

Contribution of peripheral versus central EP1 prostaglandin receptors to inflammatory pain

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ABSTRACT

Prostaglandin E₂ (PGE₂) is a key mediator of exaggerated pain sensation during inflammation. Drugs targeting the PGE₂ 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 PGE₂ 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 PGE₂ 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 PGE₂ 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 PGE₂. 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.

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PGE₂ 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 PGE₂ 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. PGE₂ 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 PGE₂ injection [11]. Furthermore, experiments using local injections of presumed selective EP1 receptor blockers

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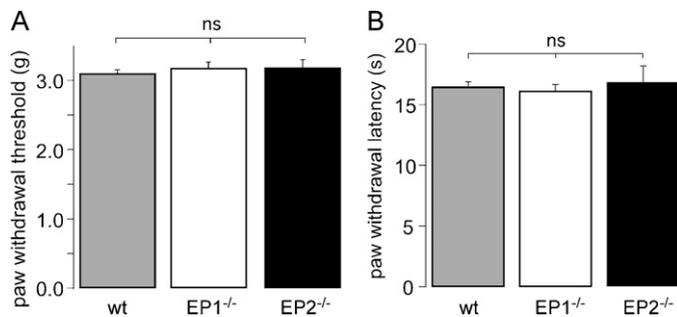


Fig. 1. Mechanical thresholds and thermal paw withdrawal latencies in naive animals. (A) Baseline thresholds (g) to mechanical stimuli in wild-type, EP1^{-/-} and EP2^{-/-} mice. Number of mice, $n=20$, 24 , and 4 , for wild-type, EP1^{-/-} and EP2^{-/-}, respectively. (B) Baseline paw withdrawal latencies (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 Basile). 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 PGE₂ injections, PGE₂ 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 PGE₂ were injected in a volume of 4 μ l. For subcutaneous PGE₂ injections, PGE₂ was dissolved in 0.1% DMSO and 99.9% saline. A total of 5 nmol PGE₂ was injected in 5 μ l 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 μ l 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. Reaction 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 naive wild-type mice, and compared with those of naive 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 \pm 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 \pm 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 PGE₂ and tested the effect of local subcutaneous injection of PGE₂ (5 nmol in 5 μ l) into one hindpaw on mechanical and heat pain thresholds. Wild-type mice displayed maximum sensitization to mechanical stimuli 30 min after injection of PGE₂ (Fig. 2A, baseline: 3.0 ± 0.1 g, sensitized: 1.5 ± 0.2 g, mean \pm SEM). This sensitization was indistinguishable from that of EP1^{-/-} mice (baseline: 2.8 ± 0.1 g, sensitized: 1.4 ± 0.2 g, mean \pm SEM). PGE₂ 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 \pm 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 \pm SEM), while EP2^{-/-} mice behaved similar to wild-type mice (baseline: 15.9 ± 1.0 s, sensitized: 1.2 ± 0.2 s, mean \pm SEM). These data confirm the role of EP1 mediated heat sensitization after local peripheral PGE₂ injection [11].

To investigate the relevance of EP1 receptors in central (spinal) pain sensitization, mice were injected with PGE₂ (0.4 nmol in 4 μ l) directly into the subarachnoid space of the spinal canal, i.e., intrathecally. Intrathecal PGE₂ 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 \pm SEM). This sensitization was the same in EP1^{-/-} animals (baseline: 3.1 ± 0.1 g, sensitized: 1.5 ± 0.3 g, mean \pm SEM). However, as previously reported [17], EP2^{-/-} animals did not show any sensitization by intrathecally injected PGE₂ (baseline: 3.2 ± 0.1 g, sensitized: 3.1 ± 0.1 g, mean \pm 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 PGE₂ 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 \pm 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 \pm 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 identical 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 PGE₂ [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 PGE₂ 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

