Neuroscience Letters 495 (2011) 98–101

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Contribution of peripheral versus central EP1 prostaglandin receptors to inflammatory pain

Torbjörn Johansson[a,b], Shuh Narumiya[c], Hanns Ulrich Zeilhofer[a,b,*]

[a] Institute of Pharmacology and Toxicology, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland
[b] Institute of Pharmaceutical Sciences, ETH Zurich, Wolfgang-Pauli Strasse 10, CH-8093 Zurich, Switzerland
[c] Department of Pharmacology, Kyoto University Faculty of Medicine, Sakyo-ku, Kyoto 606-8501, Japan

ARTICLE INFO

Article history:
Received 20 January 2011
Received in revised form 15 March 2011
Accepted 16 March 2011

Keywords:
Hyperalgesia
Spinal cord
Dorsal horn
Pain relief
Intrathecal
NSAIDs

ABSTRACT

Prostaglandin E_2 (PGE_2) is a key mediator of exaggerated pain sensation during inflammation. Drugs targeting the PGE_2 pathway by global inhibition of cyclooxygenases are well established in the treatment of inflammatory pain, but also cause significant unwanted effects. Enzymes downstream of the cyclooxygenases, or prostaglandin receptors are candidate targets possibly enabling therapeutic intervention with potentially fewer side effects. Among the PGE_2 receptors, the EP1 subtype has repeatedly been proposed as a promising target for treatment of inflammatory hyperalgesia. However its involvement in sensitization at specific (peripheral or central) sites has not been thoroughly investigated. Here, we have used mice deficient in the EP1 receptor ($EP1^{-/-}$) to address this issue. $EP1^{-/-}$ mice showed normal mechanical and heat sensitivity during baseline conditions. Local subcutaneous PGE_2 injection into one hindpaw, caused thermal and mechanical sensitization in wild-type mice and $EP1^{-/-}$ mice. Thermal sensitization in $EP1^{-/-}$ mice was less than in wild-type mice while no significant difference was seen for mechanical sensitization. Injection of PGE_2 into the subarachnoid space of the lumbar spinal cord, resulted in a similar mechanical sensitization in $EP1^{-/-}$ mice and in wild-type mice, while a tendency towards reduced reaction to noxious heat stimulation was observed in $EP1^{-/-}$ mice. These results support a major contribution of EP1 receptors to peripheral heat sensitization, but only a minor role in mechanical sensitization and in spinal heat sensitization by PGE_2. After local subcutaneous zymosan A injection, $EP1^{-/-}$ mice showed indistinguishable mechanical and heat sensitization compared with wild-type mice. Taken together, these results suggest that peripheral EP1 receptors contribute significantly to inflammation induced heat pain sensitization while evidence for a contribution to central sensitization was not obtained.

PGE_2 is a major contributor to exaggerated pain sensitivity during inflammation. Its production requires the activity of at least one of the two cyclooxygenase (COX) isoforms, constitutively expressed COX-1 or inducible COX-2, with COX-2 being particularly relevant for inflammation-induced PGE_2 formation [2,18]. Most classical non-steroidal anti-inflammatory drugs (NSAIDs) block COX-1 and COX-2 to similar degrees, while the more recently developed "coxibs" are COX-2-selective. Although these drugs often provide excellent relief from inflammatory pain, in particular their long-term use is frequently associated with side effects. Traditional NSAIDs cause upper gastrointestinal tract ulcerations [21], and the use of COX-2-selective inhibitors is associated with an increased risk of cardiovascular events [3].

For this reason, downstream components of the inflammatory pathway have been investigated in the search for alternative targets. Targeting such downstream elements might result in safer and more tolerable analgesics. PGE_2 mediates its effects through binding to four G-protein-coupled receptors (EP1-4) [14]. Although work with subtype-specific agonists has suggested that several of these EP receptors interfere with neural excitability during baseline- and inflammatory conditions either at spinal [1] or at peripheral sites [22], most drug discovery and development studies have focused on the EP1 receptor [5].

Use of $EP1^{-/-}$ mice demonstrated a role of this receptor in mediating especially peripheral heat sensitization via facilitating the activation of the transient receptor potential (TRP) channel V1 after subcutaneous PGE_2 injection [11]. Furthermore, experiments using local injections of presumed selective EP1 receptor blockers

* Corresponding author at: Institute of Phamacology and Toxicology, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland.
Tel.: +41 44 635 59 12; fax: +41 44 635 59 88.
E-mail address: zeilhofer@pharma.uzh.ch (H.U. Zeilhofer).

