Prostaglandin-mediated inhibition of serotonin signaling controls the affective component of inflammatory pain

Anand Kumar Singh, Joanna Zajdel, Elahe Mirrasekhian, Nader Almoosawi, Isabelle Frisch, Anna M. Klawonn, Maarit Jaarola, Michael Fritz, and David Engblom
Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden.

Pain is fundamentally unpleasant and induces a negative affective state. The affective component of pain is mediated by circuits that are distinct from those mediating the sensory-discriminative component. Here, we have investigated the role of prostaglandins in the affective dimension of pain using a rodent pain assay based on conditioned place aversion to formalin injection, an inflammatory noxious stimulus. We found that place aversion induced by inflammatory pain depends on prostaglandin E2 that is synthesized by cyclooxygenase 2 in neural cells. Further, mice lacking the prostaglandin E2 receptor EP3 selectively on serotonergic cells or selectively in the area of the dorsal raphe nucleus failed to form an aversion to formalin-induced pain, as did mice lacking the serotonin transporter. Chemogenetic manipulations revealed that EP3 receptor activation elicited conditioned place aversion to pain via inhibition of serotonergic neurons. In contrast to their role in inflammatory pain aversion, EP3 receptors on serotonergic cells were dispensable for acute nociceptive behaviors and for aversion induced by thermal pain or a κ opioid receptor agonist. Collectively, our findings show that prostaglandin-mediated modulation of serotonergic transmission controls the affective component of inflammatory pain.

Introduction

Pain is an adaptive warning signal under physiological conditions, but it also causes a lot of suffering during various pathological states. Pain is a complex phenomenon with many components. The sensory component provides information about location, intensity, and quality of the painful event (1). In addition, pain is inherently unpleasant and induces a negative affective state (2, 3). Converging evidence indicates that the brain regions mediating the sensory and the affective components of pain are, at least partly, distinct (1, 4–6).

Prostaglandins are key regulators of nociceptive processing during inflammation, and prostaglandin synthesis inhibitors in the form of nonsteroidal antiinflammatory drugs are widely used for their analgesic properties. Prostaglandins modulate nociceptive signaling at many levels of the neuraxis (7), but it is unclear how they are involved in the affective dimension of pain.

Results and Discussion

To investigate the affective component of pain in mice, we used conditioned place avoidance induced by inflammatory pain. This test, in which mice learn to avoid a chamber where they experienced pain induced by formalin injection to the hind paw, has been used extensively to investigate the affective component of pain in rodents (3, 5, 6, 8). To explore the role of prostaglandins, we first interfered with the cyclooxygenases COX1 and COX2 (also known as PTGS1 and PTGS2), enzymes responsible for prostaglandin synthesis. Normal (WT) mice, mice lacking COX1, and mice pretreated with a COX1 selective inhibitor (sc560, 5 mg/kg, i.p.; Figure 1B) avoided the chamber paired with formalin injections (Figure 1, A and B; for figures showing the behavioral experiments with individual values indicated, see Supplemental Figure 1; supplemental material available online with this article; https://doi.org/10.1172/JCI90678DS1). In contrast, the aversion was completely blocked in genetically modified mice lacking COX2 activity (Figure 1C) and when COX2 was inhibited ( parecoxib, 10 mg/kg) during the pain sessions (Figure 1D). To identify the cell type producing the critical prostaglandins, we next used the Cre/loxP system to delete Cox2 in specific cell types. Mice without COX2 in myeloid cells (Cox2fl/fl LysM-Cre; Figure 1E) or in brain endothelial cells (Cox2fl/fl Slo1cl-Cre mice; Figure 1F) displayed aversion comparable to that of littermates without Cre (WT mice). In contrast, mice without COX2 in neural cells (Cox2fl/fl Nes-Cre mice), including peripheral and central neurons and glia, showed no avoidance behavior (Figure 1G).