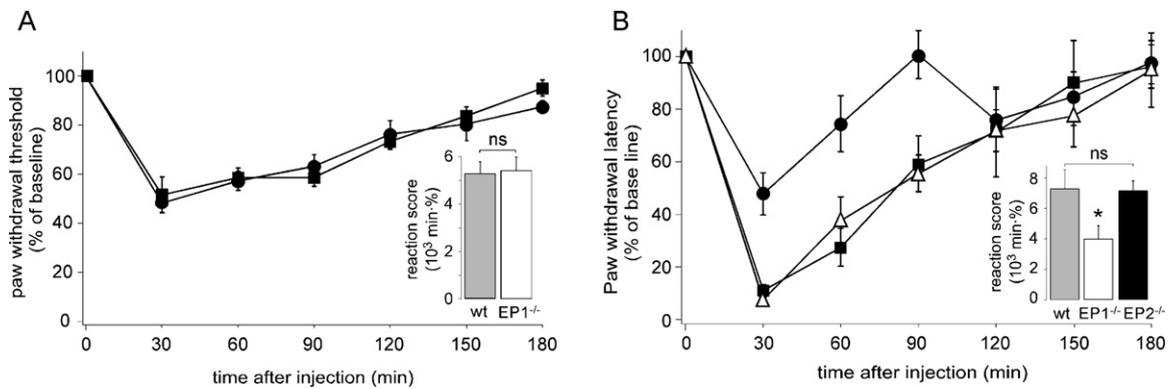


Fig. 2. Peripheral mechanical and thermal sensitization after subcutaneous PGE₂ injection into the hindpaw. (A) Mechanical baseline thresholds compared with thresholds after PGE₂ injection in wild-type (■) and EP1^{-/-} (●) mice. Inset: reaction scores calculated as changes in withdrawal thresholds integrated over time. Unpaired Student's *t*-test. (B) Paw withdrawal latencies at baseline compared with latencies after injection of PGE₂ in wild-type (■), EP1^{-/-} (●), and EP2^{-/-} (△) mice. Graphs represent reaction scores in percent of baseline values (mean ± SEM) after subcutaneous injection of PGE₂ (5 nmol in 5 μl) into the plantar side of one hindpaw. Insets: same as A but withdrawal latencies. * *P* < 0.05 significant against wild-type and EP2^{-/-} (Dunnett's Multiple Comparison Test), *n* = 5–8 mice/group.

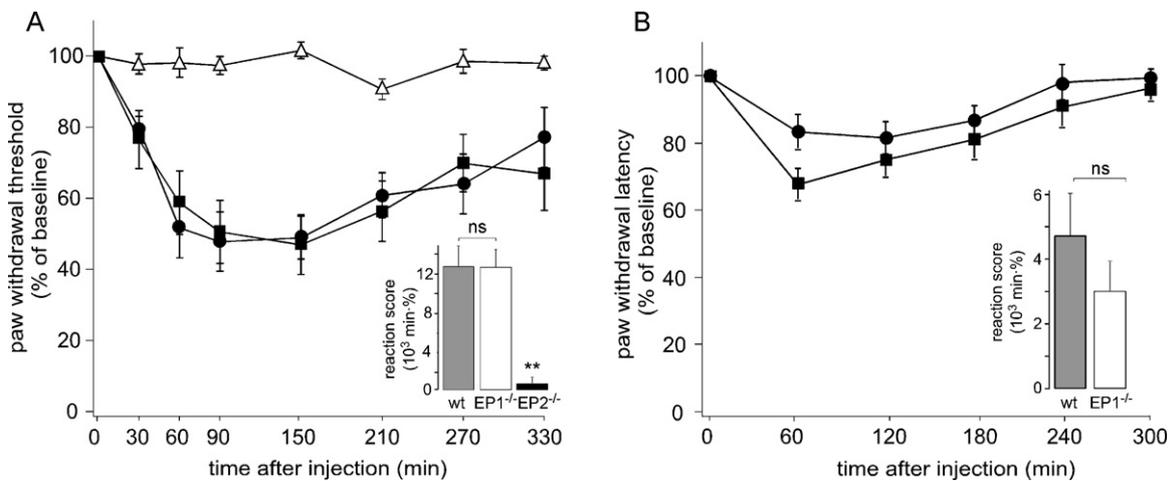


Fig. 3. Central (spinal) mechanical and thermal sensitization after intrathecal PGE₂ injection. (A) Mechanical baseline thresholds compared with thresholds after PGE₂ injection in wild-type (■), EP1^{-/-} (●), and EP2^{-/-} (△) mice. ** *P* < 0.01 (Dunnett's Multiple Comparison Test), *n* = 6, 8, and 4 for wild-type, EP1^{-/-}, and EP2^{-/-}, respectively. (B) Paw withdrawal latencies at baseline compared with latencies after injection of PGE₂ in wild-type (■) and EP1^{-/-} (●) mice. Graphs represent reaction scores in percent of baseline values (mean ± SEM) after intrathecal injection of PGE₂ (0.4 nmol in 4 μl). Insets: reaction scores calculated as changes in withdrawal thresholds/latencies integrated over time. *n* = 15, for both wild-type and EP1^{-/-}. Unpaired Student *t*-test.