0304-3940/$ – see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2011.03.046

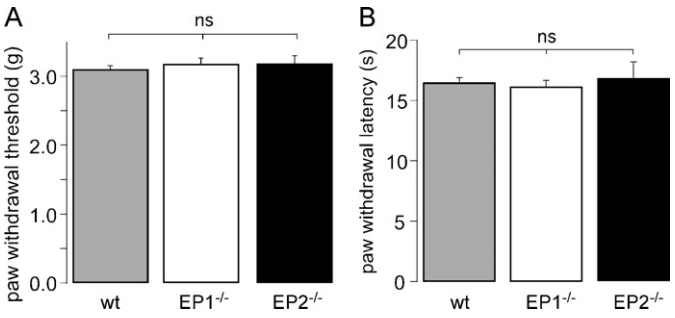

Fig. 1. Mechanical thresholds and thermal paw withdrawal latencies in naïve animals. (A) Baseline thresholds (g) to mechanical stimuli in wild-type, EP1$^{-/-}$ and EP2$^{-/-}$ mice. Number of mice, $n = 20$, 24, and 4, for wild-type, EP1$^{-/-}$ and EP2$^{-/-}$, respectively. (B) Baseline paw withdrawal latencies (s) in wild-type, EP1$^{-/-}$ and EP2$^{-/-}$ mice. $n = 21$, 21, and 5, for wild-type, EP1$^{-/-}$ and EP2$^{-/-}$, respectively. ns, not significant. Dunnett's Multiple Comparison test (panels A and B).

have suggested that EP1 receptors also are involved in mechanical sensitization at the spinal cord level. These rat studies utilized the EP1 selective antagonist ONO-8711 in the carrageenan model of inflammatory pain [13] and in a model for postoperative pain [15]. However, the contribution of this receptor to spinal inflammatory hyperalgesia is not firmly established and our own work in genetically modified mice has assigned the EP2 receptor a major role in spinal pain sensitization during inflammatory pain states [7,17]. Here, we have used EP1$^{-/-}$ mice to investigate the peripheral versus central contribution of this receptor to mechanical- and heat sensitization during inflammation.

Behavioural experiments were carried out in sex-matched groups of 7–9 weeks old EP1 (*ptger1*, EP1$^{-/-}$) [20] and EP2 (*ptger2*, EP2$^{-/-}$) [8] receptor-deficient mice, and wild-type mice (C57BL/6).

On day 1 of the experiment each mouse was tested several times to obtain baseline values for paw withdrawal latencies or mechanical response thresholds. Paw withdrawal latencies upon exposure to defined radiant heat stimuli were measured using a plantar test apparatus (Ugo Basle). Mechanical responses were obtained using an electronic von Frey anesthesiometer (IITC). Separate groups of animals were used for thermal and mechanical testing. In all behavioural experiments, the observer was blind to the genotype of the animals being tested. For intrathecal PGE_2 injections, PGE_2 was dissolved in 1% ethanol and 99% saline. Intrathecal injections were done using a Hamilton syringe (with spacer) into the lower lumbar spinal canal. In total 0.4 nmol PGE_2 were injected in a volume of 4 μl. For subcutaneous PGE_2 injections, PGE_2 was dissolved in 0.1% DMSO and 99.9% saline. A total of 5 nmol PGE_2 was injected in 5 μl subcutaneously into the hindpaw using a Hamilton syringe. For subcutaneous zymosan A injections, the baker's yeast extract from *Saccharomyces cerevisiae* was suspended in saline. A total amount of 0.06 mg in 20 μl was subcutaneously injected using a Hamilton syringe into the hindpaw. Permission for animal experiments has been obtained from the Veterinäramt des Kantons Zürich.

Sensitization to thermal or mechanical stimuli was determined as percent changes from pre-injection baselines. Reactions scores (insets in Figs. 2–4) were calculated as percent change in withdrawal latencies/thresholds integrated over time for the duration of the experiment.

We first addressed a potential role of EP1 or EP2 receptors in the maintenance of baseline nociceptive sensitivity. Baseline sensitivities to noxious heat and mechanical stimulation were determined in naïve wild-type mice, and compared with those of naïve animals lacking either the EP1 or the EP2 receptor. Mechanical sensitivity (Fig. 1A) was assessed by applying punctuate mechanical stimuli using electronic von Frey filaments. The mechanical thresholds of wild-type mice were indistinguishable from those of EP1$^{-/-}$ or EP2$^{-/-}$ mice (3.1 ± 0.07 g, 3.2 ± 0.09 g, and 3.2 ± 0.09 g, mean ± SEM). Heat sensitivity was determined by measuring the withdrawal latency in response to a defined radiant heat stimulus using a plantar test apparatus (Fig. 1B). Again, reactions of wild-type mice were not different from those of EP1$^{-/-}$ nor EP2$^{-/-}$ mice (16.0 ± 0.6 s, 15.9 ± 0.6 s, and 16.6 ± 1.0 s, mean ± SEM). These results suggest that neither EP1 nor EP2 receptors contributed to baseline nociceptive sensitivity.

