals (for review, see Fraiefeld and Kaplanski, 1998).

In contrast to PGE₂, little is known about the role of other prostanoids in the fever reaction. Prostaglandin D₂ (PGD₂) is the most abundant prostaglandin in whole brain extracts (Narumiya et al., 1982). It is produced from arachidonic acid by action of COX and prostaglandin D synthase (PGDS). PGD₂ is involved in the regulation of several physiological and pathophysiological processes such as sleep induction, attraction of inflammatory cells, allergic asthma,

platelet aggregation, smooth muscle relaxation and hormone release (Kanaoka and Urade, 2003; Urade and Hayaishi, 2000). However, the role of PGD₂ in thermoregulation is poorly understood. The purpose of this study was to investigate the effect of central PGD₂ injections on body temperature and on PGE₂ content in the CSF in order to investigate a possible involvement of PGD₂ in the fever reaction.

2. Materials and methods

2.1. Animals

Male Sprague Dawley rats (Charles River, Sulzfeld, Germany) weighing 250–300 g were used. Animals had free access to standard rat chow and tap water and were housed in a room maintained at 22 ± 1 °C with a 12:12-h light/dark cycle (lights on 07:00 am–07:00 pm). The ethic guidelines for investigations in conscious animals were obeyed and all procedures were approved by the local Ethics Committee for Animal Research.

2.2. Surgical procedures

At least 1 week before the day of an experiment, animals were anesthetized with an isoflurane/carbogen mix and a radiotelemetry transmitter (E-Mitter 4000 system, Mini Mitter, Bend, OR) was inserted into the intraperitoneal cavity for continuous recording of core body temperature (T_c). In addition, a catheter was implanted into the cisterna magna as described previously (Ram et al., 1997), with slight modifications. Briefly, a catheter was constructed by inserting a 1.5 cm long slim polytetrafluoroethylene tube (SUBL-080; ID 0.10 mm,

* Corresponding author. Tel.: +49 69 6301 7819; fax: +49 69 6301 7636.

E-mail address: Schmidtke@em.uni-frankfurt.de (A. Schmidtke).

¹ Both authors contributed equally to this study.

² Current address: Department of Traumatic Surgery, Tongji Hospital, Tongji Medical College of Huazhong University of Science and Technology, Wuhan 430030, People's Republic of China.

OD 0.20 mm; Braintree Scientific, Braintree, MA) for 1 cm into a 8 cm long polyethylene tube (PE10; ID 0.28 mm, OD 0.61 mm; Neolab, Heidelberg, Germany) and fixing of both tubes with cyanoacrylate glue (stabiloplast, Renfert, Hilzingen, Germany). The rat was fixed on a stereotaxic apparatus and a midline incision was made over the lambda skull surface. After retraction of the skin and underlying tissues, the atlanto-occipital membrane was penetrated with a 27 gauge needle and the slim tip of the catheter was inserted towards the cisterna magna. Then the catheter was fixed to the skull with cyanoacrylate glue, tunnelled under the skin through a trocar and pulled out from the dorsal neck area. Finally, the skin incision was sutured and the end of the catheter was sealed by melting. After surgery, rats were housed individually and followed up each day. Only rats without relevant disturbances of general well-being and behavior were used for the experiments. Correct placement and leakage of the catheter was verified after the experiment post mortem by injection of trypan blue followed by brain dissection. Data obtained from rats with misplaced catheters were excluded from analysis.

2.3. Animal treatment

Studies were performed on conscious, unrestrained rats at an ambient temperature of 22 ± 1 °C. One day before the experiment, each cage was placed on a receiver and radiotelemetry signals emitted by the implanted transmitter were continuously monitored in intervals of 5 min using the Vitalview software (Mini Mitter). On the day of the experiment (between 9:00 and 10:00 am), rats were shortly anesthetized with isoflurane and prostaglandins (PGE₂ or PGD₂; Cayman, Ann Arbor, MI) dissolved in artificial cerebrospinal fluid (aCSF; 141.7 mM Na⁺, 2.6 mM K⁺, 0.9 mM Mg²⁺, 1.3 mM Ca²⁺, 122.7 mM Cl⁻, 21.0 mM HCO₃⁻, 2.5 mM HPO₄²⁻, and 3.5 mM dextrose, bubbled with 5% CO₂ in 95% O₂ to adjust pH to 7.2; injection volume 5 µl) were injected into the cisterna magna (i.c.m.) through the previously implanted catheter. In experiments with subsequent injection of prostaglandins, the second prostaglandin was administered 3 h after the first prostaglandin injection. The purity of PGD₂ and PGE₂ (>99.8%) as well as the stability of the PGD₂ and PGE₂ solutions was confirmed by LC-MS/MS analyses. Average T_C values for 20-min periods were computed from T_C recorded at 5-min intervals. For each rat, a baseline temperature was defined as the mean T_C during the 60-min period immediately preceding fever induction. The change in T_C (ΔT_C) was calculated by subtracting the baseline temperature from each recorded T_C value. At the end of the experiments, rats were killed with ketamine and midazolam and the neck muscles were rapidly reflected to gain access and to withdraw a cerebrospinal fluid (CSF) sample (~120 µl) from the cisterna magna. Then the hypothalamus was excised for western blot and RT-PCR analysis. CSF and tissue samples were quickly frozen and stored at -80 °C until use.