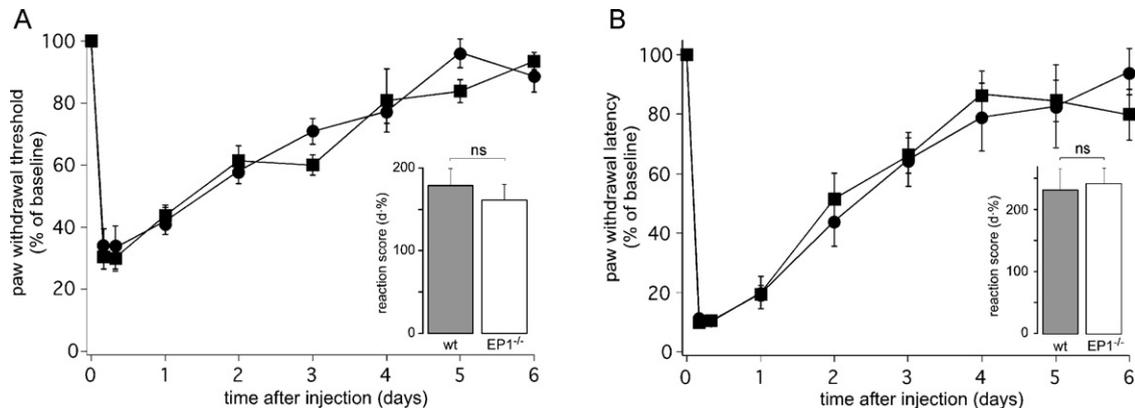


Fig. 4. Mechanical and thermal sensitization after subcutaneous zymosan A-induced inflammation. (A) Mechanical baseline thresholds compared with thresholds after injection of zymosan A (0.06 mg in 20 μl) in wild-type (■) and EP1^{-/-} (●) mice. (B) Paw withdrawal latencies at baseline compared with latencies after injection of zymosan A in wild-type (■) and EP1^{-/-} (●) mice. Insets: reaction scores calculated as changes in withdrawal thresholds/latencies integrated over time. Unpaired Student *t*-test, *n* = 6–8 mice per group.

after systemic administration [9], in rats after intrathecal injection, in the carrageenan model [13], in an incision model of postoperative pain [15], or when intrathecally co-injected together with PGE₂ [12]. The more specific ONO-8713 has been reported to reverse thermal hyperalgesia in mice when co-injected peripherally together with PGE₂ [11]. However, further data on ONO-8713 in other pain models is not available. Other EP1 receptor antagonists more recently developed by GlaxoSmithKline showed analgesic activity after systemic administration in a sub-chronic model of knee joint arthritis [4,6]. Although these studies provide significant evidence for an analgesic action of EP1 receptor antagonists, a systematic analysis of the contribution of peripheral versus central sites and on the relevance of EP1 receptors for heat and mechanical sensitization is largely lacking. This may still be clinically relevant as a significant contribution of central EP1 receptors would prompt for antagonists being able to cross the blood brain barrier, and because chronic pain patients appear to suffer more from mechanical hypersensitivity than from heat hyperalgesia.

Our present results support a major role of EP1 receptors in peripheral heat sensitization and a smaller contribution to central heat sensitization but no contribution to mechanical sensitization. While the contribution of peripheral EP1 receptors to heat hyperalgesia is in good agreement with the antagonist study by Moriyama et al. [11], its role in mechanical and spinal sensitization is more controversial and the exact reasons for the discrepant findings are difficult to determine. Possible explanations include species differences (mice versus rats), compensatory up-regulations of other EP receptors in the gene deficient mice, and off-target effects of antagonists. The latter may be particularly relevant when antagonists are injected locally, in this case intrathecally, at concentrations which exceed the K_i values at the EP1 receptor by several orders of magnitude. Reduced hyperalgesic responses to intrathecally or subcutaneously injected PGE₂ in EP2 receptor deficient mice reported in the present study and in previous studies [7,17] (for a review see [23]) clearly indicate that EP receptors different from EP1 are also involved in pain sensitization.

These findings and findings from other groups suggest that for optimal efficacy potential analgesic drugs targeting EP receptors should antagonize more than one EP receptor subtype and should also penetrate the blood brain barrier.

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