We continued to investigate the contribution of the EP1 receptor to nociceptive sensitization by utilizing its natural ligand PGE_2 and tested the effect of local subcutaneous injection of PGE_2 (5 nmol in 5 μl) into one hindpaw on mechanical and heat pain thresholds. Wild-type mice displayed maximum sensitization to mechanical stimuli 30 min after injection of PGE_2 (Fig. 2A, baseline: 3.0 ± 0.1 g, sensitized: 1.5 ± 0.2 g, mean ± SEM). This sensitization was indistinguishable from that of EP1$^{-/-}$ mice (baseline: 2.8 ± 0.1 g, sensitized: 1.4 ± 0.2 g, mean ± SEM). PGE_2 also resulted in a strong heat sensitization in wild-type mice that reached its maximum 30 min after injection (Fig. 2B, baseline: 17.1 ± 1.0 s, sensitized: 1.8 ± 0.3 s, mean ± SEM). However, as reported previously, EP1$^{-/-}$ mice were significantly less sensitized [11] (baseline: 16.2 ± 1.1 s, sensitized: 7.7 ± 1.3 s, mean ± SEM), while EP2$^{-/-}$ mice behaved similar to wild-type mice (baseline: 15.9 ± 1.0 s, sensitized: 1.2 ± 0.2 s, mean ± SEM). These data confirm the role of EP1 mediated heat sensitization after local peripheral PGE_2 injection [11].

To investigate the relevance of EP1 receptors in central (spinal) pain sensitization, mice were injected with PGE_2 (0.4 nmol in 4 μl) directly into the subarachnoid space of the spinal canal, i.e., intrathecally. Intrathecal PGE_2 injection in wild-type mice led to strong mechanical sensitization (Fig. 3A, baseline: 3.2 ± 0.1 g, sensitized: 1.5 ± 0.2 g, mean ± SEM). This sensitization was the same in EP1$^{-/-}$ animals (baseline: 3.1 ± 0.1 g, sensitized: 1.5 ± 0.3 g, mean ± SEM). However, as previously reported [17], EP2$^{-/-}$ animals did not show any sensitization by intrathecally injected PGE_2 (baseline: 3.2 ± 0.1 g, sensitized: 3.1 ± 0.1 g, mean ± SEM), pointing to the central role of EP2 in mechanical sensitization. These results suggest that EP1 receptors in the CNS are not involved in mechanical inflammatory pain sensitization in the mouse. Injection of PGE_2 into the spinal canal of wild-type animals also resulted in heat sensitization (Fig. 3B, baseline: 16.5 ± 0.6 s, sensitized: 11.3 ± 0.9 s, mean ± SEM). In EP1$^{-/-}$ mice this sensitization was slightly less compared to wild-type mice (baseline: 17.5 ± 0.6 s, sensitized: 14.4 ± 0.8 s, mean ± SEM), but this difference did not reach statistical significance ($P = 0.29$, unpaired Student t-test).

Finally, by injecting zymosan A into the hindpaw the contribution of EP1 to pain sensitization was studied in a model that resembles a more complex natural inflammation (Fig. 4). This was particularly important as the expression of EP receptors might change during inflammation. In wild-type mice, zymosan A caused local paw swelling and led to strong mechanical and thermal sensitization. EP1$^{-/-}$ mice showed virtually identically responses throughout the time course of the experiment. Because at the dose employed, zymosan A-induced pain sensitization is mainly due to sensitization induced by spinally produced PGE_2 [17], the absence of a phenotype in this test is consistent with only a minor contribution of EP1 receptors to spinal pain sensitization.

Among the four PGE_2 receptors, the EP1 subtype has been proposed as one of the most promising targets against inflammatory hyperalgesia. Early work showed that EP1$^{-/-}$ mice exhibited significantly reduced nocifensive responses to intraperitoneal injection of acetic acid and to 2-phenyl-1,4-benzoquinone (PBQ) [19], and a tendency to reduced responses in the formalin test [10,16]. Subsequent development of EP1 receptor antagonists proved analgesic activity in a variety of pain models. One of the first specific EP1 receptor antagonists that became available was ONO-8711. This compound reduced mechanical hyperalgesia in nerve injured rats