Prostaglandin E2 is the principal proinflammatory prostanoid and an important regulator of nociceptive and systemic inflammatory responses (7, 9–11). We tested mice lacking mPGES-1, the inducible form of prostaglandin E synthase, in our pain model (mPges-1 KO mice). These mice displayed significantly lower aversion scores compared with their WT littermates (Figure 1H). Prostaglandin E2 binds to 4 G protein–coupled receptors called EP, to EP4 (encoded by Ptger1-4). We tested mice lacking EP1 or EP4 receptors, since these are strongly expressed in brain structures related to motivation (12, 13) and have been shown to be implicated in nociceptive processing (14–16). Mice lacking EP1Rs (Ptger1–/– mice, referred to as EPIR KO mice) did not show any difference in their ability to avoid the aversive formalin chamber compared with WT mice (Figure 1I), indicating that EP1Rs are dispensable for place aversion induced by inflammatory pain. In contrast, mice lacking EP4Rs (Ptger4–/– mice, referred to as EPIR KO mice) showed a significant decrease in aversion scores compared with WT mice (Figure 1I), and chemogenetic manipulation showed that EP4Rs are necessary for the inflammatory-induced aversion (Figure 1J).
aversion scores compared with WT littersmate (Figure 2A). In contrast, Ptger3 knockout (EP3R KO) mice displayed no pain-induced aversion (Figure 2B).

EP3 receptors are expressed in many structures of the brain and the spinal cord. Given the important role of serotonin in the regulation of affective functions, the EP3 receptor expression in serotonergic neurons of the dorsal raphe nucleus and other serotonergic structures (12) is particularly interesting in the context of aversion. To explore the role of EP3 receptors on serotonergic neurons, we next used Ptger3fl/fl Sert-Cre mice (referred to as EP3R-SERTCre mice). In these mice, Ptger3 is deleted by Cre expressed under control of the serotonin transporter (Sert) promoter. As expected, Cre expression was specific to serotonergic neurons, identified by labeling for tryptophan hydroxylase (TpH) (Figure 2, C and D). Further, EP3R-SERTCre mice displayed a clear reduction of Ptger3 mRNA in tissue punches from the dorsal raphe region, whereas no reduction was seen in the cortex or the hypothalamus/thalamus (Supplementary Figure 2A). Subsequently, we tested the EP3R-SERTCre mice in the conditioned pain-avoidance test. We observed a complete abrogation of the aversion in EP3R-SERTCre mice (Figure 2E). Further, aversion was completely blocked in mice lacking the serotonin transporter SERT (Figure 2F).

To determine whether the ascending serotonergic pathways mediated the aversion, we deleted Ptger3 receptors in the region of the dorsal raphe nucleus. This was done by stereotaxic injection of Cre-expressing viral vectors (AAV5) into mice with floxed Ptger3. Mice with injections that affected the dorsal raphe nucleus (Figure 2G) without extending to the descending raphe nuclei showed blocked aversion (Figure 2H). Collective-

ly, these findings strongly suggest that ascending serotonergic transmission is critical for pain-induced aversion and that prostaglandin E2 can modulate such transmission by a direct effect on EP3 receptors on serotonergic neurons.

Since most EP3R splice variants are coupled to inhibitory G proteins, we investigated whether EP3R activation elicits aversion by inhibition of serotonergic neurons. To test this, we used a designer receptor exclusively activated by designer drug–based (DREADD-based) chemogenetic approach (19) to maintain firing in serotonergic cells during the painful experience and monitored the aversive response of the mice. We injected viral vectors with Cre-dependent expression of Gq-coupled DREADDs (hM4Di) or mCherry in EP3R-SERTCre mice (AAV-hM4Dq Sert-Cre and AAV-mCherry Sert-Cre mice, respectively; Figure 2I) and activated the cells by administration of clozapine N-oxide (CNO) before the formalin injections. Strikingly, chemogenetic activation of serotonergic cells blocked the aversion (Figure 2J). Further, mice without EP3Rs on serotonergic cells, which normally display no pain-induced aversion, showed a normal aversion when their serotonergic cells were inhibited during the formalin-pain session (Figure 2, K and L). This was achieved by stereotaxic injections of AAVs encoding Gq-coupled DREADDs (hM3Dq) or mCherry in EP3R-SERTCre mice (AAV-hM3Dq Sert-Cre and AAV-mCherry Sert-Cre mice, respectively; Figure 2K).