2.4. Western blot

Tissue samples were homogenized in PBS containing protease inhibitor cocktail (Complete Mini, Roche Diagnostics, Mannheim, Germany). The homogenate was centrifuged at 4 °C for 10 min at 10000 ×g and the supernatant again for 1 h at 170000 ×g. The pellet of the last centrifugation step was re-suspended in PBS containing protease inhibitor cocktail and 0.25 M sucrose. Extracted proteins (20 µg per lane) were separated by SDS-polyacrylamide gel electrophoresis and transferred onto nitrocellulose membranes by electroblotting. After blocking of nonspecific binding sites with blocking buffer (Odyssey Blocking Buffer, LI-COR Biosciences, Bad Homburg, Germany; diluted 50% in PBS), membranes were incubated overnight at 4 °C with rabbit anti-COX-2 (Cayman) or rabbit anti-mPGES-1 (Agrisera, Vännäs, Sweden) diluted 1:500 in blocking buffer containing 0.1% Tween. Mouse anti-GAPDH (Glyceraldehyde-3-phosphate dehydrogenase, 1:2000; Ambion, Austin, TX) was incubated for 2 h and used as loading control. After incubation

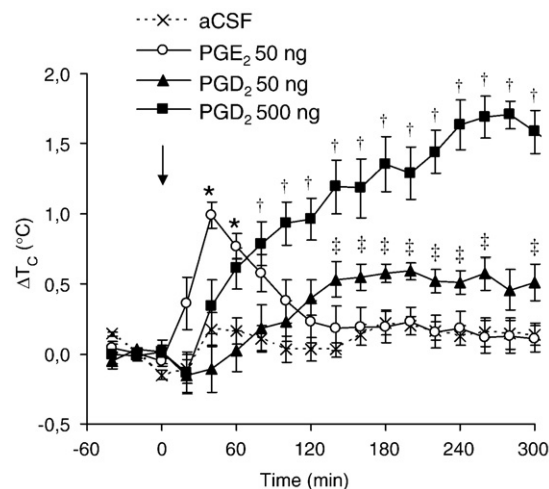


Fig. 1. Changes in core body temperature (ΔT_C) after i.c.m. injection of aCSF ($n=8$), 50 ng PGE₂ ($n=6$), 50 ng PGD₂ ($n=6$) or 500 ng PGD₂ ($n=6$). The injection time point is designated time 0. Baseline T_C were 37.4 ± 0.1 °C (aCSF), 37.6 ± 0.2 °C (PGE₂), 37.6 ± 0.2 °C (50 ng PGD₂) and 37.1 ± 0.2 °C (500 ng PGD₂). Data are expressed as mean $\pm$ S.E.M. *, †, ‡ Significantly different from aCSF group ($P<0.05$) for PGE₂, 50 ng PGD₂ and 500 ng PGD₂, respectively.

with the secondary antibody conjugated with Alexa Fluor 680 or 800 (1:10000; Invitrogen, Karlsruhe, Germany) for 2 h, blots were visualized on a Odyssey Infrared Imaging System (LI-COR Biosciences). The band densities were quantified by densitometry using the Odyssey software. All COX-2 and mPGES-1 levels were normalized relative to the level of GAPDH.

2.5. Determination of prostaglandin concentrations

CSF sample analysis was performed by using liquid chromatography–electrospray ionization–tandem mass spectrometry (LC-MS/MS) as described (Schmidt et al., 2005). The lower limit of quantification was 25 pg/ml for both PGE₂ and PGD₂.

2.6. Statistics

The results are expressed as means $\pm$ S.E.M.. Statistical analysis comprised Student's *t*-test or analysis of variance with subsequent Bonferoni post-hoc test. $P<0.05$ was considered statistically significant.

3. Results

3.1. Centrally administered PGD₂ induces fever

To assess whether PGD₂ affects the body temperature in the absence of any other stimulus, we injected PGD₂, PGE₂ (positive control) or aCSF (vehicle, negative control) into the cisterna magna (i.c.m.) of freely moving rats. Injection of PGE₂ (50 ng i.c.m.) led to a rapid and short-term increase in T_C (Fig. 1), which was similar to that after PGE₂ injection into the lateral ventricle (Oka et al., 2003), the third ventricle (Watanabe et al., 1997) or the preoptic hypothalamic area (Watanabe et al., 1995). Interestingly, administration of PGD₂ (50–500 ng i.c.m.) also increased T_C in a dose-dependent manner. However, the PGD₂-induced rise in T_C started later, developed slower and lasted for a longer time as compared to the PGE₂-induced rise in T_C (Fig. 1).

3.2. PGD₂-induced fever depends on PGE₂

In order to investigate whether the PGD₂-induced fever was mediated by PGE₂ we determined the content of PGE₂ and PGD₂ in the CSF after i.c.m. PGD₂ injection. As shown in Fig. 2, at 3 h after injection of 50 ng or 500 ng PGD₂ the concentration of PGE₂ was significantly

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