Contribution of peripheral versus central EP1 prostaglandin receptors to inflammatory pain

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Keywords: 
Hyperalgesia 
Spinal cord 
Dorsal horn 
Intrathecal 
NSAIDs

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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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-8771 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 [15]. However, the contribution of this receptor to spinal inflammatory hyperalgesia is not firmly established and our own work in [15]. However, the contribution of this receptor to spinal inflammatory hyperalgesia is not firmly established and our own work in [15]. However, the contribution of this receptor to spinal inflammatory hyperalgesia is not firmly established and our own work in

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 µl) 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 ± SEM). This sensitization was indistinguishable from that of EP1−/− mice (baseline: 2.8 ± 0.1 g, sensitized: 1.4 ± 0.2 g, mean ± 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 ± 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 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 µl) 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, 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 PGE2 (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 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 ± 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 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-8771. 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 alleviate pain [15], or when intrathecally co-injected together with PGE₂ [12]. 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 hyper-sensitivity 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 Ki 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.

Acknowledgements

We thank Robert Witschi and Ulrike Zeilhofer for help with initial experiments and Louis Scheurer and Isabelle Camenisch for genotyping of mice. This work has been supported in part by a grant from the DFG to HUZ.

References