Next, we investigated whether the prostaglandin-dependent pathway identified was specific to the affective component of pain or whether it was necessary for all aspects of pain. We monitored acute formalin-induced nociceptive behaviors (lifting, shaking, and licking the injected paw) in mice subjected to pharmacological inhibition of COX2 in mPGES-1 KO mice and in EP3R-SERTCre mice. Pharmacological inhibition of COX2, as well as lack of mPGES-1, markedly reduced the nociceptive behaviors in the second phase of the formalin test (Figure 3, A and...
displayed normal sucrose preference, normal withdrawal latencies in the hot-plate test, and normal real-time avoidance of a hot floor (Supplemental Figure 2, B–D). Thus, prostaglandin-mediated modulation of serotonergic signaling selectively controls aversion to inflammatory pain.

The finding that EP3 receptors on serotonergic neurons regulate the affective component of pain is in line with the central role of serotonin in affective functions (17) and may have relevance for the high comorbidity of pain and depression. Populations of serotonergic cells are modulated by aversive stimuli (22), including pain (23), as well as by rewarding stimuli (24). B), whereas they were unaffected in EP3R-SERTCre mice (Figure 3, C and D). This indicates that prostaglandin E2 modulates many components of pain, but that the effect on serotonergic transmission selectively controls the affective dimension. We also found a normal febrile response in EP3R-SERTCre mice (Figure 3E), indicating that responses driven by EP3Rs in nonserotonergic cells were intact (20, 21). Further, aversion induced by the κ opioid receptor agonist U-50488 (2.5 mg/kg, i.p.) was intact in EP3R-SERTCre mice (Figure 3F), and both Cox2fl/fl Nes-Cre and EP3R-SERTCre mice displayed a robust aversion to thermal pain (Figure 3, G and H). EP3R-SERTCre mice also displayed normal sucrose preference, normal withdrawal latencies in the hot-plate test, and normal real-time avoidance of a hot floor (Supplemental Figure 2, B–D). Thus, prostaglandin-mediated modulation of serotonergic signaling selectively controls aversion to inflammatory pain.

The finding that EP3 receptors on serotonergic neurons regulate the affective component of pain is in line with the central role of serotonin in affective functions (17) and may have relevance for the high comorbidity of pain and depression. Populations of serotonergic cells are modulated by aversive stimuli (22), including pain (23), as well as by rewarding stimuli (24).
Further, serotonergic neurotransmission mediates adaptive danger avoidance (25), can induce fear and anxiety (26), and regulates affective responses to stress (27, 28). The affective responses to stress are mediated by a transient increase in serotonin reuptake in the ventral striatum, leading to a local hypo-serotonergic state (29). Prostaglandin-mediated inhibition of serotonin reuptake in the ventral striatum, leading to a local hypo-serotonergic state, is not restricted to inflammatory pain (defined as 30–40 minutes after injection). See Supplemental Methods for calculation of nociceptive scores. Corresponding data for mice lacking EP3 receptors in serotonergic cells (EP3R-SERTCre; Cox2fl/fl) show means of the scores during the peak of the second phase of the formalin-induced pain (defined as 30–40 minutes after injection). See Supplemental Methods for calculation of nociceptive scores. Corresponding data for mice lacking EP3 receptors in serotonergic cells (EP3R-SERTCre; Cox2fl/fl) show means of the scores during the peak of the second phase of the formalin-induced pain (defined as 30–40 minutes after injection). See Supplemental Methods for calculation of nociceptive scores.

Methods
Additional methods are provided in the Supplemental Methods.

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Author contributions
AKS, JZ, and DE were responsible for overall study design. AKS performed the behavioral/physiological experiments except for those involving EP1-KOs (AMK and MF), fever (EM), or thermal pain and sucrose preference (NA, IF, and JZ). MF designed, implemented, and introduced others to the formalin conditioning protocol. Viral injections and histological validations were done by JZ. Quantitative PCR (qPCR) analysis was done by JZ, AKS, MJ, and IF. The manuscript was written by DE, AKS, and JZ. All authors were involved in the design of the parts of the study they executed, discussed the design and results, and commented on the manuscript.
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Address correspondence to: David Engblom, Department of Clinical and Experimental Medicine, Linköping University, 58185 Linköping, Sweden. Phone: 46.101038448; E-mail: david.engblom@liu.se.